Biochimica et Biophysica Acta, 513 (1978) 382—394

BBA 78190

SYNAPTIC MEMBRANES FROM *TORPEDO MARMORATA* ELECTRIC ORGAN

1. SEPARATION AND ANALYSIS OF NICOTINIC ACETYLCHOLINE RECEPTOR- AND ACETYLCHOLINESTERASE-CONTAINING MEMBRANE VESICLES USING AQUEOUS TWO-PHASE SYSTEMS

ANNIKA HARTMAN and EDITH HEILBRONN

Unit of Biochemistry, National Defence Research Institute, Department 4, S-172 04, Sundbyberg (Sweden)

(Received February 24th, 1978)

Summary

Two-phase systems consisting of water, dextran and poly(ethylene glycol) have been used for partition of membranes obtained from *Torpedo marmorata* electric organ. The partition behaviour of the membranes could be adjusted by using a polymer with covalently-bound charged groups. By using this method, the membranes were divided into several fractions which were analyzed for nicotinic acetylcholine receptor and acetylcholinesterase content. It was found that nicotinic receptor-enriched membranes were separated from those containing esterase in a single partition step. Receptor-enriched membranes obtained by gradient centrifugation could be further separated into two receptor fractions by the two-phase technique. The results also reveal at least two types of acetylcholinesterase-rich membranes.

Introduction

Disintegration of nervous tissue, including membranes of the electric organ from *Torpedo*, gives rise to heterogeneous membrane and organelle mixtures which can be separated by different kinds of centrifugation techniques [1—6, 26]. However, these methods are quite time consuming and rather unsatisfactory, especially for large scale preparations. An alternative method is liquid-liquid extraction in aqueous two-phase systems which separate particles mainly according to the surface properties, such as charge and hydrophobicity, rather than size and density [7].

Aqueous biphasic systems, obtained by mixing water solutions of two differ-

ent polymers (e.g. dextran and poly(ethylene glycol)) have been useful tools for analysis and separation of biological macromolecules and cell particles. Cell particles or membranes included in such a two-phase system distribute either between both the phases and the liquid-liquid interface or, in some cases, are largely confined to one of the phases. The partition of a substance between the phases and the interface can be influenced by a number of factors such as covalent coupling of different kinds of ionic or specific groups to one of the polymers used or adding different salts to the system [7—13].

In the present work, the two-phase technique has been used for studying the heterogeneity of excitable membranes from *Torpedo marmorata* electric organ as well as for separation of nicotinic-receptor- and acetylcholinesterase-enriched membranes, with or without previous centrifugation on sucrose gradients. Closer biochemical and immunological characterizations of the obtained membrane fractions will be presented in following papers.

Experimental

Material and methods

Dextran T 500, batch No. 5996 ($M_r = 5 \cdot 10^5$) was supplied by Pharmacia Fine Chemicals (Uppsala, Sweden). Poly(ethylene glycol), with M_n 4000 was obtained from Union Carbide, New York, as Carbowax 4000. Poly(ethylene glycol)-sulfonate, was a generous gift from Dr. G. Johansson, University of Lund, Sweden. All other chemicals used were of analytical grade.

Membrane fragments were prepared from the electric organ of *T. marmorata* obtained from the Marine Biological Station at Arcachon (France). 200 g electric organ (either fresh or deep frozen (- 90°C) and thawed) were minced with scissors and mixed with 100 ml 10^{-4} M benzethonium chloride containing 0.02% NaN_3. The mixture was homogenized with an Ultra Turrax 18-10 for 2 × 1 min, followed by sonication with a Branson Sonifier B-30 for 3 × 10 s. The temperature was kept near 0°C with crushed ice. The homogenate was then centrifuged at 4°C for 10 min at 5000 rev./min in a Sorvall SS-34 rotor. The supernatant solution was filtered through two layers of cheesecloth and centrifuged in the cold for 45 min at 80 000 × *g* using an SW 27 rotor (Beckman L3-50). Soluble material was removed by washing the pellet twice by homogenization in 0.01 M sodium phosphate buffer (pH 7.4) containing 0.02% NaN_3 and 10^{-4} M benzethonium chloride and recentrifugation. The final pellet was suspended in 50 ml buffer and stored at 4°C. The concentration of protein in the membrane suspension was about 5 mg/ml.

Nicotinic acetylcholine receptor-enriched membranes were prepared according to Cohen et al. [4]. 3.5 ml membrane suspension was layered on a gradient consisting of 3 ml each 1.5, 1.4, 1.3, 1.2, 1.1, 1.0 and 0.8 M sucrose followed by centrifugation for 6 h at 4°C and 80 000 × *g*. 1-ml fractions were collected and the receptor-enriched membranes were found in the gradient within 1.05—1.40 M sucrose with a maximum at 1.25 M sucrose. The acetylcholinesterase activity was mainly found where the concentration of sucrose was less than 1.0 M. The receptor-containing material, symmetrically distributed around the maximum between 1.30 and 1.20 M sucrose, was collected and the sucrose was removed by repeated high-speed centrifugation. The material obtained from 2 ×

3.5 ml membrane suspension was finally suspended in 3 ml buffer and stored at 4° C.

Assays

Protein concentrations were determined according to Lowry et al. [14]. The membrane content was calculated from the absorbance at 400 nm or 280 nm using a Beckman spectrophotometer and 1-cm cuvettes. A linear relationship exists between the amount of membranes and the absorbance for $A^{1cm}_{400nm} \leqslant 0.750$ and $A^{1cm}_{280nm} \leqslant 1.5$.

Acetylcholinesterase was measured according to Ellman [15] using acetylthiocholine as substrate. The enzyme activity was followed by the increase in absorbance at 412 nm and 30° C using an LKB 2086 Reaction Rate Ananlyzer or a Unicam SP-800 B spectrophotometer. The enzyme activity was expressed as μmol acetylthiocholine hydrolysed/min per ml or as μmol acetylthiocholine hydrolysed/min per mg protein.

Nicotinic acetylcholine receptor was measured by the binding of α-[^{125}I]-neurotoxin. Labelling with α-[^{125}I]neurotoxin from *Naja naja siamensis* was done according to Hunter [16].

150-μl aliquots of samples were incubated with 25 μl $5 \cdot 10^{-7}$ M iodinated α-neurotoxin. After 60 min, 100 μl incubation mixtures was transferred to membrane filters (Sartorius, SM 100, pore size 0.45 μm) and washed with 3 × 10 ml 0.2 M NaCl. The filters were then analyzed for ^{125}I in a Searle Model 1185 R gamma counter. For determination of non-specific binding polymers as well as 0.1% bovine serum albumin were used.

To trace the fractions obtained by partition of the membranes in two-phase systems, about 5% of the sites were labelled by α-[^{125}I]neurotoxin prior to partitioning. The low degree of labelling did not have a marked effect on the distribution of the membranes.

Two-phase systems

The two-phase system contained 6.5% dextran and 6.5% poly(ethylene glycol), including substituted poly(ethylene glycol). Phase systems with a total weight of 2.0 g were obtained by mixing membrane suspension, water solutions of 20% dextran, 40% poly(ethylene glycol) and/or a solution with a total amount of poly(ethylene glycol) being 40% but with 10% in the form of poly(ethylene glycol)-sulfonate. By changing the ratio between the solution containing poly(ethylene glycol) and the solution containing both poly-(ethylene glycol) and poly(ethylene glycol)-sulfonate a series of systems are obtained with the same polymer concentrations but differing in the amount of poly(ethylene glycol)-sulfonate. All polymer concentrations are given in percent (w/w).

The systems were mixed by 40 inversions and then allowed to separate at room temperature for 5 min; they were then mixed again and allowed to settle for 15 min. For determination of the partition between the phases and the interface, 100 μl from each phase was withdrawn. Each aliquot was diluted with 2 ml buffer (also containing 0.02% sodium azide and 10^{-4} M benzethonium chloride and analysed for membrane content, acetylcholinesterase and nicotinic acetylcholine receptor. The distribution is given as the amount of

material in each phase and at the interface, expressed as the percentage of the total amount in the system. The amount of material in each phase was calculated from the concentration of assayed substance and the volume of the phase. The amount at the interface was obtained as the difference between the total amount added and the sum of amounts in the two phases. These values agree well with experimentally calculated amounts at the interface. (The material at the interface was collected in a test tube, suspended in 2 ml buffer and analyzed).

Counter-current distribution: Multiple partition was carried out in a thin-layer counter-current distribution machine described by Albertsson [7] using 29 or 19 transfers. Using an automatic syringe each chamber was filled with 0.6 ml bottom phase and 0.8 ml top phase. The sample was included in the system in chamber 0. The interface was kept stationary during the transfers. The shaking time was 30 s and the settling time was 12 min. The experiments were carried out at 22°C. Prior to analysis 1.4 ml buffer was added to each chamber to obtain homogeneous solutions.

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis

Gel electrophoresis was performed according to a modification of the method of Stephens [17]. Samples of 1 ml (diluted to a protein concentration of about 1 mg/ml) were mixed with sodium dodecyl sulphate and 2-mercaptoethanol (final concentrations 1%). The solutions were incubated for 2 h 37°C and then dialyzed against 0.025 M Tris/glycine buffer (pH 8.3) containing 0.1% of both SDS and 2-mercaptoethanol. After dialysis, 100-μl samples were subjected to electrophoresis (3 mA/gel for 3 h) in gels containing 5% acrylamide, 0.13% bisacrylamide and 0.1% SDS. The gels were stained with amido black.

Results

Partition of the membrane suspension in two-phase systems

Extraction curves. Membranes partitioned in the poly(ethylene glycol)-dextran-water two-phase system were mainly found in the poly(ethylene glycol)-rich top phase. The distribution of the membranes between the phases and the interface may, however, be changed by replacing some of the poly(ethylene glycol) with the negatively charged poly(ethylene glycol)-sulfonate [7,10,18]. The effect of poly(ethylene glycol)-sulfonate on the distribution is shown in Fig. 1. In a system containing no poly(ethylene glycol)-sulfonate, 96% of the membranes were found in the top phase. With increasing amounts of the charged polymer in the system, the material was successively transferred from the top phase over the interface to the dextran-rich bottom phase. When 10% of the poly(ethylene glycol) was replaced by poly(ethylene glycol)-sulfonate, 85% of the membranes were found in the bottom phase. The amount of material at the interface passed through a maximum. An exchange of 5% of the poly(ethylene glycol) for poly(ethylene glycol)-sulfonate resulted in a maximal amount of 63% at the interface, while the rest was found, almost in equal amounts, in the top and bottom phase.

The membranes in each phase and at the interface were analyzed for acetylcholinesterase and nicotinic receptor (Fig. 2, which represents one of 20 similar experiments (variation ±2.5%)). The acetylcholinesterase-containing membranes

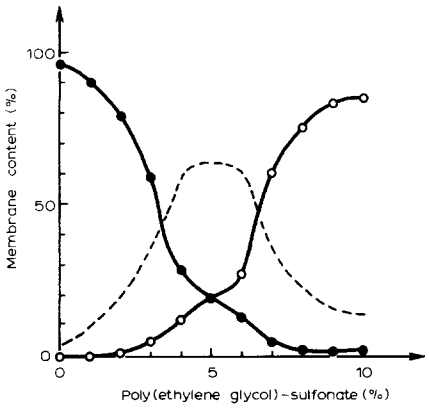

Fig. 1. The effect of poly(ethylene glycol)-sulfonate on the partition of membranes from *T. marmorata* electric organ. The two-phase system contained 6.5% dextran, 6.5% total poly(ethylene glycol), 3 mmol/kg sodium phosphate buffer (pH 7.4), 0.03 mmol/kg benzethonium chloride and 0.007% NaN_3. The amount of membranes included in the system corresponded to a protein content of 1.3—1.5 g/kg. The membrane content in each phase and at the interface is plotted versus the amount of poly(ethylene glycol) replaced by poly(ethylene glycol)-sulfonate. ●———●, membrane content in the top phase; ○———○, in the bottom phase; — — — —, at the interface.

(Fig. 2A) were extracted from the top phase via the interface to the bottom phase in two distinct steps. This may indicate the presence of at least two fractions [10,18] of acetylcholinesterase-containing membranes, differing in their partition properties. In a system where 5% of the poly(ethylene glycol) was present as poly(ethylene glycol)-sulfonate, approx. 90% of the esterase membranes were concentrated at the interface, while very small amounts (less than 1%) were found in the top phase.

The extraction profile of nicotinic receptor-containing membranes (Fig. 2B) is quite different. A larger amount of poly(ethylene glycol)-sulfonate in the system was needed for extraction of the receptor-containing membranes from

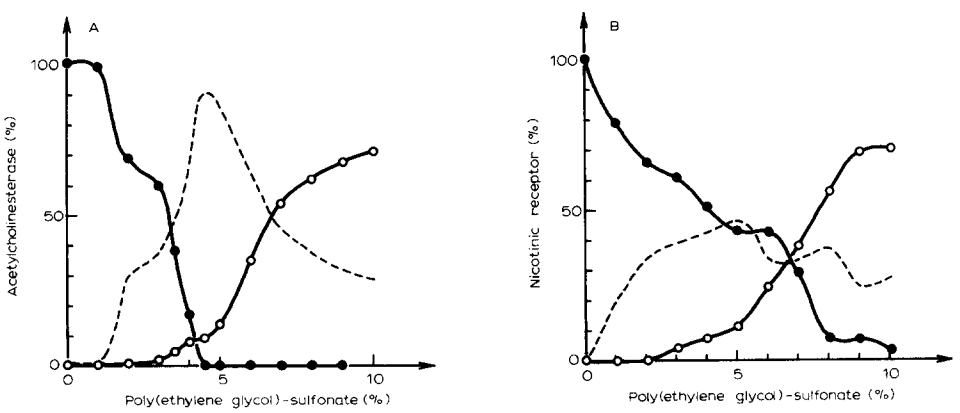

Fig. 2. Analysis of acetylcholinesterase and nicotinic acetylcholine receptor in the phases when the membrane suspension was partitioned in the same system as described in Fig. 1. ●———●, content in the top phase; ○———○, in the bottom phase; — — — —, at the interface. The distribution of (A) acetylcholinesterase-containing membranes and (B) nicotinic receptor-containing membranes is shown.

the top phase. This means that in a system with 5—6% of the poly(ethylene glycol) replaced by its charged variant, the esterase-containing membranes were found at the interface (mainly) and in the bottom phase, while more than 40% of the receptor-containing membranes were still in the top phase.

Counter-current distribution. Membrane suspensions submitted to counter-current distribution (Fig. 3A) showed a tendency to divide into three main fractions, the first two containing the acetylcholinesterase activity. Most of the nicotinic receptor (65%) was found in the first 15 tubes, but the residual 35%

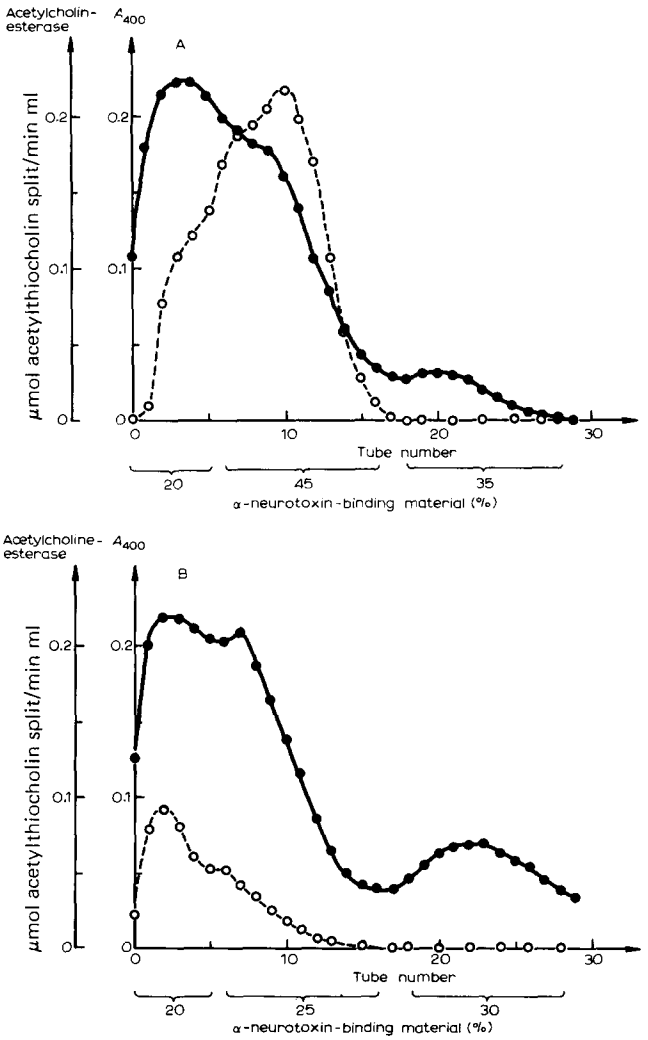

Fig. 3. Counter-current distribution of the membrane suspension obtained from *T. marmorata* electric organ. The experiment was carried out at 22°C in a two-phase system with 5.5% of the poly(ethylene glycol) replaced by poly(ethylene glycol)-sulfonate. After 29 transfers, the fractions were diluted with buffer and analyzed for membrane content (measured as the absorbance at 400 nm) (●———●) and acetylcholinesterase, (○———○). Some fractions were then pooled and assayed for nicotinic receptor (under the figure). The distribution when (A) the membrane suspension was included in the sample system in chamber 0 and (B) the material at the interface in the sample system was removed before the experiment is shown. In case of B two chambers, 0 and 1, were loaded.

was found in the small fraction (where no esterase activity was found). When the material at the interface was removed from the sample system before the counter-current distribution (Fig. 3B) about 50% of the total material was lost. This loss consisted mainly of esterase-containing membranes. Some of the receptor-containing membranes (25%) were also lost. The small membrane fraction, however, containing about 35% of the receptor, was retained and this fraction had a high affinity for the top phase.

The results obtained by counter-current distribution agreed quite well with the extraction profiles obtained by partitioning the membranes in two-phase systems containing varying amounts of poly(ethylene glycol)-sulfonate (Fig. 1 and 2).

Separation of nicotinic receptor and acetylcholinesterase enriched membranes in one partition step

It follows that there is a fraction of membranes enriched in nicotinic receptor with high affinity for the top phase. This fraction could easily be separated from the acetylcholinesterase-enriched membranes, which were mainly found at the interface. For separation of these fractions, a membrane amount corresponding to a protein content of 35 mg/20 g total weight of phase system was used. The polymers were removed by two high-speed centrifugations and each of the obtained fractions was suspended in 2.0 ml buffer. The results are given in Table I (with also a summary of the purification procedure). The specific activity of the nicotinic receptor, expressed as nmol bound α-[^{125}I]neurotoxin per mg protein, was raised from 0.04 in the crude extract to 0.5 in the membrane fraction. When the membranes were partitioned in the two-phase system, the specific activity was further increased to 3.0 in the fraction obtained from the top phase. The amount of acetylcholinesterase in this fraction was low. The material obtained from the interface also had a relatively high content of receptor, but the specific activity was low and the esterase content high.

When nicotinic-receptor-enriched membranes were prepared according to the usual method, centrifugation of membranes on sucrose gradient, the specific activity of the receptor was always found to be somewhat lower than in the top-phase fraction. Furthermore, the esterase content, as well as the total protein content, was found to be considerably higher in the sucrose gradient fraction.

Negative staining of the receptor-enriched membrane fractions (2% phosphotungstic acid, pH 7.4) and electron microscopy showed closed membrane vesicles of varying size, approx. 1000 Å being an average.

Subunit composition

Fig. 4A shows the protein subunit composition of the membrane fractions obtained from the two-phase system. It is known that the dominating subunit component in purified nicotinic receptor from *T. marmorata* has an apparent molecular weight of about 42 000 [19—22] and this component was also the dominating one in the top-phase fraction. Only weak bands of other components were seen in this fraction, with the exception of a band at about 30 000—35 000. In particular the high molecular components ($M_r > 100\,000$), which

TABLE I

PURIFICATION OF MEMBRANES FROM THE ELECTRIC ORGAN OF *TORPEDO MARMORATA*

About 7 ml of the final membrane suspension was (I) partitioned in a two-phase system containing 6.5% dextran, 6.1% poly(ethylene glycol), 0.4% poly(ethylene glycol)sulfonate, 3 mmol/kg sodium phosphate buffer (pH 7.4), 0.03 mmol/kg benzethonium chloride and 0.007% NaN_3. The material in the phases and at the interface was collected and the polymers were removed as described in Results; (II) layered on sucrose gradients and the nicotinic acetylcholine-enriched membranes in 1.30—1.20 M sucrose were collected. The sucrose was removed as described in Experimental.

Purification step	Acetylcholine receptor			Acetylcholinesterase			Protein		
	Binding of α-neurotoxin (nmol/mg protein)	Yield	(%)	μmol AthCh split/ min per mg protein	Yield	(%)	mg/ml	Yield	(%)
Crude extract	0.04	100		5.1	100		20.0	100	
Low speed supernatant	0.06	75		2.4	22		10.8	47	
High speed supernatant	—	—		2.3	17		9.0	39	
Membrane suspension	0.5	68 *	100	2.8	2.6 *	100	4.9	5.0 *	100
I. Partition in two-phase systems									
Top phase	3.0	31	46	0.5	0.1	4	3.2	0.9	18
Interface	0.8	24	35	3.4	1.9	74	9.0	2.6	51
Bottom phase	0.7	13	19	1.6	0.6	22	5.6	1.5	31
II. nAChR-enriched membranes prepared by sucrose gradient centrifugation	1.6	22	26	1.3	1.0	28	5.1	2.3	45

* The rest is found in the supernatants of the twice repeated high speed centrifugation. nAChR, acetylcholine receptor; AthCh, acetylthiocholine

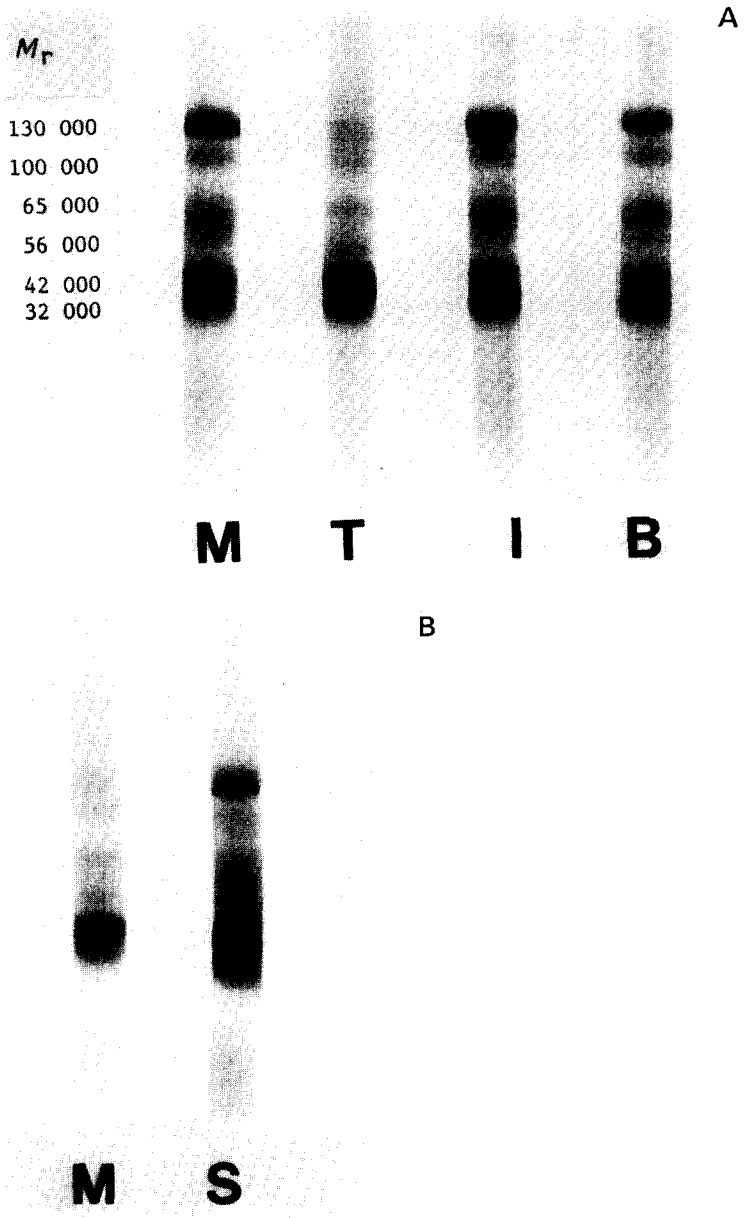

Fig. 4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of different fractions obtained from *T. marmorata* electric organ. A. The membrane suspension was partitioned in a two-phase system with a polymer composition of 6.5% dextran, 6.1% poly(ethylene glycol) and 0.4% poly(ethylene glycol)-sulfonate. The material in the phases and at the interface was collected and the polymers were removed by high speed centrifugations. The obtained fractions were treated with β-mercaptoethanol and SDS and applied to gel electrophoresis. Determination of approximate molecular weights (M_r) was made by comparison with gels containing standards. Subunit composition of: the membrane suspension (M), The membranes obtained from the top phase (T), from the bottom phase (B), and from the interface (I). B. The subunit composition of nicotinic receptor-enriched membranes prepared by two different methods, M, receptor enriched membranes obtained from the top phase (specific acticity 3.0 nmol α-neurotoxin bound/mg protein); S, respector-enriched membranes obtained by sucrose gradient centrifugation according to Cohen et al. [4] (specific activity 1.6 nmol α-neurotoxin bound/mg protein).

were the main ones in the interface and bottom phase fractions, were only very faint in the top phase fraction.

In Fig. 4B, the top-phase fraction is also compared with nicotinic receptor-enriched membranes obtained by sucrose gradient centrifugation. There are obvious differences in the subunit composition of receptor-rich membranes prepared by the two different methods. The main difference was again the decrease of the high molecular components in the top phase fraction.

Partition of nicotinic receptor-enriched membranes in two-phase systems

Fig. 5 shows the effect of poly(ethylene glycol)-sulfonate on the distribution of receptor-enriched membranes obtained by the two-phase technique (the material from the top phase) and by sucrose gradient centrifugation (the material collected between 1.20 and 1.30 M sucrose).

The partition profiles of receptor-enriched membranes obtained by the two different methods show clear differences. More poly(ethylene glycol)-sulfonate was needed for extraction of the receptor-rich membranes obtained by the two-phase technique from the top phase. Furthermore, with increasing amount of poly(ethylene glycol)-sulfonate in the system, these membranes partitioned mainly between the top phase and the interface.

With increasing amounts of poly(ethylene glycol)sulfonate in the system, the receptor-enriched membranes were extracted from the top phase in two steps, independent of the method used for preparation. The relation between these steps, however, was different in the two cases and indicated that the suspensions of receptor membranes differ in their homogeneity. This was also verified

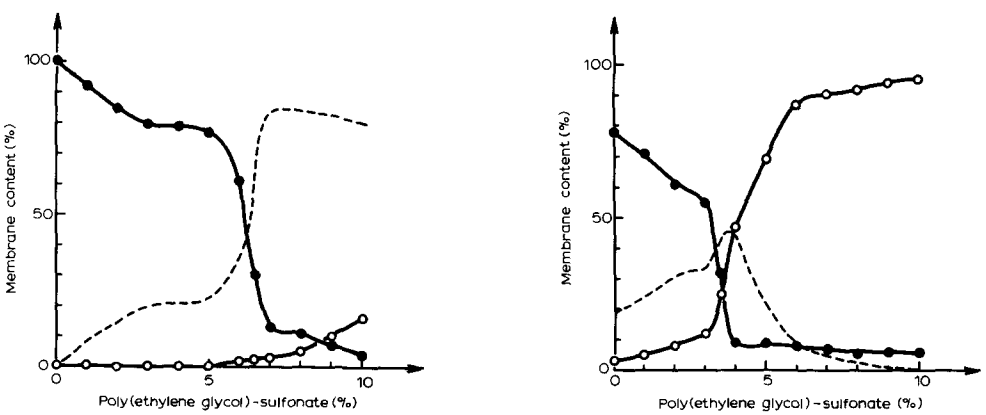

Fig. 5. The effect of poly(ethylene glycol)-sulfonate on the partition of nicotinic receptor-enriched membranes from *T. marmorata* electric organ. The two-phase system used was the same as described in Fig. 1. The amount of membranes included in the system corresponded to about 0.5 g protein/kg system. •———•, membrane content in the top phase; ○———○, in the bottom phase; — — — —, at the interface. A. The distribution of receptor-enriched membranes obtained by the two-phase technique (the top phase fraction). The specific activity of nicotinic receptor and acetylcholinesterase in this fraction was 3.0 nmol α-neurotoxin bound/mg protein and 0.5 μmol acetylthiocholine hydrolysed/min per mg protein, respectively. B. The distribution of receptor-enriched membranes obtained by sucrose gradient centrifugation according to Cohen et al. [4]. The specific activity of nicotinic receptor and acetylcholinesterase was 1.6 nmol α-neurotoxin bound/mg protein and 1.3 μmol acetylthiocholine hydrolysed/min per mg protein, respectively.

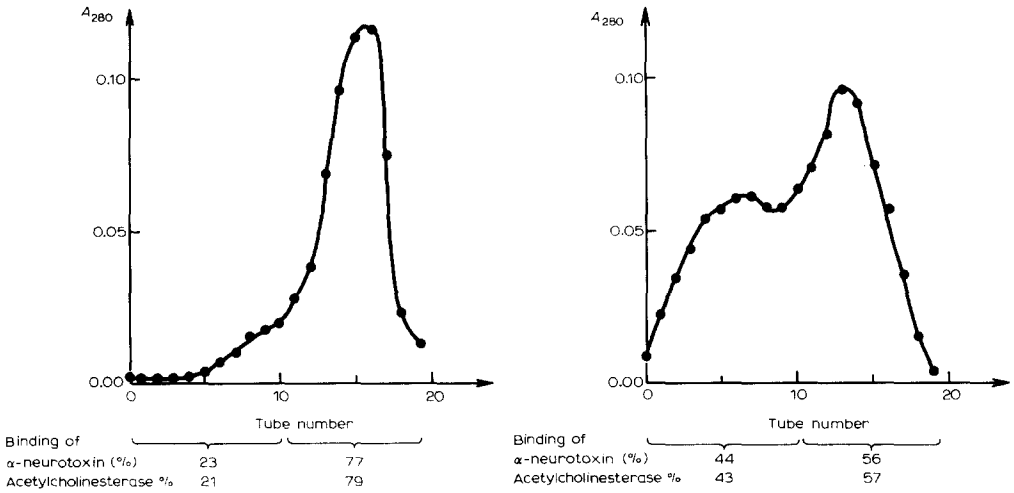

Fig. 6. Counter-current distribution of nicotinic receptor-enriched membranes obtained from the electric organ of *T. marmorata*. 19 transfers were made at 22°C using a two-phase system with 3% of the poly-(ethylene glycol) replaced by poly(ethylene glycol)-sulfonate. When the experiment was completed buffer was added to each chamber and the absorbance at 280 nm was measured in each fraction. Fractions 0—9 and 10—19 were then pooled and analyzed for nicotinic receptor and acetylcholinesterase. A. The distribution of receptor-enriched membranes obtained by the two-phase technique. The total amount of nicotinic receptor and acetylcholinesterase used in the experiment corresponded to 2.1 nmol α-neurotoxin bound/min and 0.3 μmol acetylthiocholine hydrolysed/min. B. Distribution of receptor-enriched membranes obtained by sucrose gradient centrifugation. The total amount of nicotinic receptor and acetylcholinesterase used in the counter-current distribution corresponded to 1.1 nmol α-neurotoxin bound/min and 0.9 μmol acetylthiocholine hydrolysed/min, respectively

by counter-current distribution (Fig. 6). The receptor-rich membranes obtained by the two-phase technique gave rise mainly to one peak in the counter-current distribution (Fig. 6A). One small peak can also be seen to the left of the major peak. The binding of α-[^{125}I]neurotoxin in these two peaks was 23% and 77% respectively, of the total bound α-neurotoxin. The receptor-enriched membranes obtained by sucrose gradient centrifugation gave rise to two main peaks (Fig. 6B) when applied to counter-current distribution. The binding of α-[^{125}I]-neurotoxin in these two main peaks was 44% and 56%, respectively, of the total bound toxin.

As seen in Table I, the esterase content in the two receptor-enriched fractions was low (0.1% and 1%, respectively, of that in the crude extract). When the material in the counter-current distribution was analyzed for this small esterase content it was found, in both cases, that the ratio between receptor and esterase in each fraction was about the same.

Discussion

In the two-phase system used here, closed membranes obtained from *T. marmorata* electric organ were found to have high affinity for the poly-(ethylene glycol)-rich top phase when sodium phosphate buffer was used as interfacial-determining electrolyte. The introduction of the negatively charged poly(ethylene glycol)-sulfonate resulted in a competition between the charged

polymer and the sodium phosphate buffer in determining the interfacial potential, successively changing this potential. Thus, membrane particles in the top phase with a negative net charge will, in accordance with the degree of their negative charge, more or less avoid the top phase when the amount of poly-(ethylene glycol)-sulfonate in the system is increased. It was, however, obvious from the results that membrane properties other than electric charge contributed to the membrane partition. An example of this was the partition of acetylcholinesterase-containing membranes. The slope and shape of the partition curves (Fig. 2A, mathematical analysis is not yet published) indicated differences in both electrical charge and in other surface properties. The latter were of importance in the partition, as the membranes with the lower negative net charge were the first to be transferred from the top phase.

The two different fractions of acetylcholinesterase-containing membranes may be of pre- and postsynaptic origin as an electric organ homogenate, by necessity, contains electromotor nerve-ending membranes. Other possibilities are membranes originating from different regions within the same synaptic area, or even the presence of inside-out membrane vesicles.

Our results also indicate that the bulk of acetylcholinesterase-containing membranes differs in surface properties from membranes containing acetylcholine receptor. A small amount of acetylcholinesterase seemed, however, to be firmly attached to the main receptor membrane fraction and could be functionally associated with it.

The receptor-enriched membranes in the top phase had a higher specific α-toxin binding activity and a lower acetylcholinesterase activity than those obtained by the usual gradient centrifugation technique [4]. As seen from gel electrophoresis, the acetylcholine-binding receptor subunit of 42 000 daltons was highly enriched in this fraction, other polypeptide bands had decreased in concentration and those of M_r 100 000 were very faint.

The acetylcholine-receptor-enriched membranes obtained by either gradient centrifugation or two-phase partition were different. Counter-current distribution revealed a reasonably homogeneous receptor-membrane population obtained by phase-partitioning, while those from sucrose gradients seemed to be composed of two receptor-containing fractions. This means that the latter two fractions are relatively homogeneous with respect to size and density, but not with respect to surface characteristics. Both receptor fractions contained the earlier mentioned firmly-associated, low content of acetylcholinesterase and the ratio between receptor and enzyme in the two fractions was about the same. Thus, the two receptor-enriched fractions must differ in some other aspect, such as lipid composition and/or presence of other proteins. It has earlier been reported from investigations of the electric organ of *Electrophorus electricus* that nicotinic receptor is present, in about equal amounts, in the subsynaptic and extrasynaptic areas [26] and it may be possible that the different fractions mentioned above originate from such areas present in *Torpedo* electric organ.

Finally, compared to the isolation method for membranes described by Flanagan et al. [13] the present method is quicker and easier to use on a large scale.

References

1 Jones, D.H. and Matus, A.I. (1974) Biochim. Biophys. Acta 356, 276—287
2 Salvaterra, P.M., Mahler, H.R. and Moore, W.J. (1975) J. Biol. Chem. 250, 6469—6475
3 Cotman, C.W., Lewy, W., Banker, G. and Taylor, D. (1971) Biochim. Biophys. Acta 249, 406—418
4 Cohen, J.B., Weber, M., Huchet, M. and Changeux, J.P. (1972) FEBS. Lett. 26, 43—47
5 Reed, K., Vandlen, R., Bode, J., Diguid, J. and Raftery, M.A. (1975) Arch. Biochem. Biophys. 167, 138—144
6 Bartfai, T., Berg, A., Schultzberg, M. and Heilbronn, E. (1975) Biochim. Biophys. Acta 426, 186—197
7 Albertsson, P.Å. (1971) Partition of Cell Particles and Macromolecules, 2nd edn., Almqvist and Wiksell, Stockholm, Wiley, New York
8 Johansson, G., Hartman, A. and Albertsson, P.Å. (1973) Eur. J. Biochem. 33, 379—386
9 Blomquist, G., Hartman, A. Shanbhag, V. and Johansson, G. (1974) Eur. J. Biochem. 48, 63—69
10 Johansson, G. (1974) Mol. Cell. Biochem. 4, 169—180
11 Eriksson, E., Albertsson, P.Å. and Johansson, G. (1975) Mol. Cell. Biochem. 10, 123—128
12 Andersson, B., Åkerlund, H.E. and Albertsson, P.Å. (1976) Biochim. Biophys. Acta 449, 525—535
13 Flanagan, S.D., Barondes, S.H. and Taylor, P. (1976) J. Biol. Chem. 251, 858—865
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
15 Ellman, G.L., Courtney, K.D., Andreas, Jr., V. and Featherstone, R.M. (1961) Biochem. Pharmacol. 7, 88—95
16 Hunter, W.M. (1967) Handbook of Experimental Immunology (Weir, D.M., ed.), Blackwell Scientific Publications Ltd., Oxford, 608—654
17 Stephens, R.E. (1975) Anal. Biochem. 65, 369—379
18 Hartman, A. (1976) Acta. Chem. Scand. 830, 585—594
19 Weill, C., McNamee, M. and Karlin, A. (1974) Biochem. Biophys. Res. Com. 61, 997—1003
20 Heibronn, E., Mattson, C. and Elfman, L. (1974) Proc. 9th FEBS Meet., Budapest 1974, Vol 37, 29—38
21 Mattsson, Ch. and Heilbronn, E. (1975) J. Neurochem. 25, 899—901
22 Fulpius, B.W., Zurn, A.D., Granato, D.A. and Leder, R.M. (1976) Ann. N.Y. Acad. Sci. 274, 116—129
23 Hartman, A., Johansson, G. and Albertsson, P.Å. (1974) Eur. J. Biochem. 46, 75—81
24 Johansson, G. and Hartman, A. (1976) Eur. J. Biochem. 63, 1—8
25 Bourgeois, J.P., Popot, J.L., Ryter, A. and Changeux, J.P. (1973) Brain Res. 62, 557—563
26 Duguid, J.R. and Raftery, M.A. (1973) Biochemistry 12, 3593—3597