Structural Analysis and Amphiphilic Properties of a Chemically Synthesized Mitochondrial Signal Peptide

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Anionic phospholipids induce a marked conformational change in a synthetic peptide corresponding to residues 1–27 of pre-ornithine carbamyltransferase. The peptide designated, pO-(1–27)-peptide amide, becomes more α-helical in the presence of cardiolipin or dimyristoylphosphatidylglycerol but not in the presence of dimyristoylphosphatidylcholine. The greater helix-promoting action of anionic 2-mercaptoethanol lipids is predicted by helix-coil transition theory. This statistical mechanical theory also predicts that a shorter peptide, N-acetyl-pO-(16–27)-peptide amide, has less helix-forming tendency, even in the presence of sodium dodecyl sulfate, despite the fact that it has a comparable number of positive charges. The N-acetyl-pO-(16–27)-peptide amide has no helical structure in buffer with or without dimyristoylphosphatidylglycerol but it has a small (5%) helical content in methanol. Thus, the ability of anionic lipids to promote helix formation requires more than the presence of cationic groups on the peptide. The angular dependence of the hydrophobic moment of the putative helical segment of pO-(1–27)-peptide amide demonstrates that any helical structure which is formed would have some amphiphilic character. The pO-(1–27)-peptide amide disrupts large lipid aggregates to form discoid micelles about 30 to 50 nm in diameter. The ability to lyse membranes into disc-shaped micelles is characteristic of peptides containing an amphipathic helix. In the case of the mitochondrial signal peptide, this membrane-lytic behavior may contribute to the translocation of the protein into the organelle.

Recent studies, in which mitochondrial signal sequences have been fused to cytosolic proteins, have convincingly demonstrated that such sequences are responsible, first, for targeting proteins to mitochondria and, second, for triggering subsequent protein translocation into the interior of the organelle (1–4). Additional findings that a chemically synthesized matrix protein signal peptide competitively inhibits mitochondrial import of an inner membrane protein (5) and demonstrations that the signal sequence of an outer membrane protein is functionally equivalent to that of a matrix precursor protein (4) strongly suggest that a common signal recognition apparatus (i.e., receptor) may be employed to import most mitochondrial precursor proteins. Current models to explain how proteins are subsequently partitioned between the various mitochondrial subcompartments, therefore, support the idea that mitochondrial signal sequences serve to initially trigger protein translocation to the matrix while stop-transfer sequences (6), if present elsewhere in the molecule, cause translocation to be arrested in either the outer or inner membrane (4, 7, 8).

As with all membrane systems, it is not known whether protein translocation across mitochondrial membranes involves passage through an aqueous-filled pore or through the lipid bilayer itself. Despite the high net positive charge and the overall polar characteristics of mitochondrial signal sequences, we have found that a synthetic signal peptide corresponding to the first 27 amino acids of pre-ornithine carbamyltransferase has lipophilic properties; incubation with mitochondria in the absence of reticulocyte lysate, for example, results in a complete collapse of the electrochemical potential across the mitochondrial inner membrane (5), presumably because amphiphilic properties of the peptide lead to perturbation of the inner membrane bilayer. Here, we show that indeed the synthetic peptide, designated pO-(1–27)-peptide amide, has the potential to assume an amphiphilic helical conformation in the presence of anionic lipid, interacts with synthetic liposomes, and in so doing perturbs bilayer structure and causes the formation of discoid micelles. Bilayer perturbation by mitochondrial signal peptides could conceivably play an important role during protein translocation into the organelle.

EXPERIMENTAL PROCEDURES

Lipids—Dimyristoylphosphatidylglycerol (DMPG), dimyristoylphosphatidylcholine (DMPC), and egg yolk lecithin from Avanti Polar Lipids and bovine heart cardiolipin from Sigma were all high purity commercial preparations.

Peptides—The peptides used were synthesized by solid phase procedures as previously described (5).

Solutions—All aqueous solutions were made in 10 mM sodium phosphate buffer, pH 7.0. The peptide solutions were added to dried lipid, vortexed at about 45°C, and then cooled to about 15°C. For the methanol solutions, the peptide was dissolved directly in that solvent.

Circular Dichroism—CD spectra were obtained with an Aviv Model 61DS solid state CD instrument (Aviv Associates, Lakewood, NJ). The instrument is equipped with a 50-KHz photoelastic modulator and an end-on photomultiplier. The CD was measured in a 1-mm sample cell which was maintained at constant temperature with a thermostated cell holder. Five to 10 scans were averaged and were corrected for the base line. The corrected data set was multiplied by a constant to obtain the mean residue ellipticity, [θ]. Secondary structure was estimated by a nonlinear, least-squares curve-fitting program as previously described (9, 10).

Freeze-Fracture Electron Microscopy—Samples sandwiched between two thin copper plates were incubated for 3 min at room temperature and then plunged into liquid propane without cryoprotectant. The samples were fractured and replicated as previously described (11).

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1 The abbreviations used are: p0, the precursor sequence of pre-ornithine carbamyltransferase; DMPG, dimyristoylphosphatidylglycerol; DMPC, dimyristoylphosphatidylcholine.
Calculation of Helix Probability Profiles—The probability of folding into a helical conformation was calculated for each amino acid residue by the method of Matice and co-workers (12, 13). The statistical weights for each amino acid at 30°C (12, 13) were used to construct a series of 3 x 3 matrices to calculate the helical content in water and sodium dodecyl sulfate and to construct a series of 13 x 13 matrices for the conformation in zwitterionic lipids. The effects of anionic phospholipids on the helical content of the peptides are expected to be related to the effects of sodium dodecyl sulfate upon which the statistical mechanical calculations were modeled. For the peptides in the presence of zwitterionic lipids, a statistical weight of s = 4 was used for charged residues which were separated by 3 or 4 other residues from a charged residue of opposite sign.

Calculation of Hydrophobic Moment—The hydrophobic moment was calculated as a function of the angle of rotation between residues as previously described (14).

RESULTS

The pO-(1-27)-peptide amide formed visually transparent solutions with DMPG. The difference in the CD spectra of pO-(1-27)-peptide amide in aqueous buffer in the presence and absence of DMPG (Fig. 1) indicates a large lipid-induced conformational change. The secondary structure of the peptide under these conditions was calculated from the CD spectra (Table I). The peptide has considerable γ-structure but the quantitative estimation of β-structure by CD is unreliable (9). DMPG clearly induces an increase in α-helical content. The helical content is further increased somewhat by higher lipid/peptide ratios and by lower temperature (Table I). Although the DMPG/peptide molar ratio is not sensitive to the DMPG/peptide ratio nor other residues from a charged residue of opposite sign.

Anionic lipid, cardiolipin, also induces a comparable increase in helical structure when added to the peptide as sonicated liposomes containing both cardiolipin and lecithin in a 1:3 molar ratio. In this case the lipid-peptide mixture was turbid and gave noisy spectra below 205 nm. The accuracy of the secondary structure estimates (particularly β-structure) in this case are therefore more questionable. This is also true for mixtures of the peptide with DMPC. We can conclude, nevertheless, that cardiolipin induces an amount of helical structure comparable to that induced by DMPG, while DMPC causes little or no conformational change (Table I). Although anionic lipids induce helix formation in pO-(1-27)-peptide amide, helix formation is not very extensive. The peptide can, however, attain a structure of higher helical content in methanol (Fig. 1 and Table I).

The shorter peptide, N-acetyl-pO-(16-27)-peptide amide, has little helix-forming tendency even in methanol (Fig. 2 and Table I). DMPG also does not induce helix formation in this peptide although it does appear to cause a conformational change leading to increased β-structure (Fig. 2 and Table I).

The extent of β-structure formation in N-acetyl-pO-(16-27)-peptide amide is not sensitive to the DMPG/peptide ratio nor to temperature (Table I). Although the DMPG/peptide molar ratios used in this case are not as high as the highest used with pO-(1-27)-peptide amide, the DMPG/peptide weight ratios are comparable for the two cases.

In 10 mM sodium phosphate buffer, DMPG forms irregular aggregates composed of ill-formed bilayer vesicles (results not shown). The suspension is turbid. Freeze-fracture electron micrographs of the clear "solutions" of DMPG in two concentrations of the pO-(1-27)-peptide amide show discoid micelles between 30 to 50 nm in diameter (Fig. 3a). The discoid micelles appear similar to those formed by glucagon and DMPC (11). At a lower lipid/peptide ratio of 15 (Fig. 3b), the discoid shape is not as apparent as at the higher ratio of 76 (Fig. 3a), perhaps due to the presence of excess peptide which
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FIG. 3. Freeze fracture electron micrographs of pO-(1–27)-peptide amide in DMPG. The lipid/peptide molar ratio is 76 (a) and 15 (b). Samples were frozen-quenched at room temperature. Bar, 100 nm.

FIG. 4. Helix probability profile. ---, pO-(1–27)-peptide amide in SDS; ---, N-acetyl-pO-(16–27)-peptide amide in SDS; ---, pO-(1–27)-peptide amide in zwitterionic lipid. The latter curve is of slightly higher helix-forming probability than that for this peptide in buffer (not shown). The helix-forming probability of N-acetyl-pO-(16–27)-peptide amide in buffer or zwitterionic lipid is close to zero.

FIG. 5. Angular dependence of the hydrophobic moment of the helix-forming residues 6–23 of pO-(1–27)-peptide amide.

has considerable helix-forming potential in the presence of anionic lipids. In contrast, N-acetyl-pO-(16–27)-peptide amide is predicted to have less helix-forming ability (Fig. 4). These results are in qualitative agreement with our secondary structure calculations from the CD data (Table I).

If a helix were to form in the pO-(1–27)-peptide amide, it would occur in the region of residues 6–23. Such a helical segment would have some amphipathic character with a hydrophobic moment (Fig. 5) of 0.136 for an α-helix (100°). A higher hydrophobic moment can be generated over shorter regions of the peptide as previously suggested (5). The hydro-

The formation of discoid micelles in the presence of peptides is suggestive of the formation of an amphipathic helix. Helix probability calculations by the methods of Mattice and co-workers (12, 13) illustrates that pO-(1–27)-peptide amide disrupts the discoidal lipoprotein complex at the low lipid/peptide ratio.
phobic moment of residues 16–26, which is the region of N-acetyl-pO-(16–27)-peptide amide which is most likely to become helical, is 0.415 for an α-helix (100°). Although a helix formed in this peptide would be highly amphipathic, the ability of N-acetyl-pO-(16–27)-peptide amide to fold into a helical structure is very weak (Table I).

**DISCUSSION**

The amphipathic helix is a helical peptide conformation in which hydrophobic and hydrophilic residues are clustered on opposite faces of a helix. Its role in lipid-protein interactions was first suggested for serum apolipoproteins (15). One of the characteristics of peptides which form amphipathic helices is that they solubilize phospholipids in the form of discoidal particles. In addition to serum apolipoproteins, it was noted that the peptide hormone glucagon also formed discoidal lipoprotein particles with lipid (16). Recently, a role for the amphipathic helix in the binding of hormones and other biologically active peptides to membranes has been proposed (17,18). In the present case we demonstrate that the precursor sequence of pre-ornithine carbamyltransferase also has certain characteristics of an amphipathic helix-forming peptide. The pO-(1-27)-peptide amide attains a structure of higher helical content in the presence of anionic lipid and it is able to solubilize this lipid in the form of discoidal micelles. The precursor sequence is not a potent lipid-solubilizing agent. It shows no interaction with zwitterionic lipids, it forms only a relatively small amount of helical structure in the presence of anionic lipids (Table I), and there is no peak in the hydrophobic moment of the helical region of the peptide near the periodicity required for helix formation (Fig. 5). In addition to penetrating the bilayer, the maintenance of an electrochemical potential is also required for the translocation of mitochondrial proteins. Perhaps a potent lipid-solubilizing agent would dissipate this gradient. Nevertheless, the mitochondrial leader sequence has some ability to form an amphipathic helix and solubilize lipid. This property may play a role in promoting transport of the protein across the mitochondrial membrane.

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