THE MOLECULAR WEIGHT OF AN ACETYLCHOLINE RECEPTOR ISOLATED FROM Torpedo californica[1]

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SUMMARY

The molecular weight of the acetylcholine receptor (AcChR) from Torpedo californica has been determined by membrane osmometry in detergent solutions. It appears that AcChR is a globular protein of 270,000 ± 30,000 daltons which does not change state of aggregation with varying detergent (0.1 to 1%) or protein (2 to 15 mg/ml) concentrations. Because of differences in the values obtained for protein concentration by amino acid analysis and colorimetric methods on the one hand and dry weight values on the other, it is calculated that as much as 45% of the dry weight of preparations of AcChR may be contributed by protein-bound Triton X-100.

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[4]Abbreviations: AcChR, acetylcholine receptor; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate.

The acetylcholine receptor (AcChR)[4] from the electric ray, Torpedo californica, has been isolated in recent years (1-3). The purified preparations consist of a single protein component in polyacrylamide gel electrophoresis and contain about 5% carbohydrate (2,3). This nicotinic receptor binds a variety of cholinergic ligands and fluorescent analogues of these ligands (1-4). Ligand binding studies have led to a postulate of some nonequivalence between the sites for the binding of snake venom toxin, α-bungarotoxin, and some cholinergic ligands (3). Inorganic cations also bind to the isolated receptor (3,4) and questions remain about the relationship of these sites to those for other ligands on the receptor's surface. These studies have, however, been hampered by the lack of an accurate molecular weight value for the intact receptor. Indirect methods such as elution volumes in Sepharose columns (5,6), sedimentation coefficients in sucrose density gradients (6) and electrophoresis in SDS polyacrylamide gels (3,7,8) give values oscillating between 240,000 and 550,000 daltons.

In this communication we report the direct measurement of the molecular weight of the Torpedo californica acetylcholine receptor. The AcChR is only soluble in the presence of detergents which bind to this protein in as yet uncertain amounts. The bound detergent is a potential source of erroneous molecular weight determinations when using transport analytical methods. We, thus, have chosen membrane osmometry as the analytical procedure since the number molecular weight of proteins, as determined by this technique, is independent of protein interactions with smaller molecular weight solutes.

METHODS

Purification of AcChR

Preparation of AcChR containing a specific activity of 10 nmoles of α-bungarotoxin binding/mg prepared as previously described (3) were used throughout this work.

Membrane osmometry

Osmotic pressures were obtained using a Hewlett-Packard model 503 high-speed membrane osmometer. The molecular weights were determined at 27° using deacetylated acetylcellulose membranes type B-19. The retention value of these membranes is 20,000. Prior to use the membranes were equilibrated for several days with the solvents and 10 mg/ml BSA. Later they were washed with the solvent and placed in the osmometer. The baseline, P_o, reproduction for all solvents was 0.05 cm. Osmotic pressures, P, of the AcChR were obtained by subtracting the equilibrium pressure value, P, from P_o. The number average molecular weights were determined using the equation $M_n = RT(\pi/C)_{C=0}$. The value $(\pi/C)_{C=0}$ was obtained by extrapolation to zero concentration of the experimental points using a linear least-squares analysis of the data. C is reported as g/l and the R value is given for the particular solvent used as the ratio of $84.7/\rho$ l cm $mole^{-1}$ deg^{-1}. The density of the solvent, ρ, at the experimental temperature was calculated independently. The validity of the method in high concentration of non-denaturing detergent was tested under identical experimental conditions with other proteins which had previously been analyzed by osmometry in the absence of detergents (9,10).

Protein concentrations

The Lowry (11) and biuret (12) methods were used to determine protein concentrations using BSA solutions in Triton X-100 as standard.

Amino acid compositions of 24, 48 and 72 hr acid hydrolizates with norleucine as an internal standard were carried out in triplicate samples in a Beckman amino acid analyzer.

Dry weights of AcChR in 10 mM potassium phosphate buffer, pH 7.5, and 0.1% Triton X-100 were determined after subtraction of the values for the solvent alone.

RESULTS

Protein concentrations

Because of the dependence of the osmotic pressure molecular weight values on accurate protein concentration determination, several procedures were employed to circumvent the presence of the Triton X-100 binding. The Lowry method was utilized with

BSA as standard reference because it is routinely used as the choice method for protein determinations in this research field and, most importantly, because the AcChR and BSA contain similar percentages of tyrosyl residues (3). The biuret method was employed using both BSA and aspartate transaminase as reference standards. All reference standards contained the same percentage of Triton X-100 as the sample solvent. Amino acid compositions routinely produced over 92% recovery of the sample. The comparison of the results obtained by the three methods, related to the dry weight of similar samples in detergent, is reported in Table I. There is similarity among the results obtained by the three methods and these values are considerably lower than those

TABLE I

DETERMINATION OF PROTEIN CONCENTRATION

Samples of purified acetylcholine receptor were prepared in 0.1% Triton X-100, 0.2 M potassium phosphates buffer, pH 7.4, and 0.1 M KCl.

Method	Concentration
	mg/ml
Lowry	1.00±0.02
Biuret	1.10±0.02
Amino acid composition[a]	0.93±0.03
Dry weight	1.72±0.04

[a]After acid hydrolysis of 24, 48, and 72 hr and correction for ser and thr destruction. Norleucine used as internal standard and assuming 2.5% content of tryptophan.

obtained by the dry weight determinations where preferential binding of detergent may contribute to higher apparent weights of the protein samples. The Lowry method was thereafter used routinely for the determination of protein concentrations in the osmotic pressure measurements.

Osmotic pressure

The results of osmotic pressure changes in four independent determinations of varying concentrations of receptor preparations are shown in Fig. 1. The solvent density was 1.02 g/cm^3 and the intercept point of $\pi/C = 0.095$ (cm 1 g^{-1}) corresponds to a M_n = 270,000 ± 30,000. These experiments were carried out at two different Triton X-100 concentrations, 0.1% and 1%. No significant deviation from the above results was obtained within the two detergent concentrations. The second virial coefficient values, B, calculated from the slope of the plots of π/C vs. C using

$$B = \frac{\Delta(\pi/C)}{\Delta C} \quad \frac{1}{RT}$$

are equal to 2×10^{-5} ± 8% cc mole g^{-2} which is within the range of values expected for a globular native protein (13).

DISCUSSION

The similarities in protein values obtained by three methods dependent on different analytical or chemical parameters, give confidence to the assumption that determination of protein concentration represents the protein concentration of AcChR. This is a critical measurement since Mn estimations are highly dependent on the value chosen for C. The slight variation in protein concentration between the Lowry procedure and amino acid determinations on the one hand and the biuret on the other is as

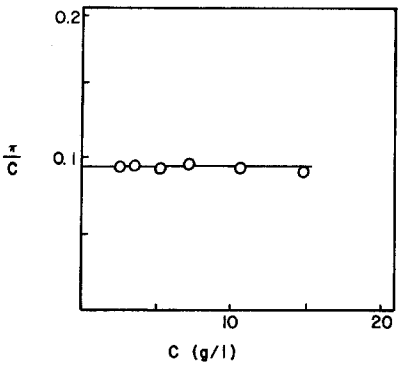

Figure 1. Plot of π/C vs C for AcChR in 0.1 M potassium phosphate buffer pH 7.4 and 0.1 M KCl, 0.1% Triton X-100 at 27°.

yet unclear and thus it is incorporated in the reported error for the Mn value. The higher weight value seen by the dry weight method reflects a 45% increase in weight due to bound Triton X-100 detergent. Since the detergent concentration used is above the critical micelle concentration (14), 45% binding is not without precedent for a membrane protein with a hydrophobic core (14).

The molecular weight obtained by membrane osmometry is highly reproducible and must reflect the molecular weight of the preparations used in ligand binding studies since these data were obtained at similar pH and detergent concentrations (10). The osmotic pressure procedure is independent of any significant detergent which may be bound to the receptor and, unlike transport procedures, gives a mean number average molecular weight rather than a weight number average molecular weight. Furthermore, any effect of detergent and/or carbohydrate on the partial specific volume, $\bar{v}$, of the protein need not be corrected because osmotic pressure measurements are independent of $\bar{v}$ or any

possible interactions with small molecular weight solutes in the solvent.

Molecular weights obtained by osmometry do not give information about dissociation of the proteins below the high concentration needed in these measurements. Yet, on the basis of similar elution volumes in Sepharose 6B columns (1 x 90 cm) for concentrations of AcChR ranging from 0.1 to 4 mg/ml, we assume that, at least within this range of concentrations, no dissociation occurs. This is the range in which most ligand binding studies have been carried out. We, thus, conclude that any future model of ligand, cation and toxin binding studies should assume a molecular weight of 270,000 ± 30,000 for AcChR. This conclusion would have to be tempered as a minimum value if Triton X-100 micelles contained more than one molecule of AcChR/micelle. However, it is known that the micelle aggregation number for this detergent is of the order of 120 molecules of Triton X-100 (molecular weight 640) and hydrophobic proteins tend to complex with detergents by insertion of their hydrophobic domains into the detergent micelles (14). Thus, a plausible interpretation of our results, which is also consistent with the independence of the Mn value on detergent concentration, is that the detergent may be bound to the hydrophobic domain of monomeric AcChR as several smaller weight (ca. 70,000) micellar aggregates. The model of a globular protein of 270,000 ± 30,000 containing several micelles of detergent is also consistent with the observation of a protein component of about 250,000 daltons in SDS polyacrylamide gels of dimethylsubirimidate crosslinked AcChR (3,7,8), the calculated value for the molecular weight of AcChR from affinity labeling of the acetylcholine binding site (15) and the large Stoke's radii observed for the receptor in

Sepharose columns in the presence of detergent (5,6).

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