Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 958–962, February 1985
Biochemistry

Anomalous interaction of the acetylcholine receptor protein with the nonionic detergent Triton X-114

(integral membrane proteins/channel-forming aggregates/phase partitioning)

PAMELA A. MAHER AND S. J. SINGER

Department of Biology, University of California at San Diego, La Jolla, CA 92037

Contributed by S. J. Singer, September 24, 1984

ABSTRACT **Integral membrane proteins that form water-filled channels through membranes often exist as aggregates of similar or identical subunits spanning the membrane. It has been suggested that the insertion into the membrane of the channel-forming domains of the subunits may impart unusual structural features to the membrane-intercalated portions of the protein. To test this proposal, we have investigated the interaction of a multisubunit channel-forming integral membrane protein, the acetylcholine receptor protein, with the nonionic detergent Triton X-114. Whereas non-channel-forming integral membrane proteins that have heretofore been studied form mixed micelles with the detergent, the acetylcholine receptor was excluded from the Triton X-114 micelles. The structural implications of this result are discussed.**

In an early thermodynamic analysis of membrane structure (1), it was predicted that a class of integral membrane proteins exists that form water-filled channels through membranes and that mediate the permeability of membranes to ions and small polar molecules. It was proposed that such a channel-forming protein generally would consist of a specific noncovalently bound aggregate of a small number n of identical or similar subunits spanning the membrane, with the channel running down the n-fold symmetry axis of the aggregate. To allow for thermodynamic stability, it was suggested that the portion of the exterior surface of the aggregate that was intercalated into the lipid bilayer would be mainly hydrophobic, but that the central channel might be lined with some of the ionic and polar amino acid residues of the subunits, in contact with the water in the channel. The purpose of such a structure would be to permit low-energy-requiring quaternary rearrangements (1, 2) of the subunits to occur, which would result in the directed translocation of specific ions and small polar molecules through the channel across the membrane. In further consideration of this model, it was pointed out (3, 4) that the insertion of such a hydrophilic channel through a membrane is not a trivial problem and, furthermore, that certain thermodynamically satisfactory mechanisms for such insertion might necessitate distinctive structural properties for channel-forming proteins, which would not be shared with other types of integral membrane proteins (see *Discussion*).

In the years since these predictions were made, several integral membrane proteins involved in membrane transport and permeability have been characterized in sufficient detail to show that they indeed consist of subunit aggregates forming transmembrane channels down their central axes. Among the best studied of these integral membrane proteins is the acetylcholine receptor (AcChoR) (for a recent review, see ref. 5). It is a pentameric aggregate of about 250 kDa made up of four homologous but nonidentical polypeptides (α–δ) in the stoichiometry $\alpha_2\beta\gamma\delta$. The binding of the ligand acetylcholine to the α chains of the aggregate changes the permeability of the central channel of the aggregate to small cations, a process that is critically involved in neuromuscular signal transmission.

To test the proposition that channel-forming proteins might have distinctive structural characteristics not shared by other types of integral membrane proteins, we have studied the partitioning behavior of AcChoR in Triton X-114 phase-separation experiments as described by Bordier (6). A 1% (wt/vol) aqueous solution of the nonionic detergent Triton X-114 is homogeneous at 4°C but separates into two phases in equilibrium at temperatures above ≈20°, one detergent-rich (≈20% detergent) and the other detergent-poor (≈0.03%). All the integral membrane proteins Bordier (6) examined strongly partitioned into the detergent-rich phase (DRP), while all the cytoplasmic and peripheral membrane proteins were found in the detergent-poor phase (DPP), providing a very simple and convenient means of distinguishing these categories of proteins. These results reflect the existence of hydrophobic domains in amphipathic integral membrane proteins (7–9), which intercalate into the micelles formed by nonionic detergents much as they do into the interior of lipid bilayers. However, upon subjecting membranes of *Torpedo californica* electroplax containing AcChoR to Triton X-114 phase separation, we obtained the surprising result that most of the AcChoR was found as the intact pentamer in the DPP (10). This observation and its significance are examined in some detail in this report.

MATERIALS AND METHODS

AcChoR. AcChoR-rich membrane vesicles prepared without detergent from *T. californica* electric organ, purified AcChoR (purified with and containing cholate), ^{125}I-labeled α-bungarotoxin, and antibodies to AcChoR used in the bungarotoxin-binding assay, all as described (11), were kindly provided by J. Lindstrom and R. Anholt (Salk Institute). Alkali treatment of the AcChoR-rich membrane vesicles was carried out by a published method (12).

Triton X-114 Phase Partitioning. Triton X-114 (Sigma) was precondensed three times to obtain a more homogeneous preparation (6). Radioactive Triton X-114 was prepared by the addition of [^{3}H]Triton X-100 (New England Nuclear) as described (6). Phase partitioning was carried out by a minor modification of the method of Bordier (6) to be described in detail elsewhere. AcChoR-rich membranes were solubilized in 1% Triton X-114 in 0.02 M Tris Cl, pH 7.4/0.15 M NaCl (Tris/NaCl) at 4°C for 10 min and centrifuged at 27,000 × g at 4°C for 20 min. A 0.3-ml aliquot of the supernatant was layered on 0.15 ml of 6% (wt/vol) sucrose/0.06% Triton X-114/Tris/NaCl, warmed to 35°C for 3 min, and centrifuged at 1300 × g for 3 min at 22°C. The upper phase, the DPP, was removed and washed twice with Triton X-114 as described (6). The lower phase, the DRP, was made to the same vol-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AcChoR, acetylcholine receptor; DRP, detergent-rich phase; DPP, detergent-poor phase.

ume as the DPP and equal volumes of the two phases were analyzed, along with the starting mixture, by $NaDodSO_4$/PAGE (13). In some cases, the α-bungarotoxin binding capacities of the starting mixture, DPP, and DRP were determined by using ^{125}I-labeled toxin in conjunction with immunoprecipitation as described (11). When phase partitioning was carried out with radioactive Triton X-114, the sucrose solution did not contain Triton X-114 and the two washes of the DPP were omitted.

Sucrose Gradient Centrifugation. Sucrose gradient centrifugation was carried out as described (14) on 5-ml linear 5–20% (wt/vol) sucrose gradients in Tris/NaCl containing 1% (starting mixture) or 0.03% (DPP) Triton X-114. Samples were pretreated with a slight excess of ^{125}I-labeled bungarotoxin for 15 min at 4°C, layered on the gradients, and centrifuged at 4°C for 5 hr at 225,000 × *g*. Seven-drop fractions were collected from the bottom of each tube and the radioactivity in each fraction was measured with a γ-counter. Beef liver catalase (11.3 S), rabbit muscle aldolase (7.3 S), and horse heart cytochrome *c* (2.5 S) were used as standards for S values.

Linoleic Acid/Triton X-114 Phase Partitioning. A 10% (wt/wt) solution of linoleic acid (Applied Science Laboratories, State College, PA) in ethanol was mixed with 1% Triton X-114 in Tris/NaCl on a rotary shaker for 3–5 days at 4°C. The AcChoR-rich membranes were solubilized directly in the mixture and subjected to phase partitioning. In some cases, [^{14}C]linoleic acid (Amersham) was used to estimate the amount of linoleic acid in the DPP and DRP.

RESULTS

Phase Partitioning of AcChoR. The results of the phase partitioning of AcChoR preparations in Triton X-114 are shown in the electrophoregrams in Fig. 1. The sets of samples shown were electrophoresed at different times in polyacrylamide gels of different concentrations, and the mobilities are therefore not directly comparable. In each set, lane 1 represents the starting mixture in Triton X-114 before partitioning, and lanes 2 and 3, respectively, the DPP and the DRP after partitioning. With *Torpedo* membranes dissolved directly in 1% Triton X-114/Tris/NaCl, Coomassie blue staining for protein (Fig. 1*C*) and periodic acid/Schiff reagent staining for oligosaccharide (Fig. 1*B*) show that all four chains of AcChoR were found in the DPP (lanes 2). A similar result was obtained with alkali-extracted membranes (Fig. 1*A*), as well as with cholate-purified AcChoR (not shown). On the other hand, a 115-kDa glycoprotein (arrow, Fig. 1 *A–C*) was found in the DRP, demonstrating behavior more typical of integral membrane proteins. In addition, a 100-kDa protein (filled arrowhead, Fig. 1 *A* and *C*), probably the α chain of the Na^+,K^+-ATPase (11, 15), was found distributed between the DPP and DRP. Another band at 43 kDa (striped arrowhead, Fig. 1*C*) was found in the DRP, but it was not clear whether the same protein was responsible for the 43-kDa band in the DPP (see ref. 16). In these experiments, the phase partitioning results were not affected by varying the protein concentration (from 0.2 mg/ml to 1.3 mg/ml with 1% Triton X-114), the detergent concentration (from 0.5% to 2.0% Triton with 0.6 mg of protein per ml), the incubation time in the 1% Triton before partitioning (from 10 min to 5 days), or the partitioning temperature (from 35°C to 56°C) or by pretreatment of the membranes with α-bungarotoxin or 3.5% 2-mercaptoethanol. In the experiment with ^{125}I-labeled α-bungarotoxin, 93% of the binding activity (11) was found in the DPP.

Phase Partitioning of the Detergent. When a 1% solution of radioactive Triton X-114 was used in the phase-partitioning experiments, in the absence of membranes the concentration of Triton in the DPP was 0.03%, and in the presence of membranes, 0.04%. Therefore, no significant redistribution of detergent accompanied the partitioning of the AcChoR protein into the DPP.

Sucrose Gradient Centrifugation. When ^{125}I-labeled α-bungarotoxin was added to membranes solubilized with Triton X-114, and this mixture was sedimented in a sucrose gradient (Fig. 2 *Top*) or when ^{125}I-labeled toxin was added to the DPP after phase partitioning of the membranes and the DPP/toxin mixture was then sedimented in a gradient (Fig. 2 *Bottom*), in both cases the radioactivity profile peaked sharply at about 13 S. This is the sedimentation rate of the dimer of the $\alpha_2\beta\gamma\delta$ aggregate (14). These results show that the AcChoR that partitioned into the DPP was present as the dimer of the original subunit aggregate and had retained its α-bungarotoxin binding activity.

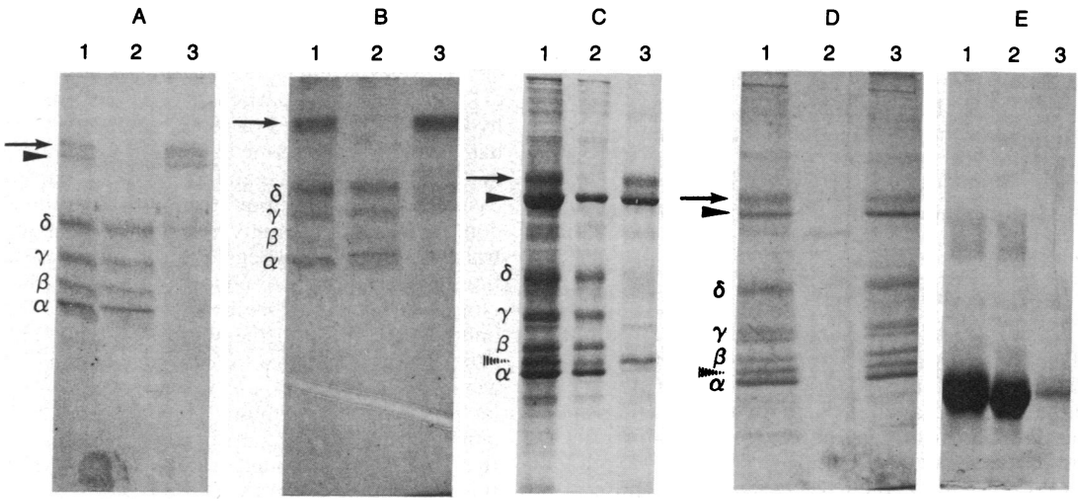

FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoregrams of AcChoR membrane preparations and controls subjected to phase partitioning in Triton X-114 detergent systems. In each set, lane 1 represents the original preparation dissolved in detergent prior to phase partitioning, and lanes 2 and 3, respectively, the DPP and the DRP after partitioning. (*A–C*) Alkali-extracted (*A*) and untreated *Torpedo* membranes (*B* and *C*), phase-partitioned in Triton X-114. (*D* and *E*) Untreated *Torpedo* membranes (*D*) and ovalbumin (*E*), phase-partitioned in the Triton X-114/linoleate mixture. *A* and *C–E* show Coomassie blue staining and *B* shows periodic acid/Schiff reagent staining. The subunits of AcChoR are designated by α, β, γ, and δ. The arrows in *A–D* designate a 115-kDa glycoprotein that invariably partitions into the DRP; the filled arrowheads in *A*, *C*, and *D* designate a 100-kDa protein that may be the α chain of the Na^+,K^+-ATPase; and the striped arrowheads in *C* and *D* designate a 43-kDa band (see text for details).

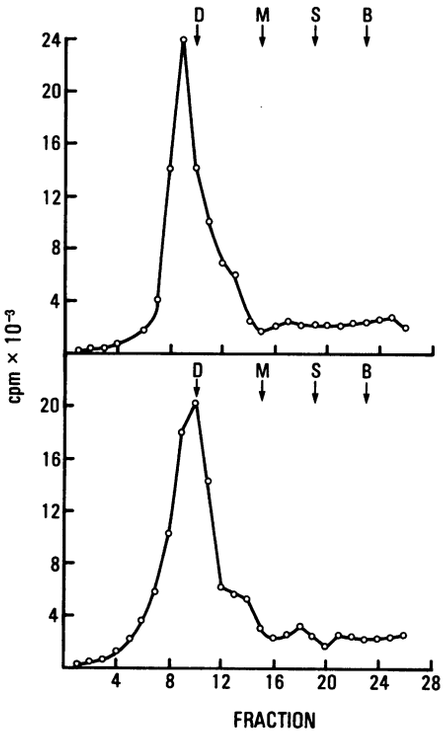

FIG. 2. Sucrose gradient centrifugation of the starting mixture of *Torpedo* membranes solubilized in Triton X-114 (*Top*) and the DPP after phase separation (*Bottom*). The samples were pretreated with ^{125}I-labeled α-bungarotoxin and the distribution of radioactivity in the gradients was measured. The letters and arrows indicate the positions in the gradient expected for the AcChoR dimer (D), the AcChoR monomer (M), the single α chain (S), and free α-bungarotoxin (B). The sedimentation profile was calibrated with standards as described in *Materials and Methods*.

Phase Partitioning with Mixtures of Triton X-114 and Linoleic Acid. When mixtures of equal parts by weight of Triton X-114 and linoleic acid were incubated at 4°C, mixed micelles were apparently produced. This was shown in experiments in which [^{14}C]linoleic acid was included. Phase separation (cloud point ≈ 15°C) resulted in 73% of the initial radioactive linoleic acid being distributed into the DRP. When the Triton/linoleate mixture was used to solubilize *Torpedo* membranes at 4°C, and phase separation was carried out, the AcChoR was found in the DRP (Fig. 1*D*), in contrast to the situation with Triton X-114 alone (Fig. 1*C*). As a control (Fig. 1*E*), ovalbumin was shown to partition into the DPP in the Triton X-114/linoleate mixtures as it does in Triton X-114 alone.

DISCUSSION

The partitioning of the integral membrane protein AcChoR into the DPP in the Triton X-114 two-phase system is a highly anomalous result, because all known integral membrane proteins that have been examined partition into the DRP (6). To assess the significance of this finding, we discuss first the nature of the phase-separation method and then some relevant structural information about the AcChoR protein.

Phase Partitioning of Triton X-114 Solutions. Triton X-114 is a polydisperse nonionic detergent, *p*-(1,1,3,3-tetramethylbutyl)phenoxypolyoxyethylene glycol, containing an average of 7.5 oxyethylene units per molecule. Above its critical micelle concentration at 4°C, it forms micelles each containing about 140 detergent molecules (9) with the *p*-octylphenyl groups sequestered in a hydrophobic core and the hydrophilic polyoxyethylene glycol residues in a random-coil conformation in the outer shell of the micelle (17). The micelle probably has the overall shape of an oblate ellipsoid (18). At temperatures above 20°C, equilibrium separation into two phases occurs, a DRP denser than water and concentrated into about 0.05 of the total volume and a DPP constituting the remainder. [The closely homologous but more hydrophilic detergent Triton X-100 shows a similar phase separation, but only at temperatures above 60°C (19)]. The DRP above 20°C contains Triton X-114 micelles much like those in the homogeneous phase at 4°C, but in a highly aggregated form (20), whereas the small amount of detergent in the DPP is probably in a nonmicellar state.

Integral membrane proteins generally form mixed micelles with nonionic detergents such as Triton X-114 (9), in which a single molecule of a protein freed of membrane lipid is intercalated into a detergent micelle, with the hydrophobic domain of the protein embedded within the hydrophobic core of the micelle. Thus, since the DRP in the Triton X-114 two-phase system contains aggregates of such mixed detergent/protein micelles, the partitioning of amphipathic integral membrane proteins into the DRP is understandable. Correspondingly, cytoplasmic proteins and peripheral membrane proteins, which do not possess hydrophobic domains that can intercalate into the micelles of nonionic detergents, are expected to partition into the DPP.* Indeed, in Bordier's experiments (6), the discrimination of several known integral membrane proteins from other kinds of proteins by their partitioning into the DRP was perfect. This was true despite wide variations in the sizes of the respective hydrophilic and hydrophobic domains of the integral membrane proteins examined. In related experiments done using a different technique, charge-shift electrophoresis (21), there was again perfect discrimination between the integral membrane proteins and other kinds of proteins that were examined with respect to the capacity of the integral membrane proteins to be incorporated into the micelles formed by nonionic detergents. However, known channel-forming proteins generally were not included among the proteins that were investigated in these studies.

The Structure and Assembly of the AcChoR Protein. The channel-forming integral membrane protein AcChoR is a pentameric aggregate of four types of glycoprotein subunits, with the stoichiometry $\alpha_2\beta\gamma\delta$ (5). Although the subunits have somewhat different molecular weights, they show striking homologies in their amino acid sequences as deduced from the nucleotide sequences of the corresponding cDNAs (22, 23). In particular, each subunit possesses four hydrophobic stretches of sequence that very likely individually span the membrane as α-helices, three clustered in the middle of the sequence and one near the carboxyl terminus. Symmetry considerations strongly suggest that each subunit contributes a homologous region to the formation of the central channel of the aggregate, but it is not known which portions of the amino acid sequence are involved in the channel. Noda *et al.* (22) and Devillers-Thiery *et al.* (23) propose that one of the four transmembrane hydrophobic α-helices of each subunit combines to form the channel, whereas Finer-Moore and Stroud (24) and Guy (25) speculate that a particular region of each subunit sequence, which could form an amphipathic α-helix, contributes a fifth transmembrane helix that defines the channel. An important structural finding in this connection is that the carboxyl termini of the chains are exposed on the cytoplasmic side of the membrane (26).

*Electrophoregrams such as those in Fig. 1 reflect the relative *masses* of a given protein that have partitioned into the DRP and DPP. The *concentration* in the DRP is about 20-fold greater than indicated in such figures. Proteins that appear to partition "completely" into the DRP therefore have partition coefficients, K, ≥ 200. For proteins that, like AcChoR, appear to partition "completely" into the DPP, $K \leq 1$.

The membrane intercalation of the active AcChoR molecule is a complex multistage process. It begins with the cotranslational insertion, via a signal peptide-mediated mechanism, of each subunit individually into the membranes of the rough endoplasmic reticulum (27). The assembly of these subunits into the AcChoR molecule in mammalian muscle, however, does not occur immediately after subunit insertion but only at a considerably later time via several intermediate stages which are not understood in detail (28). One intermediate stage appears to be dimer, α_2, which is the first stage at which α chains acquire high-affinity α-bungarotoxin binding activity and which forms before α chains are found associated with the other subunits (28). α-Bungarotoxin binding activity does not arise *in vivo* until 15–30 min after subunit insertion occurs. The functional integration of the channel of the intact AcChoR molecule must therefore occur even later, but the time, the intracellular location, and the mechanisms are not known.

With these brief remarks in mind, we now analyze the behavior of AcChoR in the Triton X-114 phase-partitioning system.

What is the Basis for the Anomalous Phase-Partitioning Properties of AcChoR? We conclude that the partitioning of AcChoR into the DPP means that intact AcChoR molecules, unlike most other integral membrane proteins (6, 9, 21), do not form mixed micelles with Triton X-114. Membranes containing AcChoR dissolve in 1% Triton X-114 to form a homogeneous solution at 4°C, reflecting the dissolution of the bilayer and the incorporation of the membrane lipids into the Triton micelles; the intact AcChoR molecules, however, most likely are released into the bulk aqueous medium. [This presumably also occurs in the dissolution of AcChoR-rich membranes in Triton X-100, which also was found to solubilize the AcChoR in a form that binds α-bungarotoxin (29).] As a result, when phase separation is induced by raising the temperature, the AcChoR is found in the DPP. It is probable that some of the small amount of Triton in the DPP is bound to the AcChoR molecules, but not in a micellar form.

Why are AcChoR molecules largely excluded from Triton X-114 micelles? A number of possible explanations are eliminated or rendered unlikely by our results. The partitioning of the AcChoR into the DPP is not attributable to the following:

(*i*) A denaturation of the AcChoR, or its breakdown into subunits. The codistribution of α-bungarotoxin, added to *Torpedo* membranes solubilized in Triton X-114, with the AcChoR into the DPP and the cosedimentation (Fig. 2) of the toxin and AcChoR of the DPP at rates characteristic of the intact AcChoR dimer (14) show that the AcChoR molecules in the DPP largely retain their native subunit structure and conformation.

(*ii*) A significant shift of Triton X-114 into the DPP along with the AcChoR. No such shift was detected.

(*iii*) A strong association of the AcChoR with peripheral membrane proteins. The AcChoR in membranes stripped of peripheral proteins by alkali treatment (Fig. 1*A*), as well as cholate-purified AcChoR, also partitioned into the DPP.

(*iv*) The dumbbell shape of the AcChoR dimer, which might be unfavorable for intercalation into the DRP micelles. In *Torpedo* membranes, the AcChoR pentameric aggregate is dimerized by disulfide-bridge formation between δ subunits (14). The partitioning of AcChoR, however, was unaffected by the presence of 2-mercaptoethanol throughout the procedure, under conditions that generated the AcChoR monomer.

(*v*) An excessive size of the membrane-intercalated hydrophobic domain of the AcChoR pentamer that does not allow it to fit into a Triton micelle. Freeze–fracture electron microscopic images (30) of AcChoR molecules show intramembranous particles of about 85 Å maximum diameter. The integral membrane protein retinal rod rhodopsin, with intramembranous particles of 110 Å diameter (31), partitions strongly into the DRP in Triton X-114 phase-separation experiments (unpublished observations), so the size of the hydrophobic domain alone cannot be the determining factor.

After eliminating these possibilities, however, we can only speculate about the correct reasons for the anomalous exclusion of AcChoR molecules from Triton micelles. It is possible that this exclusion is attributable to the large hydrophilic membrane-protruding domains of the AcChoR molecule (32), but this seems unlikely because the hydrophilic shell of the Triton micelle, consisting of the polyoxyethylene glycol residues in random-coil configuration (17), should be able to accommodate a wide range of sizes and characteristics of such domains. We cannot, however, rule out this explanation. Another possibility, which was in fact the basis for undertaking this study in the first place, is that a channel-forming protein like AcChoR might have a hydrophobic domain with a somewhat irregular external surface where it is in contact with the lipid bilayer, whereas the hydrophobic domains of non-channel-forming integral membrane proteins might generally have smooth surfaces (for reasons that are discussed briefly below). Domains with irregular surfaces might not pack well into the hydrophobic core of the Triton micelle. The importance of geometrical factors and packing considerations for the stability of detergent micelles and related structures has been emphasized (33). The essential point for our present purposes is that the hydrophobic core of a Triton micelle, packed with oriented *p*-octylphenyl residues (18), can hardly be thought of as an isotropic liquid; it must instead have considerable structure, and this structure might not allow the intercalation of a hydrophobic domain of an integral membrane protein if that domain were of sufficiently irregular shape. It would follow from these speculations that, if the hydrophobic core of the Triton micelle were rendered less structured and more fluid, the AcChoR might then intercalate into the micelle. To make the Triton micelle core less structured, we sought a compound with a flexible long-chain hydrocarbon moiety that would form mixed micelles with Triton X-114 with phase-separation characteristics similar to those of micelles of Triton X-114 alone. Linoleic acid served this purpose. When a mixture of linoleic acid and Triton X-114 was used to dissolve *Torpedo* membranes and phase separation was then carried out, the AcChoR partitioned into the DRP (Fig. 1*D*) as predicted. While this result certainly does not prove that the hydrophobic domain of the AcChoR molecules has an irregular exterior surface, it is at least consistent with that proposal.

Why might the exterior surface of the hydrophobic domain of a channel-forming integral membrane protein differ from that of other kinds of integral membrane proteins? Without going into great detail here, the structural difference could be related to different mechanisms for the stable insertion into the membrane of amino acid sequences that are, on the one hand, mainly hydrophobic or, on the other hand, substantially ionic and hydrophilic. Hydrophobic stretches are thought to be inserted via a mechanism, not yet well-defined, by which the polypeptide chain is threaded through the membrane, initiated via a signal peptide at its amino terminus (34). Such a threading mechanism may require hydrophobic domains to consist of one or more continuous stretches of about 20 nonionic and mainly hydrophobic amino acid residues, with each stretch forming a transmembrane α-helix (1). Glycophorin (35), with one transmembrane helix, and bacteriorhodopsin (36), with seven, are examples of integral membrane proteins having hydrophobic domains composed exclusively of helices. A hydrophobic domain that consisted of one helix or of several nearly parallel helices would exhibit an exterior surface that was rather regular, even approximately cylindrical.

A hydrophilic amino acid stretch, however, could not be

stably inserted into the hydrophobic interior of the membrane by the same chain-threading mechanism that operated to insert a hydrophobic amino acid stretch. It was therefore suggested (3, 4) that, in cases where each subunit of a multisubunit aggregate contributes a hydrophilic surface to the channel, the integration of the functional aggregate into the channel might require at least two stages. First, non-channel-forming hydrophobic portions of the individual subunits might become integrated into the membrane, after which the channel would be intercalated by a simultaneous concerted insertion of all the channel-forming surfaces into the membrane. An important feature of such a channel-intercalation mechanism is that it would not require the channel to consist of transmembrane helices but could permit the channel-forming domains to have a range of chain conformations in different orientations (4). Such nonhelical chain conformations within the channel could in turn generate irregularities on the exterior surface of the membrane-intercalated domain of the aggregate.

In connection with this proposal, two experimental results are relevant. One is the finding (28), referred to above, that the integration of the channel is a late event in the process of AcChoR assembly, occurring a considerable time after the membrane-association of the individual subunits. The other interesting result is that the integral membrane protein porin, which is a trimeric channel-forming aggregate in bacterial outer membranes, contains no single stretch of entirely nonionic amino acids longer than five residues throughout its entire sequence (37) and therefore almost certainly does not exhibit any transmembrane α-helices.

It should also be pointed out that if the proposals either of Noda *et al.* (22) and Devillers-Thiery *et al.* (23) or of Finer-Moore and Stroud (24) and Guy (25) for the composition and structure of the AcChoR channel were correct, the membrane-intercalated domain of the molecule would consist entirely of either 20 or 25 nearly parallel transmembrane helices, and the exterior hydrophobic surface of that domain should have a quite regular, nearly cylindrical geometry.

The anomalous partitioning of AcChoR into the detergent-poor phase in Triton X-114 phase-separation experiments is not the only such case we have encountered. Three other integral membrane proteins involved in transport, namely, the α chain of the Na^+,K^+-ATPase of kidney microsome membranes (and possibly the α chain of the *Torpedo* Na^+,K^+-ATPase, see Fig. 1 *C*), the Ca^{+2}-ATPase of sarcoplasmic reticulum membranes, and the Band 3 anion-transport protein of erythrocyte membranes, each show a degree of partitioning into the DPP that is significant, although not as complete as that shown by AcChoR (unpublished observations; see also ref. 10). A significant, although preliminary, correlation appears to exist, therefore, between anomalous partitioning behavior in Triton X-114 phase separation experiments and the probable channel-forming property of integral membrane proteins, a correlation that needs to be explored further.

Whatever the validity of these speculations, at least two interesting points emerge from this study. First, the Triton X-114 phase partitioning of a protein into the DPP does not necessarily rule out the possibility that it is an integral membrane protein. Second, it is possible to discriminate some integral membrane proteins from others by this method, and this discrimination must reflect some significant differences in the structures of these proteins.

We are very grateful to Drs. Jon Lindstrom and Robert Anholt for the gifts of the AcChoR materials used in this work and for many helpful discussions. This work was supported by U.S. Public Health Service Grant AI06659 (to S.J.S.). P.A.M. was a Postdoctoral Fellow of the Anna Fuller Fund. S.J.S. is an American Cancer Society Research Professor.

1. Singer, S. J. (1971) in *Structure and Function of Biological Membranes*, ed. Rothfield, L. I. (Academic, New York), pp. 145–222.
2. Jardetzky, O. (1966) *Nature (London)* **211**, 969–970.
3. Singer, S. J. (1977) *J. Supramol. Struct.* **6**, 313–323.
4. Singer, S. J. (1982) in *Membranes and Genetic Disease*, eds. Sheppard, J. R., Anderson, V. E. & Eaton, J. W. (Liss, New York), pp. 3–24.
5. Anholt, R., Lindstrom, J. & Montal, M. (1984) in *Enzymes of Biological Membranes*, ed. Martinosi, A. (Plenum, New York), 2nd Ed., Vol. 3, pp. 335–401.
6. Bordier, C. (1981) *J. Biol. Chem.* **256**, 1604–1607.
7. Lenard, J. & Singer, S. J. (1966) *Proc. Natl. Acad. Sci. USA* **56**, 1828–1835.
8. Wallach, D. F. H. & Zahler, P. H. (1966) *Proc. Natl. Acad. Sci. USA* **56**, 1552–1559.
9. Helenius, A. & Simons, K. (1975) *Biochim. Biophys. Acta* **415**, 29–79.
10. Maher, P. A. & Singer, S. J. (1983) *J. Cell. Biochem.*, Suppl., **7B**, 374 (abstr.).
11. Lindstrom, J., Anholt, R., Einarson, B., Engel, A., Osame, M. & Montal, M. (1980) *J. Biol. Chem.* **255**, 8340–8350.
12. Neubig, R., Krodel, E., Boyd, N. & Cohen, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 690–694.
13. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–684.
14. Chang, H. W. & Bock, E. (1977) *Biochemistry* **16**, 4513–4520.
15. Schwartz, A., Lindenmayer, G. E. & Allen, J. C. (1975) *Pharmacol. Rev.* **27**, 3–134.
16. Gordon, A. S., Milfay, D. & Diamond, I. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5862–5865.
17. Tanford, C., Nozacki, Y. & Rohde, M. F. (1977) *J. Phys. Chem.* **81**, 1555–1560.
18. Robson, R. J. & Dennis, E. A. (1977) *J. Phys. Chem.* **81**, 1075–1078.
19. Maclay, W. N. (1956) *J. Colloid Sci.* **11**, 272–285.
20. Staples, E. J. & Tiddy, G. J. T. (1978) *J. Chem. Soc. Faraday Trans. 1* **74**, 2530–2541.
21. Helenius, A. & Simons, K. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 529–532.
22. Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., Miyata, T. & Numa, S. (1983) *Nature (London)* **302**, 528–532.
23. Devillers-Thiery, A., Giraudat, J., Bentaboulet, M. & Changeux, J.-P. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2067–2071.
24. Finer-Moore, J. & Stroud, R. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 155–159.
25. Guy, H. R. (1984) *Biophys. J.* **45**, 249–261.
26. Ratnam, M. & Lindstrom, J. (1984) *Biochem. Biophys. Res. Commun.* **122**, 1225–1233.
27. Anderson, D. J. & Blobel, G. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48**, 125–134.
28. Merlie, J. P., Sebbane, R., Gardner, S., Olson, E. & Lindstrom, J. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48**, 135–146.
29. Meunier, J. C., Olsen, R. W., Menez, A., Fromageot, P., Boquet, P. & Changeux, J.-P. (1972) *Biochemistry* **11**, 1200–1210.
30. Heuser, J. E. & Salpeter, S. R. (1979) *J. Cell Biol.* **82**, 150–173.
31. Hong, K. & Hubbel, W. L. (1973) *Biochemistry* **12**, 4517–4523.
32. Klymkowsky, M. W. & Stroud, R. M. (1979) *J. Mol. Biol.* **128**, 319–334.
33. Israelachvilli, J. N., Mitchell, D. J. & Ninham, B. W. (1976) *J. Chem. Soc. Faraday Trans. 2* **72**, 1525–1568.
34. Blobel, G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1496–1500.
35. Tomita, M., Furthmayr, H. & Marchesi, V. T. (1978) *Biochemistry* **17**, 4756–4770.
36. Henderson, R. & Unwin, P. N. T. (1975) *Nature (London)* **257**, 28–32.
37. Chen, R., Krämer, C., Schmidmayr, W. & Henning, U. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5014–5017.