A spectroscopic study of the mitochondrial transit peptide of rat malate dehydrogenase

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A peptide corresponding to the N-terminal sequence of the rat malate dehydrogenase, comprising the transit sequence and two residues of the mature protein (MLSALARPVGAALR-RSFSTSAQNNAK) has been chemically synthesized, and its structural characteristics investigated by Fourier-transform i.r. (FT-IR), c.d. and ^1H -n.m.r. spectroscopy. FT-IR and c.d. spectra of the peptide were recorded in a variety of environments (aqueous solution, trifluoroethanol) and after incorporation into phospholipid bilayers. The peptide was found to be mainly in aperiodic or undefined conformation in aqueous solution. However, in trifluoroethanol a marked increase in α -helical content was observed. An increase in α -helical content was also observed in negatively charged lipids (dimyristoylphosphatidylglycerol

and cardiolipin). However, when reconstituted in a zwitterionic phospholipid (dimyristoylphosphatidylcholine), no α -helical structure was observed. N.m.r. spectroscopy was used to characterize the helical structure in greater detail in trifluoroethanol. The ^1H -n.m.r. spectrum of the peptide in this solvent was assigned using standard homonuclear two-dimensional methods. The observed patterns of nuclear Overhauser enhancements confirmed the deductions obtained from c.d. and FT-IR spectroscopy concerning the solution conformation, suggesting a region of flexible nascent helix between Ala-4 and Ser-18. This structure is discussed in terms of the possible function of the peptide.

INTRODUCTION

The majority of mitochondrial proteins are encoded in the nucleus, synthesized in the cytoplasm, then imported after termination of synthesis. Most proteins that are translocated into the matrix are synthesized as precursors containing N-terminal transit sequences of between 17 and 60 residues in length, which are cleaved off by specific peptidases after import. Import is dependent on the precursor protein being in an unfolded state, and requires membrane potential across the inner membrane (Hartl et al., 1989). There is evidence that import is mediated by proteins in the cytosol, receptors in the membrane and chaperons in the matrix [reviewed by Schatz (1993)].

The primary structures of mitochondrial transit peptides are not conserved, but show common features, suggesting that secondary- or tertiary-structural elements may be recognized by the import machinery. Transit sequences are relatively rich in positively charged residues (mainly arginine), lack acidic side chains and have a high content of serines and threonines and small clusters of adjacent hydrophobic groups. Theoretical predictions have suggested that transit sequences have the ability to form amphipathic helices similar to those found in surface-seeking peptides such as melittin, with one face of high hydrophobicity and one face highly positively charged (von Heijne, 1986). Physical methods are required to investigate whether transit sequences adopt structures that fit these predictions.

Several transit peptides of fungal origin have been synthesized. C.d. data show that in aqueous solutions the peptides exist in random conformations, whereas in hydrophobic environments likely to mimic those encountered as the preprotein passes from the cytoplasm into membranes, they form repeating secondary

structure (Roise et al., 1986; Epand et al., 1986; Pak and Weiner, 1990; Tamm and Bartoldus, 1990). Although c.d. gives an indication of the percentage of secondary structure, it does not provide evidence about those parts of the molecule that adopt it.

The transit peptide of rat malate dehydrogenase has been synthesized previously and shown to inhibit import of precursor proteins into mitochondria (Chu et al., 1989). In order to ascertain the structural features of the peptide that are important for function, we have applied n.m.r., c.d. and Fourier-transform i.r. (FT-IR) spectroscopy to investigate its structure.

EXPERIMENTAL

Materials

Trifluoroethanol (TFE), $^2\text{H}_2\text{O}$, dimyristoyl-L- α -phosphatidyl-DL-glycerol (DMPG), dimyristoyl-L- α -phosphatidyl-DL-choline (DMPC) and cardiolipin (bovine heart) were purchased from Sigma. [^3H]TFE ($\text{CF}_3\text{C}^3\text{H}_2\text{OH}$) was from Fluorochem Ltd. Reagents for peptide synthesis were obtained from Milligen. All other reagents were of analytical grade.

Peptide synthesis

The N-terminal sequence of the precursor of rat malate dehydrogenase comprising the transit sequence and two residues of the mature protein was synthesized. The sequence of the peptide (Grant et al., 1986) is as follows: MLSALARPVGAALR-RSFSTSAQNNAK. The synthesis was performed on a Milligen 9050 automated synthesizer using protocols from the manufacturers and standard fluorenylmethoxycarbonyl methods. The purified peptide was shown to be homogeneous by reversed-phase

Abbreviations used: FT-IR, Fourier-transform i.r.; TFE, trifluoroethanol; n.O.e., nuclear Overhauser enhancement; DMPC, dimyristoyl-L- α -phosphatidyl-DL-choline; DMPG, dimyristoyl-L- α -phosphatidyl-DL-glycerol.

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h.p.l.c., amino acid analysis and fast-atom bombardment m.s. ($M+H = 2721$).

Preparation of peptide/phospholipid mixtures

The peptide and phospholipids were co-dissolved in a mixture of chloroform and methanol (1:1, v/v) and the solvent removed under dry nitrogen and then under high vacuum for 24 h. The peptide/lipid molar ratio used was 1:40. Either H_2O or 2H_2O was added to the dried film of phospholipid and peptide. This was subsequently thoroughly mixed using a Vortex mixer. The samples were incubated at 30 °C for about 1 h before spectroscopic measurement.

FT-IR spectroscopy

I.r. spectra were recorded on a Perkin-Elmer 1750 FT-IR spectrometer connected to a Perkin-Elmer data station. Spectra of samples in 2H_2O and $[^2H]TFE$ were recorded in a temperature-controlled Specac cell fitted with a 50 μm Teflon spacer. The spectrometer was continuously purged with dry air to eliminate water vapour interference. Solvent spectra (2H_2O and $[^2H]TFE$) were recorded under conditions identical with those used for sample spectra. In 2H_2O , peptide spectra were obtained in the concentration range 1–10 mg/ml, whereas in $[^2H]TFE$ the concentration range was 5–10 mg/ml. All spectra were recorded at 20 °C. Second-derivative spectra were generated from the peptide absorbance spectra using the Perkin-Elmer DERIV routine as described previously (Haris et al., 1986).

C.d. spectroscopy

C.d. spectra were recorded on a Jasco J600 spectrometer in the wavelength range 185–250 nm using a 0.05 cm pathlength cell. Spectra were recorded at room temperature (about 20 °C) and a peptide concentration of 1 mg/ml for samples in H_2O and TFE. Background spectra of the solvents H_2O and TFE were recorded under the same experimental conditions as the sample spectra. Difference spectra of the peptide were generated by subtracting the background spectra from the corresponding sample spectra. Spectra were smoothed using a Fourier-transform smoothing package supplied by Jasco. Analysis of the secondary-structure content of the peptide was carried out using a fitting method developed by Provencher and Glockner (1981).

N.m.r. spectroscopy

A sample for n.m.r. spectroscopy was prepared by dissolving 12 mg of the peptide in 0.4 ml of $[^2H]TFE$, giving a peptide concentration of approx. 11 mM. N.m.r. spectra were recorded at 400 MHz on a Bruker AMX 400 at a temperature of 300 K, with presaturation of the solvent resonance. Nuclear Overhauser enhancement (NOESY) (Jeener et al., 1979) and total correlation (TOCSY) (Braunschweiler and Ernst, 1983; Bax and Davis, 1985) spectra were acquired in the phase-sensitive mode, with quadrature detection in F1 provided by time proportional phase incrementation (Drobny et al., 1979; Bodenhausen et al., 1980). The phase cycles of the NOESY and TOCSY spectra were modified to allow acquisition of data with sinusoidal modulation in t_1 to reduce noise (Otting et al., 1986). The spin-lock period in the TOCSY spectra was achieved using a DIPSI (decoupling in the presence of scalar interactions) sequence (Shaka et al., 1988). The mixing time in the n.O.e. spectrum was 150 ms, and in the total correlation spectra, 14 ms and 63 ms, to reveal short- and long-range connectivities respectively. Some 512 free induction decays (FIDs) of 2048 complex data points were acquired over a sweep width of 4032 Hz in each experiment, giving digital

resolutions of 1.96 Hz/point and 13.5 Hz/point in F2 and F1 respectively. The data were zero-filled once in F2 and three times in F1 (to yield final matrices of 2048 $\times$ 2048 real points) and multiplied by a sine bell window function, shifted by $\pi/3$, before Fourier transformation. All data processing was performed on a Silicon Graphics Indigo workstation, using software provided by Tripos Associates. Chemical shifts are referenced to the residual protonated methylene signals from the solvent at 3.85 p.p.m.

Computational methods

The hydrophobicity moment was calculated as described by Eisenberg et al. (1984), using the quoted 'normalized consensus scale' for the hydrophobicity scale. The total hydrophobic moment μH was calculated over the residues Arg-7 and Ser-15 for a variety of precession angles, and also over all ten-residue stretches over the entire 26-residue sequence using a fixed precession angle of 100°.

RESULTS

FT-IR and c.d. measurements

Information on the structure of proteins and polypeptides can be derived from analysis of the amide bands present in their i.r. spectra [for a recent review see Haris and Chapman (1992)]. The most useful of these for secondary-structural analysis is the amide I band, located between approx. 1615 and 1695 cm^{-1} . Figure 1(a) presents the absorbance spectra of the mitochondrial transit peptide in $[^2H]TFE$ and 2H_2O showing this spectral region. More detailed analysis of the amide I band can be made by generation of the second derivative (Figure 1b) or deconvoluted spectra from the absorbance spectra. It can be seen that there is a difference between the amide I maxima of the polypeptide in the two solvents. The position of the band is at 1649 cm^{-1} for the sample in $[^2H]TFE$ and 1644 cm^{-1} for the sample in 2H_2O .

Figure 1 (a) FT-IR absorbance spectra of the mitochondrial transit peptide in 2H_2O (—) and $[^2H]TFE$ (---) and (b) second-derivative spectra of the mitochondrial transit peptide in 2H_2O (—) and $[^2H]TFE$ (---)

Figure 2 (a) C.d. spectra of the mitochondrial transit peptide in H_2O (----) and TFE (—) and (b) c.d. spectra of the mitochondrial transit peptide in phospholipid bilayers composed of DMPC (----) and DMPC (—), in 50 mM sodium phosphate, pH 7.4, at a peptide/lipid molar ratio of 1:40

The amide I frequency (1644 cm^{-1}) for the peptide in aqueous solution is consistent with a random-coil or undefined structure (Haris and Chapman, 1992). The c.d. spectrum of the polypeptide in aqueous solution presented in Figure 2 shows a single negative band at about 197 nm. This is also characteristic of peptides and proteins in a random-coil structure.

For the peptide in TFE the amide I maximum (1649 cm^{-1}) is close to the frequency normally associated with α -helical structure (Jackson et al., 1991; Haris and Chapman, 1992). The c.d. spectrum of the peptide in this solvent is shown in Figure 2(a), and displays a minimum at 207 nm with a shoulder at 222 nm, highly characteristic of α -helix. Thus both the FT-IR and c.d. data are consistent with the presence of an α -helical structure for this peptide when dissolved in TFE. The c.d. and FT-IR spectra of peptides often differ in band shape from those of large proteins. In this respect, quantitative analysis using methods that are normally used for large soluble proteins can sometimes give misleading results when applied to small peptides. This is because calibration sets of known crystal structures used for quantitative analysis are mainly based on large soluble proteins and not on small peptides. We have, nevertheless, carried out some quantitative analysis from the c.d. spectra of the peptide in water and TFE. This analysis shows that the helical content of the mitochondrial transit peptide increases from 0% in water to 40% in TFE. The result is consistent with the qualitative interpretation of the data presented above.

Spectra of the mitochondrial transit peptide in aqueous phospholipid vesicles were also obtained. The change in conformation of the polypeptide is most clearly evident from the c.d. data presented in Figure 2(b). As mentioned above, the peptide in aqueous solution is mainly in an undefined or random-coil conformation, but, on transfer to phospholipid vesicles composed of negatively charged phospholipids, there is a significant increase in α -helical content. The α -helical content estimated from the c.d.

Table 1 ^1H chemical shifts (p.p.m.) of the mitochondrial transit peptide

‡, Not detected.

Residue	NH	H α	H β	H γ	H δ	H ϵ	H ξ	NH(t)
Met-1	—	4.12	2.19	2.63; 2.6		2.08		
Leu-2	8.15	4.37	1.63	1.62	0.92; 0.89			
Ser-3	7.81	4.32	3.96; 3.80					
Ala-4	7.83	4.19	1.43					
Leu-5	7.57	4.25	1.65	1.66	0.92; 0.85			
Ala-6	7.49	4.25	1.43					
Arg-7	7.70	4.45	1.90	1.78; 1.67	3.22			7.06
Pro-8		4.43	2.22; 2.0	2.13	3.72; 3.64			
Val-9	7.55	3.78	2.11	1.01; 0.93				
Gly-10	8.12	3.88; 3.80						
Ala-11	7.88	4.09	1.47					
Ala-12	7.85	4.05	1.53					
Leu-13	8.41	4.05	1.87; 1.57	1.77	0.86			
Arg-14	8.14	4.06	1.95; 1.91	1.64	3.16			7.02
Arg-15	8.41	4.07	2.00	1.84; 1.77	3.17			7.02
Ser-16	8.33	4.21	4.06; 3.95					
Phe-17	8.65	4.34	3.21		7.22	7.24	7.27	
Ser-18	8.28	4.19	4.05					
Thr-19	7.90	4.13	4.32	1.26				
Ser-20	‡	‡		‡				
Ala-21	8.08	4.18	1.33					
Gln-22	‡	‡	‡	‡				
Asn-23	‡	‡	‡					
Asn-24	7.84	4.34	3.98; 3.82					
Ala-25	7.76	4.32	1.40					
Lys-26	7.32	4.64	1.94; 1.80	1.45	1.69; 1.24	2.99		7.26; 7.13

spectrum of the peptide in DMPG vesicles was 20%. C.d. spectra of the peptide in DMPG show double minima at 207 and 222 nm. This is highly characteristic of the presence of α -helical structure. Similar results were observed for the peptide in another acidic phospholipid, cardiolipin. The polypeptide in the zwitterionic lipid DMPC shows a c.d. profile which is characteristic of a random-coil structure (Figure 2b).

The results demonstrate that the mitochondrial transit peptide undergoes a conformational change on transfer from an aqueous solution to more hydrophobic media represented by either an organic solvent (TFE) or phospholipids. In addition, there is a lipid specificity that determines this conformational change: an increase in α -helical structure is only observed with negatively charged lipids.

N.m.r. spectroscopy

Unlike c.d. and FT-IR, high-resolution structural information is accessible from high-resolution two-dimensional n.m.r. spectroscopy in suitable solvents. Our justification for taking measurements in TFE is based on studies that indicate that it does not necessarily induce helical structure in sequences that do not already show a preference for this conformation (Sonnichsen et al., 1992; Dyson et al., 1992).

The ^1H -n.m.r. spectrum of the peptide was assigned by the standard homonuclear methods of sequential assignment (Wuethrich, 1986). Extensive spectral overlap precluded identification of the spin systems of Ser-20, Gln-22 and Asn-23, and the unambiguous interpretation of a number of n.O.e.s observed in the n.O.e. spectrum. A summary of chemical shifts in the observed spin systems is given in Table 1.

Regions of the n.O.e. spectrum of the peptide, corresponding to the 'fingerprint' region ($\text{NH-H}\alpha$ and $\text{NH-H}\beta$) and the NH-NH region, are shown in Figure 3. Observed n.O.e.s are summarized diagrammatically in Figure 4. From Figures 3 and 4 it may be seen that, besides several sequential [$\text{NH-H}\alpha$ or $d_{\alpha\text{N}}(i, i+1)$] connectivities, a number of NH-NH or $d_{\text{NN}}(i, i+1)$ n.O.e.s are observed. Unfortunately many of these cannot be identified unambiguously because of the extensive spectral overlap. There is a prominent stretch of medium to strong $d_{\text{NN}}(i, i+1)$ n.O.e.s from Ala-4 to Ser-18, with ambiguities at Leu-5, Ala-11, Leu-13 and Arg-14 caused by the overlap. The sequential $d_{\alpha\text{N}}(i, i+1)$ n.O.e.s for this stretch of the peptide are weak, absent or ambiguous, except for that from Val-9, which is medium to strong. Medium to strong $d_{\beta\text{N}}(i, i)$ and $d_{\beta\text{N}}(i, i+1)$ n.O.e.s were observed throughout this region. In addition, a few medium-range n.O.e.s [$d_{\alpha\text{N}}(i, i+2)$, $d_{\alpha\text{N}}(i, i+3)$, $d_{\alpha\beta}(i, i+3)$] were observed for this region of the sequence, around Pro-8-Gly-10. No $d_{\alpha\beta}(i, i+4)$ and $d_{\text{NN}}(i, i+2)$ n.O.e.s were positively identified. The C-terminal region from Thr-19 to the C-terminus is largely uncharacterized because the spin systems of Ser-20, Gln-22 and Asn-23 were not identified.

The type and relative intensity of sequential and long-range n.O.e.s observed for a peptide sequence may give information on the secondary-structure elements present, as the observation of an n.O.e. between a pair of protons indicates the presence of at least a threshold population of conformers between which the distance is relatively short [e.g. about 0.35 nm (3.5 Å) for flexible peptides in solution]. Thus, for example, the minimum requirements for the deduction of the presence of an α -helix would be the presence of a continuous series of strong $d_{\text{NN}}(i, i+1)$ connectivities, weak or absent $d_{\alpha\text{N}}(i, i+1)$ connectivities, and the presence of medium-range $d_{\alpha\text{N}}(i, i+2)$, $d_{\alpha\text{N}}(i, i+3)$, $d_{\alpha\beta}(i, i+3)$, $d_{\alpha\beta}(i, i+4)$ and $d_{\text{NN}}(i, i+2)$ connectivities; the α -helical confor-

Figure 3 (a) 'Fingerprint' region ($\text{NH-H}\alpha$) of the 400 MHz n.O.e. spectrum (150 ms mixing time) of the mitochondrial transit peptide and (b) NH-NH region of the same n.O.e. spectrum

Inter-residue connectivities are labelled with one-letter amino acid codes.

mation would potentially be further supported by small $^3J_{\text{HN}\alpha}$ coupling constants and independent observations from c.d. and FT-IR spectroscopy. On the basis of these criteria, it may be concluded that the only stretch of sequence that displays n.O.e.s consistent with an overall α -helical conformation is that from Pro-8 to Gly-10, and the general lack of medium- to long-range n.O.e.s argues for considerable conformational heterogeneity. Dyson and Wright (1991) have reviewed the criteria for defining the conformation of linear peptides in solution, in terms of local conformation at each residue, and more ordered secondary-structural elements. The observation of strong $d_{\alpha\text{N}}(i, i+1)$ n.O.e.s in the absence of $d_{\text{NN}}(i, i+1)$ n.O.e.s indicates a population of conformers with backbone dihedral angles in the β (extended chain) region of ϕ, ψ space; conversely, observation of strong

Figure 4 Diagrammatic summary of inter-residue n.O.e.s observed in the 150 ms mixing time n.O.e. spectrum of the mitochondrial transit peptide

Strong, medium and weak n.O.e.s are indicated by the size of the black shading. Dotted lines indicate regions where n.O.e.s present could not be identified unambiguously. * indicates that the n.O.e. is referred to the $2H\delta$ of Pro-8 in place of NH. n/d indicates that the relevant resonances were not detected. n/a indicates that the n.O.e. is meaningless.

Figure 5 (a) Plot of the hydrophobicity moment of ten-residue spans of the mitochondrial transit peptide sequence as a function of residue number and (b) plot of the total hydrophobicity moment of the segment Arg-7-Ser-16 as a function of precession angle

$d_{NN}(i,i+1)$ connectivities alone indicates the presence of conformers with local structures with ϕ, ψ backbone dihedral angles in the so-called α_L or α_R minima ($\pm 90^\circ, 45^\circ$), possibly suggesting

the presence of a nascent helix. Where stretches of $d_{NN}(i,i+1)$ and $d_{\alpha N}(i,i+1)$ n.O.e.s occur together, this implies conformational averaging between the two states. Thus the presence of strong $d_{NN}(i,i+1)$ n.O.e.s from approximately Ala-4 to Ser-18, as well as some $d_{\alpha N}(i,i+1)$ n.O.e.s, may suggest the presence of, overall, a more flexible nascent helical-type structure.

Inspection of chemical-shift values for $H\alpha$ resonances may provide an alternative simple qualitative estimation of the secondary-structural content of peptides (Wishart et al., 1991a,b). A helical conformation contributes an upfield shift of approx. 0.4 p.p.m. from the random-coil shift for $H\alpha$ resonances. Fast exchange between helical and random-coil conformations would result in a single resonance appearing at or near the average chemical shift for the two conformations, thus chemical shifts that are intermediate between those predicted for random coil and α -helix indicate some degree of conformational flexibility. The chemical shifts of the $H\alpha$ resonances reported in Table 1 are all intermediate between those expected for random coil and pure α -helix; although it is inadvisable to place too much reliance on chemical-shift values, which may be strongly affected by local steric and electronic influences and other variables such as solvent and temperature, this observation does suggest that a significant population of helical conformers is present. A simple qualitative analysis of the chemical shifts (Wishart et al., 1991a) gives an estimate of the helical content of the sequence of approx. 12 residues or 46%, which is in remarkable agreement with the c.d. analysis of the peptide conformation.

Computational methods

A hydrophobicity-moment calculation was performed for ten-residue spans over the 26-residue sequence, using a precession angle of 100° (Eisenberg et al., 1984). The result is shown in Figure 5(a). The span from Arg-7 to Ser-16 was calculated to have high hydrophobicity moment (μ per residue of 0.7), at similar levels to those calculated for the N-terminal sections of many other mitochondrial transit peptides (von Heijne et al., 1989) and membrane-active peptides such as melittin (von Heijne,

1986). A second calculation of hydrophobicity moment was performed over Arg-7–Ser-16 with various precession angles (Figure 5b). The result shows maximum amphipathicity at a precession angle of 100°, which is the angle of a classical α -helix. A lower peak of hydrophobicity moment was found for the ten-residue span Leu-13–Gln-22 at a 100° precession angle.

DISCUSSION

The present n.m.r. results suggest that the malate dehydrogenase transit sequence in TFE adopts a flexible nascent helix-type structure from Ala-4 to Ser-18 (approx. 55% of the sequence) with a more well-defined helical region from Pro-8 to Gly-10. The conformation of the C-terminal region was not able to be deduced. An alternative analysis of the secondary structure based on chemical shifts of the H α resonances suggests a significant population of helical conformers, with approx. 46% of the sequence predicted to adopt a helical conformation. The c.d. and FT-IR results for the peptide in TFE solution suggest an α -helical conformation, and quantitative analysis of the c.d. results estimates the α -helical content to be approx. 40%. Clearly, it is not evident whether this refers to 40% of a unique conformer displaying an α -helical conformation or the presence of a population of conformers whose overall c.d. spectrum shows the presence of 40% α -helix. Thus the c.d. results are in general agreement with, and support the conclusions drawn from, the n.m.r. data.

Hydrophobicity-moment calculations show that an N-terminal α -helix in this region would be highly amphipathic. Leu-13, which lies on the hydrophobic side of the N-terminal amphipathic helix, has been shown previously to play an important role in import into mitochondria (Chu et al., 1987b). Mutation of this residue to a more polar residue such as glutamine reduces binding to the mitochondrial surface, and mutation to asparagine, glutamate or proline also reduces import of the precursor to the matrix. Mutation of Arg-14 to an uncharged or negatively charged residue decreases the rate of import (Chu et al., 1987a), indicating that amphipathicity is important in function.

In the presence of either TFE or anionic phospholipids the mitochondrial transit peptide is α -helical, whereas in water or the presence of a zwitterionic phospholipid it is largely random coil. The α -helix content in TFE and DMPG is 40% and 20% respectively. The higher helical content in TFE is probably due to the peptide being constrained by the homogeneous hydrophobic environment of the organic solvent. In the case of aqueous lipid bilayers, the peptide is less constrained in a bipolar environment consisting of the hydrophobic acyl chain region and the hydrophilic environment of the polar headgroups and the aqueous phase. Thus the peptide is more conformationally flexible. It is possible that the hydrophobic face of the short amphipathic N-terminal helical region is inserted into the lipid bilayer, with the polar surface and non-helical portions exposed to the hydrophilic environment.

The proposed amphipathic helical structure is in agreement with the observations of others working with similar peptides (Tamm and Bartoldus, 1990; Karlslake et al., 1990), as is the significance of anionic phospholipids (Tamm and Bartoldus, 1990; Keller et al., 1992). In view of the relatively high concentrations of anionic phospholipids in mitochondrial membranes, the amphipathic structure is likely to be important for the translocation process. The mechanisms by which newly

synthesized mitochondrial proteins translocate from their sites of synthesis in the cytoplasm across two membranes in the matrix has not been clarified. Evidence suggests that several protein receptors bind precursor proteins on the mitochondrial surface and feed them into a central import channel (Schatz, 1993). However, these receptors are not essential for protein import either *in vivo* or *in vitro*, and have been classified as functionally redundant (Baker and Schatz, 1991). Thus other pathways involving direct interactions with membrane phospholipids may be important.

We can speculate that several precursor proteins may act in concert, possibly first forming a multivalent complex with hsp70 (Deshaies et al., 1988). Within this complex, transit sequences of several precursors may come together to form a micelle. As the aggregate approaches the mitochondrial membranes, anionic phospholipids may be attracted from the membrane to form a mixed micelle, causing local disruption of the membranes and leading to a pore through which the precursors may pass. Such a concerted action of precursor proteins suggested by this model is open to experimental verification.

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