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A STUDY OF RHODOPSIN-DETERGENT MICELLES BY TRANSIENT ELECTRIC BIREFRINGENCE

A. Kent WRIGHT*
Department of Biochemistry, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163, USA

The hydrodynamic method of transient electric birefringence has been used to study bovine rhodopsin solubilized in two detergents, 0.02% Ammonyx LO and 0.045% digitonin. All measurements are interpreted as the sum of two exponentials by which the relaxation times yield the rotary diffusion coefficients for ellipsoids of revolution. The semi-major and minor axes for prolate ellipsoid models have been calculated and their axial ratio, 6.8, in both detergents, is in line with recent reports on the structure of rhodopsin. Studies on bleached rhodopsin showed a large increase in axial ratio in 0.02% Ammonyx LO.

1. Introduction

This paper describes the results of hydrodynamic studies on purified bovine rhodopsin, solubilized in two detergents. The purpose of this study is to determine the shape envelope of the rhodopsin-detergent micelles.

The hydrodynamic method of transient electric birefringence developed by Benoit [1] and O'Konski and Zimm [2] was chosen for this study. The most general theory for this method describes the transient birefringence for a homogeneous suspension of spheroids in terms of two exponential functions of time, with time constants $\tau_s = 1/6R_1$ and $\tau_f = 1/2(R_1 + 2R_3)$ [3]. R_1 and R_3 denote the distinct rotary diffusion constants for the spheroid corresponding to rotation about the short and long axes of the spheroid, respectively. Knowledge of the magnitude of these constants, combined with the inversion procedure of the Perrin equations developed by Wright et al. [4], allows the dimensions of the semi-major and minor axes of the spheriod to be determined. This method has been successfully used to determine the hydrodynamic dimensions of several proteins including bovine serum albumin, *Helix pomatia* hemocyanin, and a series of seven proteins in the detergent sodium dodecyl sulfate and therefore was chosen for this study [5–7].

2. Materials and methods

Dissected bovine retinas obtained from G. Hormel and Company (Austin, Minn.) were stored at −70°C until used. All operations were conducted under dim red light and in ice buckets with the exception of column chromatography and birefringence measurements, which were done at room temperature under dim red light. Rod outer segments (ROS) were isolated using the sucrose gradient procedure of Ebrey [8].

Purified rhodopsin was prepared from the ROS obtained by the above procedure by solubilization of the ROS in 2% Ammonyx LO, 0.067M phosphate buffer, pH 7.0 and chromatographed on a calcium phosphate column according to the method of Ebrey. Further purification was accomplished by chromatography on hydroxylapatite according to Applebury et al. [9].

Purified rhodopsin was concentrated when necessary by ultrafiltration (Amicon, Lexington, Mass.). The purity criteria is based on the ratio of the optical densities at 280 nm and 500 nm (OD 280/500).

Prior to measuring the birefringence, the samples were dialyzed three times against 0.02% Ammonyx LO,

* These experiments were conducted while the author was a visiting faculty member of the Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.
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0.004% dithiothreitol (DTT), in distilled water. With concentrations less than this amount of detergent, the rhodopsin was no longer soluble, but could be resolubilized by addition of detergent [9].

Digitonin samples were prepared by dialysis of purified rhodopsin against 0.045% digitonin, 0.004% DTT in distilled water.

Bleached rhodopsin was prepared by exposing rhodopsin to an incandescent lamp taken from a lantern projector for periods of 30 s followed removal from the light to insure against heating. This process was continued for 10 min. The birefringence apparatus was constructed by R.C. Williams, Biophysics Department, Medical College of Virginia. The optical system is similar to that described by O'Konski and Zimm and O'Konski and Haltner [2,10]. The pulsing circuit was designed by Williams and produced a pulse with a resolution time of 4 ns. The analysis of the birefringence data has been described by Wright and Thompson [5].

3. Results

The measured birefringence relaxation for all samples showed curvature when plotted on semi-logarithmic paper. Estimation of relaxation times by the usual peeling off method showed all samples could be described within experimental error by two relaxation times; τ_s and τ_f (corresponding to slow and fast relaxation processes, respectively), which suggests a spheriodal model for the rhodopsin-detergent micelles used in this study.

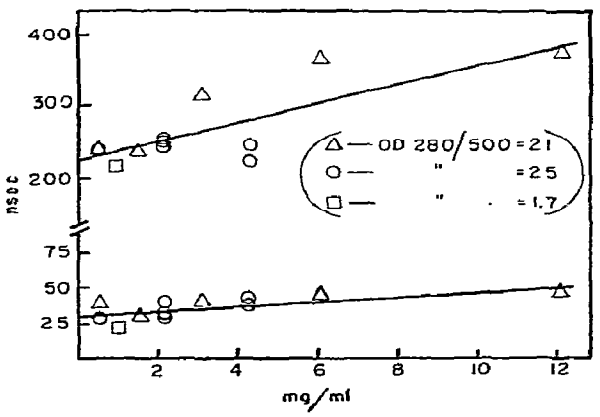

Fig. 1. Birefringence relaxation times versus rhodopsin in 0.02% Ammonyx LO.

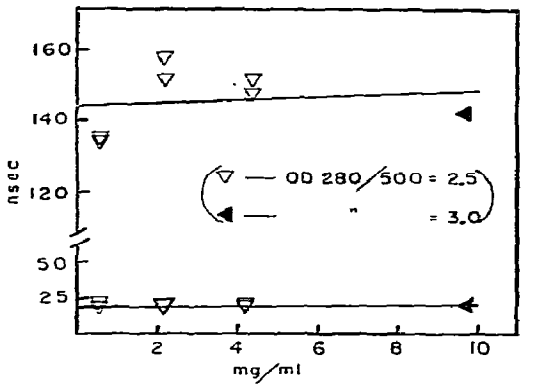

Fig. 2. Birefringence relaxation time versus rhodopsin in 0.045% digitonin.

The measured relaxation times are shown in fig. 1 through 3. The concentrations were calculated from measured optical densities at 500 nm on stock solutions, assuming a molar extinction of 43 250 M^{-1} cm^{-1} and the molecular weight for rhodopsin to be 35 000 daltons [9,11,12].

Fig. 1 shows the concentration dependence of τ_f and τ_s for rhodopsin in 0.02% Ammonyx LO, measured for three different rhodopsin preparations, with OD 280/500 ranging from 1.7 to 2.5. Since the several samples tend to extrapolate to the same relaxation times at zero concentration, the data are pooled to calculate the linear least squares lines from which the relaxation times extrapolated to zero concentration

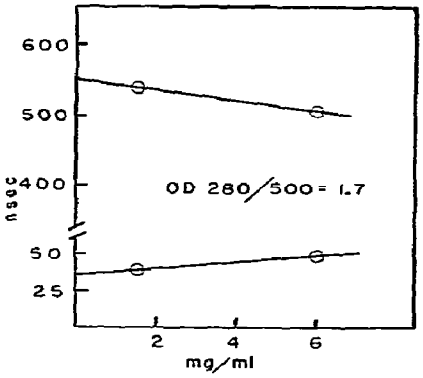

Fig. 3. Birefringence relaxation times versus bleached rhodopsin in 0.02% Ammonyx LO.

Table 1
Calculated rotary diffusion coefficients, semi-axial lengths, and axial ratios for ellipsoidal models of rhodopsin-detergent micelles based on transient birefringence data

Rhodopsin/Detergent	$R_1 \times 10^{-4}$ (s^{-1})[a]	$R_3 \times 10^{-4}$ (s^{-1})[a]	Semi-major axis (Å)	Semi-major axis (Å)	Axial ratio
0.02% Ammonyx LO	75.1 ± 5.1	796 ± 65	117 ± 4	17.1 ± 0.8	6.8 ± 0.5
0.02% Ammonyx LO (bleached)	30.3 ± 1.5	699 ± 47	169 ± 4	15.3 ± 0.6	11 ± 0.6
0.045% Digitonin	116 ± 5	1211 ± 45	100 ± 2	14.9 ± 0.3	6.7 ± 0.2

[a] Extrapolated to zero concentration.

are obtained. The resulting intercepts and standard errors are $\tau_s = 222 \pm 15$ ns and $\tau_f = 20 + 2.3$ ns [13].

Fig. 2 shows the concentration dependence of τ_f and τ_s for rhodopsin in 0.045% digitonin. As in the above discussion, the data from the two samples are pooled in the linear regression. The resulting intercepts and standard errors are $\tau_s = 144 \pm 5.7$ ns and $\tau_f = 19.7 \pm 0.7$ ns.

Fig. 3 shows the results of bleaching with white light. In the case of bleaching in the presence of 0.02% Ammonyx LO, the long relaxation time approximately doubles in duration, with no significant increase in the short relaxation, as shown in fig. 3. In this case, the relaxation times, extrapolated to zero concentration, are determined by the intercept of the straight line drawn between the two data points. The resulting values are $\tau_s = 550 \pm 27.5$ ns and $\tau_f = 35 \pm 2.3$ ns. The errors in this case are calculated assuming 5% errors in accord with the above two sets of data.

The birefringence relaxation curves for the solvents 0.02% Ammonyx LO and 0.045% digitonin appeared the same as that for distilled water and therefore solvent correction was unnecessary [5]. The solvent viscosities for 0.02% Ammonyx LO and 0.045% digitonin, measured with an Ostwald viscometer thermostated at 25°C, are 0.8704 and 0.8782 cP, respectively.

The laser light source did not bleach the rhodopsin samples since the relaxation times were unaffected after 1/2 hour and 2 1/2 laser exposures of two unbleached samples in each detergent.

The calculated rotary diffusion coefficients and semi-axial lengths of prolate ellipsoidal models for rhodopsin-detergent micelles used in this study are shown in table 1. The standard errors appearing in table 1 were calculated by propagating the relaxation time errors through the inversion procedure discussed in the Introduction, and calculating the pooled root mean square values which are obtained. The resulting axial ratios are also shown in table 1.

It is possible to rule out an oblate ellipsoid model based on the rotary diffusion coefficients for such a model since their ratio is greater than the upper limit for the oblate case [3,4].

4. Discussion

Equivalent prolate ellipsoids of revolution have been calculated for micelles of bovine rhodopsin solubilized in 0.02% Ammonyx LO and 0.045% digitonin. In both cases, the ellipsoids have an axial ratio of 6.8 within experimental error. The differences in the dimensions of the semi-axes are probably due to the different detergents and not due to conformation changes in rhodopsin, since the absorption spectra are unchanged in these two detergents.

Recent observations on rhodopsin by other techniques have implied considerable asymmetry in the structure of rhodopsin. A thorough review of these findings has been published by Ebrey and Honig [14]. More recent observations on the asymmetry of rhodopsin solubilized in detergents are given by Lewis et al. [12], and Yeager [15]. Lewis et al. computed the dimensions of prolate and oblate ellipsoidal models for rhodopsin, based on area and volume data they obtained from binding studies of Triton X-100 with rhodopsin and partial specific volumes. The semi-axial lengths for their prolate ellipsoid are 55.5 Å and 13.5 Å, and for their oblate ellipsoid, 32 Å and 10 Å.

Yeager measured the radius of gyration for rhodopsin in 1% Ammonyx LO and in the detergent dodecyltrimethylammonium bromide using the method of neutron scattering. His values in these detergents are 29 ± 2 Å and 26 ± 3 Å, respectively. He states that these values are incomparable with a spherical model of rhodopsin which he reports would have a radius of gyration of 17.3 Å. In both of these cases, the observations are on the rhodopsin core of the detergent micelle.

The radius of gyration for the prolate model of Lewis et al. is calculated to be 26.2 Å, which is in agreement with those reported by Yeager. The oblate model of Lewis et al. yields a radius of gyration of 15.6 Å, which is not supported by Yeager's data. These comparisons are supportive of our findings that an oblate model for rhodopsin can be discounted.

The equation for the frictional ratio for a prolate ellipsoid as a function of axial ratio is given by Tanford [16]. For the prolate ellipsoid of axial ratio 6.8 a frictional ratio of 1.36 is calculated. This value is in good agreement with the value 1.2 to 1.4 reported by Hubbard for rhodopsin in 2% digitonin [17]. This agreement is supportive of the prolate ellipsoid models for the micelles of rhodopsin in the two detergents used.

The effect of bleaching rhodopsin in the presence of 0.02% Ammonyx LO shows a large conformational change. The axial ratio increases to 11. This observation supports other data which show that considerable conformation changes occur in the protein moiety of rhodopsin following light absorption. Such changes have thoroughly been reviewed by Ebrey and Honig [14]. Additionally, since the review by Ebrey and Honig. Santillan and Blasie have reported that the scattering mass of rhodopsin in ROS membrane multilayers changes dramatically upon rhodopsin bleaching, based on electron density profile changes at 8A resolution X-ray diffraction. This shows that rhodopsin undergoes a conformational change, due to bleaching, in ROS membranes as well as in detergents.

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