HYDRODYNAMIC STRUCTURE OF BOVINE SERUM ALBUMIN DETERMINED BY TRANSIENT ELECTRIC BIREFRINGENCE

A. KENT WRIGHT *and* MICHAEL R. THOMPSON

From the Departments of Biochemistry and Biometry, Medical University of South Carolina, Charleston, South Carolina 29401, and the Biophysics Department, Medical College of Virginia, Richmond, Virginia 23220. Dr. Wright's present address is the Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.

ABSTRACT Birefringence relaxation studies on bovine serum albumin (BSA) reveal transient decay described by a double exponential process. The values of the relaxation times lead to estimation of the size of the equivalent ellipsoid of revolution for BSA. Previous measurements of transient birefringence for BSA have shown a single relaxation process, since the apparatus used in obtaining those data was not fast enough to detect the faster relaxation process.

INTRODUCTION

According to the theory of Scheraga and Mandelkern (1), two hydrodynamic parameters are necessary to allow the calculation of the dimensions of the equivalent ellipsoid of revolution model for a protein in solution. The most general theory for transient electric birefringence, following the sudden removal of an orienting electric field, states that prolate ellipsoids relax in a double exponential decay curve with relaxation times $\tau_s = 1/6R_1$ and $\tau_f = 1/2(R_1 + 2R_3)$, where the R's denote the principle rotary diffusion coefficients (2). It should therefore be possible to determine the dimensions of the equivalent ellipsoid from transient electric birefringence measurements made on a protein solution for which two decay processes exist.

Bovine serum albumin (BSA) is an example of a protein which is well described hydrodynamically as a prolate ellipsoid (3–5). The dimensions have been given as 140 Å for the major axis and 40 Å for the minor axis (4). These values along with temperature and solvent viscosity may be substituted in the Perrin equations yielding values for the rotary diffusion coefficients R_1 and R_3, according to Eqs. 1, 2, and 3 given by Wright et al. (6). Assuming $T = 25°C$ and solvent of water; $\eta_w^{25°}$, the expected relaxation times are $\tau_s = 71$ ns and $\tau_f = 25$ ns for the above stated dimensions of BSA. Previous electric birefringence experiments on BSA yielded a single relaxation time of 76 ns, where the measuring apparatus had a resolution time of 50 ns, as shown with propanyl carbonate (3). Thus, the faster relaxation process could not be resolved.

The purpose of the present communication is to report measurements on BSA which yield two relaxation times for transient birefringence and to report the corresponding dimensions for the equivalent ellipsoid of revolution model for BSA.

EXPERIMENTAL

BSA obtained from Dade, Lot No. LB620 FC/EL (Dade Div., American Hospital Supply, Miami, Fla.), was diluted in distilled water to three concentrations 0.75, 1.2, and 2.5 g/100 ml, pH 7.5–7.7, for which the transient birefringence was measured. Gel filtration chromatography on Sephadex G-100 showed a single, symmetrical profile at 278 nm, which was taken as evidence of a homogeneous sample. Centrifugation at 60,000 rpm in the Model E Analytical Ultracentrifuge (Beckman Instruments, Inc., Palo Alto, Calif.) showed a single, symmetrical schlieren pattern after 110 min. SDS gel electrophoresis on a thin slab showed a single band migrating at the same position as standard crystallized BSA obtained from Mann Research Labs, Inc., New York. These observations support our assumption concerning homogeneity. The resolving ability of the birefringence apparatus was measured by the transient birefringence of 1,2-propanediol carbonate.

ANALYSIS

The analysis of the optic system, with polarizers crossed, such that the polarizer axis makes an angle of 45° with the direction of the orienting field of the Kerr cell, yields the equation $I = I_o \sin^2(\delta/2)$, where I denotes the light intensity at the photomultiplier, I_o the intensity when the analyzer is oriented parallel to the polarizer, and δ denotes the birefringence in radians (optical retardation). For small retardations, as in the present case, the approximation $I \propto \delta^2$, may be used. Thus, in the linear region of the photomultiplier response, the output voltage is proportional to the square of the birefringence. When plotted on a log scale, the slope of the voltage vs. time is a factor of two greater than that for the birefringence. Birefringence relaxation times are therefore twice as large as voltage relaxation times.

RESULTS

Fig. 1 shows the data obtained for 1,2-propanediol carbonate. The relaxation time, defined as the time required for the pulse to decay to $1/e$ of its initial value, is seen to

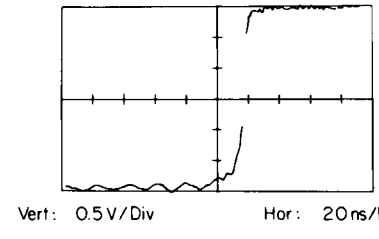

FIGURE 1 Birefringence relaxation for 1,2-propanediol cyclic carbonate.

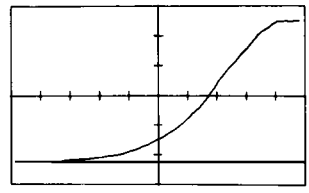

FIGURE 2 Birefringence relaxation for 0.75% BSA.

be 5 ns. This corresponds to the resolution time of the apparatus. Thus, the instrument should be able to resolve a birefringence relaxation time on the order of 25 ns with no correction for instrument response. Fig. 2 shows the data for BSA at the concentration indicated.

The data are obtained from a linear photographic enlargement of the voltage output pulse displayed on a Tektronix Type 454 oscilloscope (Tektronix, Inc., Beaverton, Ore.). Four repeated exposures on the same photograph showed a single pulse, indicating reproducibility of the signal. The error in data obtained from such a reproducible pulse is limited to one's ability to read values of the pulse at various times. The pulse displayed on the oscilloscope screen has a finite thickness, and the tracing shown in Fig. 2 corresponds to the middle of the pulse width. The error for this tracing is ±0.05 divisions.

Fig. 3 shows the analysis of the relaxation times for the data of Fig. 2. The open

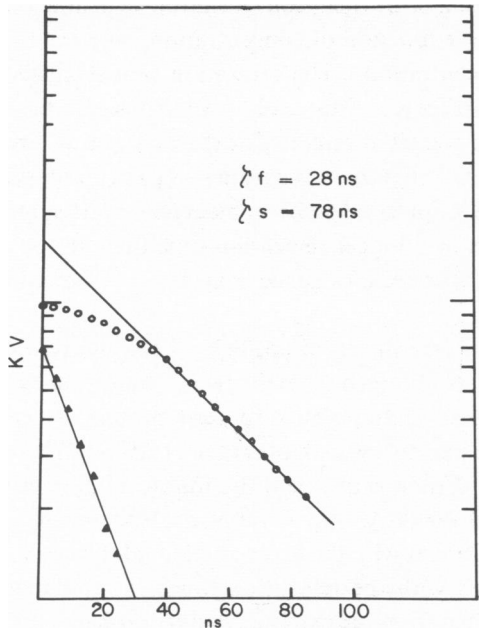

FIGURE 3 Analysis of relaxation on times for 0.75% BSA.

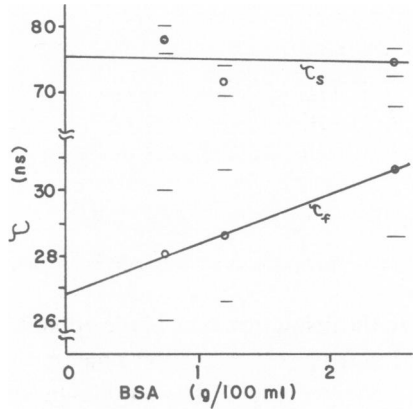

FIGURE 4 Concentration dependence of relaxation times.

circles denote scaled voltage values taken from Fig. 2, where K denotes the scale factor. The usual graphical analysis of peeling off the longer relaxation process is used to obtain the faster relaxation process. This process is determined by the triangles, which represent differences between the actual data, open circles, and the longer relaxation process, extrapolated to early times. The birefringence relaxation times were obtained according to the relation presented in the Analysis section. The error in all measured relaxation times is ± 2 ns.

Fig. 4 shows the results of the relaxation analysis for the two remaining concentration runs for BSA, along with their concentration dependence. The fast relaxation time is seen to be a linear function of concentration, whereas the slow relaxation time is independent of concentration. The relaxation times extrapolated to zero protein concentration are $\tau_f = 26.9 \pm 3.6$ ns, and $\tau_s = 75.5 \pm 1.5$ ns. These values are exceptionally close to the predicted values. The slow relaxation time also agrees with the previously reported value of 76 ns. According to purity criteria discussed in the Experimental section, BSA appeared to be homogeneous. If aggregation had occurred, the aggregate would show a longer relaxation time than 76 ns, which corresponds to the monomer. Thus, BSA birefringence relaxation is described by two relaxation processes.

The values for the rotary diffusion coefficients for the terms extrapolated to zero protein concentration are obtained from the two equations shown in the Introduction. Since the data have been extrapolated to zero protein concentration, the solvent viscosity is properly taken to be that of water at 25°C. These data, applied to the inversion procedure of Wright et al., yield the following dimensions for the equivalent prolate ellipsoid model for BSA: $2a_3 = 140.9 \pm 4.9$ Å and $2a_1 = 41.6 \pm 3.6$ Å. These values are in good agreement with those reported in references 3, 4, and 5.

The errors associated with the relaxation times extrapolated to zero protein concentrations were obtained from the extreme values consistent with the individual measurement errors of ± 2 ns. Thus, the errors associated with the dimensions of the equivalent ellipsoid of revolution represent maximum values.

If least squares criteria were used, then the errors on the extrapolated relaxation times would be greatly reduced because of the linear trend of the relaxation times measured at several concentrations. For example, the standard error for $\lim_{c \to 0} \tau_f$ based on least squares criteria is ± 0.068 ns (7). The use of this small error would reduce the errors associated with the estimated dimensions of the equivalent ellipsoid of revolution.

Because of the smallest of the sample sizes, with respect to protein concentration, it was decided to present errors based on the worst case, i.e. ignoring the reduction in error due to linearity. Even in this extreme case, the estimates of the dimensions of the ellipsoid of revolution are respectfully precise.

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