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CHARACTERIZATION OF ACETYLCHOLINE RECEPTOR ISOLATED FROM *TORPEDO CALIFORNICA* ELECTROPLAX THROUGH THE USE OF AN EASILY REMOVABLE DETERGENT, β-D-OCTYLGLUCOPYRANOSIDE

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Summary

Non-ionic detergents used for the solubilization and purification of acetylcholine receptor from *Torpedo californica* electroplax may remain tightly bound to this protein. The presence of detergent greatly hinders spectrophotometric and hydrodynamic studies of the receptor protein. β-D-Octylglucopyranoside, however, is found to be effective in solubilizing the receptor from electroplax membranes with minimal interference in the characterization of the protein. The acetylcholine receptor purified from either octylglucopyranoside- or Triton X-100-solubilized extracts exhibits identical amino acid compositions, α-Bungarotoxin and (+)-tubocurarine binding parameters, and subunit distributions in SDS-polyacrylamide gels. The use of octylglucopyranoside allows for the assignment of a molar absorptivity for the purified receptor at 280 nm of approx. 530 000 $M^{-1} \cdot cm^{-1}$. Additionally, successful reconstitution of octylglucopyranoside-extracted acetylcholine receptor into functional membrane vesicles has recently been achieved (Gonzalez-Ros, J.M., Paraschos, A. and Martinez-Carrion, M. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1796—1799).

Removal of octylglucopyranoside by dialysis does not alter the specific toxin and antagonist binding ability of the receptor or its solubility at low protein concentrations. Sedimentation profiles of the purified acetylcholine receptor in sucrose density gradients reveal several components. Sedimentation coefficients

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Abbreviations: SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride.

obtained for the slowest sedimenting species agree with previously reported molecular weight values. Additionally, the different sedimenting forms exhibit distinctive behavior in isoelectric focusing gels. Our results suggest that both the concentration and type of detergent greatly influence the physicochemical behavior of the receptor protein.

The nicotinic acetylcholine receptor is an oligomeric membrane protein involved in neuromuscular transmission. The specific interaction of the neurotransmitter (acetylcholine) with the membrane-bound receptor increases ion permeability of the postsynaptic membrane. Our own recent findings [1], as well as those of others [2], have illustrated how the presence of lipid is required for the maintenance of the receptor in a native 'sensitized' state. However, the most commonly used methods to purify the acetylcholine receptor generally involve non-ionic detergent solubilization of electroplax membranes from a variety of electric fish, followed by affinity chromatography using cholinergic ligands [3]. The presence of detergent has always been reported as a requirement for maintaining the protein in solution. Nevertheless, the precise characterization of purified acetylcholine receptor is hindered by the inherent qualities of the more commonly used non-ionic detergents which (a) consist of a mixture of different chemical species, (b) exhibit low critical micelle concentration values, (c) interfere with spectrophotometric and spectrofluorometric measurements, and (d) bind tightly to the receptor protein [4,5], thus modifying the hydrodynamic properties of the receptor protein [6], and, presumably, interfering with attempts at reincorporation of the receptor into a simulated membrane environment.

Recently, a non-ionic detergent, β-D-octylglucopyranoside, has been successfully utilized in membrane studies [7—10]. This compound appears to be advantageous over other non-ionic detergents in solubilizing certain membrane proteins [7,11]. In addition, octylglucopyranoside has been examined in detergent inactivation [12] and activation [8] studies of membrane enzymes, and in previous attempts at reconstitution of membranes [11,13]. The solubilization of *Torpedo* electroplax membrane proteins with octylglucopyranoside, and subsequent purification of the acetylcholine receptor by affinity chromatography, has provided preparations which have been functionally reconstituted into a lipid environment with regeneration of the properties associated with native receptor-enriched electroplax membranes [1]. The use of octylglucopyranoside appears to allow the preparation of acetylcholine receptor which retains many of the properties found for the more popular Triton X-100-extracted protein. Nevertheless, certain other characteristics of octylglucopyranoside-solubilized receptor must somehow facilitate its functional reconstitution. It is therefore of interest to investigate the properties of octylglucopyranoside-solubilized and -purified receptor.

This paper reports the effective solubilization of *Torpedo* electroplax membrane proteins with octylglucopyranoside. Furthermore, the purification of acetylcholine receptor in the presence of this detergent and the effects of detergent removal are discussed. Finally, we examine some of the structural and functional properties of the purified receptor in both its detergent-associated and detergent-depleted forms.

Materials and Methods

Torpedo californica electroplax was purchased from Pacific Biomarines Supply Co. Protein standards for molecular weight determinations by SDS-gel electrophoresis were from Pharmacia. β-D-Octylglucopyranoside was from Calbiochem. Carrier ampholytes for isoelectric focusing gels were purchased from Bio-Rad. α-Bungarotoxin was purified from *Bungarus multicinctus* venom (Sigma Chemical Co.) following published procedures [14]. ^{125}I-labelled α-bungarotoxin and β-D-octyl-[U-^{14}C]glucopyranoside were purchased from New England Nuclear. (+)-Tubocurarine di-[^{14}C]methyl ether iodide was obtained from Amersham. α-Cobratoxin was purified from *Naja naja siamensis* venom (Sigma) by the method of Ong and Brady [15]. Beef liver catalase and rabbit muscle aldolase were from Sigma and aspartate transaminase isozymes were kindly prepared by J.R. Mattingly [16]. PGO (glucose-oxidase/lactoperoxidase) enzymes and *O*-dianisidine dihydrochloride for glucose determinations were purchased from Sigma Chemical Co.

Purified acetylcholine receptor preparation. Solubilized acetylcholine receptor was purified by α-cobratoxin affinity chromatography from *Torpedo californica* electroplax as previously described [17], except that octylglucopyranoside replaced Triton X-100 throughout the procedure. The octylglucopyranoside concentration for solubilizing electroplax proteins was 30 mM, unless indicated otherwise. Protein concentration was determined by the method of Lowry et al. [18] and ^{125}I-labelled α-bungarotoxin binding to acetylcholine receptor was determined using a DEAE-cellulose filter disk assay procedure [19]. The recovery of the acetylcholine receptor was 20—40% from the electric tissue. Specific activities of purified receptor preparations were approximately 9 nmol of α-bungarotoxin binding sites per mg protein. A typical preparation under optimum conditions yields approximately 1 nmol of α-bungarotoxin binding sites per g (wet weight) of electroplax crude membranes.

The absorption spectra of purified acetylcholine receptor were recorded with a Cary 210 spectrophotometer. Molar absorptivity of the purified protein was calculated on the basis of absorption spectra, assuming a molecular weight of 270 000 [4]. Once the absorption spectra were recorded, aliquots from either purified acetylcholine receptor or different fractions from sucrose density gradients were hydrolyzed in sealed, evacuated tubes containing 0.3 ml of constantly boiling HCl for 24 h at 110°C. Protein concentration values for these samples were determined by amino acid analysis performed in a Durrum MBF amino acid analyzer. The method of Lowry et al. [18], using bovine serum albumin as a standard, provides protein concentration values approximately 10% lower than the amino acid analysis.

Detergent determination. Octylglucopyranoside was hydrolyzed under vacuum with 1 M HCl for 3 h at 90°C. By comparison with glucose standards, it was found that approximately 70% of the initial amount of detergent became hydrolyzed under the specified conditions. After hydrolysis, samples were neutralized and glucose was determined by the glucose-oxidase/lactoperoxidase method (Sigma), using *O*-dianisidine dihydrochloride as a chromogen. Alternatively, β-D-octyl-[U-^{14}C]glucopyranoside was used as a radioactive tracer in order to estimate the removal of detergent upon dialysis.

(+)-[^{14}C]Tubocurarine binding to purified acetylcholine receptor. Aliquots of 0.15 ml of purified receptor ((4—6) · 10^{-6} M in α-bungarotoxin-binding sites) from either Triton X-100- or octylglucopyranoside-solubilized preparations were placed in 1 cm dialysis tubing. Equilibrium dialysis was conducted against receptor buffer (10 mM sodium phosphate buffer, pH 7.4/1 mM EDTA/10^{-5} M PMSF/0.02% NaN_3) containing either 0.1% Triton X-100, 1 mM octylglucopyranoside, or no detergent in the presence of increasing concentrations of radioactive (+)-tubocurarine (0.01—1.8 μM). After 16—18 h, 50-μl aliquots were taken from the inside and outside of the dialysis bags and the extent of binding was calculated by the difference in radioactivity. α-Bungarotoxin binding activity of the different samples remained unchanged, indicating that neither sample dilution nor receptor inactivation occurred during the dialysis. Acetylcholine receptor samples in the presence of a 10-fold molar excess of a α-bungarotoxin were processed as blanks to correct for possible non-specific (+)-tubocurarine binding.

Sucrose density gradient centrifugation. Aliquots (0.3 ml) of purified acetylcholine receptor from either Triton X-100- or octylglucopyranoside-solubilized extracts were layered on top of continuous sucrose gradients (2—25% (w/v) sucrose in receptor buffer containing 0.2 M NaCl) in the presence of either 1% Triton X-100, 30 mM octylglucopyranoside, 1 mM octylglucopyranoside or no detergent. Samples were centrifuged for 3.5 h in a Beckman SW-50.1 rotor at 47 000 rev./min. Beef liver catalase [20] and mitochondrial aspartate transaminase [21] were run as either internal or external standards for sedimentation coefficient determinations.

Attempts at reducing the acetylcholine receptor were performed by either (a) preincubating the purified protein with 10 mM dithiothreitol for 10—180 min at 4—25°C or (b) solubilizing the electroplax tissue in the presence of 10 mM β-mercaptoethanol and maintaining the concentration of the reducing agent throughout the purification of the receptor. The presence or absence of reducing agent within the sucrose gradients had no effect on the sedimentation profile of the treated samples. These gradients were monitored either by absorbance at 280 nm or by radioactivity due to preincubation with ^{125}I-labelled α-bungarotoxin. In the latter method, inactivation of excess dithiothreitol was conducted with 10 mM *N*-ethylmaleimide prior to α-bungarotoxin labelling of the acetylcholine receptor.

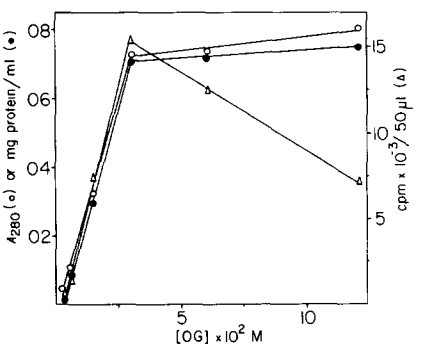
The sedimentation coefficients ($s_{20,w}$) and molecular weights for the purified receptor were calculated according to Eqns. 1 and 2, respectively, from Gibson et al. [22]. The partial specific volume of the receptor protein-detergent complex ($\bar{v}$ = 0.746 cm^3/g) in 1 mM octylglucopyranoside was determined by the differential centrifugation procedure as described [22]. The rates of sedimentation through 5—20% sucrose gradients for the acetylcholine receptor, catalase ($\bar{v}$ = 0.725, $s_{20,w}$ = 11.3 S) and aldolase ($\bar{v}$ = 0.742, $s_{20,w}$ = 7.9 S) were compared in water and in ^2H_2O.

Gel electrophoresis of the solubilized proteins. Exponential gradient (5—20%) SDS-polyacrylamide gel electrophoresis was performed using the discontinuous buffer system of Laemmli [23]. After staining with Coomassie Blue, the molecular weights were determined by comparison of the electrophoretic mobility with protein standards. Isoelectric focusing was performed on 5%

polyacrylamide gels containing 2% carrier ampholytes with variable Triton X-100 or octylglucopyranoside concentrations. Stable pH gradients were obtained by the addition of 5% glycerol to the gel mixture. The upper reservoir contained 20 mM NaOH and the lower 10 mM H_3PO_4. Gels were run overnight at 4°C. Following each run, the gels were stained as described by Gianazza and Righetti [24]. p*I* values were estimated from the gradients as described by O'Farrell [25]. Reproducibility was demonstrated using either bovine serum albumin or the cytoplasmic isozyme of aspartate transaminase as internal or external standards.

Results

Solubilization of Torpedo californica electroplax. The protein solubilization profile of *Torpedo* electroplax with increasing concentration of octylglucopyranoside (3—140 mM) is shown in Fig. 1. The total amount of solubilized protein, monitored by either the method of Lowry et al. or by absorbance at 280 nm, reached a plateau at a detergent concentration of approximately 30 mM. At the same detergent concentration the α-bungarotoxin-binding capacity of the solubilized preparation reached a maximum. Conversely, as the detergent concentration was increased above 35 mM, α-bungarotoxin-binding decreased. In addition, within the range of 3—30 mM octylglucopyranoside, the specific activity in terms of α-bungarotoxin-binding sites per mg protein remained constant. Regardless of the detergent concentration, the solubilized preparations produced the same subunit distribution in SDS-polyacrylamide gels (Fig. 2). Consequently, we utilized 30 mM octylglucopyranoside for preparative purposes since this provided both maximal protein yields and specific activities similar to those obtained in previous studies with 1% Triton X-100 as the



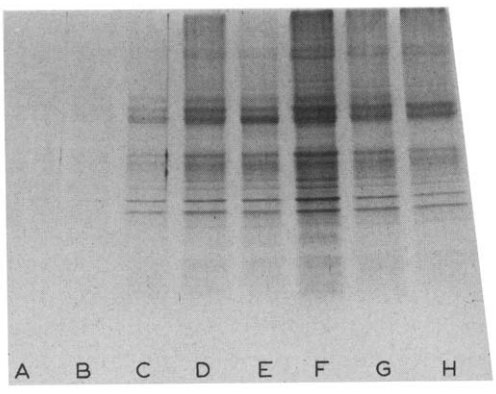
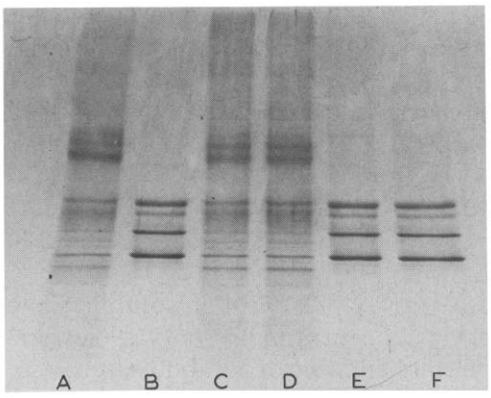


Fig. 1. Protein solubilization profile of *Torpedo californica* electroplax. Aliquots from a suspension of electroplax membranes were incubated in the presence of increasing concentrations of β-D-octylglucopyranoside (OG) and centrifuged at 100 000 × *g* for 1 h. Protein appearance in the supernatant was monitored either by the method of Lowry et al. [18] (●) or by absorbance at 280 nm (○) and ^{125}I-labelled α-bungarotoxin binding (△) was determined.

Fig. 2. Polypeptide patterns on SDS-polyacrylamide gels of β-D-octylglucopyranoside-solubilized protein extracts under different solubilizing concentrations (mM) of the detergent. A, 3; B, 6, C, 15; D, 25; E, 30; F, 35, G, 70; H, 140.



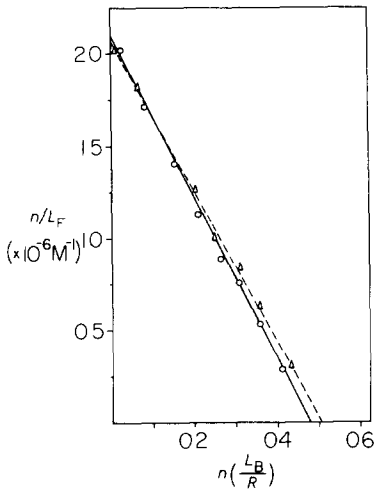


Fig. 3. Polypeptide patterns on SDS-polyacrylamide gels of crude extracts and purified acetylcholine receptor from β-D-octylglucopyranoside- or Triton X-100-solubilized preparations. A, 1% Triton X-100-solubilized extract; B, acetylcholine receptor purified from (A); C, 30 mM β-D-octylglucopyranoside-solubilized extract; D, 30 mM β-D-octylglucopyranoside-solubilized extract after removal of the detergent by extensive dialysis; E, acetylcholine receptor purified from (C); F, purified acetylcholine receptor after removal of β-D-octylglucopyranoside by extensive dialysis.

Fig. 4. Representative Scatchard plot for the binding of [^{14}C]dimethyl ether (+)-tubocurarine to purified acetylcholine receptor. ○, Equilibrium dialysis of β-D-octylglucopyranoside-solubilized acetylcholine receptor in the presence of 1 mM detergent. △, Triton X-100-solubilized acetylcholine receptor in the presence of 0.1% Triton X-100. L_B and L_F represent molar concentrations of bound and free (+)-tubocurarine, respectively. R represents the molar concentration of receptor in terms of α-bungarotoxin-binding sites.

solubilizing agent [17]. Furthermore, subunit patterns observed in SDS-polyacrylamide gels of total protein, solubilized by either 30 mM octylglucopyranoside or 1% Triton X-100, were identical (Fig. 3).

Purification of acetylcholine receptor. The acetylcholine receptor was purified by α-cobratoxin affinity chromatography from either 30 mM octylglucopyranoside- or 1% Triton X-100-solubilized extracts. In both cases, the specific activities of the purified preparations were approximately 9 nmol of α-bungarotoxin-binding sites per mg protein. The purified receptor protein exhibited four major different polypeptide chains in SDS-polyacrylamide gels with estimated molecular weights of 40 000, 50 000, 60 000 and 65 000 (Fig. 3). Regardless of the solubilizing agent (octylglucopyranoside or Triton X-100), the four subunits were apparently present in the same relative abundance (Coomassie Blue stain uptake) in purified preparations, with the 40 000 and 65 000 molecular weight subunits always more heavily stained than the 50 000 and 60 000 molecular weight subunits. As many as four different polypeptide bands with apparent molecular weights within the 40 000—50 000 range were always observed in solubilized crude extracts. These polypeptides are absent in the preparation after affinity chromatography. The amino acid compositions of purified acetylcholine receptor from either Triton X-100- or octylglucopyranoside-solubilized extracts were identical (data not shown), and in agreement with previously published results [26].

Ligand-binding features of purified acetylcholine receptor were determined by using the [^{14}C]dimethyl ether derivative of the cholinergic antagonist, (+)-tubocurarine. After equilibrium dialysis in the presence of the radioactive antagonist, ligand-binding parameters were analyzed through Scatchard plots (Fig. 4). Purified receptor, isolated from either Triton X-100- or octylglucopyranoside-solubilized extracts, exhibited twice the number of α-bungarotoxin sites as (+)-tubocurarine-binding sites. The apparent dissociation constant for (+)-tubocurarine was $2 \cdot 10^{-7}$ M. Binding parameters for (+)-tubocurarine, such as the ones described above, have been reported previously in detergent-solubilized acetylcholine receptor preparations from a variety of electric fish [27—32].

The molar absorptivity at 280 nm of the octylglucopyranoside-solubilized and -purified receptor was calculated to be $533\,000 \pm 10\,000\ M^{-1} \cdot cm^{-1}$ and is independent of the amount of detergent present.

Removal of octylglucopyranoside from solubilized preparations. Properties of detergent-depleted acetylcholine receptor. The effect of dialysis against two 1-l changes of buffer in both the solubilized crude extract and the purified receptor is shown in Table I. In either case, the total protein and the specific activity (α-bungarotoxin-binding sites/mg protein) were almost entirely recovered. In addition, the removal of octylglucopyranoside in the two preparations was greater than 98%. The amount of detergent remaining in the dialyzed purified receptor was equivalent to approx. 50 μg octylglucopyranoside/mg protein. At this extremely low detergent concentration, the receptor protein remained soluble when the protein concentration was maintained below 0.8—1 mg/ml. Furthermore, the detergent-depleted acetylcholine receptor exhibited the same amino acid composition and SDS-gel pattern (Fig. 3), and displayed a distinctive protein ultraviolet absorption spectrum. The purified acetylcholine receptor absorption spectrum was essentially identical before and after dialysis. An interesting finding was that the (+)-tubocurarine-binding features of the purified receptor were unaltered after the exhaustive removal of octylglucopyranoside, which is comparable to the results obtained with sodium cholate-depleted receptor purified from *Electrophorus electricus* [33].

TABLE I

EFFECT OF DIALYSIS ON CRUDE SOLUBLE EXTRACTS AND PURIFIED ACETYLCHOLINE RECEPTOR

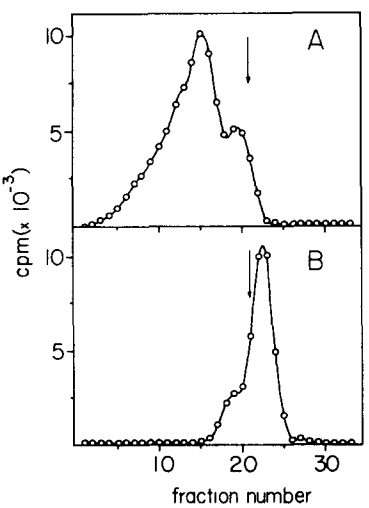
Data are expressed as percentage of the initial value. Solubilized protein samples in 10 mM sodium phosphate buffer, pH 7.4, containing 1 mM EDTA, 10 μM freshly dissolved PMSF, 0.02% NaN_3 and different octylglucopyranoside concentrations, were extensively dialyzed against the same buffer containing no detergent.

	Total protein	Specific α-bungarotoxin binding	Total octylglucopyranoside
Initial soluble extract	>90	85—90	<2 *
Purified acetylcholine receptor	85—90	>95	<2 **

* Initial octylglucopyranoside concentration, 34 mM.
** Initial octylglucopyranoside concentration, 1.4 mM.

(a) Sedimentation behavior. The sedimentation pattern of both the octylglucopyranoside-purified and the detergent-depleted (Fig. 5A) acetylcholine receptor in sucrose density gradients, containing 1 mM octylglucopyranoside or no detergent, respectively, was examined. Monitoring of either absorbance at 280 nm, radioactivity in receptor pre-labelled with either trace or saturating amounts of ^{125}I-labelled α-bungarotoxin, or ^{125}I-labelled α-bungarotoxin-binding of the different fractions from the gradients produced identical profiles with two main components: a slow (11.8 S) and a major rapid (15.3 S) sedimenting peak. In addition, the presence of several other components with higher sedimentation coefficients led to broadening of the sedimentation profile of the rapid sedimenting species. All of the above-mentioned components and the intact purified acetylcholine receptor exhibited identical amino acid compositions and SDS-polyacrylamide gel patterns under reducing conditions. On the other hand, only the 11.8 S peak did not exhibit appreciable amounts of higher molecular weight bands (112 000 and 132 000) in SDS-gels under non-reducing conditions.

The sedimentation profile depicted in Fig. 5A was not altered when either octylglucopyranoside-purified or detergent-depleted receptor protein was treated with an excess of reducing agents for extended periods of time. On the other hand, a significant conversion from rapid to slow sedimenting forms was achieved when 10% β-mercaptoethanol was maintained throughout the purification of the acetylcholine receptor starting from the detergent solubilization step, in agreement with the results of Sobel et al. [34]. Similarly, if the sucrose



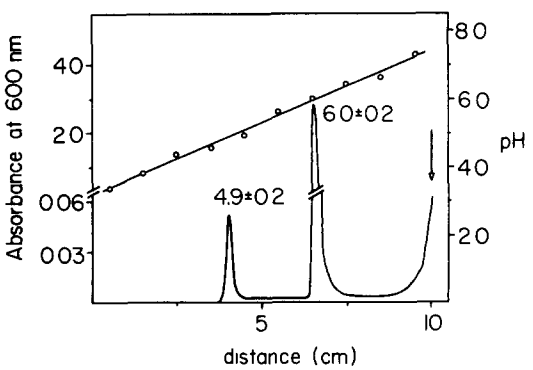


Fig. 5. Sedimentation profiles of (A) purified acetylcholine receptor from 30 mM β-D-octylglucopyranoside extracts in detergent-free sucrose gradients, and (B) purified acetylcholine receptor from 1% Triton X-100 extracts in sucrose gradients containing 1% Triton X-100. The sedimentation profiles were determined by ^{125}I-labelled α-bungarotoxin binding on the fractionated samples. The arrow denotes the position of beef liver catalase used as an internal standard.

Fig. 6. Isoelectric focusing profile of β-D-octylglucopyranoside-solubilized acetylcholine receptor. Numbers within the figure indicate estimated p*I* values for the absorbance maxima in the stained gel. The β-D-octylglucopyranoside concentration in the gel was 3.4 mM. The arrow denotes top of the gel.

gradients contained a high concentration of detergent (either 1% Triton X-100 or 30 mM octylglucopyranoside), there was almost complete conversion from rapid to slow sedimenting forms with the concommitant disappearance of the higher sedimenting minor peaks. Furthermore, in sucrose gradients containing high concentrations of Triton X-100 (but not high concentrations of octylglucopyranoside), the distance travelled by the acetylcholine receptor from the meniscus was consistently lower due to the probable increase in the buoyant density of the receptor protein-detergent complex, as suggested also by the results of Meunier et al. [35].

The sedimentation behavior of purified acetylcholine receptor from Triton X-100 extracts was also examined in sucrose gradients containing a high concentration of Triton X-100 (Fig. 5B). The results revealed a more conventional sedimentation profile [22,36,37] with a major slow sedimenting peak and a well-defined rapid sedimenting peak. Components of higher sedimentation coefficients were not detected.

(b) Isoelectric mobility. Isoelectric focusing of purified acetylcholine receptor in low-detergent-containing gels (3.4 mM octylglucopyranoside or 0.03% Triton X-100) revealed a major band at pH 6 ± 0.2 and a minor band at pH 4.9 ± 0.2, regardless of whether the receptor was solubilized with octylglucopyranoside or Triton X-100 (Fig. 6). When the octylglucopyranoside concentration in the gels was elevated to 34 mM, the amount of octylglucopyranoside-solubilized protein focusing at pH 4.9 increased (to approx. 25%), but did not equal the amount of conversion (approx. 40%) seen in gels containing 1% Triton X-100. Only Triton X-100-solubilized acetylcholine receptor in 1% Triton X-100-containing gels focused completely at pH 4.9, in agreement with the value reported by Raftery et al. [37]. Approximately equal amounts of the two isoelectric forms could be obtained from Triton X-100 receptor protein in 0.1% Triton X-100 gels. Furthermore, at high detergent concentrations, the protein focusing at pH 4.9 could be resolved into two distinct bands of similar (0.2–0.3 pH units difference) isoelectric points as described by Teichberg and Changeux [38].

The electrofocusing profile observed by protein staining (Fig. 6) was reproduced by measuring the radioactivity of electrofocused ^{125}I-labelled α-bungarotoxin-prelabelled acetylcholine receptor. The ratio of radioactivity between the low and high isoelectric forms was similar to that observed in the sucrose gradients for the slow and fast sedimenting forms, respectively, of the acetylcholine receptor.

The ratio of protein focusing at pH 4.9 or pH 6 seemed to be dependent on the detergent contained in the gel. For instance, acetylcholine receptor with the lower sedimentation coefficient from the sucrose density gradients electrofocused at pH 4.9 ± 0.2 in 0.1% and 1% Triton X-100 gels, but focused at pH 6.0 in gels containing a low amount of octylglucopyranoside (3.4 mM). Acetylcholine receptor with higher sedimentation coefficients focused in what appeared to be a single band with a p*I* value of 6 ± 0.2 in low-detergent-containing gels, but was partially converted (approx. 40%) to the pH 4.9 form in high Triton X-100 gels. High octylglucopyranoside was ineffective in this conversion.

Discussion

The structural and functional properties of many integral membrane proteins are intimately associated with the lipid matrix. Ideally, detergents should be capable of solubilizing these proteins without impairing their native physicochemical characteristics. Many investigators are studying the acetylcholine receptor as a detergent-solubilized protein by employing non-ionic poly(oxyethylene ether)-type detergents (Triton, Brij, Lubrol, etc.), presumably due to their non-perturbing effects. These detergents, however, may bind tightly to proteins, alter hydrodynamic properties, interfere with precise spectroscopic measurements, and hinder attempts at reincorporating the receptor into a simulated membrane [6].

Octylglucopyranoside apparently has some technical advantages over the above-mentioned detergents [7,9,10,39]. Octylglucopyranoside is a mild, nonionic detergent that does not appear to denature even certain soluble proteins [9]. It can be obtained as a single chemical species and has an unusually high critical micelle concentration of 25 mM [40] which facilitates its easy removal by dialysis. 30 mM octylglucopyranoside was found to be effective in solubilizing electroplax membrane proteins. At this detergent concentration, the molar ratio of detergent to phospholipids in the crude solubilized extract was approximately three times the molar ratio at the highest octylglucopyranoside concentration in which *Electrophorus* $(Na^+ + K^+)$-ATPase preparations remained stable [12]. This apparent discrepancy might be due to a difference in the susceptibility of both proteins (acetylcholine receptor and $(Na^+ + K^+)$-ATPase) to alteration by detergent. The specific activities of the acetylcholine receptor obtained after solubilization with either 1% Triton X-100 or 30 mM octylglucopyranoside were identical. At higher octylglucopyranoside concentrations, the specific activity of the solubilized preparations decreased, due, probably, to the formation of micellar structures which might be inaccessible to α-bungarotoxin-binding. The possibility of irreversible inactivation by high octylglucopyranoside concentrations was eliminated by removal of the detergent back to the previous levels (30 mM), with concommitant recovery of maximal specific activity.

Properties of the acetylcholine receptor purified by affinity chromatography from octylglucopyranoside-solubilized extracts are similar to those of conventional receptor preparations from Triton X-100 extracts. Electroplax membrane proteins solubilized by either octylglucopyranoside (3—140 mM) or 1% Triton X-100 displayed identical subunit patterns in SDS-polyacrylamide gels. Furthermore, the purified acetylcholine receptor solubilized with either detergent exhibited identical amino acid compositions and polypeptide patterns in SDS-polyacrylamide gels. Regardless of the detergent used, the purified receptor preparations never exhibited a polypeptide band between the 40 000 and 50 000 molecular weight range, which apparently rules out the existence of a 43 000 molecular weight component as an integral constituent of the acetylcholine receptor [3]. In addition, purified receptor, isolated from either octylglucopyranoside- or Triton X-100-solubilized extracts, exhibited identical binding features for the cholinergic antagonist, (+)-tubocurarine.

Basic spectral parameters of octylglucopyranoside-solubilized and -purified

acetylcholine receptor, such as the absorption spectrum and molar absorptivity (approx. 530 000 $M^{-1} \cdot cm^{-1}$), were determined since this detergent does not interfere with optical measurements within at least the 250—700 nm range. The use of ultraviolet absorption, therefore, obviates previously tedious colorimetric procedures of protein monitoring, and simplifies purification procedures and spectroscopic measurements of the receptor protein. It was also comforting to find that bovine serum albumin-based method of Lowry et al. [18] produces protein determination values similar to those assessed by absorption at 280 nm.

Greater than 98% of the total octylglucopyranoside content could be dialyzed away from both the solubilized crude tissue extract and purified acetylcholine receptor. The residual detergent in the purified receptor corresponded to roughly 50 μg detergent/mg protein which is similar to results obtained with viral membrane proteins [11,39]. This finding is surprising since dilution of Triton X-100 to molar concentrations much greater than the final octylglucopyranoside concentration shown above is reported to result in aggregation and precipitation of the acetylcholine receptor from the electric eel [28]. In contrast, extensive dialysis of octylglucopyranoside-solubilized crude extracts or purified acetylcholine receptor did not alter the α-bungarotoxin binding, total protein content, and protein subunit profile on SDS-polyacrylamide gels. Furthermore, the octylglucopyranoside-solubilized and purified acetylcholine receptor exhibited the same (+)-tubocurarine-binding parameters and amino acid composition before and after dialysis. These results indicate that conventional techniques used to ascertain the hydrodynamic properties of water-soluble proteins may be applicable to studies of the purified acetylcholine receptor in its octylglucopyranoside-depleted form.

Based upon evidence from sucrose density gradients, a number of investigators have proposed the existence of two forms of the acetylcholine receptor; a monomeric and a dimeric species [37,41—44]. Sedimentation profiles of our octylglucopyranoside-purified and octylglucopyranoside-depleted receptor in sucrose density gradients containing no detergent revealed a slow 11.8 S and a less well-defined rapid 15.3 S sedimenting peak which could conceivably correspond to the species previously reported as monomer and dimer, respectively. The molecular weight calculated (see Materials and Methods) for the slower sedimenting form (282 000 ± 20 000) agrees closely with the value of 270 000 obtained previously for the monomer by osmometry [4] and by sedimentation equilibrium methods [44]. The value estimated for the higher sedimenting form by this method is almost double that of the monomer. The term 'monomer' is retained in this discussion to imply a unit (11.8 S) one-half the size of the 15.3 S 'dimer' as determined by ultracentrifugation *. These findings, along with evidence that both peaks contain the same amino acid composition and produce the same SDS-gel pattern typical of acetylcholine receptor, support

* Since several authors have utilized the terms 'monomer' and 'dimer' in describing the major oligomeric forms of the acetylcholine receptor, we also have chosen to use them in order to be consistent and to enable the reader to make comparisons with previously published results. 'Monomer' and 'dimer' in this discussion are strictly operational terms which refer specifically to the different sedimenting (11.8 S and 15.3 S) and electrofocusing (p*I* of 4.9 and 6.0) forms of the acetylcholine receptor.

the idea of a monomer-dimer or higher aggregate relationship. Our sedimentation values, on the other hand, do not agree with values previously determined with Triton X-100-solubilized receptor [22,37]. These discrepancies are probably best explained by the inherent physiochemical properties of both detergents. Since the partial specific volume of Triton X-100 ($\bar{v}$ = 0.937—0.952) is much greater than that exhibited by most globular proteins ($\bar{v}$ = 0.73), the binding of this detergent to the acetylcholine receptor is thought to manifest itself by a significant increase in the buoyant density [35]. Another contributing factor to the discrepancies described above may be the micellar size. Octylglucopyranoside forms unusually small micelles of 8000 molecular weight [8], whereas Triton X-100 exhibits micelles of 90 000 molecular weight [45]. It is thought that the use of detergents with micelles of a small uniform size facilitates the physical study of solubilized membrane proteins [8,46,47]. We observed that the octylglucopyranoside-solubilized and -purified acetylcholine receptor sedimented with lower sedimentation coefficients in sucrose gradients containing 1% Triton X-100 than in gradients containing either no octylglucopyranoside or 1 mM or 34 mM octylglucopyranoside. Furthermore, the observed sedimentation behavior of the Triton X-100-solubilized receptor (Fig. 5B) is not likely due to proteolytic or other enzymatic degradation processes. Degradation of isolated or membrane-bound receptor leads to a marked alteration of the subunit patterns see in SDS gels, with resultant appearance of low (approx. 27 000) molecular weight components [36,48,49]. As shown in Fig. 3, our purified receptor preparations (octylglucopyranoside- or Triton X-100-extracted) contain only the characteristic 40 000, 50 000, 60 000 and 65 000 molecular weight components.

Recently, several authors [41,43,50] have suggested that a dimeric form of the receptor protein is formed through disulfide cross-links between δ-subunits which are accessible to reduction. Under our experimental conditions, we observed what could be reductive dissociation only when reducing conditions were maintained throughout most of the purification procedures for the receptor. Similarly, a large conversion to the slower sedimentating form was obtained in sucrose gradients containing high concentrations of detergent (34 mM octylglucopyranoside or 1% Triton X-100). Interestingly, acetylcholine receptor purified from Triton X-100-solubilized extracts behaves similarly to octylglucopyranoside-solubilized receptor in sucrose gradients containing no detergent, even though no initial removal of Triton X-100 from the receptor preparation was attempted. However, Triton X-100-solubilized receptor appears nearly completely as the monomeric form in high detergent sucrose gradients. These results are broadly similar to those previously described by several groups [22,36,51,52], in which oligomeric forms were observed which could be dissociated by increasing the detergent concentration and which reconfirm the notion that detergents alter the aggregation state of the acetylcholine receptor. Data also suggest that in addition to disulfide crosslinks, electrostatic and/or hydrophobic interactions may contribute significantly to the 'native' quaternary structure of the acetylcholine receptor.

The different sedimenting forms of the acetylcholine receptor observed in sucrose density gradients are further characterized by distinctive behavior in isoelectric focusing gels. The acetylcholine receptor purified from either Triton

X-100- or octylglucopyranoside-solubilized extracts in low-detergent-containing gels produced a major band at pH 6.0 and a minor band at pH 4.9. Isoelectric focusing of the monomeric (11.8 S) and dimeric (15.3 S) components of the receptor from the sucrose gradients showed that they corresponded to the pH 4.9 and pH 6.0 isoelectric species, respectively. Additionally, elevating the detergent concentration was shown to increase the amount of protein focusing with the lower p*I*, and Triton X-100 appeared to be somewhat more efficient than octylglucopyranoside in causing this conversion. These data suggest that the two species of the acetylcholine receptor not only differ in molecular weight, but also in their charge properties, which furthers the possibility of surface interactions involving electrostatic or hydrophobic forces.

In conclusion, the acetylcholine receptor purified from octylglucopyranoside-solubilized extracts exhibited most of the physiochemical properties inherent to the more conventional purified protein from Triton X-100 extracts. Differences were observed however in the hydrodynamic behavior of the two preparations depending upon the type and concentration of detergent present. Octylglucopyranoside was less efficient than comparable concentrations of Triton X-100 in causing dissociation to the monomeric form, as determined by ultracentrifugation and isoelectric focusing. Use of octylglucopyranoside in extraction and purification of the acetylcholine receptor seemed, then, to favor the dimeric state in detergent solutions, even though other properties of the purified receptor were retained. The significance of this finding is not yet understood, although it may be an important factor since reconstitution of a functional acetylcholine receptor membrane vesicle was inconclusive prior to the use of octylglucopyranoside throughout the receptor protein purification procedure [1]. Octylglucopyranoside appeared technically advantageous over the more commonly used detergents, since it did not interfere with optical measurements and was easily removed by dialysis from solubilized protein. This detergent has considerable potential in future research involving either the precise monitoring of spectroscopic probes or the reconstitution of excitable acetylcholine receptor membrane-complexes from detergent-solubilized preparations which may be affected by the presence of large amounts of tightly bound detergent.

When this manuscript was being written two other publications [53,54] appeared showing the importance of lipids in preserving the functional properties of the acetylcholine receptor. These reports emphasize the perception [1,2] that lipids maintain and, perhaps, modulate acetylcholine receptor function as an integral membrane component.

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