Acidic pH requirement for insertion of colicin E1 into artificial membrane vesicles: Relevance to the mechanism of action of colicins and certain toxins

(polypeptide-membrane binding/acidic amino acid protonation/amphipathic helices/membrane channels)

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ABSTRACT The channel-forming activity of colicin E1 in artificial membranes is known to increase at low pH values and to have a maximum near pH 4 in such membrane vesicles. The present work demonstrates that this pH dependence of activity can be attributed to membrane binding. Maximal binding of colicin E1 and a more slowly binding channel-forming carboxyl-terminal tryptic peptide occurred at acidic pH values, with the effective pK values for binding equal to 4.6 and <4.0, respectively. The binding did not require imposition of a transmembrane potential. Insertion of the tryptic peptide into the membrane was shown by retention of bound [³H]leucine-labeled peptide by vesicles after digestion with protease, as well as by retention of the peptide in salt-washed vesicles. The retention after protease treatment was also used to estimate the amount of carboxyl-terminal peptide inserted into the membrane. Approximately 12 of the 21 leucines present in the carboxyl-terminal peptide were retained after Pronase treatment at pH < 4. Reversibility of the insertion at low pH values was seen after an alkaline shift of pH to 6.0, resulting in a decrease of the protease-inaccessible fraction of the bound protein. A model is presented describing a mechanism in which protonation of one or more carboxyl residues is necessary for effective binding and insertion into the membrane by the channel-forming domain of colicin E1. This model may also be relevant to the mechanism of membrane insertion by certain toxins.

Colicin E1 is a 522-residue bactericidal protein, known to exert its lethal effect through an ability to depolarize the cytoplasmic membrane of sensitive *Escherichia coli* (1–3). Studies of colicin E1 action using artificial membranes have shown that the deenergization results from an ion channel formed by the colicin molecule, which dissipates the cellular membrane potential (ref. 4, reviewed in refs. 5–7). Colicin E1 requires an acidic pH *in vitro* to form ion channels (8), and its action is dependent upon the sign and magnitude of the membrane potential (4, 8, 9). The channel-forming activity of colicin E1 has been shown to reside in a *M_r* 20,000 carboxyl-terminal tryptic peptide (10). The tryptic peptide and colicin required a low pH for *in vitro* activity. The effective pK values for activity, assayed by using artificial vesicles, were 4.6 and 3.8, respectively, for the colicin and the tryptic peptide (8). This requirement of an acidic pH for colicin E1 action resembles the pH dependence *in vitro* for the actions of diphtheria toxin (11–14) and tetanus toxin (15), and it suggests that an explanation of this pH effect may bear on a general molecular mechanism for insertion into membranes of other colicins (2–4, 7, 16, 17), as well as the above-mentioned channel-forming toxin molecules (11–15).

The purpose of the present work was to examine the mechanism of binding and of insertion into artificial membranes by the channel-forming domain of colicin E1, and specifically its dependence on pH and protonation of one or more acidic amino acids in this domain.

EXPERIMENTAL PROCEDURES

Preparation of Colicin E1 and its Carboxyl-Terminal Peptide. Colicin E1 was purified from *E. coli* strain JC411(ColE1) by the method of Cleveland *et al.* (18), except that 0.1 M potassium phosphate buffer, pH 7.0, was used for the dialysis step. Growth conditions for cells used in the preparation of [³H]leucine-labeled colicin E1 were identical except that leucine was present at a concentration of 5 mg/liter and [³H]leucine (ICN, 50 Ci/mmol; 1 Ci = 37 GBq) was added at 1 mCi/liter 10 min after induction with mitomycin. The carboxyl-terminal tryptic peptide of colicin E1 was prepared according to Dankert *et al.* (10).

Preparation of Colicin A. Colicin A was made from *E. coli* strain 610(ColA), obtained from S. E. Luria and J. Suit, grown in L-broth, and mitomycin was added at 1.0 µg/ml to induce colicin production. Cells suspended in 0.1 M potassium phosphate, pH 7.0/1.0 M NaCl were disrupted in a French pressure cell. The resulting supernatant was passed through a Sephadex G-100 column in 0.1 M potassium phosphate, pH 7.0. Fractions containing colicin A, determined from NaDodSO₄ gel electrophoresis and cytotoxic activity, were passed through a DEAE-Sephadex column equilibrated in 0.1 M potassium phosphate, pH 7.0, with colicin A eluting in the void volume.

Preparation of Artificial Lipid Vesicles. Freeze-thaw and fused unilamellar vesicles were prepared according to Davidson *et al.* (8) from asolectin (a phospholipid mixture from soybean; Associated Concentrates, Woodside, NY) purified by the method of Kagawa and Racker (19), or a mixture of lipids resembling *E. coli* membranes composed of 70% (wt/wt) phosphatidylethanolamine, 25% phosphatidylglycerol, and 5% cardiolipin (Sigma). The buffer in which the freeze-thaw vesicles were prepared contained 10 mM dimethylglutaric acid, 90 mM NaCl, and 10 mM KCl, with the final pH adjusted to 3.6 or 4.0 with NaOH. Fused vesicles were prepared from freeze-thaw vesicles by the addition of 10 mM CaCl₂ and 40 mM EDTA (8).

Chloride Efflux Measurements. Efflux from asolectin membrane vesicles was assayed (8) with a Radiometer chloride-specific electrode (F1012Cl Chloride Selectrode).

Binding Assays. Vesicles were incubated with ³H-labeled colicin E1 or ³H-labeled tryptic peptide for the indicated times, keeping in mind the slower time course for binding of

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FIG. 1. Kinetics of binding of colicin E1 and tryptic peptide to asolectin vesicles. Colicin E1 and tryptic peptide were incubated with fused asolectin vesicles for the indicated lengths of time at the following pH values: colicin E1, pH 4.0 (○), 4.5 (□), and 5.5 (Δ); tryptic peptide, pH 3.6 (●), 4.0 (■), and 4.8 (▲).

the tryptic peptide (Fig. 1). Unless otherwise stated, the incubation buffer was identical to that in which the vesicles were prepared, and the protein-to-lipid ratio was approximately 1:100 (wt/wt), with the protein concentration typically 5–10 $\mu\text{g}/\text{ml}$. Assuming an average diameter of 1000 Å for vesicles prepared by the freeze-thaw method (8), there were approximately five colicin or tryptic peptide molecules per vesicle. Samples were filtered through 0.2- μm pore diameter membrane filters (Millipore type EG or Gelman Metrical GA-8) that had been soaked in a 2 mg/ml solution of bovine serum albumin to reduce nonspecific binding of colicin and tryptic peptide to the filter. ^3H retained on filters was determined by counting in a Searle Isocap 300 scintillation counter. The maximum binding observed for colicin E1 and carboxyl-terminal peptide was 80–100% and 40–75%, respectively, of the labeled protein added to vesicles.

Protease Digestion. Freeze-thaw vesicles were incubated with colicin E1 or the tryptic peptide at pH 4.0 and 3.6, respectively, for at least 30 min to ensure maximal binding. Pronase (protease type XIV, Sigma), a relatively nonspecific protease (20), was freshly dissolved in incubation buffer and then used at a final concentration of 0.8 mg/ml, which digested colicin E1 or the tryptic peptide in solution. Digestions were terminated after 1 hr by filtration and immersion of filters into scintillation fluid. For digestions at pH 6.0 after binding at pH 3.6 or 4.0, 10 mM dimethylglutaric acid/90 mM NaCl/10 mM KCl, pH 10, was added to raise the pH.

Extraction of Membrane-Inserted Tryptic Peptide. Tryptic peptide (0.4 mg) that had been dialyzed 20 hr in 10 mM dimethylglutaric acid/90 mM NaCl/10 mM KCl, pH 3.6, was added to 5 ml of vesicles prepared by the freeze-thaw method in the same buffer. After incubation for 30 min at room temperature to ensure maximal insertion, the fragment/vesicle mix was treated with NaCl (final NaCl concentration = 0.5 M) to remove any peripherally bound fragment from the vesicles. Vesicles were sedimented by treatment with 20 mM CaCl_2 followed by centrifugation for 10 min at $12,000 \times g$. The membrane pellet was extracted with 7 ml of 2:1 (vol/vol) chloroform/methanol, followed by the addition of 4 ml of 10% (wt/vol) trichloroacetic acid in water. The organic phase was separated from the aqueous phase by centrifugation, and the precipitated protein that formed at the interface was collected. The precipitate was treated with chloroform/methanol and trichloroacetic acid a

second time, and the protein obtained from this step was washed with acetone. The final precipitate was reconstituted in 8 M urea/4% (wt/vol) NaDodSO₄/4% (vol/vol) mercaptoethanol/50 mM Tris-HCl, pH 8.6, and the products were analyzed on a 12.5% acrylamide/NaDodSO₄ gel.

Retention of Vesicles on Filters. Freeze-thaw vesicles (1 ml) were prepared in buffer at pH 3.6 as previously described, except 0.25 μCi of 1,2-di[1- ^{14}C]palmitoyl L-phosphatidylcholine (Amersham, 112 mCi/mol) was included in the lipid mixture. Thirty microliters of the vesicles was added to 4 ml of incubation buffer, pH 3.6, to give a final lipid concentration of 150 $\mu\text{g}/\text{ml}$, and 20 μg of tryptic fragment was then added. After a 30-min incubation, samples were withdrawn for filtering and scintillation counting. To test for possible effects of various treatments on vesicle retention, either vesicle/peptide suspensions were treated with Pronase at 0.8 mg/ml and pH 3.6 for 1 hr, or the pH was raised to 6.0 with or without Pronase addition for 1 hr. Approximately 30–50% of the colicin and tryptic peptide appeared to be lost prior to Pronase treatment after a shift of pH to 6.0. Analysis of ^{14}C -labeled vesicle retention indicated that most of the loss was due to a decrease in retention of vesicles on the filters after this pH shift (data not shown).

RESULTS

Effect of pH on the Binding of Colicin E1 and Its Tryptic Peptide to Artificial Membrane Vesicles. Since the activity of colicin E1 and its carboxyl-terminal tryptic peptide has been assayed in fused asolectin vesicles as a function of pH (8), the dependence of binding on pH was compared under the same conditions after a 1-min incubation (Fig. 2). Binding of colicin E1 was greatest at about pH 4.0, with an apparent pK of 4.5–4.6, and decreased below pH 4.0. Binding of the tryptic peptide increased continuously, with an effective pK of 4.0, as the pH was lowered from 4.5 to 3.6, a value below which the vesicles were unstable. The pH dependence of the binding of colicin E1 and tryptic peptide to asolectin vesicles prepared by the alternate freeze-thaw method was similar, as was that for the binding of colicin E1 to vesicles composed of an *E. coli* membrane-like mixture of lipids, phosphatidylethanolamine/phosphatidylglycerol/cardiophospholipin (70:25:5, wt/wt) (data not shown). The titration curves for binding of colicin E1 or tryptic peptide to asolectin vesicles were nearly identical to those for the activity of colicin E1 and the peptide (Fig. 2), measured previously through the rate of Cl^- release from the vesicles (8). Consistent with the data shown in Fig. 1, the pH profile for the binding of colicin E1 was the same for incubation times of 1 and 30 min (Fig. 3), although after the 30-min incubation colicin E1 did not show a decrease in binding below pH 4. For the tryptic peptide, however, the pH profile was slightly shifted in the alkaline direction after the 30-min incubation (Fig. 3). Thus, the titration of binding at acidic pH values was not sensitively dependent on incubation time, method of vesicle preparation, or lipid composition of the vesicles.

Effect of Membrane Potential on Binding of Colicin E1 and the Tryptic Peptide to Vesicles. The activity of colicin E1 and the peptide in artificial membranes has been shown to depend upon the potential imposed across the membrane (4, 9). Binding of colicin E1 and the tryptic peptide was compared in the presence of initially imposed K^+ -diffusion potentials of -60 and $+42$ mV, and in the presence and absence of the protonophore carbonyl cyanide *m*-chlorophenylhydrazone to dissipate any residual membrane potential (21). No significant differences in binding, which was typically 80–100% of the added colicin and 40–75% of the tryptic peptide, were observed as a function of membrane potential (data not shown). All binding assays were then conducted in the absence of an imposed diffusion potential.

FIG. 2. Effect of pH on the binding of colicin E1 and its tryptic peptide to asolectin vesicles and on their ability to induce Cl^- efflux from Cl^- -loaded vesicles. Binding was assayed for fused vesicles incubated with [^3H]leucine-labeled colicin E1 (○) or tryptic peptide (●) for 1 min. Relative binding of 100% corresponds to the maximal absolute binding observed in a given set of experiments. This value was 80–100% and 40–75%, respectively, of the colicin and peptide added to vesicles. Activity data were taken from ref. 8 and are the initial rate of Cl^- efflux caused by addition of colicin E1 (Δ) or the tryptic peptide (▲).

The Extent of Insertion of Colicin E1 and Its Tryptic Peptide into Artificial Membrane Vesicles. To distinguish between peripheral binding and insertion, vesicles to which ^3H -labeled colicin E1 or peptide had bound were incubated in high ionic strength buffer containing 2 M NaCl, which itself did not displace any of the colicin or peptide bound to the lipid vesicles. After this exposure to high ionic strength, the tryptic peptide could be extracted from the separated mem-

FIG. 3. Effect of incubation time on the binding of colicin E1 and its tryptic peptide to asolectin vesicles. Fused vesicles were incubated with [^3H]leucine-labeled colicin E1 for 1 (●) and 30 (○) min or with tryptic peptide for 1 (▲) and 30 (Δ) min prior to filtration. The broken line indicates the decrease in binding of colicin E1 below pH 4.0 for a 1-min incubation.

FIG. 4. Analysis by NaDodSO₄/polyacrylamide gel electrophoresis of membrane-inserted tryptic peptide and the products of digestions of colicin E1 and tryptic peptide with Pronase in solution. A 12.5% acrylamide gel was run with the following: lane 1, molecular weight standards [bovine serum albumin (68,000) and lysozyme (14,000)]; lane 2, colicin E1 and tryptic peptide; lane 3, tryptic fragment extracted from membranes; lane 4, as in lane 3, 2× the protein concentration; lane 5, 1-hr Pronase digest of colicin E1 in buffer, pH 3.6, 3× colicin used in lane 2; lane 6, 1-hr Pronase digest of tryptic peptide in buffer, pH 3.6; lane 7, 1-hr Pronase self-digest, pH 3.6.

brane fraction (Fig. 4, lanes 3 and 4). Thus, the binding of colicin to the vesicles must involve more than an ionic interaction between protein side chains and the lipid head groups. The fraction of the carboxyl-terminal domain of the colicin that is inserted into the membrane after binding can be defined by treatment of vesicles containing [^3H]leucine-labeled colicin or tryptic peptide with Pronase, a nonspecific protease (20) that should digest the regions of the protein not protected by the membrane. The radioactivity retained in the vesicles reflects the number of leucine residues protected by the membrane. Treatment with Pronase subsequent to binding resulted in the loss of all but approximately 12 leucines of the 21 and 44 leucine residues known from the sequence (ref. 22; Fig. 5) to be present in the tryptic peptide and colicin (Table 1). Treatment of colicin E1 and tryptic peptide with Pronase in the absence of vesicles under these conditions resulted in digestion of the protein to small peptides (Fig. 4, lanes 5 and 6). Because the vesicles were found to be permeable to Cl^- after treatment with high ($\geq 100 \mu\text{g/ml}$) concentrations of Pronase, the Pronase may have access to the inserted protein from both sides of the membrane.

Reversibility of Protein Insertion. The fraction of the

FIG. 5. Sequence of the tryptic peptide of colicin E1 (10, 22). The standard one-letter code is used. The 21 leucine residues present in the tryptic peptide, which begins at residue 336 (10), are underlined.

Table 1. Accessibility of bound colicin E1 or tryptic peptide to Pronase digestion

Sample	pH	Pronase	Vesicle-associated label, %	No. of leucines retained per molecule
Colicin E1	4.0	—	100	44
	4.0	+	27.0 ± 2.0	12.0 ± 1.0
	6.0	—	100	44
	6.0	+	11.5 ± 3.5	5.0 ± 1.5
Tryptic peptide	3.6	—	100	21
	3.6	+	59.0 ± 7.0	12.5 ± 1.5
	6.0	—	100	21
	6.0	+	25.5 ± 7.5	5.5 ± 1.5

Subsequent to binding of [³H]leucine-labeled colicin E1 and its tryptic peptide at pH 4.0 and 3.6, respectively, for 30 min, freeze-thaw vesicles were treated with and without Pronase at the pH indicated, and incubated for 1 hr. The number of leucines inaccessible to Pronase digestion was determined from the amount of ³H retained on filters relative to controls at the same pH that were not treated with the protease. Results are mean ± SD.

inserted colicin E1 or tryptic peptide that was inaccessible to Pronase decreased as the pH was increased, indicating some reversibility in the insertion process. When the pH was increased to 6.0 subsequent to binding at pH 3.6 or 4.0, and prior to Pronase treatment, only about 5 leucine residues of the tryptic peptide or colicin were retained in the membrane vesicles (Table 1). The number of leucines retained at pH 6.0 was calculated relative to the fraction of colicin E1 or tryptic peptide associated with the vesicles after the 1-hr incubation at this pH. The smaller number of bound leucines after Pronase treatment at pH 6.0, relative to the untreated control, shows that the insertion of part of the protein containing 5–10 leucine residues is reversible. These residues in the bound colicin and peptide are initially inaccessible to Pronase at pH 4.0 and 3.6, and they become accessible when the pH is increased to 6.0. Segments of the protein are apparently moving out of the membrane when the pH is increased. The detailed time course and the possibility of discrete stages in this extrusion have not yet been investigated. For both the colicin and the peptide, a segment of the protein containing approximately 5 leucines remains anchored in the membrane.

DISCUSSION

The requirement of an acidic pH for the *in vitro* action of toxins and toxin-like molecules is documented for diphtheria (11–14) and tetanus (15) toxin. The channel-forming colicin A exhibits an effective pK ≤ 4.0 for activity with asolectin membranes (data not shown). A low pH is also required by proteins of certain viruses to penetrate biological membranes and induce membrane fusion (23–26), and a pK of about 4 has been reported for the pH-dependent vesicle fusion induced by G protein from vesicular stomatitis virus (26).

Colicin E1 requires a low pH, near 4.0, for maximal activity in artificial vesicles (8). It is evident from the present work that, for colicin E1 and its carboxyl-terminal tryptic peptide, the pH dependence of activity reflects an acidic pH requirement for binding and insertion into the membrane by the protein. The pH titrations associated with binding of colicin E1 and the carboxyl-terminal peptide are nearly identical to titrations of activity as a function of pH (Fig. 2). The effective pK values of 4.6 and ≈4.0 for binding of colicin E1 and the peptide imply that one or more acidic amino acid residues must be protonated for effective binding and inser-

tion into the membrane. A possible involvement of histidine residues can be ruled out because chemical modification of the two histidines in the tryptic peptide did not alter the pH dependence of binding or activity (27). Circular dichroism data indicating that the tryptic peptide of colicin E1 in nonionic detergent undergoes a pH-dependent change in α -helical content, with an effective pK of about 4.0 (28), support the concept that protonation of one or more acidic amino acids is necessary to allow the protein to achieve a favorable conformation in a nonpolar environment.

The data presented on the Pronase accessibility of the colicin and carboxyl-terminal peptide, coupled with a knowledge of the primary amino acid sequence of the carboxyl-terminal domain (Fig. 5), allow testing of models (5, 18, 29) for the colicin E1 channel. A simple model has been proposed which defines the channel as being composed of five membrane-spanning amphipathic α -helices, the polar faces of which line an aqueous pore (5). The first helix in this model begins at approximately residue 383, and the fifth helix ends near residue 504. Most of the residues 383–504, once inserted, would then be inaccessible to the aqueous medium because they are in the bilayer. This model predicts that 7 of the 21 leucines present in the tryptic peptide, those in the regions flanking residues 383 and 504, and leucine 504, might be digested by added Pronase. In addition, 4 leucines are predicted to be in one helix-connecting loop, which may or may not be accessible to Pronase digestion. Thus, 10–14 residues are predicted to be inaccessible to Pronase. Within the limits of experimental accuracy, this corresponds to the results presented in Table 1.

A hypothesis for the involvement of acidic pH in binding and insertion into membranes of the colicin channel-forming domain is presented in Fig. 6. The acidic pH requirement for binding implies that one or more acidic amino acids in this carboxyl-terminal domain must be protonated. It is inferred that such residues reside in an otherwise nonpolar region of the inserted protein that is in contact with the membrane lipid. The thermodynamics for insertion of an acidic residue into the lipid are much more favorable when the residue is neutralized (30). In Fig. 6, the segment of the protein containing the relevant carboxyl group exists in an unspecified conformation in solution (A and B), but is proposed to form an amphipathic helix upon association with the phospholipid (C and D). If the carboxyl group is protonated (D), the amphipathic helix would change to an energetically more favorable inserted state (E), with the nonpolar face of the helix associated with lipid chains and the polar face with the lipid head groups. A suggestion for the identity of one such acidic amino acid, Glu-468, was derived from the amphipathic helical model for the colicin E1 channel mentioned above, in which Glu-468 resides on an otherwise nonpolar face of an amphipathic helix (5). Insertion of the other amphipathic helices that comprise the channel, and have true nonpolar faces, would not require an acidic pH. However, as essentially no binding is seen at neutral pH, this model requires that the protonation of the critical carboxyl(s) on a particular helix is required for the cooperative insertion of all of the amphipathic helices that make up the colicin E1 channel. This would allow shielding of the polar faces on the inside of the channel from the lipid environment. The faster binding of the colicin, relative to the tryptic peptide (Fig. 1), suggests that regions of the molecule not contained in the tryptic peptide help to stabilize the C and D states. This would occur through surface interactions between the head groups of the acidic phospholipid, known to enhance colicin action *in vitro* (6), and the side chains of the basic (pI = 9–10) colicin E1 molecule.

The insertion of colicin E1 and its tryptic peptide into the membrane is at least partly reversible after initial binding at pH 4.0, so that deprotonation of one or more amino acid

FIG. 6. Schematic model for the mechanism of pH-dependent insertion of the channel-forming domain of colicin E1 into membrane vesicles. It is proposed that protonation of at least one critical carboxyl residue is required for insertion into the membrane. A and B represent the segment of the protein surrounding this residue in an unspecified conformation in the aqueous environment. Polar and nonpolar residues are indicated, respectively, by solid and broken lines. This segment is assumed to acquire an amphipathic helical conformation on association with the phospholipid vesicle (C and D). If the carboxyl is protonated (D), the helix will change to a more energetically favorable state (E) which will allow insertion of the entire channel-forming domain. In E the nonpolar face is associated with the hydrophobic acyl chains and the polar face with the phospholipid head groups. Partial reversibility of the D $\rightarrow$ E transition is indicated.

residues causes a structural change that results in partial extrusion of the inserted protein from the membrane. Interestingly, the measurement of approximately 5 leucines retained in the membrane after the pH has been increased to 6.0 (Table 1) corresponds fairly closely to the number of leucines, 6, present in a hydrophobic sequence of 35 amino acids extending from residue 474 to residue 508 (Fig. 5). It has been proposed that this 35-residue sequence is responsible for the initial binding of colicin E1 to the membrane (18). While the data presented here do not support the latter argument, they might imply that once the channel has inserted, the 35-residue hydrophobic sequence could act as an anchor that prevents complete dissociation of the channel-forming domain from the membrane.

While structural details of the proteins may vary among colicin E1, other channel-forming colicins, and toxin molecules such as diphtheria and tetanus, the similar pH dependence of the action of these molecules *in vitro* suggests that the concept of acidic amino acid protonation and resultant conformational change in the presence of membrane lipid, as described in Fig. 6, is relevant to the mechanism of insertion into membranes for all of these proteins.

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- Gould, J. M. & Cramer, W. A. (1977) *J. Biol. Chem.* **252**, 5491-5497.
- Tokuda, H. & Konisky, J. (1978) *J. Biol. Chem.* **253**, 7731-7737.
- Weiss, M. J. & Luria, S. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2483-2487.
- Schein, S. J., Kagan, B. L. & Finkelstein, A. (1978) *Nature (London)* **276**, 159-163.
- Davidson, V. L., Brunden, K. R., Cramer, W. A. & Cohen, F. S. (1984) *J. Membr. Biol.* **79**, 105-118.
- Cramer, W. A., Dankert, J. R. & Uratani, Y. (1983) *Biochim. Biophys. Acta* **737**, 173-193.
- Konisky, J. (1982) *Annu. Rev. Microbiol.* **36**, 125-144.
- Davidson, V. L., Cramer, W. A., Bishop, L. J. & Brunden, K. R. (1984) *J. Biol. Chem.* **259**, 594-600.
- Bullock, J. O., Cohen, F. S., Dankert, J. R. & Cramer, W. A. (1983) *J. Biol. Chem.* **258**, 9908-9912.
- Dankert, J. R., Uratani, Y., Grabau, C., Cramer, W. A. & Hermanson, M. (1982) *J. Biol. Chem.* **257**, 3857-3863.
- Donovan, J. J., Simon, M. I., Draper, R. K. & Montal, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 872-876.
- Draper, R. K. & Simon, M. I. (1980) *J. Cell Biol.* **87**, 849-854.
- Kagan, B. L., Finkelstein, A. & Columbini, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4950-4954.
- Sandvig, K. & Olsnes, S. (1980) *J. Cell Biol.* **87**, 828-832.
- Boquet, P. & Duflot, E. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7614-7618.
- Pattus, F., Martinez, C., Dargent, B., Cavard, D., Verger, R. & Lazdunski, C. (1983) *Biochemistry* **22**, 5698-5703.
- Morlan, J., Llonbés, R., Verenne, S., Chartier, M. & Lazdunski, C. (1983) *J. Mol. Biol.* **170**, 271-285.
- Cleveland, B. M., Slatin, S., Finkelstein, A. & Levinthal, C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3706-3710.
- Kagawa, Y. & Racker, E. (1971) *J. Biol. Chem.* **246**, 5477-5487.
- Narahashi, Y. (1970) *Methods Enzymol.* **19**, 651-664.
- Heytler, P. G. (1979) *Methods Enzymol.* **55**, 462-472.
- Yamada, M., Ebina, Y., Miyata, T., Nakazawa, T. & Nakazawa, A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2827-2831.
- Skehel, J. J., Bayley, P. M., Brown, E. B., Martin, S. R., Waterfield, M. D., White, J. M., Wilson, I. A. & Wiley, D. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 968-972.
- White, J. & Helenius, A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3273-3277.
- White, J., Kartenbeck, J. & Helenius, A. (1982) *EMBO J.* **1**, 217-222.
- Eidelman, O., Schlegel, R., Tralka, T. S. & Blumenthal, R. (1984) *J. Biol. Chem.* **259**, 4622-4628.
- Bishop, L. J., Davidson, V. L. & Cramer, W. A. (1984) *Biophys. J.* **45**, 62a (abstr.).
- Brunden, K. R., Uratani, Y. & Cramer, W. A. (1984) *J. Biol. Chem.* **259**, 7682-7687.
- Guy, H. R. (1983) *Biophys. J.* **41**, 363a (abstr.).
- von Heijne, G. & Blomberg, C. (1979) *Eur. J. Biochem.* **97**, 175-181.