AN ANALYSIS BY LOW-ANGLE NEUTRON SCATTERING OF THE STRUCTURE OF THE ACETYLCHOLINE RECEPTOR FROM *TORPEDO CALIFORNICA* IN DETERGENT SOLUTION

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ABSTRACT The acetylcholine receptor from the electric tissue of *Torpedo californica* is a large, integral membrane protein containing four different types of polypeptide chains. The structure of the purified receptor in detergent solution has previously been investigated by sedimentation analysis and gel filtration. Sedimentation analysis yielded a molecular weight of 250,000 for the protein moiety of the receptor monomer-detergent complex; hydrodynamic characteristics such as the Stokes radius, however, refer to the receptor-detergent complex. In this paper we report the results of our use of low-angle neutron scattering to investigate the shape of the receptor-detergent (Triton X-100 from Rohm & Haas Co., Philadelphia, Pa.) complex and separately of its protein and detergent moieties. By adjustment of the neutron-scattering density of the solvent with D_2O to match that of one or the other of the moieties, its contribution to the scattering can be nearly, if not completely, eliminated. Neutron scattering from Triton X-100 micelles established that this detergent is contrast matched in ~18% D_2O. Scattering measurements on the receptor-detergent complex in this solvent yielded a radius of gyration of the acetylcholine receptor monomer of 46±1Å. The radius of gyration and molecular volume (305,000 Å^3) of the receptor are inconsistent with a compact spherical shape. These parameters are consistent with, for example, a prolate cylinder of dimensions (length × diameter) ~150 × ~50 Å or an oblate cylinder, ~25 × ~130 Å. More complex shapes are possible and in fact seem to be required to reconcile the present results with previous electron microscopic and x-ray analyses of receptor in membrane and with considerations of the function of the receptor in controlling ion permeability.

The neutron-scattering data yield, in addition, an independent determination of the molecular weight of the receptor protein (240,000±40,000), the extent of Triton X-100 binding in the complex (~0.4 g/g protein), and from the extended scattering curve, an approximation to the shape of the receptor-Triton X-100 complex, namely an oblate ellipsoid of axial ratio 1:4.

INTRODUCTION

Nicotinic acetylcholine receptors are postsynaptic membrane proteins that alter the ion permeability of the membrane as a consequence of binding acetylcholine. These receptors have been solubilized from the membrane in nonionic detergents and extensively purified by

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affinity chromatography (reviewed in Karlin, 1976; Heidmann and Changeux, 1978). In isolation, the receptors are identified by their binding of acetylcholine and congeners, and curarimimetic snake neurotoxins, and by their covalent reaction with affinity labels.

The acetylcholine receptor purified from the electric tissue of *Torpedo californica* is obtained as a mixture of monomer and dimer species with molecular weights of 250,000 and 500,000, respectively (Reynolds and Karlin, 1978), and contains four types of chains (Weill et al., 1974). Native dimer is formed through disulfide cross links between δ subunits (Suarez-Isla and Hucho, 1977; Chang and Bock, 1977; Hamilton et al., 1977, 1979), the largest of the four different polypeptide chain components. The detergent-solubilized monomer and dimer species of *Torpedo* receptor have sedimentation coefficients of ~9 and 13 S (Raftery et al., 1972; Potter, 1973; Carroll et al., 1973; McNamee et al., 1975; Reynolds and Karlin, 1978) and Stokes radii of ~70 (Raftery et al., 1972; Potter, 1973; Reynolds and Karlin, 1978) and 90 Å (Potter, 1973; Reynolds and Karlin, 1978).

The determination of the size and shape of solubilized amphiphilic proteins, like the acetylcholine receptor, is complicated by the necessary presence of detergent (Tanford and Reynolds, 1976). Conventional sizing techniques, such as gel filtration or sedimentation methods, useful for water-soluble proteins, generally cannot distinguish the contributions of the protein and the bound detergent components to the hydrodynamic properties of the complex.

Neutron-scattering analysis, however, permits the blanking out of the contribution of bound detergent by contrast matching (Yeager, 1976; Osborne et al., 1978). In contrast matching, the neutron-scattering density of the solvent is adjusted to equal that of one component of the complex (Engelman and Moore, 1975). When this condition prevails, no net scatter derives from the matched component; it is effectively rendered invisible. (Strictly, this is true only when the matched component has a uniform scattering density distribution.)

The particular advantage of neutrons over other sources of scattering radiation (e.g., x rays) in such experiments lies in the large difference between the coherent neutron-scattering lengths of hydrogen and deuterium (Bacon, 1962). This allows the scattering density of the solvent to be varied over a wide range, which encompasses the scattering densities of most biological molecular species and of many detergents, by adjusting its relative content of H_2O and D_2O (Schoenborn, 1976; Jacrot, 1976).

In this paper we describe a neutron-scattering analysis of the size and shape of the monomer form of the acetylcholine receptor from *Torpedo californica* electric tissue and its complex with Triton X-100 detergent (Rohm & Haas Co., Philadelphia, Pa.). Our results are compared with dimensions obtained with other structural methods on purified and membrane-bound acetylcholine receptors. Some of our results have appeared in a preliminary report (Wise et al., 1978).

LIST OF SYMBOLS

- A Cross-sectional area of sample in beam (cm^2)
- a See Eq. 7
- b See Eq. 8
- c Sample concentration (g/cm^3)
- h $(4\pi \sin \theta)/\lambda$ ($Å^{-1}$)

l_i Neutron-scattering length of atom i (cm)
M Molecular weight
N Number of particles per unit volume (cm^{-3})
N_A Avogadro's number
R Radius of gyration of neutron-scattering density (Å)
R_s Radius of gyration of particle shape (Å)
$\mathbf{r}$ Vector of arbitrary origin
$\mathbf{r}_\rho$ Vector whose origin is center of gravity of particle neutron-scattering density distribution
$\mathbf{r}_s$ Vector whose origin is center of gravity of particle shape assuming a uniform density distribution
V Particle volume ($Å^3$)
$\bar{v}$ Particle specific volume (cm^3/g)
x Volume fraction
z Sample thickness (cm)
α Axial ratio (length:diameter)
β Volume fraction of D_2O in solvent
γ Fractional exchange of exchangeable hydrogens
δ Mass fraction
ϵ Attenuation factor = $\exp(-\Sigma z)$
2θ Scattering angle (radians)
λ Neutron wavelength (Å)
$\rho(\mathbf{r})$ Neutron-scattering density distribution of particle ($cm/Å^3$)
$\bar{\rho}$ Average neutron-scattering density of particle ($cm/Å^3$)
ρ_0 Average neutron-scattering density of solvent ($cm/Å^3$)
$\Delta\rho(\mathbf{r}) = \rho(\mathbf{r}) - \rho_0$ Neutron-scattering density contrast distribution($cm/Å^3$)
Σ Total neutron-scattering cross section per unit volume (cm^{-1})
$d\sigma/d\Omega$ Differential coherent scattering cross section (cm^2)
ϕ $1 - c\bar{v}$
Φ_0 Incident neutron flux ($neutron/cm^2/s$)
$\Delta\Omega$ Solid angle of detector (steradians)

THEORY

The coherent neutron-scattering intensity as a function of scattering angle for a solution of identical, noninteracting particles is given by:

$$I(2\theta) = \Phi_0 \exp(-\Sigma z)\, NAz\, (d\sigma/d\Omega)\, \Delta\Omega, \tag{1}$$

where, in the limit of small scattering angles, the differential scattering cross section takes the form (Guinier and Fournet, 1955; Jacrot, 1976):

$$d\sigma/d\Omega = V^2(\bar{\rho} - \rho_0)^2 \exp[-(1/3)h^2R^2], \tag{2}$$

where R is a function of ρ.

Average neutron-scattering densities for a particle or solvent are computed using the relationship:

$$\bar{\rho} = (\Sigma \eta_i l_i)/V = \int \rho(\mathbf{r})\, d\nu/V, \tag{3}$$

where η_i is the number of atoms of type i (i.e., C, N, O, etc.) in the molecule, and l_i is the corresponding neutron-scattering length for that species of atom (Bacon, 1962). The difference between the average scattering densities of a particle and the solvent, $\overline{\Delta\rho} = \bar{\rho} - \rho_0$, is the average neutron-scattering density contrast.

Radius of Gyration

The angular dependence of the scattering intensity is contained in the exponential term in Eq. 2 and reflects the structure of the scattering particle. Specifically, $R(\rho_0)$ is the radius of gyration of the particle in solvent with scattering density ρ_0.

$$R(\rho_0) = [\int (\mathbf{r} - \mathbf{r}_\rho)^2\, \Delta\rho(\mathbf{r})\, d\nu / \int \Delta\rho(\mathbf{r})\, d\nu]^{1/2} \tag{4}$$

The integral is taken over the volume of the scattering particle. $\mathbf{r}_\rho$ defines the center of gravity of the particle's neutron-scattering density contrast distribution, namely:

$$\mathbf{r}_\rho = \int \mathbf{r}\Delta\rho(\mathbf{r})\, d\nu / \int \Delta\rho(\mathbf{r})\, d\nu. \tag{5}$$

In general, the particle will possess a nonuniform scattering density (i.e., $\rho(\mathbf{r}) \neq$ constant) so that its radius of gyration will vary with contrast. The explicit functional dependence of the radius of gyration on contrast is given by (Stuhrmann, 1974; Luzzati et al., 1976):

$$R^2(\rho_0) = R_s^2 + a/\overline{\Delta\rho} - b/(\overline{\Delta\rho})^2, \tag{6}$$

where R_s is the radius of gyration of the scattering particle at infinite contrast, that is, the radius of gyration of the particle taken as having a uniform scattering density. (We shall use "particle shape" to indicate this uniform scattering density likeness of the particle.) The parameters a and b are defined as:

$$a = \int (\mathbf{r} - \mathbf{r}_s)^2 [\rho(\mathbf{r}) - \bar{\rho}]\, d\nu / V \tag{7}$$

and

$$b = \{ | \int \mathbf{r}[\rho(\mathbf{r}) - \bar{\rho}]\, d\nu | / V \}^2, \tag{8}$$

where the integrals are over the volume of the scattering particle and $\mathbf{r}_s$ is the vector from the origin of the coordinate system to the center of gravity of the particle shape. Qualitatively, a indicates the relative orientation of high and low density regions within the particle. Thus, $a > 0$ indicates that with respect to the average scattering density of the particle the distribution of densities higher than the average lies further from the center of gravity than the distribution of densities lower than the average; $a < 0$ indicates the opposite. b is a measure of the asymmetry of the neutron-scattering density distribution; namely, the distance separating the center of gravity of the particle shape and the center of gravity of the particle's neutron-scattering density contrast distribution is given by: $|\mathbf{r}_\rho - \mathbf{r}_s|^2 = b/(\overline{\Delta\rho})^2$.

If the distribution of the deviations from the average scattering density of the particle, $\rho(\mathbf{r}) - \bar{\rho}$, is arbitrarily divided into two nonoverlapping regions which have, respectively,

volumes V_1 and V_2, distributions of the deviations from $\bar{\rho}$, $\rho_1(\mathbf{r})$ and $\rho_2(\mathbf{r})$, and centers of gravity of $\rho_1(\mathbf{r})$ and $\rho_2(\mathbf{r})$ located at $\mathbf{r}_1$ and $\mathbf{r}_2$, then we obtain from Eq. 8 that

$$b = |\mathbf{r}_1 - \mathbf{r}_2|^2[x(1-x)(\bar{\rho}_1 - \bar{\rho}_2)]^2, \tag{9}$$

where $\bar{\rho}_1$ and $\bar{\rho}_2$ are the average neutron-scattering densities of regions 1 and 2, respectively, and $x = V_1/V$ is the volume fraction of region 1. If $\rho_1(\mathbf{r})$ and $\rho_2(\mathbf{r})$ are each either centrosymmetric or uniform, $\mathbf{r}_1$ and $\mathbf{r}_2$ are vectors to the centers of gravity of the shapes of V_1 and V_2, i.e., $\mathbf{r}_1 = \mathbf{r}_{1s}$ and $\mathbf{r}_2 = \mathbf{r}_{2s}$, so that b measures the distance separating the centers of shape of the two volumes.

R_s, a, and b are evaluated from measurements of the particle's radius of gyration $R(\rho_0)$, at different contrasts by fitting the data, plotted as $R^2(\rho_0)$ vs. $(\Delta\rho)^{-1}$ (Stuhrmann plot), to a parabolic curve (Eq. 6). This analysis assumes that the structure of the particle is unchanged as ρ_0 is varied, that is, $\rho(\mathbf{r})$ is independent of the solvent-scattering density. (The effects of deuterium exchange with solvent on the evaluation of a and b is treated in Ibel and Stuhrmann, 1974.)

Zero-Angle Intensity

If the scattered intensity is extrapolated to zero angle, we find that

$$\lim_{2\theta \to 0} I(2\theta) = I(0) = \Phi_0 \exp(-\Sigma z) A z \bar{v}^2 c M N_A^{-1} (\bar{\rho} - \rho_0)^2 \Delta\Omega \tag{10}$$

where we have reformulated the expression in terms of the molecular weight, partial specific volume, and weight concentration of the particle. It follows from Eq. 10 that when the scattering density of the solvent equals the average scattering density of the particle, there will be no net zero-angle scatter. The particle is said to be contrast matched in solvent having this

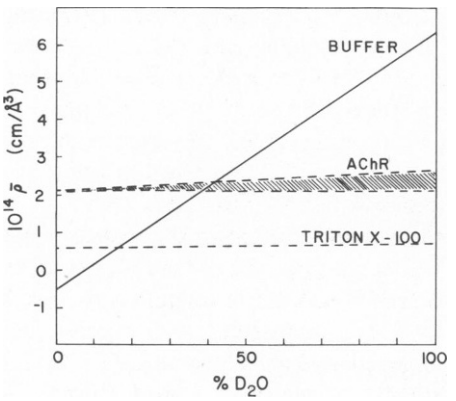

FIGURE 1 Average neutron-scattering density as a function of solvent D_2O. Average neutron-scattering densities were evaluated based on the atomic compositions of the *Torpedo californica* acetylcholine receptor and Triton X-100 (see Methods, Eqs. 11–13). For the receptor, the upper and lower lines defining the hatched region represent, respectively, conditions of complete ($\gamma = 1$) and zero ($\gamma = 0$) H $\rightleftharpoons$ D exchange with solvent by noncarbon bonded amino acid hydrogens. At any given solvent D_2O concentration, the average neutron density contrast of a component is the difference between the scattering density of that component and the scattering density of the buffer (solvent), $\bar{\rho} - \rho_0$. The intersection of the buffer scattering density line with a component's scattering density line defines the contrast-match point of that component; namely, the solvent D_2O content which yields zero average contrast.

scattering density. If M and $\bar{\nu}$ are independent of the solvent-scattering density, a plot of $[I(0)/c]^{1/2}$ vs. ρ_0 falls on a straight line which intersects the ρ_0 axis at the average scattering density of the particle, $\bar{\rho}$. The slope of this line is proportional to $M^{1/2}$.

Contrast Matching

The contrast variation method (Stuhrmann, 1974; Ibel and Stuhrmann, 1975) is founded on the premise that different regions of a structural complex will, in general, have different neutron-scattering densities. Consider, specifically, the protein and detergent components of a solubilized amphiphilic protein. We can obtain structural information about each of the two components and their geometrical relationship to one another by measuring neutron-scattering profiles under different contrast conditions (Fig. 1). In essence, we are obtaining different views of the structure as one region or another is rendered invisible by contrast matching.

Exact contrast matching of either the protein or detergent, however, requires that the matched component have a uniform scattering density. If this condition is not satisfied, then even at the average contrast match point of one of the components, unmatched regions of that component, regions which have scattering densities above and below the average, will still contribute to the net scattering intensity and to the radius of gyration (Eq. 4). Contrast matching of a component, in the strict sense, is achieved only in the limit of the zero-angle intensity when Eq. 2 is exact.

METHODS

Acetylcholine Receptor

Receptor was purified from the electric organ of *Torpedo californica* and converted to the monomer form by reduction as described previously (Hamilton et al., 1977). Receptor monomer was dialyzed against 30 ml TNP50 (0.2% Triton X-100, 50 mM NaCl, 10 mM $NaPO_4$, 1 mM EDTA, 0.02% NaN_3, pH 7.0), made up either in H_2O or in D_2O (pD = 7.0, where pD = pH [meter reading] + 0.4; Bates, 1964). Dialysis was for 4 h at 4°C with a change of dialysate every hour. In each case, the final outside solution was saved for use as a solvent blank. After dialysis, dithiothreitol was added to each receptor solution to a final concentration of 0.4 mM. The receptor solutions were stored in liquid N_2 until used.

In some cases the receptor was alkylated after the reduction step; thus, after reduction in 2 mM DTT at pH 8.0, 25°C, for 30 min, the pH was lowered to 7.4 and *N*-ethylmaleimide added to a final concentration of 5 mM. After reduction and alkylation, ~90% of the receptor sedimented as monomer (~9 S) in a sucrose density gradient. The monomer peak was isolated and dialyzed for 3 h at 4°C against ~50 vol TNM buffer (0.2% Triton X-100, 10 mM NaCl, 5 mM 3-[N-morpholino] propanesulfonic acid, 1 mM EDTA, 0.02% NaN_3, pH 7.4). The receptor monomer was concentrated on Whatman DE-52 (Whatman, Inc., Clifton, N.J.) suspended in TNM. Receptor (2–3 mg in ~14 ml buffer) was applied to an 0.5-cm-diam column containing 1–1.5 ml of DE-52 at a flow rate of 100 μl/min. The receptor was eluted at the same flow rate with TNP1000 (like TNP50 but containing 1 M NaCl) and collected in 0.4-ml fractions. Typically, the peak fraction contained 1–1.5 mg protein. Finally, the concentrated receptor monomer was dialyzed against TNP50 made up with the desired proportion of D_2O.

Total protein concentration was determined colorimetrically by the method of Lowry et al.

modified for the presence of Triton X-100 by the addition of 100 λ of 10% sodium dodecylsulfate to 50 λ of initial sample. The binding of siamensis 3 neurotoxin by receptor was measured by a DEAE-filter method as described by Damle and Karlin (1978). The specific activities of the reduced receptor preparations ranged from 6–7 nmol toxin binding sites per mg protein. Reduction of the receptor results in an approximately 20% decrease in toxin binding, as measured by this filtration method.[1]

Sucrose-Density Gradient Determination of Detergent Binding

Duplicate samples of receptor monomer, 150 mg in 75 μl of TNP50 were layered over 17 ml of a 5–20% (wt/vol) sucrose density gradient made up in either 0.02 or 0.04% ^{3}H-Triton X-100 (ring labeled to 0.3 mCi/g, a gift from Rohm & Haas Co.) in NP50 (50 mM NaCl, 10 mM $NaPO_4$, 1 mM EDTA, 0.02% N_aN_3, pH 7.0). These were centrifuged at 27,000 rpm in a SW 27.1 rotor for 36 h at 5°C. Duplicate samples of TNP50 were layered over identical gradients and centrifuged at the same time. Fractions (0.2 ml) were collected and the radioactivity and protein concentrations determined. The Triton X-100 bound to the 9-S receptor was determined from the excess radioactivity in the receptor containing gradients over that in the blanks.

Neutron-Scattering Densities

Average neutron-scattering densities for the acetylcholine receptor, Triton X-100, and the solvent (NP50) were computed (Eq. 3) based on atomic composition and tabulated values of the atomic scattering lengths (Bacon, 1962). They are:

$$\bar{\rho}_{prot} = (2.09 + 0.57\beta\gamma) \times 10^{-14}\ \text{cm/Å}^3, \tag{11}$$

$$\bar{\rho}_{det} = (0.59 + 0.11\beta) \times 10^{-14}\ \text{cm/Å}^3, \tag{12}$$

$$\rho_0 = (-0.55 + 6.89\beta) \times 10^{-14}\ \text{cm/Å}^3, \tag{13}$$

where β is the volume fraction of D_2O in the solvent and γ is the fraction of receptor hydrogens not covalently bonded to carbon which exchange with solvent. The atomic composition of the receptor was evaluated from its amino acid composition (Karlin et al., 1975). $\bar{v}_{prot}$ = 0.73 ml/g was calculated from the receptor's amino acid composition and the tabulated partial specific volumes of the amino acids (Cohn and Edsall, 1943); $\bar{v}_{det}$ = 0.908 for Triton X-100 (Tanford et al., 1974). Triton X-100 is an ethoxylated octylphenol containing an average of 9.5 ethylene oxide residues per molecule (Rohm & Haas Co.).

Data Collection

Neutron-scattering measurements were conducted at the High Flux Beam Reactor facility at Brookhaven National Laboratory, Upton, N.Y. The experimental configuration was essentially as described by Moore et al. (1977). Neutrons of wavelength 2.37Å ($\Delta\lambda/\lambda$ = 0.02 full width at half maximum) were used with an incident neutron flux at the sample of 6.4 ± 0.2 × 10^5 neutrons/cm^2 per s.

Samples were contained in either aluminum cells with quartz windows or quartz cells, manufactured to close tolerances, with nominal dimensions of 7-mm diam × 2-mm thickness. The sample cells were mounted in a temperature-controlled, automatic sample changer, maintained at 8 ± 2°C. Scattered neutrons were detected using a two dimensional position-sensitive detector (Alberi et al., 1975) located 2 m from the sample.

Four different samples were mounted on the sample changer for each measurement. These were the primary receptor or detergent sample itself; a sample of buffer solution to correct for solvent scatter; an empty sample cell to evaluate the scatter arising from the specimen cell alone; and a plug of neutron

[1]Damle, V. N. Unpublished results.

opaque BC_4 which completely blocked any neutron scatter, to provide a measure of the ambient background. In addition, a fifth cell containing an incoherent scattering sample (Lucite [Du Pont de Nemours & Co., Inc., Wilmington, Del.] or 8% D_2O) was often included to evaluate the uniformity of the detector response. During the course of an experiment, the samples were cyclically exposed to the neutron beam, each for a fixed data collection interval. Total time of exposure of a sample to the neutron beam reanged from ~2.5 h in D_2O to ~6 h for samples in H_2O.

The primary data from each scattering measurement was first summed for repetitive scans, then ring integrated about a center defined by the direct beam, and normalized to constant monitor counts. The resultant data are reported as neutrons detected per square millimeter of detector area per 10^8 monitor counts. The net coherent scattering intensity was obtained from (Yeager, 1976):

$$I(2\theta) = \{[I_1(2\theta) - I_4(2\theta)]/\epsilon_1 - [I_3(2\theta) - I_4(2\theta)]/\epsilon_3\} - \phi\{[I_2(2\theta) - I_4(2\theta)]\epsilon_2 - [I_3(2\theta) - I_4(2\theta)]/\epsilon_3\}. \quad (14)$$

The subscripts designate the contents of the different cells, namely: (1) sample, (2) buffer, (3) empty sample cell, and (4) blocked beam. The factors ϵ_i account for the exponential attenuation of the neutron flux in traversing samples of finite thickness due to all scattering and absorption processes; specifically, $\epsilon_i = \exp(-\Sigma_i z)$ (Schmatz et al., 1974). The ϵ_i were evaluated by measuring the intensity of the direct neutron beam through each sample after moving the beam stop out of position and normalizing the values obtained to a total neutron-scattering cross section of zero for the empty sample cell ($\epsilon_3 = 1$). (The total neutron-scattering cross section of a sample cell itself was found to be negligible.) The total scattering cross section is dominated by the scatter of the solvent; thus, ϵ_1 and ϵ_2 should be essentially identical. $\phi = 1 - c\bar{v}$ corrects for the volume of solute in cell 1, where c is the concentration of solute and $\bar{v}$ is its partial specific volume.

RESULTS

The acetylcholine receptor exists in solution as a complex with detergent. The neutron scattering of detergent alone and of receptor-detergent complex were determined at various D_2O concentrations. Low-angle, neutron-scattering data were collected for the detergent, Triton X-100, in NP50 solvent buffer; and for the monomeric form of the acetylcholine receptor-detergent complex, in TNP50 buffer.

Guinier plots, namely log $I(2\theta)$ vs. $(2\theta)^2$ (Eq. 1), were constructed from the results of the individual scattering experiments, after treatment of the raw experimental data through ring integration, background subtraction, and normalization (see Methods). A typical family of such curves for both the detergent and the receptor-Triton X-100 complex, each curve representing a different H_2O:D_2O ratio in the solvent buffer, is presented in Fig. 2. Only the linear region of the Guinier plot for each sample is shown. The data within this region are adequate in each case to determine the slopes and intercepts of the lines.

Zero-angle Scattering Intensity

The forward scattering intensity, $I(0)$, in each H_2O/D_2O mixture was evaluated from the zero-angle intercept of the least squares line fit to the data points in the Guinier plot. The square root of the zero-angle intensity is proportional to the contrast. In turn, the contrast, $\bar{\rho}$-ρ_0, is directly related to the fractional D_2O content of the solvent, β, through ρ_0, and $\bar{\rho}$ (see Eqs. 11–13). Contrast curves were constructed by plotting $(I(0)/c)^{1/2}$ as a function of β (Fig. 3). The points define a straight line whose intercept with the abscissa is the contrast-match point of the solute.

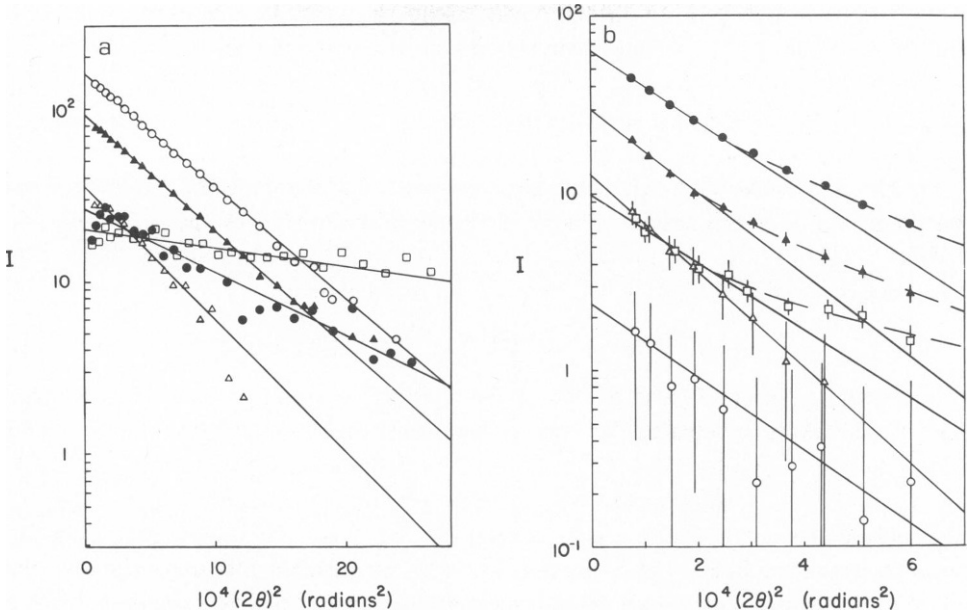

FIGURE 2 Guinier plots obtained at different solvent contrast conditions. (*a*) Representative Guinier profiles for Triton X-100 detergent micelles in NP50 buffer containing: 100% (○); 80% (▲); 60% (△); 40%, (□); and 0% (•) D_2O. The lines are least-squares fits to the experimental data. For the data shown, the concentration of Triton X-100 was 0.2% (wt/vol) except for the 0% D_2O data, which is for a 1% (wt/vol) Triton X-100 solution. (*b*) Guinier profiles for the acetylcholine receptor in TNP50 buffer containing: 100% (•); 80% (▲); 60% (□); 20% (○); and 0% (△) D_2O.

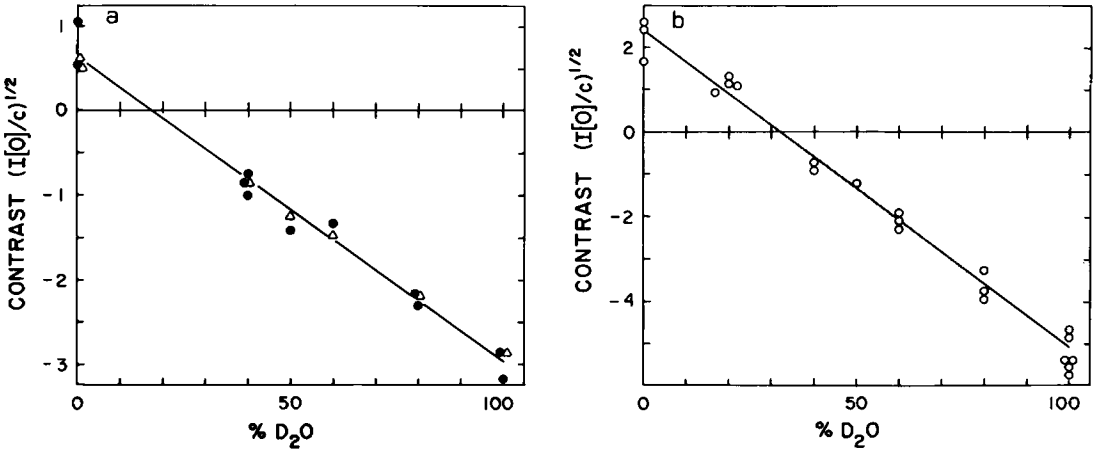

FIGURE 3 Contrast curves. (*a*) Triton X-100 detergent micelles. 0.2% Triton X-100, (•) and 1% Triton X-100, (△). (*b*) Acetylcholine receptor-Triton X-100 complex. Data for receptor concentrations ranging from 0.5 to 4 mg/ml are included in this plot.

TRITON X-100 CONTRAST-MATCH POINT The line in Fig. 3 *a* crosses the β-axis at 17.7 ± 2.2% D_2O. Thus, Triton X-100 is contrast matched in solvent with an absolute neutron-scattering density, ρ_0 (0.177) = $\bar{\rho}_{det}$ = 0.67 × 10^{-14} cm/Å^3. The observed value is in excellent agreement with the theoretical contrast-match point, 17.3% D_2O, computed from Eqs. 12 and 13.

MOLECULAR WEIGHT OF THE DETERGENT MICELLE The slope of the contrast curve is proportional to the partial specific volume of the solute multiplied by the square root of its molecular weight. Taking the square root of Eq. 10, normalized with respect to exp $(-\Sigma z)$ (see Methods), and differentiating with respect to β, we obtain:

$$\partial[I(0)/c]^{1/2}/\partial\beta = [\Phi_0 A z \Delta\Omega/N_A]^{1/2}\bar{\nu}M^{1/2}[\partial(\bar{\rho} - \rho_0)/\partial\beta] \quad (15)$$

The first quantity in brackets is related to the incident neutron flux and the experimental geometry. This expression places the scattering intensity measurements on an absolute scale; it is essentially invariant for all of the experiments reported here. The last term, $\partial(\bar{\rho} - \rho_0)/\partial\beta$ was calculated directly using the theoretical expressions for $\bar{\rho}$ and ρ_0 (Eqs. 12 and 13).

The molecular weight of a detergent micelle is 5.5 ± 0.3 × 10^4, where the values of the parameters appearing in Eq. 15 are listed in Table I. The micelle molecular weight we obtain for Triton X-100 is smaller than values reported elsewhere. Possible explanations for this discrepancy will be considered in the Discussion.

ACETYLCHOLINE RECEPTOR–TRITON X-100 COMPLEX CONTRAST-MATCH POINT The zero-angle intensity data for the receptor-detergent complex (Fig. 3 *b*) indicate that the complex is contrast-matched in TNP50 containing 31.9 ± 2.2% D_2O; thus the complex has an average neutron-scattering density, $\rho_0(0.319) = \bar{\rho}_{com} = 1.65 \times 10^{-14}$ cm/Å^3.

The scattering density of the receptor-detergent complex is simply related to the scattering densities of its protein and detergent components. We obtain directly from Eq. 3 that

$$\bar{\rho}_{com}(\beta) = x\bar{\rho}_{det}(\beta) + (1 - x)\,\bar{\rho}_{prot}(\beta), \quad (16)$$

where x is the volume fraction of detergent comprising the complex. An explicit indication of the dependence of the component scattering densities on β is included in Eq. 16 to emphasize

TABLE I
QUANTITIES USED IN THE CALCULATION OF MOLECULAR WEIGHTS

Φ_0	$2.5 \times 10^9\, n$ cm^{-2} (10^8 monitor counts)$^{-1}$
A	0.28 cm^2
z	0.21 cm
$\Delta\Omega$	2.5×10^{-7} steradians
$[\partial(I(0)/c)^{1/2}/\partial\beta]_{com}$	$-236.4 \pm 6.3\, n^{1/2}$ cm g$^{-1/2}$
$[\partial(I(0)/c)^{1/2}/\partial\beta]_{det}$	$-113.3 \pm 3.6\, n^{1/2}$ cm g$^{-1/2}$
$\partial(\bar{\rho}_{det} - \rho_0)/\partial\beta$	-6.78×10^{-14} cm Å^{-3}
$\partial(\bar{\rho}_{com} - \rho_o)/\partial\beta$	-6.50×10^{-14} cm Å^{-3} ($\gamma = 1$; $x = 0.379$)
$\partial(\bar{\rho}_{com} - \rho_0)/\partial\beta$	-6.86×10^{-14} cm Å^{-3} ($\gamma = 0$; $x = 0.302$)
$\bar{\nu}_{det}$	0.908 ml g^{-1}
$\bar{\nu}_{prot}$	0.73 ml g^{-1}
$\bar{\nu}_{com}$	0.79 ± 0.01 ml g^{-1} ($\gamma = 1$; $x = 0.379$)
$\bar{\nu}_{com}$	0.78 ± 0.01 ml g^{-1} ($\gamma = 0$; $x = 0.302$)

n = number of neutrons.

the effect of labile H ⇌ D exchange with solvent on the component neutron-scattering densities. Solving for the volume fraction of bound detergent leads immediately to:

$$x = [\bar{\rho}_{com}(\beta) - \bar{\rho}_{prot}(\beta)]/[\bar{\rho}_{det}(\beta) - \bar{\rho}_{prot}(\beta)]. \quad (17)$$

In principle, Eq. 17 could be evaluated at any value of β, provided the absolute scattering densities of the components were known. However, we avoid the problem of placing the contrast curves on an absolute scale by evaluating x at the contrast-match point of the complex; that is at $\beta = 0.319$. Note that it is impossible to determine $\bar{\rho}_{prot}(\beta)$ in any straightforward scattering experiment since the receptor, in solution, is always complexed with detergent.

In 31.9% D_2O, the scattering density of the solvent, ρ_0, is equal to the average scattering density of the complex, so that $\bar{\rho}_{com}$ is obtained directly: $\bar{\rho}_{com}(0.319) = \rho_0(0.319) = 1.65 \times 10^{-14}$ cm/Å^3. The values of $\bar{\rho}_{det}(0.319) = 0.63 \times 10^{-14}$ cm/Å^3 and $\bar{\rho}_{prot}(0.319) = 2.09 \times 10^{-14}$ cm/Å^3 ($\gamma = 0$) or $\bar{\rho}_{prot}(0.319) = 2.27 \times 10^{-14}$ cm/Å^3 ($\gamma = 1$) were computed using the theoretical expressions for their neutron-scattering densities (Eqs. 12 and 13). Substitution of these values into Eq. 17 then gives $x = 0.30 \pm 0.10$ ($\gamma = 0$). This value represents a lower limit on the volume fraction of bound detergent since we have assumed that none of the noncarbon-bonded hydrogens exchanges with the solvent. If, on the other hand, all of the labile receptor hydrogens reach equilibrium with the solvent ($\gamma = 1$), $x = 0.38 \pm 0.09$.

MOLECULAR WEIGHT OF THE RECEPTOR-DETERGENT COMPLEX By placing the zero-angle scattering data on an absolute scale, we could evaluate the molecular weight of the receptor-detergent complex from the slope of the contrast-curve, just as was done for the detergent micelles (see Molecular Weight of the Detergent Micelle). The partial specific volume of the complex was calculated from the volume fraction of bound detergent, reported above, and the partial specific volumes of the detergent and receptor, assuming volume additivity $\bar{v}_{com} = [(x/\bar{v}_{det}) + (1 - x)/\bar{v}_{prot})]^{-1}$. The values of the other parameters appearing in Eq. 15 are listed in Table I. The lower and upper limits found for the molecular weight of the acetylcholine receptor-Triton X-100 complex are $3.2 \pm 0.3 \times 10^5$ ($\gamma = 0$) and $3.5 \pm 0.3 \times 10^5$ ($\gamma = 1$), depending on the degree of hydrogen exchange.

MOLECULAR WEIGHT OF THE ACETYLCHOLINE RECEPTOR MONOMER The volume fraction of bound detergent together with the molecular weight of the receptor-detergent complex, define the number of detergent monomers bound to the receptor, namely,

$$n = xV_{com}/V_{det} = x\bar{v}_{prot}M_{com}/\{[(1 - x)\bar{v}_{det} + x\bar{v}_{prot}]M_{det}\}, \quad (18)$$

where V_{det} is the volume of a detergent monomer. Eq. 18 is valid if the packing of the detergent and protein are not altered in forming a complex. Using the values of x and M_{com} determined above, we find that the number of detergent monomers bound to the receptor lies in the range $n = 133 \pm 50$ ($\gamma = 0$) to $n = 183 \pm 44$ ($\gamma = 1$), depending upon the actual extent of hydrogen exchange. Finally, we subtract the mass of bound detergent to obtain the molecular weight of the receptor, leaving 230,000 ± 40,000 ($\gamma = 1$) – 240,000 ± 40,000 ($\gamma = 0$) for the molecular weight of the acetylcholine receptor monomer.

At a concentration of 0.2%, Triton X-100 is thus bound to the extent of 0.35 ($\gamma = 0$)–0.49 ($\gamma = 1$) g per g of receptor protein. At lower concentrations of Triton X-100, the quantity

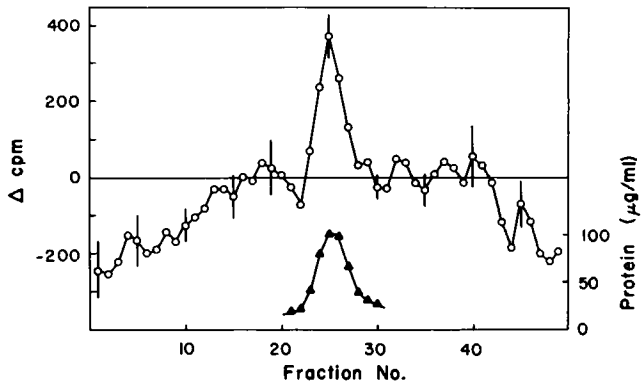

FIGURE 4 Binding of Triton X-100 to the acetylcholine receptor. Purified receptor in TNP50 was centrifuged into 5–20% sucrose density gradients containing 0.02% ^{3}H-Triton X-100 in NP50. Background counts were evaluated from parallel gradients onto which samples of TNP50 alone were layered. Δ (sample-background) cpm, (○) and protein, (▲) were evaluated. Representative propagated errors are indicated.

bound was directly determined (Fig. 4); at 0.02% we obtained 0.34 ± 0.06 g/g and at 0.04%, 0.37 ± 0.08 g/g.

Radii of Gyration

Values obtained for the radii of gyration of the detergent micelle and the receptor-detergent complex, in each H_2O/D_2O mixture, are summarized in Table II. The radii of gyration were evaluated from the slopes of the least-squares fit lines to the scattering intensity data in Guinier plots.

RADIUS OF GYRATION OF TRITON X-100 DETERGENT MICELLES. Fig. 5 *a* shows the radii of gyration data for the detergent plotted as R^2 vs. $(\overline{\Delta\rho})^{-1}$ (Stuhrmann, 1974). The data points were fit to a parabola using a least-squares procedure, and the parameters R_s, a, and b were evaluated (Eq. 6). Their values are listed in Table III. For both concentrations of Triton X-100 studied, a is small. This indicates that the detergent micelle has a nearly uniform

TABLE II
RADII OF GYRATION AS FUNCTION OF D_2O CONCENTRATION

[D_2O]	Triton X-100	Receptor
% vol/vol	*Å*	
100	25.9 ± 1.0 (3)*	46.4 ± 0.8 (6)
80	24.9 ± 0.4 (3)	44.4 ± 1.5 (3)
60	25.7 ± 1.6 (2)	41.3 ± 1.6 (3)
50	25.6 ± 1.0 (2)	36.2 (1)
40	17.9 ± 2.0 (5)	11.2 ± 4.8 (3)
20	—	46.0 ± 1.4 (4)‡
0	17.1 ± 2.6 (4)	50.3 ± 0.5 (3)

*Mean ± SEM (number of independent determinations).
‡The results at 16.7, 20, and 22% D_2O were averaged.

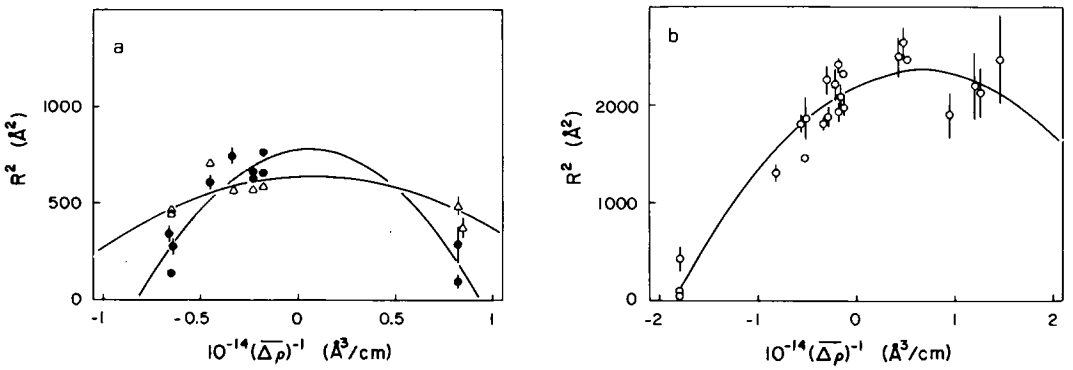

FIGURE 5 Variation of the square of the radius of gyration with inverse contrast. (*a*) Triton X-100 micelles measured at 0.2% (•) and 1% (△) detergent concentrations. The data were fit, separately, to a quadratic equation from which the parameters R^2, *a*, and *b* were evaluated (see Eq. 6 and Table III). (*b*) Stuhrmann plot for acetylcholine receptor-Triton X-100 complex.

neutron-scattering density distribution; and therefore, that its scatter can be completely eliminated by contrast matching.

The form of the detergent micelle is postulated to be comprised of a peripheral hydrophilic region surrounding a hydrophobic core (Tanford, 1974). The theoretical scattering density calculated for the hydrophobic portion of the Triton X-100 micelle is 0.40×10^{-14} cm/Å^3, based on a partial specific volume of 1.17 ml/g for the octylphenyl residue (Weast, 1966). It is theoretically contrast matched in 13.7% D_2O. The remaining hydrophilic portion of the micelle then has a scattering density of $\sim(0.69 + 0.17\beta) \times 10^{-14}$ cm/Å^3 and is contrast matched in solvent containing 18.4% D_2O. This predicted small difference between the contrast-match points of the hydrophilic and hydrophobic portions of the detergent is substantiated by the observed uniformity of the neutron-scattering density within the micelle.

The plots in Fig. 5 *a* both exhibit significant curvature which is reflected in nonzero values of *b* (Table III). The curvature decreases with increasing detergent concentration; *b* is ~70% smaller for the 1% Triton X-100 data. The interpretation of the nonzero *b* values for these curves in terms of an asymmetrical distribution of regions with different scattering densities

TABLE III
COEFFICIENTS OF THE STUHRMANN EQUATION* CALCULATED BY A LEAST-SQUARES METHOD

	Triton X-100‡		Receptor-Triton X-100 Complex§
	0.2%	1%	
	wt/vol		
R_s (Å)	28.0	25.3	46.9
a	1.19×10^{-6}	5.21×10^{-7}	4.99×10^{-6}
b (Å^{-2})	1.02×10^{-9}	3.17×10^{-10}	3.70×10^{-10}

*Eq. 6.
‡Triton X-100 solutions were in NP50 (see Methods).
§Concentrations of receptor varied from 0.5 to 4 mg/ml; no variation of *R* with receptor concentration was observed; and all results were averaged.

within the detergent micelle (Osborne et al., 1978) is problematical since the amphiphilic character of the detergent monomer dictates an inherently symmetrical density distribution, that is, the centers of gravity of the hydrophobic and hydrophilic regions of the micelle necessarily coincide (Tanford, 1974). Moreover, we have shown above that the Triton X-100 micelle has a nearly uniform neutron-scattering density distribution (see Discussion).

RADIUS OF GYRATION OF RECEPTOR-DETERGENT COMPLEX If the packing of the detergent in the acetylcholine receptor-Triton X-100 complex is the same as in a free detergent micelle, then in solvent containing ~20% D_2O, the net scattering intensity will originate predominantly from the protein. Our results (Table II) show that the acetylcholine receptor monomer has a radius of gyration of 46.0 ± 1.4 Å at the contrast-match point of the detergent.

To examine the radius of gyration of the bound detergent, we made neutron-scattering measurements on the receptor-detergent complex in solvent containing 40% D_2O. The theoretical estimate of the contrast-match point of the receptor is 41.8% D_2O, taking $\gamma = 1$ (see Methods; Eqs. 11 and 13). If the scattering density fluctuations within the protein component at its match point are small, the determined radius of gyration will reflect the structure of the bound detergent. The radius of gyration of the complex measured in solvent containing 40% D_2O is 11.2 ± 4.8 Å. The large error is likely due to the fact that the concentration of bound detergent is approximately equal to the concentration of unbound detergent in the solvent.

The Stuhrmann plot (R_ρ^2 vs. $(\overline{\Delta\rho})^{-1}$) for the receptor-detergent complex is shown in Fig. 5 *b*, and the least-squares fit parameters R_s, a, and b are listed in Table III. These parameters can be interpreted in terms of the structure of the complex provided the shape of the complex is invariant with the D_2O content of the solvent and the scattering particles comprise a homogeneous system. The parameter a is positive for the receptor-detergent complex. Qualitatively this means that overall, the portions of the complex whose scattering densities lie above $\bar{\rho}_{com}$ have a larger radius of gyration than those regions of the complex whose scattering densities fall below $\bar{\rho}_{com}$. Low-density regions would include hydrophobic regions of the protein in addition to the detergent.

The curve in Fig. 5 *b* is nonlinear, which indicates that the centers of gravity of the different neutron-scattering density regions of the complex do not coincide. If we assume uniform or centrosymmetric density distributions for protein and detergent moieties we can estimate the distance separating the centers of gravity of the protein and detergent scattering density distributions from the value of b. Substituting x, $\bar{\rho}_{det}$, $\bar{\rho}_{prot}$, and b into Eq. 9, we find that $|\mathbf{r}_{s,prot} - \bar{\mathbf{r}}_{s,det}| = 60 \pm 15$ Å for the receptor-detergent complex.

Extended Scattering Curves

Extended scattering data were collected for the Triton X-100 micelles and the receptor-detergent complex in solvent containing 100% D_2O. Since the contrast in this solvent is high, the intensity term originating from the particle shape dominates the net scattered intensity. In addition, the signal:noise ratio is improved since D_2O has a relatively small incoherent scattering cross section.

TRITON X-100 DETERGENT MICELLES An extended scattering curve for the detergent is plotted in Fig. 6 *a* as $\log(I/I[0])$ vs. $\log(hR_s)$. This type of plot facilitates comparison with

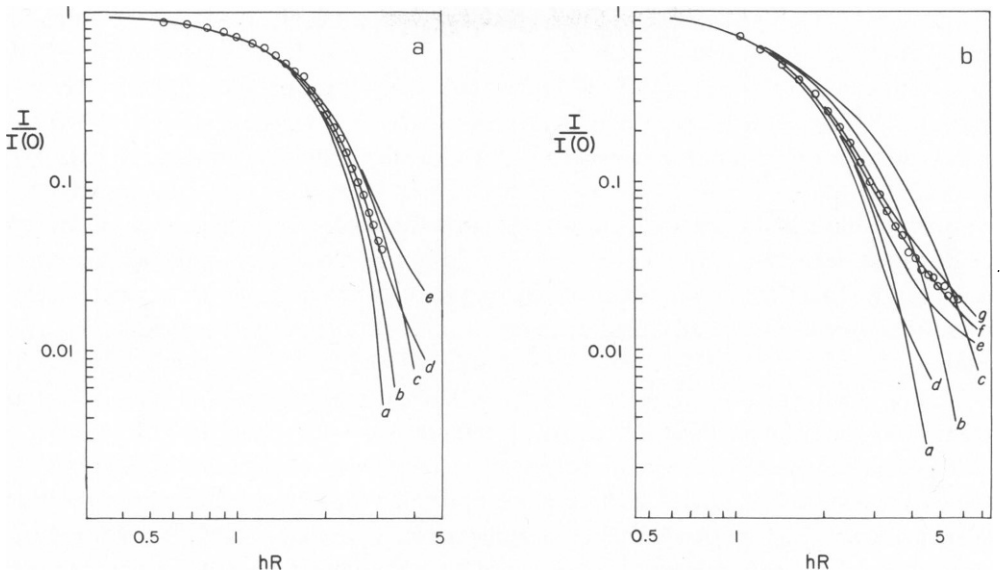

FIGURE 6 Extended scattering curves. (*a*) Higher angle scattering data for the Triton X-100 detergent micelles in 100% D_2O is plotted as described by Beeman et al. (1957). The experimental points are compared with theoretical scattering curves computed for ellipsoids of revolution with axial ratios: curve *a*, 1 (sphere); curve *b*, 1.5 (prolate) and 1:1.5 (oblate); curve *c*, 2 (prolate); curve *d*, 1:2 (oblate); curve *e*, 1:3 (oblate). (*b*) Extended scattering data for the receptor-detergent complex in TNP50 at 100% D_2O. The theoretical curves are for ellipsoids of revolution with axial ratios: curve a, 2 (prolate); curve b, 3 (prolate); curve c, 4 (prolate); curve d, 1:2 (oblate); curve e, 1:3 (oblate); curve f, 1:4 (oblate); curve g, 1:6 (oblate).

the theoretical scattering profiles for scattering objects of different shapes (Beeman et al., 1957). The experimental data nearly superimpose on the predicted scattering curve for an oblate ellipsoid with 1:2 axial ratio. However, the data do not extend out far enough to absolutely rule out a prolate ellipsoid shape with an axial ratio of ~1.75 $h_{max} = 0.13$ Å^{-1}

ACETYLCHOLINE RECEPTOR-TRITON X-100 COMPLEX Neutron-scattering data for the receptor-detergent complex were collected out to $h_{max} = 0.13$ Å^{-1} (Fig. 5 *b*). For the complex, the best-fit model curve is that for an oblate ellipsoid with an axial ratio of 1:4.

DISCUSSION

Triton X-100 Micelles

The molecular weight of Triton X-100 micelles has been measured previously in the temperature range 20–60°C by ultracentrifugation, light-scattering, and fluorescence spectroscopic techniques (summarized in Robson and Dennis, 1977). There is considerable variation in the reported values which may be due to differences in the composition of the Triton X-100 (Biaselle and Millar, 1975); nevertheless, on average these values are larger than the result of 55,00 daltons that we obtain. The molecular weights of detergent micelles, however, are temperature dependent (Tanford, 1974), and our estimate of the molecular weight of Triton X-100 micelles at 8°C is consistent with a smaller micelle size at lower

temperatures (Dwiggens and Bolen, 1961; Corti and Degiorgio, 1975). The present molecular weight estimate is in good agreement with the value of 65,520 measured at 5°C by ultracentrifugation (Lewis et al., 1974). Our preliminary results on the neutron scattering of Triton X-100 micelles at 25°C indicate a radius of gyration at this temperature in D_2O of 30 Å compared to 26 Å at 8°C, consistent with a higher molecular weight at the higher temperature.

An oblate ellipsoidal shape (axial ratio, 1:2) of the Triton X-100 micelles is consistent with the extended scattering curve (Fig. 6 *a*) and with the theoretical treatment of micelle formation by Tanford (1974). Based on hydrodynamic measurements and model building, Robson and Dennis (1977) also suggest an oblate ellipsoidal shape (axial ratio 1:1.9) for the Triton X-100 micelle. An oblate ellipsoid with an axial ratio of 1:2 and radius of gyration of 26 Å has a volume of 120,000 $Å^3$ and, at $\bar{\nu}_{det} = 0.908$, a molecular weight of 80,000 rather than the molecular weight of 55,000 obtained from the contrast curve (Fig. 3 *a*).

The nonlinearity seen in the Stuhrmann plot for the detergent micelles (Fig. 5 *a*) is puzzling. The curvature cannot in this case be accounted for by an asymmetric, nonuniform scattering density distribution within the micelle, as we have noted above. Nonlinearity in a plot of this type would also result if the average scattering densities of all micelles were not equal or if the size or shape of the detergent micelles varied with the D_2O content of the solvent. Scattering density heterogeneity is an unlikely explanation since the partial specific volume of the detergent does not appear to vary with concentration above the critical micelle concentration (Tanford et al., 1974). Moreover, we find that an increase in the detergent concentration results in a decrease in the curvature of the Stuhrmann plot, although the micelle size distribution is predicted to broaden with increasing detergent concentration (Tanford, 1974). Variation in the size of the scattering particle with contrast would also result in nonlinearity of the plot of $(I[0]/c)^{1/2}$ vs. ρ_0, which we do not observe. Possibly, either the shape or distribution of sizes of the micelles changes with the H_2O/D_2O content of the solvent, without any concomitant variation in average size. Theoretical calculations indicate that H ⇌ D exchange with solvent by the detergent will not, in itself, lead to the observed nonlinearity in the Stuhrmann plot.

Acetylcholine Receptor and Its Complex with Triton X-100

The molecular parameters of the acetylcholine receptor evaluated from the neutron-scattering experiments include a determination of the receptor's molecular weight, the amount of detergent it binds, and its radius of gyration.

DETERGENT BINDING AND MOLECULAR WEIGHT Our analysis indicates that ~40% of the receptor-detergent complex is comprised of detergent so that the partial specific volume of the complex is $\bar{\nu}_{com} = 0.78$–0.79 ml/g. Specification of the exact volume fraction of bound detergent and hence $\bar{\nu}_{com}$ depends upon the extent of H ⇌ D exchange with the solvent by labile hydrogens of the receptor, and this is undetermined. For completeness, we have expressed our results as a range of values corresponding to the limits of no exchange and complete exchange of labile hydrogens.

$\bar{\nu}_{com} = 0.77$ ml/g has been reported for the predominant forms of the *Torpedo* receptor-toxin complex in 1% Triton X-100 (Gibson et al., 1976); a similar determination gave $\bar{\nu}_{com} = 0.78$ ml/g for the *Electrophorus electricus* receptor-toxin complex also in 1% Triton X-100

(Meunier et al., 1972). However, the analysis of amphiphilic, detergent-binding proteins by comparison with the sedimentation rates of standard proteins that do not bind detergent is problematical (Reynolds and Tanford, 1976; Reynolds and Karlin, 1978).

The mass of detergent bound per mass of protein, δ_{det}, is obtainable from x (or $\bar{\nu}_{com}$), given $\bar{\nu}_{det}$ and $\bar{\nu}_{prot}$. We find based on the neutron-scattering results that $\delta_{det} = 0.35$–0.49. Edelstein et al. (1975) examined detergent binding directly by measuring the amount of ^{3}H-Triton X-100 remaining after extraction and purification of the receptor in the radioactive detergent. They report $\delta_{det} = 0.113$, but receptor aggregation with consequent reduced detergent binding may have occurred under their conditions of minimal detergent content. Gibson et al. (1976) report $\delta_{det} = 0.23$ for both monomer and dimer forms of *T. californica* receptor, and Meunier et al. (1972) find $\delta_{det} = 0.21$ for the receptor protein isolated from electric eel. In each case, the conclusions are based on values of $\bar{\nu}_{com}$ obtained from sedimentation velocity in comparison with nondetergent binding proteins. We note that small errors in the partial specific volumes will profoundly influence the outcome for δ_{det}. Gibson et al. (1976) take $\bar{\nu}_{det} = 0.94$ ml/g, whereas Meunier et al. (1972) use $\bar{\nu}_{det} = 0.99$ ml/g. $\delta_{det} = 0.45$ was obtained in a membrane osmometry study of the molecular weight of the receptor (Martinez-Carrion et al., 1975), but the preparation was not characterized with regard to its monomer/dimer content and the two forms of receptor may bind different amounts of detergent.

The molecular weight of the monomer form of the *T. californica* acetylcholine receptor, determined by neutron scattering, is found to be 230,000–240,000 in near agreement with the value of 251,000 obtained by rigorous sedimentation analysis (Reynolds and Karlin, 1978). The neutron-scattering result has a much larger uncertainty, however, due in part to the inaccuracy in estimating the amount of detergent bound.

STRUCTURE OF THE RECEPTOR-DETERGENT COMPLEX Two methods were used to evaluate the shape of the receptor-detergent complex; the results are in close agreement. When the radius of gyration and molecular volume of the complex are used to evaluate its shape, we obtain two ellipsoid solutions, one of which is oblate with a 1:3.4 axial ratio. Evaluating the extended scattering profile for the receptor-detergent complex, we find that it is fit at low angles by the theoretical intensity curve for an oblate ellipsoid with an axial ratio of ~1:4. The oblate ellipsoid model for the complex has approximate dimensions: 40 × 140 × 140 Å.

This axial ratio differs significantly from that predicted on the basis of hydrodynamic measurements of the receptor-detergent complex (Reynolds and Karlin, 1978). Reynolds and Karlin (1978) report $R_{stokes}/R_{min} = 1.6$ (see Eq. 10 in Tanford et al., 1974) for the monomeric form of the acetylcholine receptor in the detergent Brij 58. Assuming a rigid ellipsoid of revolution shape this yields (Tanford, 1961) axial ratios of 12 (prolate) or 1:14 (oblate) for the complex. These rather large axial ratios are decreased only slightly if we presume Reynolds and Karlin have underestimated the amount of detergent bound and hence R_{min}. Allowing for an equal mass of Brij 58, bound as the amount of Triton X-100 found by neutron scattering, results in axial ratios of 9 (prolate) and 1:12 (oblate).

Solvent hydration of the complex was not considered in the calculation of R_{min}, but this seems unlikely to account for the entire difference between the hydrodynamic and neutron-scattering results because an enormous amount of bound water would be required to achieve parity. It is possible that the two detergents do not associate equivalently with the receptor.

Brij 58 is a polyoxyethylene cetyl ether with a molecular weight nearly twice that of the average Triton X-100 monomer (Helenius and Simons, 1975). However, the micelle molecular weight of Brij 58 is similar to that reported for Triton X-100 (Helenius and Simons, 1975). Ultimately, we must question the validity of the assumption that the receptor-detergent complex behaves hydrodynamically like a rigid ellipsoid of revolution. Apparently aberrant hydrodynamic behavior has been noted for other nonionic detergent-protein complexes (Helenius and Simons, 1975).

STRUCTURE OF THE BOUND DETERGENT Detergent binding to membrane proteins is postulated to occur in a micellar fashion; that is, the structure of the bound detergent should be similar to that of a free detergent micelle (Robinson and Tanford, 1975; Osborne et al., 1978). Support for this postulate comes from the observation that the number of bound detergent monomers in a protein-detergent complex is typically close to the average number of monomers in a free micelle (Osborne et al., 1974; Clarke, 1974; Helenius and Simons, 1975; Robinson and Tanford, 1975). Additionally, at least for rhodopsin-detergent complexes, the radius of gyration of the bound detergent approximately equals the radius of gyration of free detergent micelles (Yeager, 1975; Sardet et al., 1976; Osborne et al., 1978).

If these conditions apply to the acetylcholine receptor-Triton X-100 complex, then the amount of detergent bound suggests that there are the equivalent of two Triton X-100 micelles bound to each receptor monomer. The available data are insufficient to determine whether the detergent does, in fact, bind in a micellar form, though it is difficult to construct a model of the receptor-detergent complex containing two separate and distinct detergent micelles. Given the amount of detergent bound, the small radius of gyration (11 ± 5 Å) observed with the protein component contrast matched is an unrealistic estimate of the radius of gyration of the bound detergent for any conceivable packing configuration. This value may be in error because of the low coherent scattering intensity, arising from the bound detergent, roughly equivalent to that from the TNP50 solvent background. An alternative but less likely possibility is that there are two micelles of detergent bound to the receptor, but they are located relatively far apart ($>\sim 60$ Å). In this eventuality the interference between the two micelles would not be observable in the low-angle limit of our experiments. The measured radius of gyration would then appear the same as if the two micelles were completely dissociated (Guinier, 1963). The radius of gyration of the complex measured with the protein contrast-matched does appear to approximate the radius of gyration of free Triton X-100 micelles measured at the same solvent contrast (see Table II).

STRUCTURE OF THE ACETYLCHOLINE RECEPTOR The neutron-scattering results for the acetylcholine receptor-Triton X-100 complex with the detergent contrast-matched show that the receptor alone has a radius of gyration of 46 Å. Because of the low coherent scattering intensity and high incoherent background, an analysis of the extended scattering curve was not possible at this contrast. Since a single parameter cannot specify shape, the structure of the receptor is indeterminate based on the radius of gyration measurement alone. Alternative simple shapes consistent with the radius of gyration and molecular volume of the receptor are the oblate and prolate ellipsoids and cylinders listed in Table IV (see also Fig. 7). These dimensions disregard any nonuniformity present in the scattering density distribution within the protein in that we assume that the radius of gyration measured at the detergent contrast-match point is identical to the radius of gyration of the receptor shape. The radius of

TABLE IV

SIMPLE SHAPES CONSISTENT WITH MOLECULAR VOLUMES AND RADII OF GYRATION

	Dimensions*	α‡	α§
	Å		
Detergent			
Prolate ellipsoid	40 × 100	2.5	1.7
Oblate ellipsoid	80 × 25	1:3.2	1:2
Complex			
Prolate ellipsoid	65 × 180	2.7	
Oblate ellipsoid	140 × 40	1:3.5	1:4
Prolate cylinder	60 × 140	2.2	
Oblate cylinder	130 × 30	1:3.8	1:4
Receptor			
Prolate ellipsoid	55 × 190	3.4	
Oblate ellipsoid	30 × 140	1:5.1	
Prolate cylinder	50 × 150	2.8	
Oblate cylinder	130 × 25	1:5.2	

Ellipsoid: $V = (4/3)\pi p^2 q$, where $2p$ is the "diameter" of the elipsoid and $2q$ is its length (i.e., axis of rotation). $\alpha = q:p$ is the axial ratio. For a prolate ellipsoid, $\alpha > 1$, and for an oblate ellipsoid, $\alpha < 1$. The radius of gyration of the ellipsoid is given by $R_s^2 = (2p^2 + q^2)/5$. Cylinder: $V = \pi p^2 q$, where p is the cylinder's radius and q its length. For a cylinder, $R_s = p^2/2 + q^2/12$. In each case, the two simultaneous equations were solved for p and q. For the detergent the value of R_s used was the value of R measured in 100% D_2O (see text); for the complex, R_s was obtained from the Stuhrmann plot (Table III); for the receptor alone R_s was taken as the value of R measured at the contrast-match point of the complex. In the latter case, the more accurate estimate of $M_{prot} = 250{,}000$ was used (Reynolds and Karlin, 1978).

*Diameter × length (rounded to the nearest 5 Å).

‡Axial ratio evaluated from computed dimensions.

§Axial ratio obtained from extended scattering-curve profiles.

gyration and molecular volume values do rule out a compact spherical shape for the receptor since the radius of gyration of the receptor's equivalent spherical volume is only 32 Å.

Any realistic model of the acetylcholine receptor is further constrained by the following structural criteria: there must exist a portion of the receptor that interacts hydrophobically with membrane and with detergent. We know that detergent is necessary for the solubilization of the receptor from the membrane and that the solubilized receptor binds a fixed amount of detergent. In addition the magnitude of the single-channel ion conductance (Katz and Miledi, 1972; Anderson and Stevens, 1973; Neher and Sakmann, 1976; Sheriden and Lester, 1977) indicates the likely presence of a pore associated with the receptor that spans the lipid bilayer (Karlin, 1973). An ion shuttle could not accommodate the large conductances observed.

Extension of the receptor ~50 Å beyond the extracellular membrane surface is indicated by the spacing of ferritin-conjugated, anti-receptor antibodies from the labeled membrane surface (Karlin et al., 1978), by the thickness of the outer dense lamina of the electroplax (Rosenbluth, 1975), and by direct observation of edge particles in negatively stained preparations of receptor-rich membranes[2] (Cartaud et al., 1978; Klymkowsky and Stroud, 1979). The dimension of the receptor perpendicular to the membrane plane therefore must be

[2]Wise, D. S. Unpublished observations.

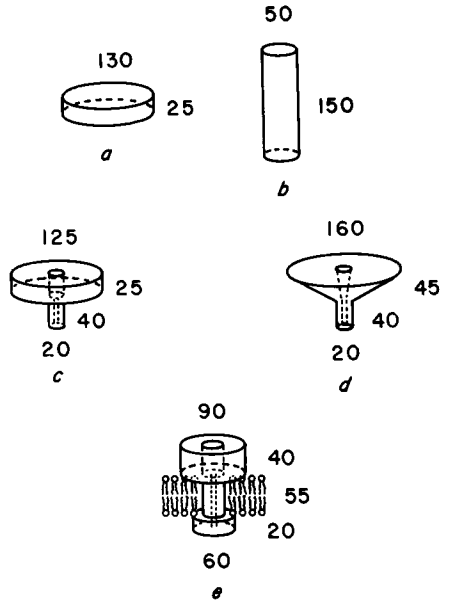

FIGURE 7 Hypothetical models of the average distribution of the mass of the acetylcholine receptor monomer. The indicated dimensions (dimensions indicated in the figure are rounded off to nearest 5 Å) of all models shown yield a radius of gyration and volume that agree with the values obtained for the receptor, namely, $R = 46$ Å and $V = 3 \times 10^5$ Å^3. Although the receptor monomer is not likely to be axially symmetrical in view of its subunit stoichiometry of $\alpha_2\beta\gamma\delta$ (Reynolds and Karlin, 1978; Lindstrom et al., in press), it is represented here as cylindrically symmetrical for computational simplicity and in accord with the resolution of the analysis. (*a*) Oblate cylinder. (*b*) Prolate cylinder. (*c*) Oblate cylinder with tail. A cylindrical tail and pore were added to an oblate cylinder head piece to provide an ion channel across the membrane. The headpiece has a cylindrical pore 20 Å in diameter running through it. The pore diameter constricts to 10 Å in the tailpiece. (*d*) Funnel. This model was constructed to take into account the overall length of the receptor as indicated by electron microscopic and x-ray diffraction results (see Discussion). The funnel head is a cone with a cone-shaped pore (20 Å base diameter) running through it. The tailpiece is a cylinder with a cylindrical pore 10 Å in diameter, identical to the tailpiece in Fig. 7 *c*. (*e*) Model which is consistent with all available structural data for the receptor. The model consists of three stacked, concentric cylinders with a cylindrical pore running through them. The pore has a diameter of 30 Å within the top cylinder, then constricts in size to 10 Å through the middle and bottom cylinders.

at least 100 Å to account for this extension plus the thickness of the bilayer. Rosenbluth (1975) and Potter and Smith (1977) report an average thickness of 120 Å for the postsynaptic membrane of *Torpedo*, with some structures in the membrane extending out to 155 Å in length (Potter and Smith, 1977). X-ray scattering profiles from membranes containing no more than 50% of their protein as receptor have also been interpreted as indicating an overall receptor length of ~115 Å, extending ~50 Å from one side of the membrane and ~15 Å from the opposite side (Ross et al., 1978).

The dimension of the receptor in the plane of the membrane is estimated from negative stain electron micrographs of receptor-rich membranes. These typically reveal a surface covered with ring-shaped particles 60–90 Å in diameter with a prominent central pit (Nickel

and Potter, 1973; Cartaud et al., 1973; Reed et al., 1975; Chang et al., 1977; Cartaud et al., 1978; Ross et al., 1978; Klymkowsky and Stroud, 1979).

These considerations have led us to some models for the receptor which we present for their heuristic value (Fig. 7). Each model has a radius of gyration of ~46 Å and volume of $\sim 3 \times 10^5$ Å^3, i.e., identical to the receptor's. The model in Fig. 7 *c* adds an ion channel and a hydrophobic tail onto a simple oblate cylindrical shape. Note that these additions have only a small influence on the dimensions of the oblate head. Fig. 7 *d* was constructed to increase the extension of the receptor beyond the membrane surface. The overall diameter of this model, however, is more than twice that observed by negative stain electron microscopy, although stain penetration about the perimeter of the protein as shown could result in a smaller observed diameter. The large diameter however also seems to be inconsistent with the average center-to-center spacing of the receptor of 90–100 Å as measured in *Torpedo* receptor-rich membranes (Dupont et al., 1973; Cartaud et al., 1978). The model shown in Fig. 7 *e* takes into account all the known structural features of the receptor outlined above. We note that this model additionally allows for a 60-Å separation between the centers of gravity of the protein and detergent, in agreement with the neutron-scattering result (see Results). The detergent is presumed to replace the lipid bilayer in the model.

We have premised our model building on the assumption that the structure of the receptor in detergent solution is similar to its structure in the membrane, at least at low resolution. In support of this contention is the fact that the morphology of the receptor particles appears identical when receptor-rich membranes and solubilized receptors are examined by negative-stain electron microscopy (Cartaud et al., 1973; Chang et al., 1977; Cartaud et al., 1978). Further, our initial neutron-scattering measurements on receptor dimers in solution indicate that these particles have a radius of gyration of ~60 Å. Applying the parallel axis theorem (Goldstein, 1959; Engelman and Moore, 1975) to this result indicates a center-to-center spacing of ~80 Å between the monomers, in good agreement with the average separation observed by electron microscopy in the membrane (Dupont et al., 1973; Cartaud et al., 1978). On the other hand, there are differences in the functional properties of receptor in solution and in membrane (Moody et al., 1973; Sugiyama and Changeux, 1975; Eldefrawi et al., 1975; O'Brien and Gibson, 1975; Chang and Bock, 1979).

Recently, Klymkowsky and Stroud (1979) have proposed some cylindrically symmetrical three dimensional models of the receptor derived from the x-ray diffraction profiles of receptor-rich, but nevertheless, heterogeneous membranes (Ross et al., 1978). The molecular weight of the receptor obtained (280,000–310,000) is significantly larger than the value measured by Reynolds and Karlin (1978); moreover, the radius of gyration of these models is only 38 ± 1 Å, significantly smaller than the neutron-scattering result. A less compact shape of the receptor as in the examples in Fig. 7, is likely.

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