Electrooptical Properties of Reduced Protein–Sodium Dodecyl Sulfate Complexes†

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ABSTRACT: Two independent electrooptical properties, the specific Kerr constants and the electric birefringence relaxation times, of the saturated sodium dodecyl sulfate complexes of a series of reduced polypeptides of known molecular weight are reported. Both the Kerr constants and the relaxation times are unique functions of the molecular weight of the polypeptide chain. The specific Kerr constants depend upon the square of the polypeptide molecular weight. The relaxation times of the complexes, which are proportional to the rotational diffusion constants, are dependent on the molecular weight to approximately the first power. The latter finding is inconsistent with the compact prolate ellipsoid model for sodium dodecyl sulfate–protein complexes proposed by Reynolds and Tanford ((1970) *J. Biol. Chem. 245,* 5161) in which the cross section is constant and the length depends linearly on molecular weight; for this model the relaxation times would depend on approximately the 2.5 power of the molecular weight in the range of sizes investigated. Combination of the present results with other properties in the literature rules out a number of other models characterized by compactness and near inflexibility. No firm conclusions can be drawn as to the suitability of the free-draining flexible model of Shirahama, Tsujii, and Takagi ((1974) *J. Biochem. (Tokyo) 75,* 309).

Electric birefringence studies provide a useful means of obtaining several kinds of independent information about macromolecules (for review, see Fredericq and Houssier, 1973). The Kerr constant, related to the magnitude of the birefringence induced by the electric field, is a function of the optical and electrical properties of the molecule. In addition the rotational diffusion coefficient of the molecule can be obtained directly from the relaxation of the birefringence induced by the pulsed electric field. Since the rotational diffusion coefficient depends upon both the size and shape of the macromolecule, electric birefringence may be used to study aggregation processes. We are using this method to characterize the pre-gelling aggregation of sickle-cell hemoglobin; because of the rapidity of measurement by this technique, it can be used to follow the kinetics of aggregation as well as to characterize the products. In the course of calibrating the method for the analysis of the hemoglobin work (to be published elsewhere), we have obtained unexpected results on protein–sodium dodecyl sulfate complexes which are relevant to their structure; these results are reported here.

The complexes formed between reduced polypeptide chains and sodium dodecyl sulfate have received considerable attention recently because of the widespread use of sodium dodecyl sulfate–polyacrylamide gel electrophoresis as a method of determining polypeptide molecular weights (Shapiro et al., 1967; Weber and Osborn, 1969). In studies aimed at understanding the success of this method, it was reported that reduced unfolded proteins uniformly bind 1.4 g of sodium dodecyl sulfate/g of protein (Pitt-Rivers and Impiombato, 1968; Reynolds and Tanford, 1970a) at saturation. Viscometric (Reynolds and Tanford, 1970b) and gel filtration studies (Fish et al., 1970) also are consistent with a uniform structure for these complexes. Although it is now generally accepted that many reduced proteins form uniform complexes with the same saturating amounts of sodium dodecyl sulfate, agreement as to the nature of these complexes has not been reached. Two significantly different models for the structure of these complexes have been proposed. On the basis of viscosity studies, Reynolds and Tanford (1970b) proposed a compact prolate ellipsoid model in which the cross section is constant and the length is linearly dependent upon molecular weight. On the other hand, Shirahama et al. (1974) have proposed, on the basis of free boundary electrophoresis results, a "necklace" model in which the sodium dodecyl sulfate is bound in clusters along a flexible free-draining polypeptide chain. The results in this paper provide additional information which contributes to distinguishing between these models.

The present paper reports measurements of both the specific Kerr constants and electric birefringence relaxation times of a series of well-characterized sodium dodecyl sulfate–protein complexes. The results presented demonstrate the usefulness of electric birefringence as a tool for molecular characterization and provide new information about sodium dodecyl sulfate–protein complexes. During the preparation of this manuscript, a report of the electric birefringence relaxation times alone of some sodium dodecyl sulfate–protein complexes appeared (Wright et al., 1975). These results, which are not entirely in agreement with ours, are discussed later.

Materials and Methods

Proteins. The proteins used in this study were obtained from the following sources and were used without further purification: bovine serum albumin, ovalbumin, transferrin, β-lactoglobin from Nutritional Biochemicals; chymotrypsinogen A, and pepsin, from Worthington; whale myoglobin from Sigma; and fumarase from Calbiochem. Hemoglobins A and S were prepared from human serum by the method of Hinson and McMeekin (1969). Except for hemoglobin and myoglobin, the detergent–protein complexes were prepared by dissolving the proteins at about 1 g/100 cm^3 concentration in water containing 1.5 to 2 times their weight of sodium dodecyl sulfate,

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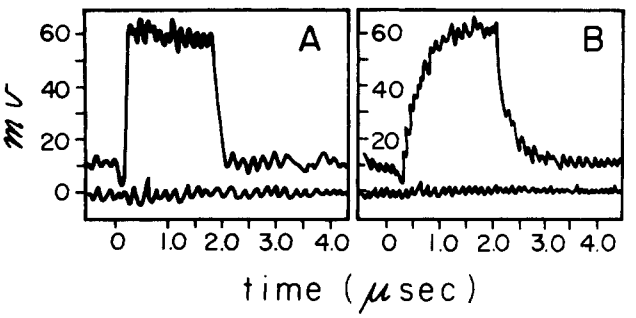

FIGURE 1: Examples of oscilloscope tracings showing the birefringence pulses obtained for (A) urea and (B) a sample of bovine serum albumin–sodium dodecyl sulfate complex. The analyzer angle is positive in these examples.

and 1% β-mercaptoethanol; the pH was adjusted to 8.5 with NaOH. The solutions were kept at either 20 °C for 20 h or at 37 °C for 2 h; they were then dialyzed at 25 °C against two changes of 100 volumes of 10 mM sodium dodecyl sulfate, 0.1% EtSH,[1] and 0.002% NaN_3 (to retard spoilage) for at least 48 h. The preparation of hemoglobin and myoglobin complexes was the same, except that EtSH was omitted. This omission had no effect on the results. The solutions were then diluted with the last dialysate to the concentration desired for the electric birefringence measurement. Concentrations were determined spectrophotometrically where possible, or by the Lowry method (1951) standardized using appropriate proteins. Appropriate extinction coefficients in sodium dodecyl sulfate were determined in a separate experiment, using published extinction coefficients in the native solvent, and comparing with the absorption in sodium dodecyl sulfate. The values used for native proteins were (all at their absorption maxima near 279 nm): bovine serum albumin $E_{1\%}^{1cm} = 6.67$, chymotrypsinogen $E_{1\%}^{1cm} = 20.4$, ovalbumin $E_{1\%}^{1cm} = 7.4$, transferrin $E_{1\%}^{1cm} = 11.4$, pepsin $E_{1\%}^{1cm} = 11.4$, fumarase $E_{1\%}^{1cm} = 5.1$ (Sober, 1972). The Lowry method (1951) was used for hemoglobin A and S and for myoglobin; it was standardized using both myoglobin and hemoglobin.

Sodium Dodecyl Sulfate. In some experiments, sodium dodecyl sulfate from Theo. Schuchardt (Munich), lot 26005, was used; this material was shown to be over 98% pure sodium dodecyl sulfate and free of tetradecyl sulfate by gas chromatography (unpublished results from this laboratory). Two lots of lauryl sodium sulfate Schwarz/Mann No. 902696 (lot Y1973 and lot X3366) were also used, although (in spite of the manufacturer's specification of 99.5% purity) these were both found by gas chromatography to contain 25–31% of the tetradecyl sulfate, and small amounts of the decyl compound. The experimental results obtained using these two lots did not differ from those obtained with the pure material.

Electric Birefringence Apparatus. The electric birefringence apparatus consisted of a pulse generator, an optical system and detector circuit, and the Kerr cell. The pulser was built after the design of Krause and O'Konski (1959); it provides square pulses of 2-μs duration, with a decay time of approximately 25 ns, made possible by a 50-ohm resistance in parallel with the Kerr cell. The pulse magnitude can be varied from 2000 to 8000 V, providing with the Kerr cell, having an electrode gap of 0.17 cm, electric fields ranging from 12 000 to 47 000 V/cm. The pulses were triggered singly or at 1-s intervals.

The optical system consisted of a 3-mW polarized helium–neon laser (Hughes) light source, followed by a Glan-Thompson polarizing prism, the Kerr cell, a ¼ wave device (in this case a double Fresnel rhomb), an analyzing polarizer, and a photomultiplier tube (EMI 9698B), the output of which after amplification was displayed on an oscilloscope (Techtronix 7704) and photographed. Viewed from the source, the first polarizer is at 45°, the Fresnel rhomb has its fast direction at 45°, and the moveable analyzer is near 135°. With these optics the scattered light transmitted through the analyzer in the extinction position is less than 2×10^{-5} of the original light intensity. The analyzer is mounted in a gear–wheel–worm gear arrangement (Charles Lohr, Inc., Bethesda, Maryland) such that one complete turn of the external calibrated barrel corresponds to a change of angle of 2°. The external barrel can be read to 0.005°. With this arrangement, optical retardations down to 0.1° can be measured. The Kerr cell consists of gold-plated brass electrodes mounted in a Teflon assembly which is inserted into a commercial rectangular cuvette with internal dimensions of 1.0 × 0.3 cm. The light path is 0.3 cm.

Data Analysis. The retardation δ is obtained from the relation

$$\Delta I_\delta = I_0[\sin^2(\alpha + \delta/2) - \sin^2\alpha] \quad (1)$$

where ΔI_δ is the change in light intensity during the pulse, α is the angle of the analyzer with respect to the extinction position, and I_0 is the light intensity observed when the polarizer and analyzer are parallel. I_0 is determined in each experiment from Malus law $I_0 = I/\sin^2\alpha$ by measuring I over a wide range of both positive and negative α. Finding a constant I_0 confirms the linearity of the detection system, and the validity of assuming that the voltage output is proportional to the light intensity. The angle of the analyzer which corresponds to the extinction position was measured directly in each experiment; this procedure automatically corrects for the small but significant residual birefringence present in some of the cuvettes. The optical retardation δ during the pulse was calculated from data such as are shown in Figure 1 using the complete eq 1; the linear approximation used by other authors (Krause and O'Konski, 1959) is only valid if $\alpha \gg \delta$, which was not the case in our experiments. At a given field strength, data were obtained at several values of α, both positive and negative, and good agreement among these measurements was obtained. Correction was made for the small but significant electric birefringence of the solvent.

The oscilloscope tracing obtained in kinetic experiments was expanded and calculated as above, as a function of time. In each experiment, data were obtained at different field strengths, different angles α, and different sensitivities, and good agreement in the decay times was obtained. Figure 1A shows the results obtained with urea; this pulse gives an apparent decay time of 0.025 μs, which is thus the limit of the instrumental time resolution. Figure 1B shows an example of data obtained for the bovine serum albumin–sodium dodecyl sulfate complex.

Results

Sodium Dodecyl Sulfate–Protein Complexes. The object of this investigation was to characterize the saturated complexes between reduced polypeptide chains and sodium dodecyl sulfate which, according to Reynolds and Tanford (1970a) and Pitt-Rivers and Impiombato (1968), contain 1.4 g of sodium dodecyl sulfate/g of protein. Control experiments were performed to determine the free detergent concentration needed to obtain the saturated complexes under our conditions of no added electrolyte; the electric birefringence results depended

[1] Abbreviations used: EtSH, β-mercaptoethanol; PVP, polyvinylpyrrolidone.

TABLE I: Electrooptical Properties of Sodium Dodecyl Sulfate-Protein Complexes.

Protein	Mol wt	τ (μs)	K_{sp} ($\times 10^7$) statvolts^{-2} cm^2
Sodium dodecyl sulfate complexes			
Transferrin	76 000	0.26	0.83
Bovine serum albumin	65 000	0.28	0.99
Fumarase	50 000	0.22	1.10
Ovalbumin	43 000	0.15	0.61
Chymotrypsinogen	25 700	0.12	0.36
Pepsin	35 000	0.16	0.55
β-Lactoglobulin	18 000	0.070	0.10
Hemoglobin S	16 000	0.086	0.095
Hemoglobin A	16 000	0.088	0.097
Myoglobin	16 000	0.073	0.087
Native Proteins			
Bovine serum albumin[a]		0.084	0.11
Ovalbumin[b]		0.073	0.17

[a] Extrapolated to zero concentration. [b] Pertains to 0.6% concentration.

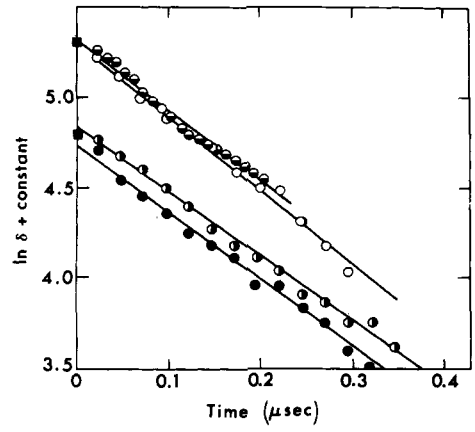

FIGURE 2: Example of birefringence decay data obtained for bovine serum albumin-sodium dodecyl sulfate complex at 0.13% protein, 0.01 M sodium dodecyl sulfate (free), and 0.1% EtSH. (⊖) $\alpha = 1.181°$, $E = 34\,120$ V/cm; (○) $\alpha = -3.174°$, $E = 34\,120$ V/cm; (◑) $\alpha = 0.773°$, $E = 24\,700$ V/cm; (●) $\alpha = -1.92°$, $E = 24\,700$ V/cm. Squares represent steady-state value of electric birefringence at 34 120 and 24 700 V/cm.

upon the free sodium dodecyl sulfate concentration up to 6 mM, but above this concentration no effect of additional sodium dodecyl sulfate was observed. Therefore the experiments reported here were done at a concentration of free sodium dodecyl sulfate of 10 mM, or 2.88 g/l. Since the critical micelle concentration of pure samples of sodium dodecyl sulfate with no added salt is 8 mM, micelles were present in our experiments. However, electric birefringence experiments on 10 mM sodium dodecyl sulfate gave a Kerr constant only slightly larger than water, so that the contribution of micelles to the results was negligible and easily accounted for. So small a contribution from the micelles is consistent with the expectation that at low ionic strength, sodium dodecyl sulfate micelles are nearly spherical (Tanford, 1972); thus they have little optical or electrical asymmetry, the two requirements for exhibiting electric birefringence.

Relaxation of Birefringence. The decay of the induced birefringence at the end of the electric field pulse was measured as described under Methods. Figure 2 shows an example of the data obtained (sodium dodecyl sulfate–bovine serum albumin), plotted as a first-order plot of ln δ (eq 1) against time. The four sets of data pertain to four angles α and two field strengths; as seen here, the slope, and thus τ, is independent of both field strength and α. In each of the experiments, similar linear results were obtained, corresponding to a single decay time τ, where $\delta_t/\delta_0 = e^{-t/\tau}$, δ_0 and δ_t being the retardations at time zero and time t, respectively.

The measured decay times of a series of sodium dodecyl sulfate-protein complexes are listed in Table I. Each listed value represents an average of at least five experiments on at least two independent preparations. Several protein concentrations in the range from 0.05 to 0.6% were examined for each complex. Within this concentration range, the concentration dependence of the relaxation time was found to be within the scatter of the experimental values (10–20%). Also shown in the table are values for *native* ovalbumin and bovine serum albumin. It is seen that the decay times for all of the complexes are larger than those of the native proteins.

Benoit (1951a,b) has shown that the electric birefringence relaxation time is related to the rotational diffusion coefficient Θ by the relation $\tau = 1/6\Theta$. The rotational diffusion coefficient of a macromolecule depends on its shape and size. For example, for a rigid prolate ellipsoid of revolution with $a/b > 5$

$$\Theta = \frac{3kT}{16\pi a^3 \eta}\left[\left(2 \ln \frac{2a}{b} - 1\right)\right] \tag{2}$$

where a is the major semi axis and η is the solvent viscosity (Perrin, 1934). For a sphere

$$\Theta = \frac{kT}{8\pi r^3 \eta} \tag{3}$$

where r is the radius of the particle. If it is assumed that molecular weight M is proportional to the molecular volume, then for an ellipsoid, $M \propto V = \frac{4}{3}\pi a b^2$. If b^2 is a constant, then $a \propto M$, and eq 2 can be written

$$\Theta \propto (1/M^3)\left[2 \ln \frac{2a}{b} - 1\right]$$

and

$$\tau \propto M^3 \Big/ \left[2 \ln \frac{2a}{b} - 1\right] \tag{4}$$

Thus for this model the relaxation time is approximately proportional to a high power of M (approximately 2.5 in the range of M examined here). On the other hand, if the axial ratio a/b is constant, then $M \propto a^3$. From eq 2 we then have

$$\Theta \propto 1/M \text{ and } \tau \propto M \tag{5}$$

For a solid sphere, again we have $M \propto r^3$, and from eq 3 we have

$$\Theta \propto 1/M \text{ and } \tau \propto M \tag{6}$$

These equations show that the nature of the functional dependence of relaxation time on molecular weight can distinguish between certain models for the shapes of a series of similar molecules.

Figure 3 shows a double logarithmic plot of the relaxation times listed in Table I against molecular weight; the slope is 0.87. This value is clearly inconsistent with a rigid ellipsoid with constant cross section, for which an exponent near three is expected (eq 4). (The term in brackets brings the slope down

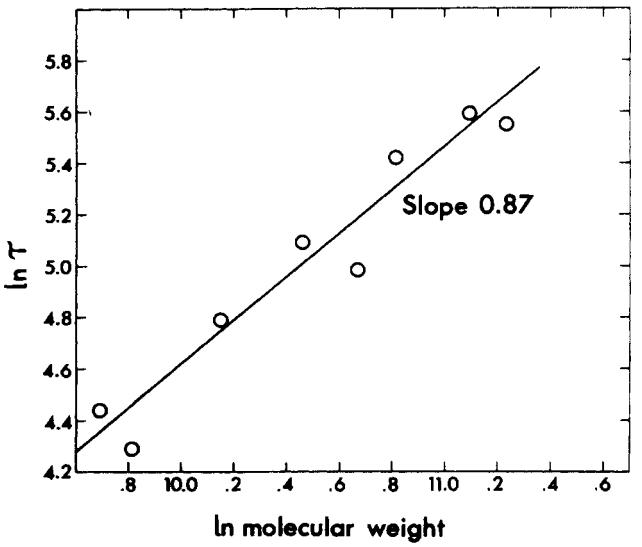

FIGURE 3: Double logarithmic plot (natural logarithms) showing the dependence of relaxation time on the molecular weight of the polypeptide chain.

to 2.5 for the size range of interest.) An exponent near one would be consistent with an ellipsoid with an axial ratio independent of M (eq 5), or a solid sphere (eq 6). The expected molecular weight dependence of the relaxation times for other alternative models such as flexible and free draining structures obviously depends in part on a number of segmental rotation time constants, but no adequate theory is immediately available for such models (see Discussion).

Rudd and Jennings (1974) have recently reported the electric birefringence of the sodium dodecyl sulfate complex of a polyvinylpyrrolidone (PVP) sample with an average molecular weight of 700 000. A range of relaxation times from 4 (±2) to 33 (±10) μs was obtained. The extrapolation of our curve of Figure 3 gives a relaxation time of 2 μs for this molecular weight, indicating that this unstructured polymer–sodium dodecyl sulfate complex has a relaxation time with a molecular weight dependence somewhat within the range expected on the basis of the polypeptide–sodium dodecyl sulfate complexes. The long extrapolation required for this comparison dictates caution in interpreting this result.

Specific Kerr Constants. According to the Kerr law, the birefringence induced by an electric field depends linearly on the square of the electric field strength at the limit of low field strength:

$$\lim_{E \to 0} \left(\frac{\Delta n}{nE^2} \right) = K_{sp} C_v$$

where n is the isotropic index of refraction, E is the field strength, C_v is the volume concentration of macromolecule, and

$$\Delta n = \frac{\delta \lambda}{2\pi l}$$

(δ is the optical retardation, and l is the length of the optical path length between the electrodes). Theoretical treatments (Fredericq and Houssier, 1973) give for the Kerr constant

$$K_{sp} = \frac{2\pi(g_a - g_b)}{15n^2}(P + Q) \qquad (7)$$

where $(g_a - g_b)$ represents the optical anisotropy of the molecules and $P + Q$ is the orientation function, P being the permanent dipole term, and Q the induced dipole term.

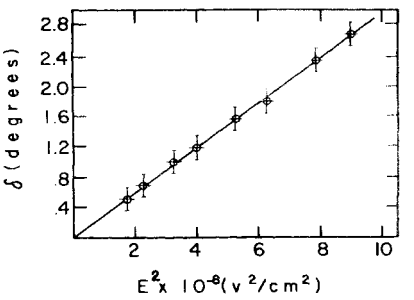

FIGURE 4: Example of retardation plotted as a function of the square of the electric field strength. The concentrations are 0.08% bovine serum albumin, 0.01 M sodium dodecyl sulfate (free), and 0.1% EtSH. Each point represents the average of four measurements at different angles (α) of the analyzer, both positive and negative.

The steady-state electrical birefringence of the protein–sodium dodecyl sulfate complexes was measured as a function of field strength. Figure 4 shows an example of a plot of the electric birefringence against the field strength squared for a bovine serum albumin–sodium dodecyl sulfate complex. Each point represents an average of measurements at four angles α, analyzed by using eq 1. It is seen that Kerr law behavior is obtained throughout the accessible field strength range, and that no saturation effects are visible. Similar linear plots were obtained for all of the sodium dodecyl sulfate–protein complexes examined. The specific Kerr constants obtained from the slopes of plots similar to Figure 4 are summarized in Table I. Each listed value represents the average of at least five, and usually more, experiments done on several preparations and concentrations. Within the concentration range examined (0.1–0.6% in protein), no concentration dependence was observed. Also shown in Table I are values for K_{sp} for ovalbumin and bovine serum albumin in the native state. For native bovine serum albumin, our value is in good agreement with those of Krause and O'Konski (1959) and Moser et al. (1966). For ovalbumin, our value of 0.17×10^7 statvolt^{-2} cm^2 agrees well with that of Riddiford and Jennings (1967), although it is considerably smaller than that of Krause and O'Konski (1963).

Table I shows that the specific Kerr constants of the sodium dodecyl sulfate complexes are considerably larger than those of the native proteins. The double logarithmic plot of specific Kerr constants of the complexes against molecular weight in Figure 5 is linear, and has a slope of 2.0, indicating a strong molecular weight dependence. According to eq 7, the specific Kerr constant is a function of the optical anisotropy and the electrical dipole moment which can be either permanent or induced. We know that the complexes are highly charged, and that the total charge increases with molecular weight; the polarization of the counterion cloud is probably a significant factor in the mechanism of orientation of the complexes. Optical anisotropy must also be present for the observation of electrical birefringence. In the sodium dodecyl sulfate–protein complexes, the characteristic properties of the individual proteins are presumably subordinate to the effects produced by the large amounts of bound sodium dodecyl sulfate; therefore the optical anisotropy term in eq 7 probably originates from molecular asymmetry which produces form birefringence. That is, the optical anisotropy of the oriented solution is due to alignment of asymmetric molecules which differ in index of refraction from the solvent, as opposed to being due to intrinsic optical anisotropy of symmetrical particles. Thus, the large specific Kerr constants of the complexes indicate that they are asymmetrical in shape. The strong molecular weight

dependence does not necessarily indicate that the degree of asymmetry increases with molecular weight since we know the charge, and the accompanying mobile counterion cloud, increases with molecular weight.

Consideration of the electric birefringence measurements of Rudd and Jennings (1974) on the sodium dodecyl sulfate-PVP complex shows that the Kerr constant calculated from their data fits reasonably well on the curve of Figure 5 for the appropriate molecular weight of 700 000. Although it is a rather long extrapolation, when combined with other data (see below), it suggests that the sodium dodecyl sulfate-PVP and sodium dodecyl sulfate-polypeptide complexes are similar in nature.

Discussion

We have shown that both the specific Kerr constants and the electric birefringence relaxation times of the saturated complexes of reduced proteins and sodium dodecyl sulfate are unique functions of the molecular weight of the polypeptide chains. This finding is consistent with other hydrodynamic measurements on these complexes, including gel electrophoresis (Shapiro et al., 1967; Weber and Osborn, 1969), gel chromatography (Fish et al., 1970), and viscosity (Reynolds and Tanford, 1970b). Our data thus support the use of hydrodynamic measurements of sodium dodecyl sulfate-polypeptide complexes for determining polypeptide molecular weights. Electric birefringence measurements could be used for this purpose; by this technique, two independent properties, the Kerr constant and the relaxation time, can be obtained in a few hours using a relatively small sample. Since the specific Kerr constants depend on the square of the molecular weight and the relaxation time depends on only the first power of the molecular weight, Kerr constant measurements provide better resolution of molecular weights. However, use of this technique, like gel electrophoresis and gel chromatography, is subject to the condition that the polypeptide chains of interest all bind the same amount of sodium dodecyl sulfate on a per gram basis. Since some proteins have recently been reported to bind anomalous amounts of sodium dodecyl sulfate at saturation (e.g., Grefrath and Reynolds, 1974; Robinson and Tanford, 1975), it is advisable to combine the technique with other measurements.

Two inherently different models have been proposed for the conformation of the reduced polypeptide-sodium dodecyl sulfate complexes. On the basis of viscosity data in which the intrinsic viscosity of the complexes was found to depend on the 1.2 power of the molecular weight, Reynolds and Tanford (1970b) proposed a *compact* prolate ellipsoid in which the length of the major axis depends on molecular weight and the minor axis is constant. More recently, on the basis of the finding that the free boundary electrophoretic mobility of the complexes (unlike the mobilities in gels) is independent of molecular weight, Shirahama et al. (1974) proposed a "necklace model", in which the sodium dodecyl sulfate is bound in micelle-like clusters at intervals along a flexible polypeptide chain. The essential difference between these models is in the degree of compactness, and in the statistical nature of the conformation proposed by Shirahama et al. The ellipsoidal model is based on an interpretation of viscosity data using Simha shape factors, a treatment which is applicable only to solid compact structures (non-free-draining) (Tanford, 1961). The flexible polyelectrolyte model proposed by Shirahama et al. (1974) is free draining, and presumably includes considerable solvent within the domain of the complex. There is also a difference in flexibility between the two models: whereas the

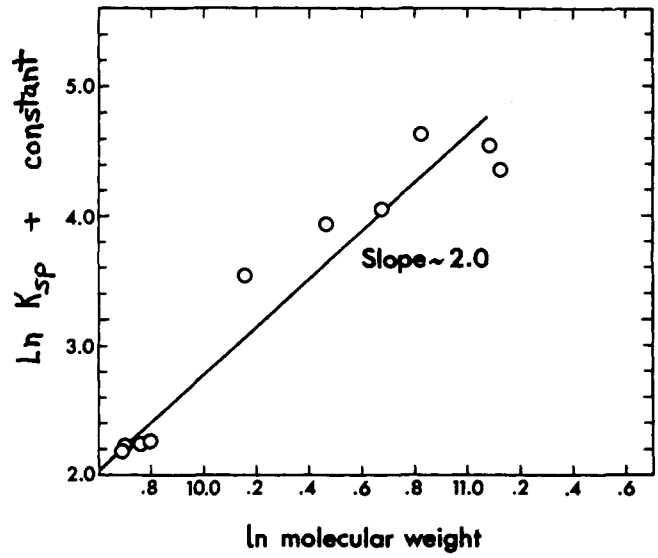

FIGURE 5: Double logarithmic plot (natural logarithms) showing the dependence of the specific Kerr constants on the polypeptide molecular weight.

"necklace" model is a flexible coil, the ellipsoid model is more constrained, although there is a suggestion that the rod-like particles may be "not entirely rigid" (Reynolds and Tanford, 1970b).

The most important result of our study is the finding that the relaxation times of the sodium dodecyl sulfate-polypeptide complexes depend approximately linearly on the molecular weight of the polypeptide chains. This finding is inconsistent with a rigid compact prolate ellipsoid or any similar elongated form (such as a cylinder) in which the short axis is constant and the long axis depends on molecular weight. According to eq 4 such a structure would give a molecular weight dependence approaching the third power (the dimensions given by Reynolds and Tanford (1970b) would give a slope of 2.5), as compared with our value of 0.9. Also, the decay time expected for bovine serum albumin from the ellipsoid model is 1.4 μs as compared with the experimental value of 0.255 μs. Compact rigid structures for which a linear dependence of relaxation time on molecular weight is predicted include rigid spheres, or ellipsoids with constant axial ratios. However, a spherical model is ruled out by the asymmetry indicated by the large Kerr constants, as well as by the viscosity data and gel chromatography results (Reynolds and Tanford, 1970b; Fish et al., 1970). A compact ellipsoid with a constant axial ratio would be consistent with our results, but would appear to be inconsistent with the Simha shape factors obtained by Reynolds and Tanford (1970b). An alternative to these compact structures is the "necklace" model of Shirahama et al. (1974) in which the sodium dodecyl sulfate is bound in clusters at intervals along a flexible polypeptide which then behaves like a flexible polyelectrolyte. Such a flexible polyelectrolyte, e.g., polyglutamic acid or polylysine, is expected to expand at low ionic strengths (Tanford, 1961), and would thus possess the asymmetry indicated by the experimental data, including the viscosity molecular weight exponent of 1.2 obtained by Reynolds and Tanford (1970b). Several attempts at theoretical treatments of rotary diffusion of random flexible or stiff chain structures have been made (see Zimm, 1956; Hearst, 1963; Stockmayer and Baur, 1964), which deserve further evaluation. The applicability of these treatments to the Shirahama model is questionable, however, due to the deviation of the

latter from average spherical symmetry; moreover, since some of the segments of the polyelectrolyte in the "necklace" model are comparable in size to sodium dodecyl sulfate micelles, the number of segments in the sodium dodecyl sulfate–polypeptide complexes would not suffice for the statistical treatments used in describing the properties of polymers in solution.

The interpretation of the specific Kerr constants is less straightforward; no theory is available other than the observation from literature values (see Fredericq and Houssier, 1973) that in a very general way the larger the particle, the larger the Kerr constant tends to be. We have shown that the specific Kerr constants of the sodium dodecyl sulfate–protein complexes are larger than those of the native proteins, and are strongly dependent on molecular weight, in contrast to those of the native proteins. This finding supports the concept that the characteristic properties of the individual proteins are overwhelmed by the large amounts of bound sodium dodecyl sulfate. The large specific Kerr constants indicate qualitatively that the complexes are asymmetric in shape since it is unlikely that they possess any *intrinsic* optical anisotropy (see Results). The birefringence induced by the electric field is most likely *form* birefringence rather than *intrinsic* birefringence; that is, it is due to alignment of asymmetric particles which have an index of refraction different than that of the solvent. A large asymmetry is characteristic of the prolate ellipsoid model of Reynolds and Tanford (1970b), but as discussed above it would also be consistent with the model of Shirahama et al. (1974) in which expansion of the coil at low ionic strength, due to charge repulsions, is expected (Tanford, 1961).

An examination of our results for evidence of flexibility is pertinent to the elucidation of the structure of the complexes. First, the possibility of flexibility raises the question of whether the observed electric birefringence is due to orientation of the complex as a whole, or to orientation of segments of the complex. Our relaxation times are appropriate for molecular rotations. Thus, if eq 3 is used to calculate an "equivalent radius" R_e, defined as the radius of a sphere which would have the same relaxation time as the one measured, one obtains a range from 45 Å for hemoglobin subunits to 67 Å for bovine serum albumin. A similar "equivalent sphere" treatment of viscosity data gave a range of 27 to 83 Å, respectively, for these complexes (Reynolds and Tanford, 1970b). The strong molecular weight dependence of the specific Kerr constants also suggests that each complex orients as a whole in the electric field. However, it should be noted that the orientation of the complex as a whole does not rule out flexibility (see Stockmayer and Baur, 1964). Second, we have observed strictly Kerr law behavior with our complexes. Non-Kerr law field strength dependence of birefringence of long rod-like molecules, such as DNA, has been attributed to their flexibility (Fredericq and Houssier, 1973). However, Rudd and Jennings (1974) observed Kerr law field strength dependence of the electric birefringence of sodium dodecyl sulfate–PVP complexes, which are known to be flexible polymers. Thus, while our results do not provide any evidence in favor of flexibility, they cannot rule it out.

It is remarkable that both the specific Kerr constant and the relaxation time of a PVP–sodium dodecyl sulfate complex of average molecular weight 700 000 (Rudd and Jennings, 1974) obey the empirical functions of molecular weight which we have determined for the polypeptide molecular weight range from 16 000 to 70 000. Shirahama et al. (1974) found that the electrophoretic mobilities of PVP–sodium dodecyl sulfate complexes were similar to those of the sodium dodecyl sulfate–polypeptide complexes, and independent of molecular weight, and suggested that sodium dodecyl sulfate interacts with the unstructured homopolymer and the polypeptides in a similar way. Our results on two additional independent properties of the sodium dodecyl sulfate–protein complexes, combined with the measurements of Rudd and Jennings (1974) on sodium dodecyl sulfate–PVP complexes, support this suggestion.

During the preparation of this manuscript, the electric birefringence relaxation times (but not the Kerr constants) of some protein–sodium dodecyl sulfate complexes were reported (Wright et al., 1975). These authors report observing two relaxation processes in each experiment, an observation which we cannot substantiate. In all of our experiments a single exponential which corresponds to the "slow" process of Wright et al. describes over 80% of the decay. Figure 2 shows that our time data extrapolate back to the *steady state* value of the retardation at zero time, indicating that there is no faster process which has been missed. Processes faster than our instrumental limitation of 30 ns would still be resolved if present, but would give inaccurate apparent decay times of about 30 ns, as in the case of urea.

The "slow" relaxation times reported by Wright et al. (1975) are in reasonable agreement with our relaxation times for those proteins which were examined in both laboratories. A brief discussion of their interpretation of this data is pertinent because they have used data which is essentially in agreement with ours to arrive at a different conclusion. Assuming Reynolds and Tanford (1970b) model dimensions, Wright et al. (1975) use eq 2 to calculate a quantity proportional to the theoretical decay time, which is then plotted as a double logarithmic plot against the experimental decay time. The resulting plot is not linear, but has two linear regions with a break around 25 000 molecular weight. The theoretical quantities calculated for the proteins with molecular weights below 25 000 are probably incorrect, however, because the small axial ratios of these (Reynolds and Tanford, 1970b) require the use of the complete Perrin equation rather than the approximate eq 2. The experimental relaxation times for the proteins above molecular weight 25 000, in agreement with ours, differ markedly from the theoretical ones based on Reynolds and Tanford's model (1970b) (e.g., for bovine serum albumin, the measured value is 260 ns compared with a theoretical one of 1400 ns). This discrepancy is attributed to flexibility. Despite the author's conclusions to the contrary, however, so marked a deviation may imply a degree of flexibility which is not compatible with the compact solid implied by the Reynolds and Tanford model (although the latter allows for some flexibility). Indeed, so great a degree of flexibility might be inconsistent with the compact-solid treatment on which the prolate ellipsoid model is based (Reynolds and Tanford, 1970b). Thus we cannot agree that the results of Wright et al. (1975) support the compact rigid ellipsoid model for sodium dodecyl sulfate complexes; rather their results agree with our data and are thus incompatible with this model. It is clear that an alternative model is required to describe the properties of sodium dodecyl sulfate–protein complexes; the flexible free draining polyelectrolyte model of Shirahama et al. (1974) is one such attempt.

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Purification and Chemical Characterization of Salt-Extractable Glycoproteins from Porcine Mitral Valve†

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ABSTRACT: Four major glycoproteins were extracted by dilute salt solution from porcine mitral valvular tissue. Two of these major glycoproteins, porcine valve glycoprotein I and porcine valve glycoprotein III, were isolated and purified by fractionation of salt extract with ammonium sulfate followed by column chromatography on DEAE-cellulose. The purified glycoproteins appeared to be homogeneous by polyacrylamide disc electrophoresis in several buffer systems, and by Sephadex filtration. The porcine valve glycoprotein I has a molecular weight of approximately 120 000. Isoelectric focusing yielded a single band, pI = 5.8. The glycoprotein contained large amounts of acidic amino acids, and amide nitrogen. The carbohydrate moiety was composed of fucose, mannose, galactose, glucose, glucosamine, and galactosamine in the molar ratio of 5:10:15:12:7:2 per mole of glycoprotein. The second major glycoprotein, porcine valve glycoprotein III, has an approximate molecular weight of 72 000. This glycoprotein gives two bands upon analytical isoelectric focusing with isoelectric points of pI = 4.1 and 4.3. Porcine valve glycoprotein III contained large amounts of acidic amino acids and low amounts of amide nitrogen. Its carbohydrate moiety was composed of glucose, galactose, mannose, fucose, glucosamine, and sialic acid in the ratio of 3:3:2:1:4:1 mol/mole of glycoprotein. This glycoprotein was similar to a glycoprotein preparation isolated from porcine aortic intima by P. V. Wagh and B. I. Roberts (1972), *Biochemistry 11,* 4222.

Mesenchymal glycoproteins are considered as major components of vascular tissue. The isolation of glycoproteins from vascular tissue was achieved by Robert and his co-workers (Robert et al., 1965; Moczar and Robert, 1970) using trichloroacetic acid to solubilize tissue collagen, followed by extraction with urea. Barnes and Partridge (1968) utilized cold alkali to extract glycoproteins from human thoracic aorta. A simple salt extraction procedure for the isolation and purification of glycoproteins from bovine and porcine aortas was reported by Radhakrishnamurthy et al. (1964, 1966) and by Wagh and Roberts (1972). This gentle extraction procedure has the advantage of solubilizing glycoproteins with the least alteration from their native form.

The biological importance of mesenchymal glycoproteins was recently emphasized by Goldstein and his co-workers (Goldstein et al., 1968) and by Kawakita and Iwamoto (1975) who reported that an antigenic cross-reactivity existed between

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