Secondary Structure and Assembly Mechanism of an Oligomeric Channel Protein†

Nancy Tobkes, B. A. Wallace, and Hagan Bayley*
Department of Biochemistry and Molecular Biophysics, Columbia University, New York, New York 10032
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ABSTRACT: The α-toxin of *Staphylococcus aureus* is secreted as a water-soluble, monomeric polypeptide (M_r 33 182) that can assemble into an oligomeric membrane channel. By chemical cross-linking, we have confirmed that the major form of the channel is a hexamer. The circular dichroism spectrum of this hexamer in detergent revealed that it contains a high proportion of β-sheet that we deduce must lie within the lipid bilayer when the protein is associated with membranes. The circular dichroism spectrum of the monomeric toxin in the presence or absence of detergent was closely similar to the spectrum of the hexamer, suggesting that the secondary structure of the polypeptide is little changed on assembly. Results of experiments involving limited proteolysis of the monomer and hexamer are consistent with the idea that assembly involves the movement of two rigid domains about a hinge located near the midpoint of the polypeptide chain. The hydrophilic monomer is thereby converted to an amphipathic rod that becomes a subunit of the hexamer.

Our knowledge of the secondary and tertiary structure of integral membrane proteins remains incomplete. Moreover, little is known about the mechanisms by which these molecules become incorporated into the lipid bilayer. Two structural classes of integral membrane proteins are often encountered. Proteins of the first class have large globular extramembranous domains anchored to the lipid bilayer by segments of ~25 hydrophobic amino acids that are thought to form membrane-spanning helices [for example, glycophorin A (Segrest et al., 1973; Schulte & Marchesi, 1979), histocompatibility antigens [e.g., Coligan et al. (1981)], and the hemagglutinin of influenza virus [e.g., Porter et al. (1979)]]. Proteins of the second class are globular and are largely buried within the lipid bilayer. They are generally believed to consist of bundles of approximately parallel α-helices oriented perpendicular to the membrane surface [for example, bacteriorhodopsin (Henderson & Unwin, 1975), rhodopsin (Nathans & Hogness, 1983), and the lactose transporter of *Escherichia coli* (Foster et al., 1983)]. Certain integral proteins may share aspects of both these structural classes [for example, the anion transporter of human red blood cells (Drickamer, 1980; Markowitz & Marchesi, 1981; Kaul et al., 1983)], while the structures of others are clearly different but as yet poorly defined (see Discussion). In the area of assembly, rapid progress has been made in understanding the processing and sorting of newly synthesized integral membrane proteins (Schatz & Butow, 1983; Walter et al., 1984), but little information exists concerning the associated changes in polypeptide conformation. Such information is also scarce for other processes involving the interaction of proteins with membranes, including the assembly of the complement pore, the fusion of viral membranes with those of eukaryotes, and the binding of blood clotting factors to membranes.

To further our knowledge in these areas, we are studying the structure and assembly of the channel formed by the α-toxin of *Staphylococcus aureus*. The toxin is secreted as a monomer. It has an M_r of 33 182 and an amino acid composition that is markedly *polar* (Six & Harshman, 1973a,b; Gray & Kehoe, 1984). On contact with susceptible cells, such as rabbit erythrocytes, the toxin assembles into an oligomeric channel with an internal diameter of about 30 Å [for reviews, see Harshman (1979), Freer & Arbuthnott (1982), and Bhakdi & Tranum-Jensen (1983)]. The structure of the channel clearly differs from the structures of integral proteins such as glycophorin and bacteriorhodopsin, and as Bhakdi and his colleagues have suggested (Bhakdi & Tranum-Jensen, 1983), knowledge of the mechanism of assembly of the relatively simple α-toxin channel might give insight into other processes wherein water-soluble proteins assemble into membranes. Here we present new information concerning both the structure and the assembly of the channel.

Materials and Methods

Materials. S. aureus (Wood strain) was obtained from the American Type Culture Collection. Vacuum dialysis was carried out with collodion bags (UH100/10) from Schleicher & Schuell. Deoxycholic acid (DOC)[1] from Sigma was recrystallized from ethanol/water. The hot solution was first treated with charcoal. Other materials were obtained as follows: controlled pore glass beads (CPG 00350) from Electro-Nucleonics, cellulose thin-layer chromatography plates from Macherey-Nagel, dimethyl suberimidate from Pierce, proteinase K from Boehringer-Mannheim, TPCK-treated trypsin from Sigma, phenylmethanesulfonyl fluoride from Sigma, and $Na^{125}I$ from Amersham International.

Preparation of α-Toxin. α-Toxin was isolated from the supernatants of cultures of *S. aureus* by a method closely similar to that of Cassidy & Harshman (1976). All steps were at 4 °C. After adsorption chromatography on controlled pore glass beads, the toxin-containing fractions were concentrated by ultrafiltration and further fractionated on a Sephadex G-50 column. The purified toxin was concentrated to ~2 mg/mL by vacuum dialysis against 10 mM Tris·HCl–150 mM NaCl, pH 8.0, and stored at −70 °C.

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* Address correspondence to this author at the Center for Neurobiology & Behavior, Columbia University, New York, New York 10032.

[1] Abbreviations: CD, circular dichroism; DOC, deoxycholate; SDS, sodium dodecyl sulfate; TPCK, N^{α}-tosylphenylalanine chloromethyl ketone; Tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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Formation of Toxin Oligomers. Oligomers were formed at room temperature according to the procedure of Bhakdi et al. (1981). DOC was added in stepwise fashion to monomeric α-toxin, over a period of 50 min, to a final concentration of 6.25 mM. The resultant oligomers were isolated by gel filtration on Sephacryl S-300 in 10 mM Tris·HCl (or 10 mM sodium phosphate), pH 8.0, containing 150 mM NaCl and 1.25 mM DOC. The oligomers were concentrated by vacuum dialysis against the appropriate buffer and stored at 4 °C.

Chemical Cross-Linking. Toxin oligomers (1.45 mg/mL) were cross-linked with dimethyl suberimidate (0.8 mg/mL) for 1 h at 25 °C. Cross-linking was carried out in the phosphate buffer, which was first brought to 0.2 M by the addition of 1 M sodium phosphate, pH 8.0. The reaction was quenched with 0.6 M ethanolamine hydrochloride (pH 8.0, 0.2 volume). Denatured portions of the reaction mixture [1.2% (w/v) SDS, 100 °C, 2 min] were electrophoresed in 4.5% (w/v) polyacrylamide gels according to Fairbanks et al. (1971) except that the buffers contained 0.2% (w/v) SDS.

Circular Dichroism Spectroscopy. All toxin samples were freshly prepared for spectroscopic measurements by gel filtration on Sephacryl S-300 eluted with 10 mM sodium phosphate–150 mM NaCl, pH 8.0, containing 1.25 mM DOC when required. If necessary, the fractions were immediately concentrated by vacuum dialysis. Some aggregation occurs when samples of monomer, in particular, are stored. These samples often gave spectra that were very noisy in the low-wavelength region.

Spectra were recorded at room temperature (~23 °C) with a Cary Model 60 spectropolarimeter equipped with a Model 6001 CD attachment. Measurements were made in 0.05-cm path length cells at toxin concentrations of 0.50–0.75 mg/mL. Protein concentrations were initially estimated from the absorbance at 280 nm, using an extinction coefficient of 1.16 (at 1 mg/mL), based on amino acid analysis. Each spectrum was recorded at least 3 times. The data were averaged, and the spectrum of the corresponding buffer (column eluant or dialysate, also averaged) was subtracted. The spectrum so obtained was fitted to a set of reference spectra derived from 15 water-soluble proteins to give the fractions of each class of secondary structure (Chang et al., 1981). The calculated fractions were positive in all cases even though the linear least-squares method used did not constrain them to be so. This indicates that the secondary structures found in α-toxin are well represented in the reference data set. The sums of the fractions were normalized to unity to correct for errors in protein concentrations (Mao et al., 1982). The data from 14 independent analyses are compiled in Table I.

Proteinase K Treatment. Samples of toxin monomer and toxin hexamer (1.0 mg/mL) were digested with proteinase K at 25 °C. The substrate:enzyme ratio was 20:1 (w/w). At the appropriate time points, the enzyme was inactivated with phenylmethanesulfonyl fluoride (2 mM). The buffers used for the digests are given in Figure 3 (legend). Portions of digested toxin were subjected to SDS gel electrophoresis after treatments that do or do not dissociate the hexameric form. "Undissociated" samples were electrophoresed in gels prepared according to Fairbanks et al. (1971), but containing 4.5% (w/v) acrylamide and 0.2% (w/v) SDS. The samples were prepared at 25 °C by adding 1 volume of electrophoresis loading buffer containing 0.4% (w/v) SDS to the digested protein. "Dissociated" samples were electrophoresed in gels prepared according to Laemmli (1970), containing 15% (w/v) acrylamide. The samples were prepared by adding 1 volume of loading buffer containing 1.5% (w/v) SDS and by heating at 100 °C for 1 min. In control experiments, it was found that 1.25 mM DOC *increased* the rate at which toxin monomer was digested by proteinase K; i.e., the detergent did not inhibit the enzyme.

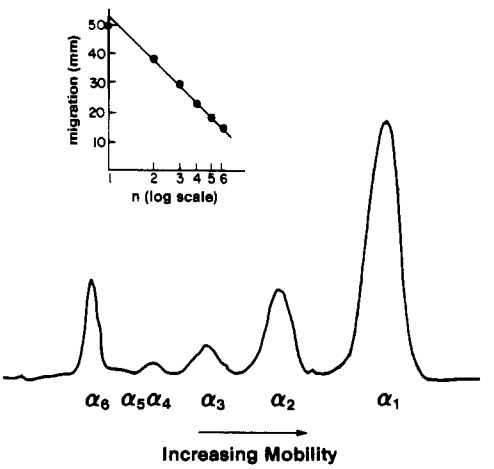

FIGURE 1: SDS–polyacrylamide gel electrophoresis of oligomeric α-toxin after cross-linking with dimethyl suberimidate. A densitometer trace of the gel after staining with Coomassie Blue is shown with the assignments of the bands (α_n). The band corresponding to the pentamer was more clearly visible when the protein was stained with silver. The spacing of the bands is according to a logarithmic progression (insert). A small deviation is seen for the monomer, which might differ in shape from the subunits in the oligomers, especially if the latter are resistant to complete denaturation in SDS (see the text).

Peptide Mapping. Tryptic maps of the fragments of monomeric α-toxin generated by proteinase K treatment were prepared according to Elder et al. (1977). α-Toxin (1.0 mg/mL) was digested with proteinase K (substrate:enzyme, 40:1) in 10 mM sodium phosphate–150 mM NaCl, pH 8.0, as described in the legend of Figure 4. The cleavage products were electrophoresed in gels prepared according to Laemmli (1970). The polypeptides were located by staining with Coomassie Blue and radiolabeled in situ with ^{125}I by using chloramine-T. The gel slices were treated with trypsin (25 μg/slice) for 20 h at 37 °C in 0.05 M NH_4HCO_3, pH 8.0 (500 μL). The supernatants from the digests were dried in a vacuum centrifuge, and the radiolabeled peptides in the residue were separated in two dimensions on cellulose thin-layer plates by electrophoresis followed by chromatography (Elder et al., 1977). Autoradiographs were made at −70 °C with Kodak X-Omat AR (XAR-5) film.

RESULTS

At the outset, we wished to reexamine the question of the number of toxin subunits in each oligomeric channel. To do so, we cross-linked the oligomeric form of α-toxin with several chemical reagents. The results obtained with dimethyl suberimidate were typical. The cross-linked protein was heated with SDS to 100 °C to dissociate the remaining noncovalent oligomers, which are stable in the presence of this detergent at room temperature (McNiven et al., 1972; Cassidy & Harshman, 1979), and subjected to electrophoresis in a SDS–polyacrylamide gel. Six bands of protein were observed, separated by distances that followed a logarithmic series (Figure 1). After more extensive cross-linking (not shown), more than 50% of the oligomers were converted to covalent hexamers without the formation of heptamers or larger covalent complexes. The hydrodynamic properties of the oligomer are those of a homogeneous species (Forlani et al., 1971; Arbuthnott et al., 1973; Cassidy & Harshman, 1979; Bhakdi et al., 1981). Therefore, the abrupt termination of the electrophoretic "ladder" after the sixth band directly confirms that

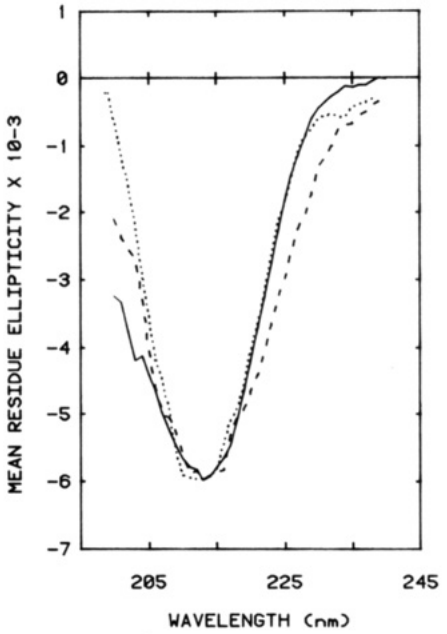

FIGURE 2: Circular dichroism spectroscopy of monomeric and hexameric α-toxin. CD spectra of monomeric α-toxin in the absence of detergent (—), monomeric α-toxin in buffer containing 1.25 mM DOC (---), and hexameric α-toxin also in buffer containing 1.25 mM DOC (···). The spectrum of the hexamer was shifted by 2 nm to shorter wavelengths as described in the text.

Table I: Computed Secondary Structure Contents of Monomeric and Hexameric α-Toxin

	α-helix	β-sheet	β-turn	random coil	N
monomer	0.05	0.57	0.11	0.27	7
monomer in 1.25 mM DOC	0.05	0.55	0.12	0.28	3
hexamer in 1.25 mM DOC	0.10	0.62	0.09	0.19	4
hexamer (shifted)	0.07	0.52	0.16	0.25	4

the predominant oligomeric form is a hexamer in accord with the sedimentation equilibrium measurements of Bhakdi et al. (1981). Thus any doubts we had concerning the subunit stoichiometry, based on the experimental and systematic errors involved in hydrodynamic methods for determining molecular weights (Tanford & Reynolds, 1976), were dispelled.

The secondary structures of monomeric toxin in the presence and absence of DOC and of the hexamer in DOC were examined by circular dichroism spectroscopy (Figure 2). Care was taken to eliminate optical artifacts caused by light scattered from protein aggregates by using α-toxin samples freshly prepared by gel filtration. Both the monomeric and hexameric forms contained a substantial fraction of β structure (sheet and turns, ~68%), little α-helix (~6%), and some random coil (or unassigned structure, ~26%) (Table I).

The CD spectra of the monomeric and hexameric toxins closely resembled each other, and when the spectrum of the hexamer was shifted by 2 nm to shorter wavelengths, the similarities were still greater (Figure 2). It should be noted that even if the conformational change that occurs during assembly involves little alteration in secondary structure, as we suggest below, the CD spectra would not be expected to be completely superimposable: light scattering could not be completely eliminated at short wavelengths, the motion of fixed domains about a hinge (see below) will involve small changes in secondary structure, and the CD of β-sheet is particularly sensitive to changes in environment (Manavalan & Johnson,

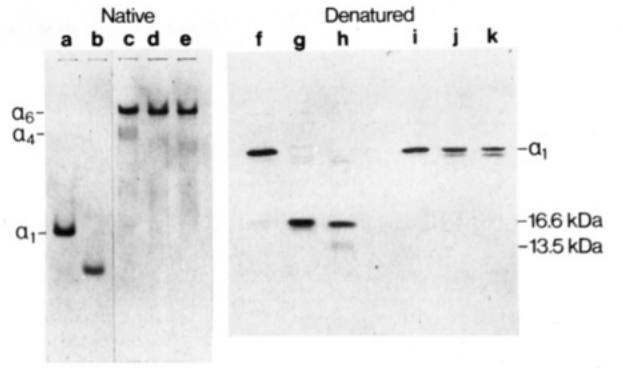

FIGURE 3: SDS–polyacrylamide gel electrophoresis of toxin monomers and hexamers after digestion with proteinase K. Digests were at 25 °C, and the substrate:enzyme ratio was 20:1 (w/w). Samples f–k were "dissociated" before electrophoresis; samples a–e were not. (a) Undigested monomer; (b) monomer, 5-min digestion; (c) undigested hexamer; (d) hexamer, 5-min digestion; (e) hexamer, 60-min digestion; (f) undigested monomer; (g) monomer, 5-min digestion; (h) monomer, 60-min digestion; (i) undigested hexamer (dissociated); (j) hexamer, 5-min digestion (dissociated); (k) hexamer, 60-min digestion (dissociated).

1983). Indeed, the red shift in the CD spectrum of the hexamer may be due to a change in the polarity of the local environment of the β structure (Wallace et al., 1984), a possibility in keeping with our model for toxin assembly (see below). The calculated secondary structures of the monomeric and hexameric forms were very similar even without taking into account the spectral shift (Table I).

Further information about the structural changes occurring when the monomer assembles to form the hexamer was gained by limited proteolysis of both species in buffer containing 1.25 mM DOC. The results obtained with proteinase K, a nonspecific protease, are shown in Figure 3. First, we found that, compared to the hexamer, the monomer was highly sensitive to the protease (Figure 3, lanes a–e). The stability of the oligomeric toxin toward other proteases had been noted earlier (Cassidy & Harshman, 1979; Füssle et al., 1981). Second, the two forms yield different fragmentation patterns. Under mild conditions,[2] the monomer yielded a band with an apparent mass of 16.6 kDa (Figure 3, lane g). We could show that this band actually contains two fragments with closely similar mobilities, together containing most of the mass of the intact toxin. First, prolonged digestion yielded two products that were relatively stable toward further digestion: one with about the same mobility as the band generated initially and the other of 13.5 kDa (Figure 3, lane h). The latter, somewhat diffuse band was formed via intermediates of 15.7 and 15.0 kDa (data not shown). Second, tryptic maps of the iodinated polypeptides from the gel bands (Elder et al., 1977) revealed that the initial 16.6-kDa species yielded almost all the tryptic peptides that could be generated from the intact toxin (Figure 4a,b). After prolonged proteolysis the 16.6-kDa species yielded a simpler map (Figure 4c). The major peptides that were now absent appeared in maps of the 13.5-kDa band (Figure 4d). In contrast, the subunits in the hexamer were not cleaved at their midpoints, while a fragment of 1.0–1.5 kDa was slowly removed (Figure 3, lanes j and k). It was also noted that the fragments of the hexamer did not dissociate in SDS at 25 °C (Figure 3, lanes d and e).

DISCUSSION

Our results are relevant to both the structure and the assembly of the channel formed by α-toxin. First, we consider

[2] In the presence of proteinase K (enzyme:substrate, 1:50) at 4 °C, the half-life of the α-toxin polypeptide (1.0 mg/mL) was 7.5 min.

Table II: Comparison of α-Toxin and Related Channels or Pores[a]

	subunit composition	appearance in electron microscope	predominant secondary structure	gated pore	SDS resistant	posttranslational assembly	resistant to proteolysis
α-toxin[b]	α_6	pore, projects from membrane surface	β	no	yes	yes	yes
gap junction connexon[c,d]	α_6	pore, no projection visible	β	yes	no	no	yes
acetylcholine receptor[e-g]	$\alpha_2\beta\gamma\delta$	pore, projects from membrane surface	αβ	yes	no	no	yes
complement membrane attack complex[h-k]	α_{12-18}	pore, projects from membrane surface	αβ	no	yes	yes	yes
porin (*E. coli* and mitochondrial)[l-n]	α_3	clearly differs from above, e.g., three channels per trimer	β	yes	yes	yes	yes

[a] This table is intended only to outline the similarities between these proteins and not to provide a detailed comparison. Resistance to proteolysis implies that the pore structure remains intact. In some cases cleavage of polypeptide chains does occur quite readily, although the structure is maintained. Here, posttranslational assembly implies that the polypeptide chains exist in a water-soluble form after translation. The subunits of the nicotinic acetylcholine receptor are highly homologous. Porin differs in gross structure, but it does share several unusual properties with α-toxin. [b] From this work and references cited in text. [c] Makowski et al., 1982. [d] Unwin & Zampighi, 1980. [e] Moore et al., 1974. [f] Karlin, 1980. [g] Anderson & Blobel, 1981. [h] Tschopp et al., 1984. [i] Tschopp et al., 1982. [j] Podack & Tschopp, 1982a. [k] Podack & Tschopp, 1982b. [l] Schatz & Butow, 1983. [m] Rosenbusch, 1974. [n] Garavito et al., 1983.

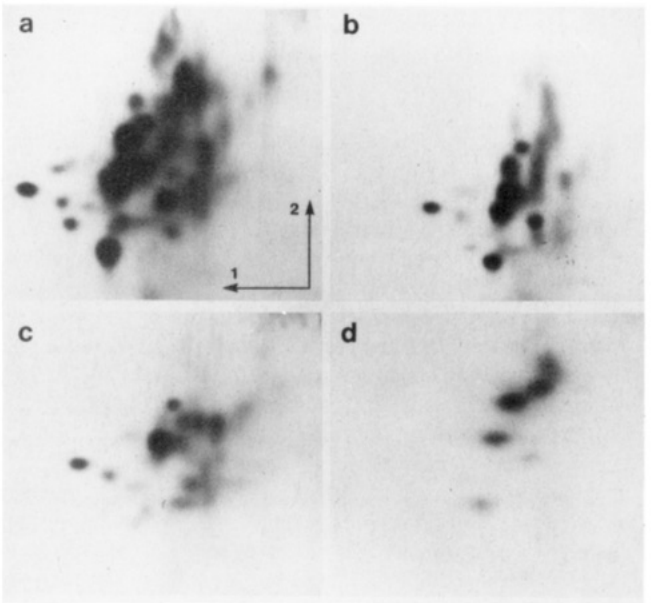

FIGURE 4: Tryptic maps of iodinated proteinase K fragments derived from α-toxin. Autoradiograms are shown of maps from the following: (a) intact toxin; (b) the 16.6-kDa band after 7.5-min digestion at 37 °C; (c) the 16.6-kDa band after 60-min digestion at 37 °C; (d) the 13.5-kDa band after 60-min digestion at 37 °C. The directions of electrophoresis (1) and chromatography (2) are shown in panel a.

the structure of the channel, which we have confirmed by the chemical cross-linking experiment described here to be a hexamer. The CD measurements indicate that the secondary structure of the hexamer in detergent differs considerably from the structure present in those membrane proteins in which α-helix is found in association with the lipid bilayer.[3] It has even been proposed that only helical polypeptide chains exist in lipid bilayers [e.g., Engelman & Steitz (1981)], but this is an extreme view [e.g., Tanford & Reynolds (1976) and Unwin & Henderson (1984)]. According to our estimate, each toxin monomer contains barely enough α-helix to form a membrane-spanning rod (6–10% helix ≃ 17–29 residues of length 25.5–43.5 Å).[4] Further, it is not possible to construct a channel 30 Å in diameter lined by only six helices, and we

[3] The hexamer in detergent is identical with the assembled channels recovered from erythrocyte membranes (Füssle et al., 1981).

[4] A lower limit for the length of such a helix might be the width of the hydrocarbon domain of a lipid bilayer composed of dipalmitoylphosphatidylcholine, which is 26 Å (Lewis & Engelman, 1983). An upper limit might be the length of the α-helices in bacteriorhodopsin, which is 45 Å (Agard & Stroud, 1982).

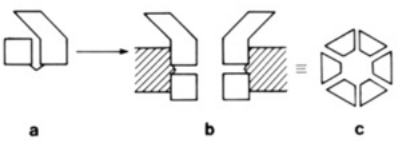

FIGURE 5: Model for the assembly of α-toxin. (a) The monomer consists of two domains containing primarily β structure linked by a hinge. (b) On assembly the monomer opens up about the hinge, revealing an occluded hydrophobic surface. In the hexamer this surface is in contact with the lipid bilayer (or detergent), while the interior surface of the channel comprises residues that were originally on the surface of the monomer. (c) Top view of hexamer.

suggest that the intramembranous part of the hexamer is composed of domains of β-sheet surrounding the central pore. These strands are unlikely to be oriented in parallel fashion because they would have to be connected by equally long segments of polypeptide located within the bilayer. In soluble proteins parallel β-strands are often connected by α-helix (e.g., in triosephosphate isomerase or adenylate kinase), but α-toxin does not contain enough helix to perform this function. The strands might also be connected by random coil, but this possibility is unattractive on energetic grounds as the peptide bonds would neither be solvated nor participate in intramolecular hydrogen bonding (Engelman & Steitz, 1981). Therefore, we favor domains of antiparallel β-sheet as the predominant structure within the bilayer. These domains might be similar to those found in the immunoglobulins or in trypsin. Regions at the edges of the sheets or at turns, which do not form intramolecular hydrogen bonds and therefore find the hydrocarbon phase inhospitable, could be accommodated if they were located at domain–domain interfaces or if the domains extended into the aqueous phase. Our results reinforce both the theoretical arguments (Tanford & Reynolds, 1976; Unwin & Henderson, 1984) and the limited experimental evidence (Rosenbusch, 1974; Makowski et al., 1982; Table II) for structures other than helix in those regions of integral proteins lying within the lipid bilayer.

α-Toxin bears a remarkable structural likeness to three eukaryotic channel proteins: the membrane attack complex of complement, the gap junction connexon, and the nicotinic acetylcholine receptor (Table II). The similarities include morphology in the electron microscope, intraspecies subunit identity or strong homology, the existence of β-sheet structure, resistance to SDS (for α-toxin and complement), and resistance to proteolysis. How close this resemblance is at a detailed structural level remains to be explored experimentally.

On the basis of our findings, a simplified working model for the assembly of α-toxin into lipid bilayers has been devised. Because there is little or no change in secondary structure on

assembly, it seems reasonable to suggest that, correlated with the hydrophilic to amphiphilic transition, a change in tertiary structure takes place. In our model (Figure 5), the water-soluble monomer contains two domains connected by a hinge. The protein turns partly inside out before forming hexamers in which the hydrophilic residues that were formerly on the surface of the monomer line the channel or take part in subunit–subunit and domain–domain interactions. Hydrophobic residues that were occluded between the two domains in the monomer are revealed during assembly and form the outer surface of the channel, which is in contact with the hydrocarbon core of the bilayer. When α-toxin interacts with biological membranes, the conformational change would be induced by the toxin receptor. A minor, protease-sensitive, tetrameric form that we frequently observe in our preparations of hexameric toxin (e.g., Figure 3c) might be a late intermediate in the assembly process. It may be noted that there are several water-soluble proteins in which domains of antiparallel β-sheet are separated by hinges comprising a single length of polypeptide chain (e.g., immunoglobulins and tomato bushy stunt virus coat protein).

The pattern of cleavage of α-toxin with proteinase K is in keeping with our model, although such experiments can only provide support and not proof as changes in the position and rate of attack by proteases can originate from relatively subtle conformational changes [e.g., Jørgensen (1975)]. By analogy with the action of proteases on many other proteins containing more than one domain [e.g., Mihalyi (1972) and Speicher et al. (1980)] we suggest that the highly sensitive initial site of cleavage in the monomer is at a hinge region. Further support for this is provided in the very recent sequence analysis of the α-toxin gene by Gray & Kehoe (1984). The region from residues 119–143, which contains 32% glycine, might form the hinge, and it is positioned such that fragments of $\sim$16.5 kDa would be yielded if the first peptide bond cleaved lay toward the C-terminal end of this region. As expected, this cleavage site is absent or occluded in the hexamer.

Conformational changes of the sort suggested here may be important in the assembly of other membrane pores such as the membrane attack complex of complement [Tschopp et al. (1984) and other references cited in Table II], the post-translational integration of proteins into membranes of organelles (Schatz & Butow, 1983; the relevance may even extend to steps in cotranslational assembly), and the fusion of viral and host-cell membranes (Skehel et al., 1982; Hsu et al., 1982).

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Membrane Protein Conformational Change Dependent on the Hydrophobic Environment†

M. Lisa Wilson‡ and Frederick W. Dahlquist*
Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403-1229
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ABSTRACT: Two conformational states of the coat protein of the filamentous bacteriophage M13 have been detected in detergent solution by using magnetic resonance techniques. When 3-fluorotyrosine is incorporated in place of the two tyrosine residues in the protein, four ^{19}F nuclear magnetic resonance signals are observed, two for each conformer of the protein. The equilibrium between the two forms can be modulated by pH, temperature, and detergent structure. The rate of interconversion of the isomers is rapid on the minutes time scale but is slow relative to the T_1 relaxation time of the fluorine resonances of ~50 ms. The conformational change between the conformers results in the perturbation of a basic residue in the protein such that this group has a pK_a of ~9.5 in one state which shifts to 10.5 or more in the other conformational state. The temperature dependence of the equilibrium suggests an enthalpy difference of about 10 kcal/mol which is offset by entropy to give nearly zero free energy difference between the states at pH 8.3 in deoxycholate solution at room temperature. This suggests a substantial reorganization of the noncovalent interactions defining the two conformational states. The conformational equilibrium is strongly dependent on detergent structure and the presence of phospholipid in the detergent micelle. The results are not consistent with a strong, specific lipid binding to the protein but appear to be consistent with a more general effect of the overall micelle structure on the conformational state of the protein.

The interactions of lipids and proteins have been examined by a number of physical and biochemical techniques. The classic electron spin resonance studies of Jost et al. (1973) using spin-labeled lipids detected a motionally restricted component of the lipids in the presence of membrane proteins and coined the term "boundary lipid" to describe this motionally restricted component. More recent deuterium magnetic resonance studies of labeled lipids in the presence of proteins suggest that the interaction between most lipids and membrane proteins is characterized by rapid exchange [on the nuclear magnetic resonance (NMR)[1] time scale of 10^{-5}–10^{-6} s] of lipid molecules between the boundary lipid environment and the bulk lipid environment (Oldfield et al., 1978; Paddy et al., 1981). These measurements suggest that this exchange rate is similar to that of lipid diffusing in the absence of protein. Such a result implies that the interactions between lipid and protein are likely to be weak and generally lack the binding specificity that one might associate with the presence of well-defined lipid binding sites along the protein surface. Thus, it appears that the bulk of lipid–protein interactions reflect a nonspecific solvation of the hydrophobic exterior region of membrane proteins by the hydrophobic lipid.

Detergents often mimic the effects of lipid in mediating activity of membrane proteins because they provide the hy-

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‡ Present address: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511.

[1] Abbreviations: DOC, sodium deoxycholate; CD, circular dichroism; SDS, sodium dodecyl sulfate; DMPC, dimyristoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DPG, bovine diphosphatidylglycerol; NMR, nuclear magnetic resonance; MCI, micellar conformation I of M13 gene 8 protein; MCII, micellar conformation II of M13 gene 8 protein; TFA, trifluoroacetic acid; *L/P*, [lipid]/[protein] ratio; *L/P/D*, [lipid]/[protein]/[detergent] ratio; Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

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