TRANSLATIONAL DIFFUSION COEFFICIENT AND MOLECULAR WEIGHT OF THE ACETYLCHOLINE RECEPTOR FROM *TORPEDO MARMORATA*

Wolfgang DOSTER, Benno HESS, Diane WATTERS and Alfred MAELICKE
Max-Planck-Institut für Ernährungsphysiologie, Rheinlanddamm 201, 4600 Dortmund-1, FRG

Received 21 February 1980

1. Introduction

Since the nicotinic acetylcholine receptor was purified to apparent homogeneity, properties such as molecular weight and subunit composition have been a matter of discussion (reviewed [1,2]). The reason for this is the need for detergent to maintain the receptor in a soluble state, which results in micellar and microheterogeneous preparations. Association with detergent micelles obviously affects the apparent density of the receptor and may also lead to cosolubilization and copurification of unrelated proteins. To solve these questions we have recently applied a variety of relatively new techniques in molecular studies of the receptor protein in both detergent-containing and detergent-free solutions [3]. These results provide a close estimate of the molecular weight for purified acetylcholine receptor from the electric organs of *Torpedo* and *Electrophorus electricus*.

2. Materials and methods

Acetylcholine receptor from the electric organs of *Torpedo marmorata* and *Electrophorus electricus* was prepared as in [3,4]. Purified receptor was freed from excess detergent by chromatography on hydroxylapatite [3]. Sedimentation velocity studies were carried out using a Beckman model E analytical ultracentrifuge. Schlieren optics, which measure the refractive index gradient in the cell, were employed. Partial specific volumes of the receptor proteins were calculated from the known amino acid composition [5]. Solvent densities and viscosities were determined by means of a microbalance and a microviscometer, respectively.

Quasielastic light scattering experiments were performed by means of a modified Malvern Molecular Analyzer, System 4300, equipped with a Spectra Physics 4W Argon laser, model 165-08. At 514.5 nm, an 800 mW beam was focussed to a diffraction limited spot by means of a single line lens. A pinhole was placed in the focal plane of the lens to remove any non-colinear light from the beam. The beam was then focussed through a diaphragm to the center of the thermostated measuring cell. The protein solution was passed into the cell via 0.45 μm and 0.22 μm Millipore filters. The collecting optics consisted of a diaphragm which defines the scattering solid angle, and a lens which images the scattering region on the pinhole of a photomultiplier tube. The photocurrent was analyzed by a 72 channel digital correlator, the background was obtained by 4 delayed channels with an accuracy of 0.2%.

The autocorrelation function of the electric field scattered by a suspension of macromolecules is [6]:

$$g^1(t) = \sum_i A_i \exp(-q^2 D_i t)$$

with D_i, A_i and q denoting the translational diffusion coefficient of molecule i, an intensity factor and the magnitude of the scattering vector, respectively. Since a small amount of aggregated receptor was present under all experimental conditions, a 2-exponential analysis of the correlation function was required. A non-linear fit program (Harwell Subroutine VCO 5A) modified by Dr T. Plesser and K. H. Müller of our institute was applied.

3. Results

Acetylcholine receptor from *Torpedo marmorata*, prepared in 0.2 M $NaPO_4$, 0.05% Tween (pH 7.4) was subjected to ultracentrifuge sedimentation velocity studies using Schlieren optics. As shown in fig.1a, two major components with sedimentation coefficients ($s_{20,w}$) of 9.21 S and 14.4 S were observed. The S value of the larger particle corresponds with high accuracy to the expected theoretical value for a dimer of the smaller one. Conversion of dimers into monomers was achieved by incubation of receptor with the disulfide reducing agent, dithiothreitol (DDT) (fig.1b), proving directly the existence of a monomer–dimer equilibrium [3,7–9].

Aliquots of the preparations subjected to sedimentation velocity studies were also analyzed by the light scattering technique. With receptor preparations containing both monomers and dimers a satisfactory decomposition of scattering data could not be achieved. Since the diffusion coefficients of monomer and dimer differ by less than a factor of 2, only their z-average is obtained from an analysis of the autocorrelation function. To circumvent this complication a preparation consisting largely of only 1 population of particles was required, as is obtained in the presence of DTT (fig.1b). Even then, the autocorrelation function did not completely conform to a single exponential (fig.2).

Instead 2 exponentials were observed, the first relating to ⩾80% of all particles in solution. The correlation time of the second component (20%) was ~4-times greater than the main component's but varied somewhat from preparation to preparation. By a 2-exponential analysis we determined the diffusion coefficient of the main component, corresponding to the smallest macromolecule, (fig.2) as $D_{20,w}$ = 2.94×10^{-7} cm^2/s. This value was reproducible within 2% for different measurements and different preparations. With $s_{20,w}$ 9.21 and a partial specific volume ($\bar{\nu}$) of 0.745 we calculated a molecular weight (M) of 298 000 for the receptor monomer. For the receptor preparation with the monomer–dimer equilibrium (fig.1a), a translational diffusion coefficient (z-average) $D_{20,w}$ = 2.67×10^{-7} cm^2/s was ob-

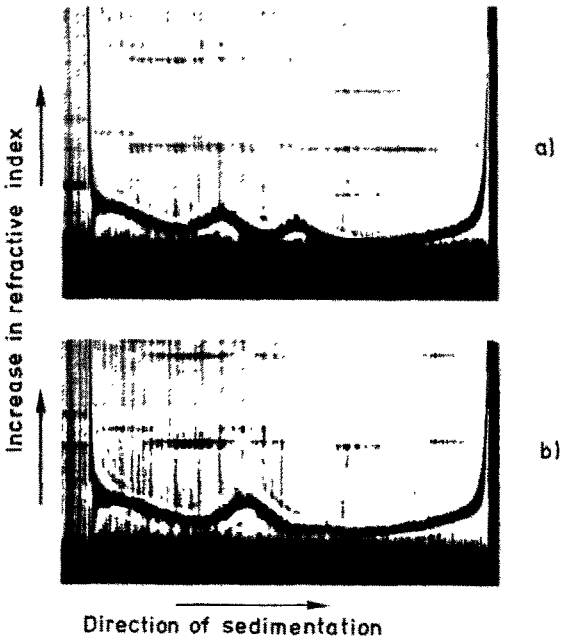

Fig.1. Sedimentation velocity pattern of purified acetylcholine receptor as determined by Schlieren optics. Acetylcholine receptor from *Torpedo marmorata* (in (a) 0.2 M $NaPO_4$, 0.05% Tween 80 (pH 7.4) and in (b) 0.2 M $NaPO_4$, 0.02 M DTT, 0.05% Tween 80 (pH 7.4)) was subjected to sedimentation velocity studies at 44 000 rev./min and 20° C. Pictures were taken at 60° observation angle after: (a) 54 min; (b) 66 min centrifugation at the designated speed. Receptor concentration 0.7 mg/ml. From a set of photographs taken at various sedimentation times the following sedimentation coefficients were calculated: (a) $s^1_{20,w}$ = 9.29 S. $s^2_{20,w}$ = 14.43 S; (b) $s^1_{20,w}$ = 9.21 S.

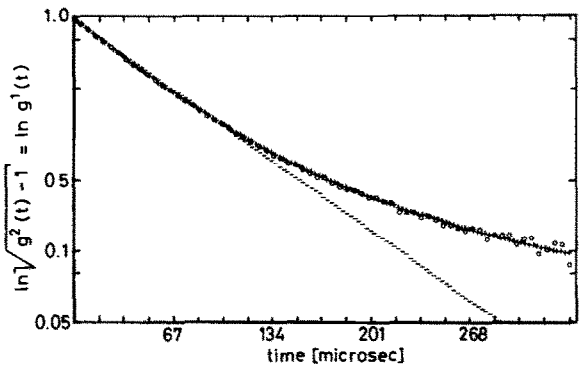

Fig.2. Quasielastic laser light scattering of purified acetylcholine receptor from *Torpedo marmorata*. Acetylcholine receptor in 0.2 M $NaPO_4$, 0.02 M DTT, 0.05% Tween 80 (pH 7.4) was subjected to laser light scattering studies at 20° C (see section 2). The autocorrelation function versus time plot of experimental data (o o o) is analyzed by a single exponential (– – –) and a 2-exponential (+ + +) fit. The main (first) component comprises ~80% of the total amplitude. The translational diffusion coefficient for the first component (smallest particles) is $D_{20,w}$ = 2.94×10^{-7} cm^2/s.

tained. For acetylcholine receptor from *Electrophorus electricus* we obtained slightly larger values for the diffusion coefficient and, consequently, slightly smaller values for the apparent molecular weight.

4. Discussion

This study employs for the first time laser light scattering together with ultracentrifuge sedimentation velocity measurements to determine the molecular weight of detergent-solubilized acetylcholine receptor. None of these methods can be affected in accuracy by unusual properties of the receptor–detergent system such as hydrophobic adsorption, viscosity or friction. S and $D_{20,w}$ values are accurate for the solute under study (i.e., the receptor micelle) and do not require estimates of other molecular parameters. To determine M_r from S and $D_{20,w}$ values, the solute(s) $\bar{v}$ must be known. In calculating this value from the known amino acid composition [5], possible errors may be introduced by the unknown contributions of the carbohydrate moieties of the receptor [21] and the asymmetrical amino acid and detergent distribution within each particle. These effects are likely to yield smaller values for the app. M_r, and we therefore consider our values to be close to the lower limit. Furthermore, the relatively low S and $D_{20,w}$ values for a molecule of this size indicate that the receptor has an asymmetrical shape and/or is partially unfolded even in solution [3].

Our value of M_r 298 000 for receptor monomer from *Torpedo marmorata* may be compared with those obtained in hydrodynamic studies [3,4,10–13], gradient electrophoresis [3], SDS–gel electrophoresis after crosslinking [3,14–16], osmometry [17] and equilibrium sedimentation studies [3,18–20] where values of M_r 170 000–>500 000 have been obtained. A considerably closer range for the minimum M_r of 230 000–330 000 has been yielded in [3,16,18]. Our data confirm this range and are considerably less sensitive to artefacts than most methods previously applied.

Acknowledgements

We thank Dr R. Rüchel for helpful discussions and U. Schacknies and H. Burghardt for excellent technical assistance.

References

[1] Maelicke, A., Fulpius, B. W. and Reich, E. (1977) Handbook of Physiology, sec. I, pt I, pp. 493–519, American Physiological Society, Bethesda, MD.
[2] Heidmann, T. and Changeux, J.-P. (1978) Ann. Rev. Biochem. 47, 317–357.
[3] Rüchel, R., Watters, D. and Maelicke, A. (1980) submitted.
[4] Klett, R. P., Fulpius, B. W., Cooper, D., Smith, M., Reich, E. and Possani, L. D. (1973) J. Biol. Chem. 248, 6841–6853.
[5] Schackman, H. K. (1957) Methods Enzymol. 4, 32–103.
[6] Cummins, H. Z. (1973) in: Photon Correlation and Light Beating Spectroscopy, pp. 285–330, Plenum Press, Oxford.
[7] Chang, H. W. and Bock, E. (1977) Biochemistry 16, 4513–4520.
[8] Hamilton, S. L., McLaughin, M. and Karlin, A. (1977) Biochem. Biophys. Res. Commun. 79, 692–699.
[9] Suarez-Isla, B. and Hucho, F. (1977) FEBS Lett. 75, 65–69.
[10] Meunier, J. C., Olsen, R. W. and Changeux, J. P. (1972) FEBS Lett. 24, 63–68.
[11] Gibson, R. E., O'Brien, R., Edelstein, S. J. and Thompson, W. R. (1976) Biochemistry 15, 2377–2383.
[12] Mennier, J. C., Olsen, R. W., Menez, A., Fromageot, P., Boquet, P. and Changeux, J. P. (1972) Biochemistry 11, 1200–1210.
[13] Potter, L. (1973) Drug Receptors, (Rang, H. P. ed) pp. 295–312, McMillan, London.
[14] Biesecker, G. (1973) Biochemistry 12, 4403–4409.
[15] Hucho, F. and Changeux, J.-P. (1973) FEBS Lett. 38, 11–15.
[16] Hucho, F., Bandini, L. and Suarez-Isla, B. A. (1978) Eur. J. Biochem. 83, 335–340.
[17] Martinez-Carrion, M., Sator, V. and Raftery, M. A. (1975) Biochem. Biophys. Res. Commun. 65, 129–134.
[18] Reynolds, J. A. and Karlin, A. (1978) Biochemistry 17, 2035–2038.
[19] Edelstein, S. J., Beyer, W. B., Eldefrawi, A. T. and Eldefrawi, M. E. (1975) J. Biol. Chem. 250, 6101–6106.
[20] Carroll, R. C., Eldefrawi, M. E. and Edelstein, S. J. (1973) Biochim. Biophys. Res. Commun. 55, 864–872.
[21] Vandlen, R. L., Wu, W. C. S., Eisenach, J. C. and Raftery, M. A. (1979) Biochemistry 18, 1845–1854.