REVIEW ARTICLE

Peptide models for membrane channels

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Peptides may be synthesized with sequences corresponding to putative transmembrane domains and/or pore-lining regions that are deduced from the primary structures of ion channel proteins. These can then be incorporated into lipid bilayer membranes for structural and functional studies. In addition to the ability to invoke ion channel activity, critical issues are the secondary structures adopted and the mode of assembly of these

short transmembrane peptides in the reconstituted systems. The present review concentrates on results obtained with peptides from ligand-gated and voltage-gated ion channels, as well as proton-conducting channels. These are considered within the context of current molecular models and the limited data available on the structure of native ion channels and natural channel-forming peptides.

INTRODUCTION

Membrane channels provide one of the major avenues of communication across cell membranes. Of these, the ion channels that are responsible for nerve excitation and control of the transmembrane potential are the most studied and will be concentrated on here. The availability of amino acid sequences for many membrane channel proteins opens up the possibility of direct structural and functional studies on synthetic peptides that correspond to putative transmembrane and channel-lining sequences [1–3]. Such studies and their relevance to the intact channel assemblies are the subject of this review. This model membrane approach represents a considerable simplification of the system that allows detailed biophysical characterization, but carries with it attendant uncertainties in extrapolation to the natural state. Amongst the critical issues are the relative importance of different secondary structures and the mode of assembly of short transmembrane peptides in reconstituted systems.

The review focuses on the following topics, taken in order. First, methods for identification of putative transmembrane domains and pore-lining sequences are dealt with, before going on to a review of the limited amount of direct structural information that is available on intact channel proteins. Then the structures of natural channel-forming peptides are considered because currently it is for these that the most detailed information is available. The remainder of the review then concentrates on synthetic peptides that correspond to the putative transmembrane domains of intact channel proteins. Topics covered are secondary structure, channel-forming activity and mode of assembly in reconstituted systems. The different natural and synthetic peptides discussed are listed in Table 1.

DEDUCTIONS FROM PRIMARY SEQUENCE AND MUTAGENESIS

Hydropathy plots

The usual method for identifying putative transmembrane segments in a channel of known sequence is from hydropathy plots of the hydrophobicity of adjacent amino acid residues averaged over a moving window of suitable length [33–35]. The hydro-

phobicity scale is defined from the transfer free energy of amino acids between organic solvents and water, and statistics on the distribution of residues in proteins of known structure. Typically, a stretch of 20–23 amino acids of high hydrophobicity is sufficient to define a transmembrane segment, if this is in an α -helical conformation. For a β -sheet conformation less than half this number would be required, if the β -strands are not tilted (e.g. see [36]). Putative transmembrane domains identified from hydropathy profiles are given in Figure 1 for the major families of ion channel proteins, both ligand-gated and voltage-gated. These schemes are consistent with the indicated segments being α -helical in conformation and they largely provide the current working models for the structure of these different families of channels. A notable exception is that of the nicotinic acetylcholine receptor for which recent structural data suggest that a large part of the transmembrane segments is not α -helical but possibly β -sheet [37]. For ionotropic glutamate receptors, recent evidence (reviewed in [38]; see also [39]) suggests that the C-terminus may be located on the opposite (i.e. cytoplasmic) side of the membrane from the N-terminus. This would imply an odd number of transmembrane segments, unlike the prototypic scheme indicated for ligand-gated channels in Figure 1 (see [39]).

Hydrophobic moment

The hydropathy profile is sufficient to identify putative transmembrane sequences if these are predominantly hydrophobic. The method has been verified for the bacterial reaction centre structure [40] and for bacteriorhodopsin [35]. In integral proteins that contain multiple transmembrane segments, however, this must not always be the case. For these more complex structures, all or part of certain transmembrane segments may contact other protein segments, rather than the hydrophobic lipid chains. In this case, depending on the internal protein contacts, the hydrophobicity may be lower [41] and, for pore-lining segments, a partly hydrophilic face will be expected. The hydrophobic moment [42,43] may be used to identify amphipathic segments, such as those lining aqueous channels, and the direction of the hydrophobic moment can then give information on the orientation of the transmembrane segment within the membrane plane. For complex, polytopic, transmembrane proteins, how-

Table 1 Natural channel-forming peptides of known structure and putative transmembrane segments of channel proteins

N, number of residues; <H>, mean hydrophobicity; < μ_α > and < μ_β >, absolute values of mean hydrophobic moment of α -helical and β -sheet structures respectively, calculated with the normalized 'consensus' hydrophobicity scale [2,42]. Sequences are given using the standard single-letter code, with U=Aib, O=hydroxyproline and J=isovaline. Alignments for voltage-activated channels are given according to [32]. *Definitions: gA, gramicidin A; alm, alamethicin; L-zrv, Leu-zervamicin; mel, melittin; δ -tx, δ -haemolysin; M1 δ AChR and M2 δ AChR, M1 and M2 segments of the δ -subunit of *Torpedo californica* nicotinic acetylcholine receptor; M2 α 4AChR, M2 segment of α_4 subunit of rat neuronal nicotinic acetylcholine receptor; MA β AChR, MA segment of the β -subunit of *Torpedo* nicotinic acetylcholine receptor (in the link between the M3 and M4 segments); M1GlyR, M2GlyR and M4GlyR, M1, M2 and M4 segments of the α_1 -subunit of rat glycine receptor; M2NMDAR, M2 segment of NMDA receptor; M1CFTR–M6CFTR, M1–M6 segments of the CFTR Cl channel; CaIVS1–CaIVS6, S1–S6 segments from repeat IV of L-type voltage-gated Ca channel; CaIS2, S2 segment from repeat II of dihydropyridine receptor Ca channel; KS2 and KS4, S2 and S4 segments of *Drosophila* Shaker voltage-activated K channel; NaIS3 and NaIS6, S3 and S6 segments from repeat I of rat brain voltage-activated Na channel; NaIS4 and NaIVS4, S4 segment of repeats I and IV of *Electrophorus electricus* voltage-activated Na channel; NaIS4(r), S4 segment of repeat I of rat brain voltage-activated Na channel; NaIVS4–S45, S4 segment with S4–S5 linker from repeat IV of *E. electricus* Na channel; CaIVH5, H5 segment from repeat IV of L-type voltage-gated Ca channel; NaIH5–NaIVH5, putative pore regions of repeats I–IV of *Electrophorus electricus* voltage-activated Na channel (designated PR-I to PR-IV); KH-5, KH-5-N, KH5 and KH5 sept., putative pore region of *Drosophila* Shaker A voltage-gated K channel; TM-minK and K26, apolar domain of rat Isk (minK) slow voltage-activated K channel; Pep-8a, central hydrophobic segment from subunit 8 of *Saccharomyces cerevisiae* proton F_1F_0 -ATPase.

Peptide* sequence	N	<H>	< μ_α >	< μ_β >	Ref
<i>natural peptides:</i>					
gA f-VGALAVVWLWLWLW-Etn	15	0.90	–	–	4
alm Ac-UPUAUAQUVUGLUPVUUEQF-OH	20	0.54	0.23	–	5
L-zrv Ac-LIQJITULUOQUUPF-OH	16	0.56	0.34	–	6
mel f-GIGAVLKVLTTGLPALISWIKRKRQQ-NH ₂	26	0.10	0.35	–	7
δ -tx f-MAQDIISTIGDLVKWIIDTVNKFTHK	26	0.12	0.60	–	8
<i>transmembrane segments (ligand-gated):</i>					
M1 δ AChR LFYVINFITPCVLISFLASLAFY	23	0.71	0.09	0.12	9
M2 δ AChR EKMSTAISVLLAQAVFLLLSQR	23	0.24	0.25	0.03	9
M2 α 4AChR EKVTLCISVLLSLTVFLLITE	22	0.52	0.11	0.31	10
MA β AChR GLTQPVTLQDLKEAVEAIKYIAEQLK	27	0.06	0.34	0.21	11
M1GlyR PYLIQMYIPSLILVILSWISFWA	23	0.68	0.20	0.03	12
M2GlyR PARVGLGITTVLMTTQSSGSRA	23	0.10	0.21	0.20	12
ARVGLGITTVLMTTQSSGSRA	22	0.10	0.22	0.22	13
M4GlyR RIGFPMFLIFNMFWIYK	20	0.52	0.31	0.17	13
M2NMDAR ALTLSSAMWFSWGVLLNSGIGE	22	0.48	0.12	0.17	3
<i>transmembrane segments (phosphorylation regulated):</i>					
M1CFTR RFMFYGIFLYLGEVTKAVQPLLLG	24	0.42	0.28	0.18	14
M2CFTR RSIAIYLGIGLCLLFIVRTLIL	22	0.55	0.20	0.19	14
M3CFTR GLALAHFVWIAPLQVALLMGLI	22	0.74	0.08	0.10	14
M4CFTR ASAFGLGLFVLVLAFLQAGLG	21	0.71	0.10	0.01	14
M5CFTR SAFFFSGFFVFLSVLPYALI	21	0.79	0.09	0.09	14
M6CFTR KGIILRKIFTTISFCIVLRMAV	22	0.33	0.04	0.34	14
<i>transmembrane segments (voltage-gated):</i>					
CaIVS1 TYFEYLMFVLILLNTICLAMQH	22	0.52	0.16	0.01	15
CaIIS2 Ac-DFANRVLLSLFTIEMLLKMYGL-NH ₂	22	0.33	0.21	0.01	16
CaIVS2 IAMNILLMLFTGLFTVEMILK	21	0.52	0.33	0.16	15
KS2 ITDPFFLIETLCIIWTFELTVRFLA	26	0.52	0.14	0.29	17
CaIVS3 DPWNVDFDLVIGSIIIDVILSE	22	0.52	0.23	0.12	15
NaIS3 DPWNWLDFTVITFAVTEFVDL	22	0.39	0.15	0.13	18
NaIS4 RTFRVLRALKTITIFPGLKTIVRA	24	0.02	0.23	0.25	19
NaIS4 (r) ALRTFRVLRALKTISVIPGLK	21	0.06	0.20	0.16	20
NaIVS4 RVIRLARIARVLRRLIRAAKGIR	22	–0.25	0.16	0.10	21
NaIVS4–S45 Ac-TLFRVIRLARIARVLRRLIRAAKGIRTLFAIMMS-NH ₂	34	0.09	0.16	0.00	22
CaIVS4 NSRISITFFRLFRVRLIKLLSR	23	–0.08	0.30	0.36	15
KS4 Me-ILRVIRLVRFIRIKLSRHS-NH ₂	20	–0.10	0.11	0.02	23
CaIVS5 YVALLIVMLFFIYAVIGMQMFGK	23	0.71	0.10	0.20	15
CaIVS6 VFYFISFYMLCAFLIINLFVAVI	23	0.85	0.20	0.00	15
NaIS6 IFFVLVIFLGSFTLILNLAVV	22	0.91	0.13	0.04	24
CaIVH5 FQTFPGAVLLLFRCATGEAWQ	21	0.32	0.28	0.10	15
NaIH5 DNFAWTFCLFRLMLQDYWENLYQMT	26	0.18	0.31	0.27	25
NaIIH5 DFFHSFLIVFRALCGEWIETMWDCME	26	0.31	0.32	0.18	25
NaIIIH5 DNAGMGYLSLLQVSTFKGWMIDIMYA	25	0.30	0.11	0.12	25
NaIVH5 KKQGVDDIFNFETFGNSMICLFEITTSAGWDGLLL	36	0.25	0.08	0.03	25
KH-5 AFWWAVVTMTTVGYGDMTPVG	21	0.49	0.10	0.07	26
KH-5-N SFFKSIPDAFWWAVVTMTTVGYG	23	0.44	0.29	0.05	26
KH5 f-DAFWWAVVTMTTVGYGDMT-NH ₂	19	0.41	0.10	0.15	27
KH5 sept. KSIPDAFWWAVVTMTTVGYGDMTPGK	26	0.26	0.21	0.04	28
TM-minK SKLEALYILMVLGFFGFFTLGIMLSYIRSKKL	32	0.40	0.06	0.06	29
K26 K_EALYILMVLGFFGFFTLGIMLSYIR	26	0.54	0.19	0.03	30
<i>transmembrane segment (proton ATPase):</i>					
Pep-8a Ac-GFLLMITLLILFSQFFLPMILR-NH ₂	22	0.66	0.24	0.07	31

Figure 1 Arrangement of the putative transmembrane segments in the sequences of ion-channel proteins, or their subunits, as identified by hydropathy analysis

The N-terminus is on the left and all putative transmembrane sequences (boxes) consist of approx. 20 or more residues. For voltage-gated Na and Ca channels, the arrangement shown is for one of the four internal repeats in the sequence. For the voltage-gated channels, segment S4 does not have a high net hydrophobicity because every third residue is positively charged; the intervening residues are mainly hydrophobic and it is thought to be a transmembrane segment contributing to the voltage sensor. The region marked H5 is identified from mutational and blocking experiments as being associated with the pore of voltage-gated channels. For ligand-gated channels, the arrangement applies to all four (homologous) subunits. nAcCh, nicotinic acetylcholine. For ATP-binding cassette (ABC) channels, only the first of the two repeats is shown. For the CFTR the two repeats are linked by the intervening regulatory phosphorylation domain that is absent from the multidrug-resistance (MDR) P-glycoprotein. For F₁F₀-type ATPases, the proteolipid subunit c, which is thought to form the proton channel, corresponds to only half of that indicated for the 16 kDa polypeptide of the V-ATPases.

ever, not all highly amphiphilic stretches are necessarily transmembrane. A case in point is the MA segment of the nicotinic acetylcholine receptor [43], which is no longer thought to be a pore-lining sequence [11]. For the latter, the more hydrophobic M2 segment is accepted as constituting the lining of the pore (see below). The mean hydrophobicities and the absolute values of the mean hydrophobic moment (calculated for both α -helical and β -sheet structures) for various membrane peptides that have been studied are given in Table 1.

Mutagenesis and labelling

The prediction methods based on the sequence can be augmented by a variety of chemical labelling [44] and/or mutational experiments, in addition to proteolysis [45] and antibody labelling [46]. Cysteine-scanning mutagenesis can be used, in which label sites are introduced systematically at positions throughout the sequence. Examples of applications are the nicotinic acetylcholine receptor [47], voltage-gated K⁺ channels [48,49] and the V-ATPase proton channel [50]. Some of these techniques, with

specific regard to channel structure, have been discussed recently [51].

In vitro mutagenesis is particularly helpful in establishing functionally important transmembrane segments. For instance, site-directed mutations and domain swapping, in conjunction with electrophysiological measurements and the use of channel blockers, have been employed to identify the pore region [52–54] and the voltage sensor [55–58] of voltage-gated ion channels. These two domains are marked by the H5 region and the S4 segment respectively in the schemes shown in Figure 1. Other details relating to voltage-gated channel structure obtained by mutagenesis are reviewed in refs. [59,60]. In the nicotinic acetylcholine receptor, and other ligand-gated channels, the M2 segment (see Figure 1) has been identified with the ion pore by similar mutagenesis and labelling experiments (reviewed in [37,61]).

On the one hand, the success of the above procedures is necessary for the identification of the correct sequences for use in the design of appropriate peptide models. On the other hand, the testing of these predictions with the isolated peptide domains in

reconstituted membranes can further provide a valuable additional check on their reliability.

STRUCTURES OF INTACT CHANNELS

Nicotinic acetylcholine receptor

The structure of an intact ion channel in a membrane environment that currently has been obtained to highest resolution (9 Å; 0.9 nm) is that of the ligand-gated nicotinic acetylcholine receptor from a muscle-like synapse [37,62]. This structure has yielded some surprises. Whereas previously the intramembranous segments of the bacterial reaction centre [63] and of bacteriorhodopsin [64] had been found to be entirely α -helical, which is also the case for the plant and bacterial light-harvesting complexes [65,66], it was found that part of the transmembrane segments of the nicotinic acetylcholine receptor are likely to be composed of β -sheet. The receptor channel structure is depicted in a sideways projection in Figure 2(a). The projection in the plane of the membrane (not shown) reveals electron density that corresponds to only one transmembrane α -helix from each of the four subunits that make up the $\alpha_3\beta\gamma\delta$ heteropentamer. These helices line the central pore in the channel structure and can be aligned with the M2 transmembrane segments in each of the subunits (cf. Figure 2b). They are enclosed on the lipid-facing side by a continuous rim of electron density that is insufficient to represent α -helices but could correspond to a β -sheet. It was suggested [37] that the overall transmembrane secondary structure resembles that of the B-subunit pentamers of the soluble enterotoxin [67] and verotoxin-1 [68] from *Escherichia coli*. Each

of these latter structures consists of a ring of five helices surrounded mainly by β -sheet, and the M1, M3 and M4 segments of the receptor subunits may form sets of three anti-parallel β -strands as are found in these proteins. Studies on the secondary structure of the nicotinic acetylcholine receptor by using IR spectroscopy have revealed that the proteinase-resistant transmembrane domain contains both α -helical and β structures [69], consistent with the proposal of a β -sheet scaffold surrounding pore-forming α -helices. Quantitatively, it was found that the intramembranous secondary structure was: α -helical, 50%; β structure and turns, 40%; random, 10%. This implies the existence of more than the four transmembrane stretches predicted by simple hydropathy plots.

The M2 helices constituting the pore are bent in the region of the highly conserved residue Leu-251 (dark pink in Figure 2b). Other (polar) residues that have been identified as lining the channel are the hydroxy-amino acids coloured light pink in Figure 2(b) and the two glutamates (E) at the lower and upper ends of M2. The conserved Leu-251, together with the leucine residues at homologous positions in the other subunits, form the gate of the closed channel, possibly as in a leucine zipper [37]. In the open channel configuration the kink in the M2 helix is preserved but has rotated away to the side, removing the gate that was formed by the hydrophobic leucine residues [62]. The narrowest constriction of the open pore is then formed by the lower face of M2, with the hydroxy-containing residues aligned almost parallel to the axis of the channel.

Bacterial toxins

The X-ray crystal structures of several bacterial toxins that are

Figure 2 Channel structure of the nicotinic acetylcholine receptor from *Torpedo marmorata*

(a) Closed channel in profile obtained from the membrane structure at 9 Å resolution. The positions of the channel-lining transmembrane α -helices are marked by heavy lines and the estimated limits of the lipid bilayer by dotted lines 30 Å apart. (b) Helical net plot of the M2 segment of the α -subunit. The dashed line identifies a face composed entirely of small residues, flanked by bulky hydrophobic side chains (dotted line) used in aligning with electron densities in the three-dimensional map. The arrow shows the estimated position of the kink in the helix, and the bilayer-spanning segment is included in the shaded rectangle. Coloured residues are discussed in the text. Reproduced from [37], with the permission of Academic Press.

found to form membrane channels have been determined. One of the most significant of these is that for pro-aerolysin from *Aeromonas hydrophila*, because a direct connection with the pore structure was established in this case [70]. Proteolytic activation of the pro-toxin exposes a large hydrophobic patch on the major lobe of the protein. This lobe contains a high proportion of β -sheet structure composed in part of very long strands. This sheet adopts a helical twist such that the hydrophilic face undergoes a 180° rotation as it reaches the C-terminal domain. Fitting the X-ray structure to electron microscope images from two-dimensional membrane crystals suggests that the aerolysin channel is a heptameric structure. The C-terminal domains, which consist almost entirely of β -strands, traverse the bilayer, with the hydrophobic faces exposed to lipid and the hydrophilic faces lining the aqueous channel. The likely topology is that of a β -barrel, as in porins, resulting in a large transmembrane pore.

The membrane insertion of a variety of bacterial toxins is activated by large changes in conformation, possibly with molten globule structures as intermediates. The crystal structures of colicin, diphtheria toxin and δ -endotoxin have revealed their translocation domains to be characteristic of 'inside-out' membrane proteins (reviewed in [71]). The common structure of this domain consists of an α -helical bundle of between seven and ten helices, some of which are hydrophobic, are of sufficient length to span the membrane and are buried in the soluble toxins. Membrane insertion is suggested to be mediated by these helical hairpins of the hydrophobic core. Because the toxins can form pores in artificial membranes the structure of the inserted domain most probably also consists of a helical bundle rearranged such that the polar surfaces of the more amphiphilic helices face the channel lumen. The assembly and pore-forming ability of a putative membrane-spanning peptide (FLTTYAQAANTHLF-LLKDAQIYG) derived from the *Bacillus thuringiensis* δ -toxin has been studied [72].

Annexin V

The annexins are a family of cytosolic, calcium-dependent, membrane-binding proteins. The X-ray structure of annexin V, which is known to form voltage-gated Ca^{2+} channels in lipid bilayers, has been determined and found to be almost entirely α -helical [73]. Although this amphiphilic protein binds peripherally to negatively charged phospholipids at the membrane surface, without major structural rearrangement, the structure reveals a prominent central hydrophilic pore that has been associated with the calcium-sensitive channel [74]. The membrane permeability is proposed to arise from a destabilization of the lipid bilayer. Four repeats are arranged around the central protein pore, which consists of a four-helix barrel contributed by two helices each from repeats II and IV. The residues lining the pore are highly conserved and many are charged. Mutational analysis revealed that Glu-95 is essential for the cation selectivity filter, Glu-17 and Glu-78 outside the pore modulate the ion conductance, and Glu-112 is the main voltage sensor controlling the gating of the channel [74,75]. Like the B-subunits of enterotoxin and verotoxin-1 (see above), annexin V is, therefore, a further example of a soluble protein for which details of the configuration of a hydrophilic pore can be deduced from the high-resolution structure.

Porins

The porins, which form aqueous channels in the outer membrane of Gram-negative bacteria, constitute one of the few classes of integral membrane proteins for which the high-resolution struc-

tures have been solved by X-ray crystallography [76–78]. The secondary structure is predominantly β -sheet (approx. 60%), with reverse turns, and the transmembrane pore is formed by the lumen of an anti-parallel β -barrel that is constricted by an internal loop. The number of β -strands in the barrel is 16 or more, although OmpA, for which the structure has not yet been determined, is predicted to consist of only eight membrane-spanning strands [79]. The β -strands in porin from *Rhodobacter capsulatus* are composed of 7–18 residues, with a mean length of 11 residues, and are tilted at angles of 30–60° with respect to the membrane normal [76,77]. The porin molecules are present as stable trimers and therefore not all of the outward-facing residues are exposed to lipid, nor is the height of the barrel necessarily as great as the bilayer thickness over the whole of its perimeter. The membrane-facing outer surface is composed of hydrophobic or non-polar residues with a girdle of aromatic residues close to each bilayer surface, and the interior of the pore has a lining similar in composition to the outer surface of water-soluble proteins. Such structures would not be identified as membrane-spanning by conventional hydropathy analysis (cf. [79,80]).

In general, porins allow the transmembrane diffusion of small water-soluble molecules. The OmpF and PhoE porins, whose structure has been determined [78], are weakly cation-selective and weakly anion-selective respectively [81]. Both these porins (and others), when reconstituted in planar bilayers, show large distinct channel conductances that are voltage-gated [81–84]. The pore size is restricted by the internal loop within the barrel lumen, and is 7 Å × 11 Å for OmpF and 7 Å × 9 Å for PhoE, which is reduced to 3 Å × 6 Å by ordered water molecules [78]. At the level of the constriction, three arginine residues stacked at the barrel wall face a cluster of acidic residues situated between the internal loop and the wall. The mutation L125E at this region in PhoE reverses the ion selectivity to cationic [84]. In general, the selectivity seems to be determined by the net electric field at the mouth of the pore [85].

Channel models

In the absence of detailed structural information on any of the voltage-gated ion channels, current experimental strategies and channel design for these proteins are based partly on molecular modelling [32,86–88]. The fact that the homologous Na and Ca channels contain four internal repeats has led to the proposal of a four-fold tetrameric structure for these channels, and also for the A-type K channel subunit that contains only a single analogous repeat (cf. Figure 1). This supposition, which was supported by electrophysiological studies on the charybdotoxin sensitivity of hetero-oligomers [89], has been substantiated recently by low-resolution images of the purified Shaker K channel [90]. A three-dimensional model has been put forward for the K channel [86] that is consistent with mutagenesis and channel-blocking data, the most salient of which is the involvement of the H5 or P-region in the channel lining. In addition to the segments S1–S6, a putative amphipathic helix S45 in the linking region between S4 and S5 is postulated to span part of the membrane, and an amphipathic helix that contacts lipid chains is also postulated in the S2–S3 link. The structure is composed of three concentric cylinders, with the outer cylinder comprising α -helical segments S1–S3 and S5, all of which are exposed to lipid and span the bilayer. The intermediate cylinder is composed of α -helical segments S4, S45 and S6. The overall twisted superhelical structure is rather different from packing motifs involving helical bundles. Voltage gating is proposed to take place by the helical screw mechanism, in which the positively charged S4 segment moves a considerable way out of the membrane. The inner

cylinder lines the pore and, at its most confined, is composed of the most conserved part of the H5 region in a short eight-stranded β -barrel which penetrates half-way through the membrane. The remaining, wider, section of the pore is composed of S45 and part of S6 from the middle cylinder. Subsequently it has been proposed that both ends of the P-segment are α -helical, forming the vestibule, and that the selectivity filter is formed by the linking sequence TTVGYGD (in Shaker) which contains β -turn and random coil conformations [91]. An anti-parallel β -hairpin model has been offered recently for the structure of the mouth of the Na channel [92]. This model, composed of decapeptides from the H5 regions of the four internal repeats, was aimed primarily at creating a structure for the toxin binding site. The hairpins do not form a barrel, but rather a cone-shaped outer vestibule. Questions of the unsatisfied hydrogen bonds in the backbone and the relationship with the rest of the protein structure were left unresolved.

Other models have concentrated on the pore region of the voltage-gated channels, most notably on long β -barrel structures. Secondary structure prediction and hydrophobic moment profiling have led to the proposal of a general β -turn- β -hairpin motif for the H5 segment of various voltage-gated and related K channels, independent of their numbers of putative transmembrane segments [88]. Not all predictions were compatible with a β -barrel of sufficient length to span the entire membrane. It is likely that there is variability amongst the different K channels. A long β -barrel model has been constructed for the pore of NGK2 (Kv3.1) K channels [87,93]. The 21-residue H5 pore segment (formerly called SS1-SS2) has the sequence PIGFWWAVVTMTTLGYGDMYP. The barrel has a right-handed twist with a 35° tilt (shear number +8) and is composed of eight anti-parallel strands contributed symmetrically by four β -hairpins, one from each subunit of the tetramer. The height of the barrel is 26 Å. The hairpin turn involves five residues centred on residue 10 of H5 (Thr-397 in NGK2; Thr-439 in Shaker) and has four unsatisfied hydrogen-bond pairs. Of the 12 possible hairpin configurations, on balance this was most in accord with the experimental data on pore-lining residues and channel blocking. It also has the most dense packing of side chains in the barrel lumen and therefore is most likely to display high ion selectivity. The channel is most constricted at the level of the aromatic residues at positions 4, 6 and 16, with the ring of hydroxy groups from the conserved tyrosine having a diameter of 3 Å. With the exception of one threonine, all polar side chains line the pore. The model provides an energetically feasible channel with plausible K⁺ permeability [93]. This model has been described in some detail because it offers an alternative structure that may have a direct relevance to experiments on isolated peptides in lipid bilayers.

NATURAL PEPTIDE CHANNELS

In following the strategy of simplifying complex, polytopic and often multisubunit intrinsic channels down to single putative transmembrane segments, it is of interest to consider the known structures of small peptide antibiotics that form channels in membranes. The principal of these are gramicidin A and alamethicin, the electrical activity of which has been studied extensively in lipid bilayers. These two peptides are found to be prototypic of two quite different modes of membrane channel formation.

Gramicidin A

Gramicidin A from the bacterium *Bacillus brevis* is a linear 15-residue hydrophobic peptide, the amino acids of which have

alternating L- and D-configurations. Both N- and C-terminal residues are blocked and both have the L-configuration; the amino acid sequence of the peptide is: HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHCH₂CH₂OH.

Gramicidin A is selective for univalent cations and gives rise to well defined single-channel events in planar lipid bilayers, with a conductance that is comparable with that of natural channels. X-ray diffraction of single crystals grown from organic solvents has yielded structures that consist of left-handed, anti-parallel, double-stranded β -helices [94–96]. These structures are of sufficient length to span the hydrophobic thickness of a lipid bilayer, and have a central pore which is large enough to transport univalent cations. The double-helical structure is, however, incompatible with a variety of functional studies which indicate that the ion-conducting channel is formed by a head-to-head gramicidin dimer in lipid bilayers (reviewed in [4]). This plasticity in the structure of small peptides, a point that will be returned to later in connection with the α - and β -secondary structure of peptides composed solely of L-amino acids, has to be borne in mind.

The structure of gramicidin A in a phospholipid bilayer matrix has been determined from the orientational constraints provided by solid-state NMR measurements on isotopically labelled peptides in oriented samples, coupled with energy refinement [97]. The channel-forming structure consists of right-handed, single-stranded β -helices with six to seven residues per turn (Figure 3). The hydrogen bonding topology is that of a β -sheet in which the carbonyl oxygens of the peptide backbone are tipped towards the channel lumen and the hydrophobic side chains are all directed outwards towards the lipid. This amphipathic feature of the structure, which results from the alternating D- and L-amino acids in gramicidin, is not possible in membrane proteins, which are composed solely of L-amino acids. For the latter, the side chains of adjacent residues in a β -helix would alternate between channel-facing and lipid-facing orientations. More residues per turn would then be required to accommodate the bulk of the side chains that are situated within the channel lumen, and correspondingly more residues per peptide would be needed to span an equivalent width of the bilayer (cf. [98]). This second constraint could be alleviated in multiple-stranded β -helices such as are found in the crystals of gramicidin A. In the bilayer structure, the side chains of the tryptophan residues in the C-terminal parts of the head-to-head gramicidin A dimer are oriented with the indole -NH groups directed towards the hydrophilic bilayer surface (Figure 3). This is a feature that is found for tryptophan (and equivalently tyrosine) residues in the known high-resolution structures of integral membrane proteins [77,99] and is thought to assist in the vertical positioning of the protein/peptide in the membrane.

Alamethicin

Alamethicin is a 20-residue peptide from the fungus *Trichoderma viride* which contains a high proportion of the unusual amino acid α -aminoisobutyric acid (Aib), in addition to two proline residues, and is capable of forming voltage-gated channels of very high conductance in lipid membranes. The N-terminus of the peptide is blocked and the C-terminal residue is L-phenylalaninol (Phl). The amino acid sequence is: Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Glu-Phl.

Such peptaibol peptides which contain several Aib residues have a strong propensity to form helices, including 3₁₀-helices [100]. The X-ray crystal structure determined for alamethicin

Figure 3 Channel structure of gramicidin A

Stereo view of the computationally refined structure in the lipid bilayer that is obtained from orientational restraints provided by solid-state NMR. The tryptophan indole-NH groups are clustered at the bilayer surface, and the N-terminus for each monomer is buried at the bilayer centre. Side chains of the central part of the helix are omitted for clarity. Reproduced courtesy of R. R. Ketchem and T. A. Cross. See also [97].

shows it to be predominantly α -helical with a bend, punctuated by a partial 3_{10} -helical turn, that is generated by the proline residue at position 14 [5] (Figure 4a). A somewhat similar structure was found by NMR for alamethicin in solution (methanol) [102]. The three polar residues, Gln-7, Glu-18 and Glu-19, have their side chains extended from the convex face of the helix, and the presence of Pro-14 causes the backbone carbonyls of Aib-10 and Gly-11 also to be exposed on this face. The latter increases the amphiphilic nature of the helix beyond that calculated from the amino acid sequence in Table 1 and contributes to its channel-forming properties.

The concentration dependence of the transmembrane current and the single-channel conductances supported by alamethicin indicate that the channel is composed of several alamethicin molecules, up to 10 or 11 (reviewed in [103]). Analysis of binding isotherms for the association of alamethicin with lipid vesicles has also indicated aggregation of the peptide within the membrane [104,105]. Based on the structural studies, it therefore seems likely that the channels are made up of helical barrels in which the polar faces of the alamethicin helices are oriented inwards to the central pore of the barrel, as proposed originally in the model of [5]. The most stable model channel structures are

Figure 4 Structure of alamethicin and channel model

(a) Crystal structure of alamethicin [5]. There are three alamethicin molecules with closely related structures in the asymmetric unit for which the kink angle varies slightly (35° to 39°). The polar side chains Glu-18 and Gln-7, and the exposed carbonyl of Gly-11, as well as the proline residue at position 14, are indicated. Reprinted from *Prog. Biophys. Mol. Biol.* **55**, Sansom, M.S.P., pp. 139–235 [2], copyright (1991), with kind permission from Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington OX5 1GB, U.K. (b) Model of a hexameric helical barrel for the channel of the E18Q alamethicin variant based on the alamethicin crystal structure. The alamethicin molecules are oriented such that their N-terminal helices are parallel. Possible channel-stabilizing hydrogen bonds involving the side chains of residues Gln-7, Gln-18 and Gln-19 are indicated. Reproduced from [101], with permission, copyright Springer-Verlag (1993).

formed with the N-terminal helices aligned parallel [5,101]. This causes the pore to widen at the C-terminus as a result of the kink induced by the Pro-14 residue (Figure 4b). The side chains of adjacent N-terminal domains interdigitate in a zipper-like structure and the channel is stabilized by hydrogen bonds between the Gln-7 side chains of adjacent monomers and by Glu-18–Gln-19 H-bonds [5,101]. The (hydrated) annulus of the Gln-7 residue produces the greatest restriction in the channel diameter. Various models for the formation of helical bundles and barrels by alamethicin in the membrane and for voltage activation are discussed in [103].

Others: zervamicins and melittin

Other peptides with a similar structure and channel behaviour to that of alamethicin include the zervamicins from *Emericelloposis salmosynnemata* and melittin from bee venom. The zervamicins are also proline-containing peptaibols, the crystal structures of which have been determined and found similarly to be composed of bent helices, with the polar nature of the convex face enhanced by unsatisfied backbone hydrogen bonds [100]. Interestingly, embryonic water-associated channels have been identified in the crystal structure of Leu-zervamicin [6], a 16-residue peptaibol that also forms voltage-activated channels in lipid membranes [106]. The polar faces were found to associate in an anti-parallel fashion in the crystal, whereas the hydrophobic faces associate non-selectively in both parallel and anti-parallel modes [6]. Melittin is a 26-residue amphiphilic peptide that also contains a proline residue in the middle of the sequence but, unlike the peptaibols, does not contain Aib residues. The crystal structure of melittin [7] consists of two α -helical segments, without 3_{10} -helical elements, and like that of alamethicin has the shape of a bent rod. The solution structures of melittin in methanol [107] and bound to detergent micelles [108] obtained by NMR are similar to that in the crystal. Melittin also forms voltage-activated channels in planar lipid bilayers, but with a concentration

dependence that suggests that the channel is composed of four monomers [109].

The finding that ion channels are induced by strongly amphiphilic and surface-active peptides such as melittin, which have a pronounced lytic activity on membranes at higher concentrations, raises the question of the exact nature of the channel formation in these cases. Amphipathic helices are capable of associating with lipids with their axis parallel to the plane of the membrane, and this is the orientation found for melittin bound to micelles ([108]; see also [110]). It is possible, however, that the amphipathic peptide may change from a surface-aligned to a transmembrane orientation on application of a membrane potential (discussed in [3]). Another example of an extremely amphipathic cytolytic peptide is δ -haemolysin from *Staphylococcus aureus*. This 26-residue peptide does not contain proline residues, and the solution structure in methanol [8] and bound to lipid micelles [111] was found to have a central region that forms an extended amphipathic α -helix. This toxin also forms ion channels, but these are only weakly voltage dependent [2,112].

PEPTIDE SECONDARY STRUCTURE

Peptides corresponding to putative transmembrane channel segments that have been studied are listed in Table 1. Crystal structures are currently not available. Information on the secondary structure so far has come mostly from circular dichroism (CD), Fourier-transform IR (FTIR) and high-resolution NMR spectroscopies, often of the peptides in organic solvents. (Extrapolation of the latter to a membrane environment is uncertain and may account for the apparent variability in some results.) The application of these methods to channel peptides and proteins has been discussed recently [51]. Given the results summarized above for the structure of the acetylcholine receptor and the fact that current models for the pore region of voltage-gated ion channels propose this to involve a β -barrel type of structure [32,86–88], characterization of the secondary structure is a topic

Figure 5 Possible secondary structures for transmembrane peptide segments

The thickness of the hydrophobic region of the membrane is taken as 32 Å. The number of residues in the hydrophobic span is: (a) 21 (α -helix), (b) 9 (untitled anti-parallel β -sheet), (c) 12 (35°-tilted anti-parallel β -sheet), (d) 20 (β -sheet hairpin).

of major importance. Possible allowed conformations and corresponding hydrophobic spans for transmembrane peptide secondary structures with fully satisfied backbone hydrogen bonding (as is required energetically in a hydrophobic environment) are indicated in Figure 5. Approx. 22 residues are required for a membrane-spanning α -helix, but only about nine residues are needed for the more extended β -sheet structure if the strands are oriented parallel to the membrane normal. However, for a β -strand tilt of 60°, or for an untitled β -hairpin structure, a comparable number of apolar residues to that for an α -helix is required. Tilts of 35° and 60° can be realized by staggering the hydrogen bonding between adjacent anti-parallel β -strands by one or two residues respectively relative to the untitled structure [36].

Ligand-gated channels

Of the peptides corresponding to putative transmembrane segments of the nicotinic acetylcholine receptor that have been studied in lipid bilayers, the CD of the M2 δ AChR peptide [113], and of the template-attached tetramers T₄M2 δ and T₄M1 δ [114], has been investigated in the helix-promoting solvent trifluoroethanol. For M2 δ AChR, corresponding to the M2 segment of the δ -subunit of the *Torpedo* receptor, the calculated secondary structural content is 57% α -helix and 43% random, and for the T₄M2 δ tetramer it is 44% α -helix, 40% β -strand and 16% random. The latter includes the 9-residue template of the whole 101-residue structure. For mutant T₄M2 δ with the single residue replacement S8A, 45% α -helix, 20% β -strand and 35% random structure was found. For the T₄M1 δ tetramer, corresponding to the M1 segment of the δ -subunit, a higher helical content was obtained: 60% α -helix, 19% β -strand and 21% turns.

Of the glycine receptor peptides, CD spectra have been obtained from M2GlyR and M4GlyR in trifluoroethanol [13]. The M2GlyR peptide, corresponding to the M2 segment of the α_1 -subunit of the rat receptor, and the variant in which the two arginine residues close to the termini were replaced by glutamic acid (R2E and R21E), had roughly 50% α -helical content, with random coil and some β -sheet conformations accounting for the majority of the residual secondary structure. The M4GlyR peptide, corresponding to the M4 segment of the α_1 -subunit, contains too many aromatic residues for reliable analysis of the CD spectra, but ordered structure was indicated. In 10% aqueous

trifluoroethanol, random coil structures prevailed for all three peptides but the secondary structure of M4GlyR was found to be more stable against the conformational changes induced by addition of buffer.

High-resolution ¹H-NOESY NMR spectra of the M2 δ AChR, M2GlyR and M2NMDAR (glutamate receptor M2) peptides in dodecylphosphocholine micelles exhibit many inter-residue connectivities characteristic of an α -helix [3]. For M2GlyR, at least 15–20 of the 23 residues were deduced to be in an α -helical conformation. Solid-state ¹⁵N-NMR of [A12-¹⁵N]M2 δ AChR and of [A11-¹⁵N]M2GlyR has been used to determine the orientation of these peptides in aligned phospholipid bilayers [3,115]. The chemical shift anisotropy demonstrated that the backbone N–H bonds of the labelled residues were parallel to the membrane normal. Assuming a helical conformation for the peptide, this would imply that the α -helices are incorporated spanning the membrane, with the helix axis perpendicular to the membrane plane. Interestingly, it was found by similar methods that magainin2, a highly amphiphilic channel-forming peptide, adopted an orientation suggesting that it lies parallel to the plane of the membrane [115].

Voltage-gated channels

Of the putative transmembrane segments of voltage-gated ion channels, the secondary structure of peptides corresponding to S2 and S3, to S4 with and without the linking region to S5, and to the H5 putative pore-lining segments, have been studied. In addition, the putative single transmembrane segment of the small minK (or IsK) K-channel protein has been investigated.

The CaIIS2 peptide, corresponding to the S2 segment of the second repeat from the dihydropyridine receptor Ca channel of skeletal muscle, has been studied by ¹H-NMR and CD spectroscopy [16]. Both indicated α -helical content for the peptide in the amphiphilic solvent methanol, although other structures also made some contribution to the CD spectra. The KS2 peptide, corresponding to the S2 segment of the Shaker K channel, has been studied by CD in trifluoroethanol and in detergent micelles [17]. In both environments the α -helical content was high, estimated to be 78% in trifluoroethanol/water (2:3, v/v) and 99% in dodecyl sulphate micelles. The NaIS3 peptide, corresponding to the S3 segment of the first repeat of the rat brain I Na channel, has been studied in trifluoroethanol by CD [113]. The calculated secondary structural content was 30% α -helix, 35% β -strand, 3% turns and 30% random coil. The CD of peptides corresponding to the two putative transmembrane segments, M1 and M2, of the ROMK1 delayed rectifier K channel has been examined in hydrophobic solvents [116,117]. Both peptides adopted a high degree of helical structure, estimated to be 88% for M1 and 73% for M2.

The secondary structure of the S4 voltage-sensor segment is of considerable interest because of the regular occurrence of positively charged residues at every third position. This motif would distribute the positive charges evenly around the face of an α -helix, as postulated in the helical screw model of voltage gating [86], but would align all the positive charges parallel to the helical axis in a ₃₁₀-helix, creating a very strongly amphipathic structure. The structure of peptide NaIS4(r), the S4 segment from the first internal repeat of rat brain I and II Na channels, has been determined in trifluoroethanol/water (9:1, v/v) by ¹H-NMR and distance geometry/restrained molecular dynamics [20]. The peptide was found to be α -helical over the region from Leu-2 to Ile-14, with the C-terminus disordered and no regions of ₃₁₀-helix.

The secondary structure of the blocked peptide KS4, based on the Shaker K-channel S4 sequence, has been investigated by FTIR spectroscopy in both aqueous and trifluoroethanol solutions, and reconstituted in phospholipid bilayers and micelles [23]. The peptide was found to be of random coil structure in water but α -helical in trifluoroethanol, the latter being consistent with the NMR results with the NaIS4(r) Na-channel peptide. The KS4 peptide was also predominantly α -helical in lysophosphatidylcholine micelles and in dimyristoyl phosphatidylcholine or phosphatidylglycerol bilayers, with relatively minor contributions from β -sheet or turn. The 34-residue NaIVS4-S45 Na-channel peptide, that contains the additional S45 section which forms the link with the S5 segment, has been examined by CD spectroscopy [22]. Calculated α -helical contents were approx. 50 % in methanol, trifluoroethanol and vesicles containing anionic phospholipids, which was decreased slightly to approx. 40 % in zwitterionic phospholipid vesicles. The remaining secondary structure was attributed to β -sheet and random coil, with the β -sheet component in lipid bilayers comprising 25 % on average.

In several models for voltage-gated channels the putative pore-lining segment, H5, is assumed to have a secondary structure other than α -helical, often β -sheet (see above). Peptides NaIH5–NaIVH5, which correspond to the H5 or P-regions from all four internal repeats of *Electrophorus* Na channels, have been examined by CD spectroscopy [25]. The peptides from the first three repeats were estimated to have approx. 30 % α -helical content both in trifluoroethanol/water (2:3, v/v) and in dodecyl sulphate micelles. For the longer NaIVH5 peptide this was decreased to approx. 15 %, roughly corresponding to the same number of residues. Studies have been carried out on peptides of different lengths from the H5 region of Shaker K channels (see Table 1). The extended N-terminal section, KH-5-N, was estimated to have a fractional helicity of 0.38 in trifluoroethanol/water (2:3, v/v) [26]. The corresponding KH-5 peptide and its highly conserved region, consisting of the first 12 residues, were found to have low α -helical content, estimated at 20 % and 15 % respectively, in SDS micelles [17]. The H5 peptide from the ROMK1 delayed rectifier K channel was also found to have little α -helical content in a hydrophobic environment [116]. The full-length KH5 peptide was found from CD to be α -helical in 95 % trifluoroethanol, but was interpreted to have a mixed secondary structure of low helical content in buffer and in the presence of liposomes [28]. In contrast, the shorter blocked peptide KH5 was found by FTIR spectroscopy to be predominantly α -helical both in lysophosphatidylcholine micelles and in phospholipid vesicles [27]. The latter method is more appropriate to vesicular systems if special corrections and precautions are not applied in CD spectroscopy [51]. It is also conceivable that blocked N- and C-termini may promote helix formation of peptide segments (cf. [31]).

The IsK (or minK) protein consists of only 130 residues and is encoded by a DNA clone that on expression induces slowly activating voltage-gated K channels [118]. It has been suggested that this may be a regulatory protein, because heterologous expression at high levels in oocytes also induces Cl channels, a property that is shared, however, with a variety of quite unrelated membrane proteins [119]. The available evidence is therefore in favour of IsK being a true channel protein. The 32-residue TM-minK peptide, which contains the single putative membrane-spanning segment of the IsK protein, has been studied by CD in methanol [29]. The α -helical content in this amphiphilic solvent was estimated to be 57 %. A 63-residue 'truncated IsK' peptide containing nine additional residues at the N-terminus and 22 at the C-terminus, relative to TM-minK, was estimated to have 31 % helicity that was attributed entirely to the putative trans-

membrane sequence [120]. Of peptides outside the putative transmembrane region, the 20-residue peptide from the N-terminus was found to have high helicity (estimated at 50 %).

A shorter putative membrane-spanning peptide, K26, which contains the whole of the apolar sequence of IsK, has been reconstituted in zwitterionic phospholipid bilayers by dialysis from the helix-forming solvent 2-chloroethanol [30]. In bilayer membranes at high peptide concentrations, the K26 peptide was found by FTIR spectroscopy to be wholly in a β -sheet conformation. The latter is a further example of the possible plasticity in secondary structure of relatively short peptides that was mentioned above in connection with gramicidin A. It has long been known, for instance, that the M13 bacteriophage coat protein may be in either an α -helical or a β -sheet conformation, depending on the method of preparation [121]. This structural polymorphism has been characterized in detail for the M13 coat protein reconstituted in lipid bilayers [122]. Factors favouring the α -helical conformation were high lipid/peptide ratios, negatively charged lipids, high ionic strength and unsaturated lipid chains [123]. A concentration- and time-dependent conversion from α -helical monomers into β -sheet polymers was observed in lipid membranes. The equilibrium configuration at low lipid (high peptide) concentration can be in favour of the polymerized β -sheet structures. In general, all factors that can lead to aggregation of the peptide, not just high concentrations but also decreases in electrostatic repulsions, higher lipid chain packing densities and phase separation, will tend to favour the formation of β -sheet structures.

Proton channels

The structure of the whole 79-residue proteolipid c-subunit of the transmembrane section of the F_1F_0 -ATPase from *E. coli* has been investigated in trifluoroethanol and in chloroform/methanol/water by two-dimensional NMR spectroscopy [124,125]. This proteolipid, which is identified with the dicyclohexylcarbodiimide (DCCD)-binding component of the proton channel, was found to contain two extended regions of predominantly α -helical character that were shown to be arranged in a hairpin configuration [125]. The 16 kDa DCCD-binding proteolipid from *Nephrops norvegicus* that corresponds to the V_0 section of the vacuolar V-ATPases was estimated from FTIR spectroscopy to contain > 60 % α -helix, with probably 20 % extended-chain structures, 9 % disordered structure and 4 % turns [126]. A peptide corresponding to the single putative transmembrane segment of the small subunit 8 proteolipid (different from the c-subunit proteolipid analogue) of the F_1F_0 -ATPase from *Saccharomyces cerevisiae* has been studied by FTIR and CD spectroscopy [31]. This proline-containing peptide, Pep-8a, was found from FTIR to be fully α -helical in the solid state (dried from chloroform or methanol), and from CD was estimated to contain approx. 60 % α -helix when dissolved in hexafluoropropan-2-ol or its 1:1 (v/v) mixture with methanol.

Considered together, all these results suggest that measurements in organic solvents cannot necessarily be taken to indicate the secondary structure in a lipid membrane. Additionally, lipid composition and other parameters such as ionic strength and peptide concentration may be significant determinants of secondary structure.

PEPTIDE CHANNEL ACTIVITY

Single-channel electrical recordings have been made on planar bilayers, or bilayers formed on patch pipettes, with incorporated peptides and with their oligomers linked covalently to a multi-

functional carrier template [24,127]. Ion selectivity was established by the reversal potentials obtained from current–voltage relationships that were determined in the presence of transmembrane salt gradients or in bi-ionic media (cf. [2]). The results for peptides from ligand-gated channels are considered first, followed by those for peptides from voltage-gated channels. Of the peptides studied, experimental data on secondary structure have not been reported in all cases (see previous section), although design principles involving prediction methods and molecular modelling have been used to identify putative helical sections and pore-lining sequences (cf. [15,113]).

Ligand-gated channels

Nicotinic acetylcholine receptor

The amphiphilic M2 δ AChR peptide, corresponding to the M2 segment of the *Torpedo* receptor δ -subunit that is believed to be one of the five homologous pore-lining segments, has been found to form cation-selective channels of heterogeneous conductance and lifetime [9,10]. Template-attached tetramers and pentamers (T₄M2 δ and T₅M2 δ), on the other hand, formed cation-selective channels of homogeneous conductance which corresponded to the two primary channel populations that were observed with M2 δ AChR monomers [10,128]. It was concluded that the channels formed by the monomeric M2 δ AChR peptide corresponded to tetrameric and pentameric arrays. The pentameric T₅M2 δ channels had the higher conductance (40 pS in 0.5 M KCl), which corresponded quite closely to that of the native reconstituted channels (45 pS) under similar conditions. Additionally, channels from the tethered peptides were blocked by the local anaesthetic analogue QX-222 and by chlorpromazine, as for the native channels. Replacing conserved residues expected to face the pore lumen (S8A and F16A) had similar effects on conductance (decrease and increase respectively) to those of site-directed mutagenesis of the native receptor. Peptides with the same amino acid composition as M2 δ AChR but with randomized sequence did not form discrete channels, although conductance was affected; neither did the hydrophobic M1 δ AChR peptide, corresponding to the highly conserved first putative transmembrane segment of the receptor, nor its tethered tetramer. Similar results were obtained with an M2 α 4AChR peptide that was based on the sequence of the M2 segment of the rat neuronal receptor α_4 subunit [10]. These findings are in rather good accord with current models, and with the structural data discussed above, in which the nicotinic acetylcholine receptor channel consists of a pentamer of bent α -helices contributed by the M2 segments from each subunit.

The peptide MA β AChR, which corresponds to the MA segment in the long sequence linking the M3 and M4 segments of the β -subunit of the *Torpedo californica* nicotinic acetylcholine receptor, has also been studied in lipid bilayers [11]. This MA-segment is not one of the putative transmembrane segments identified from hydropathy plots and is not found in the sequences of the γ -aminobutyric acid_A (GABA_A) or glycine receptors, but was considered as a candidate for lining the channel because of its strongly amphipathic α -helical periodicity. Interestingly, the MA β AChR peptide monomer was found to give rise to long-lived channels with just two discrete conductance levels, which were, however, lower than that of native receptors. This behaviour was unlike that of the monomeric M2 δ AChR peptide, which gave a rather more continuous spectrum of conductances, and resembled more that of synthetic designed channels [129]. This MA-segment is currently not thought to be a candidate for the channel lining (see above).

Glycine receptor

Peptides corresponding to the amphiphilic M2 segment of the strychnine-binding α_1 -subunit of the glycine receptor have been found to form randomly gated anion-selective channels [12,13]. In one case the channel fluctuations were not stationary, with conductances that were found to vary over a large range, and cation-selective channels were observed in addition to those that were anion-selective [13]. In the other case, the conductances of the most frequently formed anion-selective channels (25 pS and 49 pS in 0.5 M KCl) were comparable with those of the native inhibitory receptor Cl channel of the rat spinal cord [12]. Replacing the two arginine residues at the N- and C-termini of the M2 segment by glutamic acid (R2/3E and R21/22E) reversed the channel selectivity, giving rise solely to cation-selective channels. On the other hand, peptides corresponding to the more hydrophobic M1 [12] and M4 [13] segments were found not to form discrete gated channels, but M4GlyR increased the overall bilayer conductance. Both the M2 and M4 peptides, as well as M2 with the Glu substitutions, increased the permeability of lipid vesicles, prohibiting the establishment of a transmembrane potential or dissipating a pre-existing membrane potential [13].

A template-attached tetramer of the M2 peptide, T₄M2GlyR, formed homogeneous anion-selective channels with a single conductance level of 25 pS in 0.5 M KCl [12]. The primary monomer channels with lower conductance were therefore attributed to tetrameric assemblies and those of higher conductance to pentameric assemblies. The T₄M2GlyR homotetramer channels were blocked by picrotoxin, which is effective with GABA receptors, and by the anion-channel blockers 9-anthracene carboxylic acid and niflumic acid, but not by the cation-channel blocker QX-222. On this basis, it was suggested that the native glycine receptor channel is composed of a pentameric assembly of α - and β -subunits with a central pore lined by the M2 segments [12]. This is in accord with current ideas on the receptor structure and its subunit stoichiometry [130].

Cystic fibrosis transmembrane conductance regulator (CFTR) channel

The channel-forming properties of peptides (M1CFTR–M6CFTR) corresponding to all six putative transmembrane domains of the first repeat of the CFTR phosphorylation-regulated Cl channel have been investigated [14]. The M1CFTR, M3CFTR, M4CFTR and M5CFTR hydrophobic peptides were found not to elicit discrete gated channels in lipid bilayers, but displayed irregular current fluctuations that indicated incorporation into the membrane. Both the M2CFTR and M6CFTR amphipathic peptides did, however, form anion-selective channels with two primary conductances and sporadic events of both higher and lower conductance. Peptides of scrambled sequence with the same amino acid composition as the M2 and M6 segments gave rise to erratic changes in conductance but did not elicit discrete channels.

Mixtures of the M2CFTR and M6CFTR peptides exhibited discrete channels with properties different from those of the single peptides, presumably indicating the formation of heterooligomers. These channels were of more uniform conductance and were long-lived (Figure 6). Both the conductance level and the strong anion selectivity, but not the gating properties, were similar to those of authentic CFTR channels under comparable conditions. It was therefore postulated that the M2 and M6 segments are constituents of the CFTR pore, which is possibly composed of the M2, M6, M10 and M12 segments, in agreement with mutagenesis studies. Secondary-structural characterization was not carried out, but the segments are of sufficient length to

Figure 6 Channel formation and ionic selectivity in lipid bilayers containing an equimolar mixture of the M2CFTR and M6CFTR peptides corresponding to putative transmembrane segments of the CFTR Cl channel

(a) Single-channel recordings and current histogram for 100 mV membrane potential and bathing solutions of 0.15 M KCl, 1 mM CaCl_2 and 10 mM Hepes (pH 7.4). The open-channel conductance is 8 pS. (b) Current–voltage relationships for symmetrical 0.5 M KCl solutions (○) and under a 5-fold concentration gradient of KCl (●). For the latter, the reversal potential indicates that the channels are 95% anion-selective. From [14], with permission.

form a transmembrane helical bundle. Currently the CFTR channel is less well characterized than, for instance, the nicotinic acetylcholine receptor. These peptide studies therefore provide a valuable addition to the present knowledge that might also be relevant to other members of the superfamily of ABC transporters that contain conserved ATP-binding cassettes, for instance the multi-drug resistance P-glycoprotein.

Voltage-gated channels

Sodium channels

Early studies [18] on an amphipathic peptide, NaIS3, which corresponds to the S3 segment of the first repeat of the rat brain I sodium channel, showed that this was able to form cation-selective channels of heterogeneous conductance. The most frequent single-channel event had a conductance (25 pS in 0.5 M NaCl) similar to that of authentic sodium channels, but showed little discrimination between Na^+ and K^+ ions and was not sensitive to voltage. In contrast, a hydrophobic peptide (NaIS6) corresponding to the S6 segment of rat brain sodium channels did not form discrete channels [24].

The S4 channel segment has been identified with the voltage sensor and is characterized by a high density of positively charged residues, which occur at every third position in the sequence with the intervening residues being apolar. A peptide (NaIVS4) corresponding to the S4 segment in the fourth repeat of the sodium channel from *Electrophorus electricus* was found to give rise to conductances higher than those with the NaIS3 peptide [21]. A transmembrane potential induced a minimum conductance level of 300 pS with additional fluctuations of 200 pS, the relative population of which increased with increasing peptide concentration. The conductance was gated closed by prolonged negative potentials to a level of 70 pS (all in 0.5 M NaCl) and activated by positive potentials with an apparent gating charge of 3. Although this is a highly basic peptide, the conductance was found to be selective for cations, but with only slight selectivity for Na^+ over K^+ ions. As already noted, this is also the case for the NaIS3 peptide but, unlike the latter, the conductance of the NaIVS4 peptide was voltage-gated. The analogous NaIS4 peptide was found to be capable of dissipating a diffusion potential in vesicles containing anionic lipids (in which peptide aggregates were formed), but not in those com-

posed only of zwitterionic lipids (in which peptide aggregation did not take place) [19]. Similar results were obtained on leakage of calcein from vesicles.

An *E. electricus* NaIVS4 peptide that was increased in length to include the S45 region which constitutes the link with the S5 segment has also been studied [22]. The conductance elicited by this NaIVS4-S45 peptide was much reduced relative to that of NaIVS4 and was more comparable with that of reconstituted authentic Na channels. Voltage gating was retained by the NaIVS4-S45 peptide (gating charge of 3 or less) and, in contrast to the NaIVS4 peptide, the channels displayed a selectivity for Na^+ over K^+ ions, a property that is shared with intact Na channels. It was estimated that the channel was formed from 3–5 monomers. These results suggest a role for the S45 fragment in the channel lining and possibly in gating, as suggested in one of the proposed channel models [86]. It is possible that the far-reaching effects of the additional linker region could be a special feature of the unique composition of the S4 segment, which may require this additional stabilization. Other more hydrophobic putative transmembrane segments that have been studied may not prove to be so sensitive to the terminal extensions.

The finding that the NaIS3 and NaIVS4 peptides are capable of forming ion channels in lipid bilayers, although with somewhat different characteristics from those of the native Na channels, is rather at variance with the results of mutagenesis studies. The latter identify the H5 segment, and to some extent S6, as the main pore-lining elements of intact expressed channels. Structural simplification by concentrating on a single peptide segment may give rise to the formation of channels that are not realizable in the overall folding pattern of the whole polytopic protein. Conversely, peptide segments corresponding to sequences involved in the pore of native channels may lack some of the flanking elements essential to their intact three-dimensional configuration. On the other hand, direct evidence for the maintenance of native structure is lacking for the mutant proteins. Indeed, the structural information derived indirectly from mutagenesis studies often relies heavily on the perturbation of native function. Under these circumstances, the possibilities established by the peptide experiments need to be taken into account.

Potassium channels

Functional experiments on K-channel peptides have concen-

trated mostly on the putative pore region of the channel, the H5 sequence or P-region. This corresponds to the most highly conserved region of the voltage-gated K-channel family. The shorter KH5 peptide of Shaker channels in Table 1 was found to give rise to single-channel events with conductances for the monomer which ranged from 12 to 50 pS in 0.5 M KCl [27]. A tetramer that was obtained by tethering this sequence (with the additional terminal proline residues) to a branched lysine core elicited less frequent channel events with a conductance of 13 pS in 1.0 M KCl. These conductances are in the range of those found for native K channels but, as discussed above, the secondary structure of this peptide is at variance with current models for the pore lining. The longer KH5 peptide in Table 1, which contains additional residues flanking the P-region, was found to induce channels of greater conductance than that of the native Shaker channels [28]. These channels were preferentially conductive to anions ($P_{\text{K}}:P_{\text{Na}}:P_{\text{Cl}} = 1:0.4:3.2$) and were not blocked by either tetraethylammonium or the putative inactivating 'ball'-peptide. Ion selectivity was not determined for the shorter KH5 peptide. These results indicate that peptides corresponding to the H5 region are capable of producing ion channels in planar bilayers but, in one case at least, these have properties differing considerably from those of the native channels. In view of current interpretations of the mutagenesis data on this region of the channel protein, these results may imply that additional parts of the protein structure are necessary for correct assembly of the pore region and/or actually contribute to the pore lining. This is in line with at least one of the models put forward for the channel structure [86].

A peptide derived from an entirely different K channel, the small single-subunit IsK (or minK) protein, which is 130 residues in length and on expression induces a slowly activating, voltage-sensitive potassium current, has also been studied [29]. This protein has only one putative transmembrane domain and therefore is particularly suited for the study of derived peptides reconstituted in lipid bilayers. The peptide TM-minK containing the putative transmembrane sequence was found to elicit heterogeneous channels with major conductance levels of 12 and 29 pS in 0.5 M KCl, only with trans negative potentials. The latter corresponds to the rectification properties of the endogenous channels. No selectivity was found between K^+ and Na^+ ions, however. Similar results were obtained with a longer 'truncated-IsK' peptide of 63 residues in length which was extended by nine residues at the N-terminus and by 22 residues at the C-terminus, relative to the TM-minK sequence [120]. This truncated form had been expressed in oocytes and found to induce K channels that were characteristic of the mature form [131]. For this longer peptide, major single-channel conductances of 17, 38 and 50 pS in 0.5 M KCl were obtained in planar bilayers, but again with no discrimination between K^+ and Na^+ , unlike the expressed truncated form. This latter result was attributed to the IsK protein being an activator rather than a channel-forming protein but, as discussed above [119], this explanation now seems less likely.

Calcium channels

A rather comprehensive survey has been conducted on peptides corresponding to the putative transmembrane segments of the dihydropyridine-sensitive, voltage-gated, L-type Ca channel [15,132]. Of the peptides derived from the fourth internal repeat (CaIVS1–CaIVS6), those with sequences of the amphipathic S2 and S3 segments were found to form discrete cation-sensitive channels in lipid bilayers, whereas those with sequences of the S1 segment, of the hydrophobic S5 and S6 segments, and also of the

S4 and H5 segments, did not. For the latter segments, incorporation into the bilayer was indicated, however, by the production of erratic variations in the membrane current. Similarly, bivalent-cation-selective channel activity has been found for a peptide (CaIIS2) that corresponds to the S2 segment of the second repeat from the dihydropyridine receptor Ca channel of the T-system of skeletal muscle [16]. These results are all in agreement with the more limited data from Na-channel peptides and, for the H5 segment, tend even more strongly in the direction of the conclusions drawn for the corresponding K-channel peptides.

Strikingly, a template-assembled tetramer of the CaIVS3 peptide was found to emulate many of the pharmacological and conductance properties of the native channels [132]. These cation-selective $\text{T}_4\text{CaIVS3}$ channels had saturable conductances for a range of bivalent ions that were similar to those of authentic channels, and exhibited blockade by nifedine, verapamil, QX-222 and Cd^{2+} at comparable concentrations to those for authentic channels. The $\text{T}_4\text{CaIVS3}$ channels also displayed conductance for Na^+ and K^+ ions that was blocked by low (micromolar) levels of Ca^{2+} , as for authentic channels. Most notably, the stereospecific action of agonist and antagonist enantiomers of the dihydropyridine BayK 8644 was reproduced in the $\text{T}_4\text{CaIVS3}$ tetramer channels. As with the S5 monomer, the template-assembled $\text{T}_4\text{CaIVS5}$ tetramer did not elicit discrete channel activity. These extremely interesting results have to be viewed, however, in terms of the deterministic nature of the H5 segment in specifying the high-affinity Ca^{2+} -binding site [3]. The implications of somewhat similar discrepant results have been discussed already for Na-channel peptides, and this point is returned to again in the Conclusion section.

Proton ATPase

A peptide (Pep-8a) corresponding to the putative transmembrane segment of the subunit 8 proteolipid of the proton F_1F_0 -ATPase from *S. cerevisiae* has been investigated in planar and patch-clamp bilayers [31]. Subunit 8 is not the DCCD-binding F_0 -proteolipid (subunit 9) that is known to be involved in the proton channel [133], but is part of the F_0 section of the ATPase, possibly involving the link with the catalytic F_1 section. Channels of very high conductance (in 1 M KCl) that were weakly voltage dependent were formed in planar bilayers. A single conductance level was observed in any given experiment but different conductance levels (most frequently 440 or 3000 pS) were observed in separate experiments. The ion selectivity, particularly that for protons, was not determined.

ASSEMBLY OF PEPTIDES IN BILAYERS

Several approaches have been used to study the assembly of putative transmembrane segments in lipid membranes (methods are reviewed in [51]). The first and perhaps most direct has been referred to above and consists of the synthesis of oligomers attached to designed templates [24]. The others involve the assembly of independent segments, partly in the spirit of the two-stage model of independent assembly proposed and established for certain α -helical integral membrane proteins, most notably bacteriorhodopsin [134,135]. One aspect of such studies, the concentration dependence of the membrane current, has been touched on already but has not been universally applied to the channel studies reviewed above. Further strategies are discussed below.

Figure 7 Influence of lipid composition on the assembly of the NaIS4 peptide, corresponding to the S4 segment of the first repeat of *Electrophorus* Na channels, on binding to vesicles

Shown are binding isotherms at 24 °C, where X_b^* is the molar ratio of bound peptide to total lipid and C_f is the equilibrium concentration of free peptide, obtained on binding of fluorophore-labelled NaIS4 to egg phosphatidylcholine vesicles (a), or to egg phosphatidylcholine/phosphatidylserine (1:1, mol/mol) vesicles (b). The sharp increase in binding to the latter, after an initial lag, is indicative of intramembranous aggregation of the peptide [104,105]. From [19], with permission, copyright American Chemical Society (1992).

Binding and resonance energy transfer

The assembly of peptides corresponding to putative transmembrane or pore-lining segments in lipid bilayers has been studied for a variety of channel proteins. This involves analysis of binding isotherms to reveal intramembranous aggregation processes (cf. [104,105]) and determining the resonance energy transfer between donor and acceptor fluorophores attached at the N- or C-terminus of the peptides. Fluorescence resonance energy transfer is a relatively long-range process (for 50 % efficiency: $R_0 \sim 50$ Å, depending on donor–acceptor pair), which ensures the reliable detection of peptide–peptide associations in oligomeric assemblies, but specific associations must be distinguished from random ones and from possible diffusional collisions between peptides [51,72].

The NaIS4 voltage sensor peptide derived from the first repeat of the *Electrophorus* Na channel has been studied by fluorescence labelling [19]. Shifts in the fluorescence maxima indicated the incorporation of the peptide in an apolar membrane environment. Both the shape of the binding isotherms and the fluorescence energy transfer measurements demonstrated that aggregation of the peptide monomers occurred readily in vesicles containing acidic lipids but not in those composed solely of zwitterionic lipids (Figure 7). The latter finding correlated with the ability of the peptide to induce permeation of the vesicles. Neither the insertion of the peptide nor its state of membrane aggregation were influenced, however, by the presence of a transmembrane diffusion potential. The S2-segment peptide, KS2, from the Shaker K channel has also been found to self-assemble in lipid vesicles, but not to associate specifically with an unrelated membrane-bound amphipathic peptide [17]. The M2-segment peptide from the ROMK1 delayed rectifier K channel has also been reported to self-assemble in lipid membranes, but the M1 peptide did not, nor did it associate with M2 [116,117].

The ‘pore’ peptides NaIH5–NaIVH5, corresponding to the H5 regions from all four internal repeats of the *Electrophorus* Na channel, have been studied by fluorescence spectroscopy [25]. From resonance energy transfer measurements, possible associations between the different pairs of H5 peptides were established. With the exception of the NaIH5/NaIIH5 pair, all H5 peptides were found to co-assemble, but not with an unrelated

membrane-associated peptide. Fluorophore-labelled peptides corresponding to different sections of the H5 region from Shaker K channels were also studied [17,26]. It was found that the H5-derived peptides KH-5 and KH-5-N, and a peptide corresponding to the first 12 highly conserved residues of KH-5, can self-assemble in lipid bilayers, but do not co-assemble with an unrelated membrane-bound peptide. Interestingly, no association was found between the KH-5 peptides and the Na-channel peptide NaIS4. However, KH-5 and its conserved 12-mer were found to form heteroaggregates with the KS2 K-channel peptide. A peptide lacking residues 1–4 within the most conserved region of KH-5 did not bind to lipid membranes at all, and the single-residue substitution W4S reduced the partitioning of KH-5 into the membrane by a factor of five. The H5-segment peptide from the ROMK1 channel has also been found to self-assemble, and additionally to co-associate with the M2 segment, in lipid membranes [116,117].

The 32-residue TM-minK peptide, which includes the single putative transmembrane segment from the IsK channel sequence, has also been shown from energy transfer studies to self-assemble in lipid bilayers [29]. The longer 63-residue peptide corresponding to the truncated form of IsK that can be functionally expressed [131] was also found to self-associate in lipid bilayer vesicles [120].

Taken together, the above results indicate that specific association is possible between putative transmembrane peptides, which is a prerequisite for them forming functional channel structures of the type thought to be present in the natural parent proteins. It should be noted, however, that most of the unrelated control peptides that did not co-assemble with the peptides of interest were highly amphiphilic in character.

Spin-label ESR

The study of the interactions between spin-labelled lipids and integral membrane proteins and of the rotational mobility of spin-labelled integral proteins by ESR spectroscopy is well established [136]. Lipid–protein interactions are characterized by the stoichiometry and by the selectivity, where the former is determined by the intramembranous perimeter of the protein

assembly and the latter by the protein residues situated in the region of the lipid polar headgroups [36]. Rotational mobility is determined by the intramembranous cross-section of the protein and hence by its state of oligomerization. Both types of ESR experiment may therefore be used to investigate the assembly of transmembrane peptides in lipid bilayers [36,51].

The K26 peptide, corresponding to the putative transmembrane segment of the IsK K-channel protein, has been studied by ESR spectroscopy in phosphatidylcholine bilayers [30]. The peptide displays a pronounced selectivity for interaction with negatively charged lipids and a low stoichiometry of interaction of approx. 2.5 phospholipids per peptide monomer. The selectivity indicates close proximity of the positively charged residues at the peptide termini to the lipid headgroups. This and the low stoichiometry is consistent with an enclosed (e.g. barrel) structure in which the β -strands (cf. above) are either strongly tilted or have a reverse turn at their centre (see Figure 5). The oligomeric peptide exhibits rather slow rotational diffusion, which implies that the peptide units are aggregated in the lipid bilayer. Somewhat similar results have been obtained with the β -sheet form of the M13 coat protein [137]. In the latter case, marked differences were observed in the lipid-protein interactions with the α -helical and β -sheet forms of the coat protein [138].

The above ESR methods have also been used to determine the conditions under which removing the extramembranous domains of the Na,K-ATPase by extensive proteolysis preserves the structural integrity of the transmembrane domains [45]. This is an alternative but somewhat similar and complementary approach to simplifying the system to that of self-assembly of synthesized transmembrane peptides. As already mentioned, similar proteolytic shaving of the extramembranous portion has been used to determine the secondary structure of the membrane domains of the nicotinic acetylcholine receptor [69]. As expected, this was found to differ from that of the intact protein.

Lipid-protein interactions with the 16 kDa proteolipid from *Nephrops*, which corresponds to the V_o section of the V-ATPases, have been studied in membranous preparations by similar methods [139]. The lipid stoichiometry was found to be rather low (equivalent to 5.0–7.5 phospholipids per monomer), consistent with a hexameric arrangement deduced from low-resolution image reconstruction by electron microscopy. A selectivity was observed for negatively charged lipids, in agreement with the placement of positively charged residues in the vicinity of the lipid headgroups in model building. Additionally, the insertion of the channel-forming diphtheria toxin into anionic lipid bilayers has been studied with lipid spin labels [140]. The low-pH-triggered insertion was found to take place in two stages. At pH 6.2 the stoichiometry of lipid-protein interaction was 24 lipids per bound toxin, which was attributed to the insertion of only the translocation domain, whereas at pH 5.0 this was increased to 40 lipids/toxin, which was attributed to the insertion of the whole toxin. The change in folding of the translocation domain of diphtheria toxin on pH-induced membrane insertion has also been studied by ESR methods involving site-directed spin-labelling [141]. These results offer considerable promise for further studies on transmembrane channel peptides.

CONCLUSION

Peptides corresponding to putative transmembrane channel segments are capable of self-assembly in lipid bilayers. The success in identifying such sequences by hydropathy analysis, and the at least partial success of the hydrophobic core of lipid membranes in mimicking the native environment, arise from the fact that many such sequences appear to have a markedly apolar

character. This extends even to the putative pore-lining faces of some channels. The philosophy of studying membrane-associated single peptide segments as channel models is further supported both by the observation that expression of homo-oligomers can result in the formation of functional channels and by the fact that independently folded domains of some integral proteins are capable of self-assembly to give the native form.

Functional studies on single-channel formation have met with some considerable success in reproducing the characteristics of the native channels. This has been spectacular even, in the case of the pharmacological properties of some template-assembled peptides. Characterization of the secondary structure in the membrane environment has not invariably gone hand-in-hand with the functional studies (and vice versa). This remains a critical issue in view of the proposals that some channels may depart from the α -helical paradigm on the one hand, and the fact that such peptides may be capable of displaying a plasticity in secondary structure on the other. Single-channel experiments are necessarily conducted at low peptide concentrations which will tend to favour retention of α -helical conformation, as opposed to assembly into polymeric β -sheet structures. Also, the assembly of peptide oligomers on predetermined templates allows some freedom of design in directing the type of secondary structure that will be adopted. Unfortunately, however, the low concentrations employed in single-channel measurements precludes direct application of most of the standard methods for structural determination.

The voltage-gated ion channels remain among those for which direct structural information is lacking and therefore they provoke intense interest. The finding that many of the properties of Ca channels may be reproduced by template-assembled tetramers of the S3 segment alone seems somewhat at variance with blocking, mutagenesis and inactivation experiments which implicate the H5 (or SS1–SS2) region and also part of S6 [59]. It has been suggested on the basis of peptide experiments that S3 may form the pore itself and that the other regions, specifically H5, may contribute to the mouth of the pore [3]. A similar discrepancy with regard to Na channels has already been discussed. The peptide experiments are able to identify a feasible pore configuration but do not necessarily establish this as the native pore structure. The final resolution of these points may prove to be a hybrid solution, guided partly by the results with reconstitution of transmembrane peptides.

Taking a more general view, experiments with isolated putative transmembrane segments indicate some of the ways in which such sequences of amino acids in integral proteins may assemble in membranes. They also reveal the features of such defined sequences that can contribute in detail to the properties of channel pores. Additionally, the contributions of specified peptide structures in defining the interactions of side chains at the intramembranous surface of integral proteins with the lipid milieu are available from such studies. It is likely that both solid-state NMR [142] and spin-label ESR [143] will be among the techniques that will contribute materially to such studies in the future.

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