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Conformational Properties of the Complexes Formed by Proteins and Sodium Dodecyl Sulfate†

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ABSTRACT: Circular dichroism spectra have been obtained for albumin, α-chymotrypsinogen, collagen, concanavalin A, elastase, hemoglobin, histone f_2b, α-lactalbumin, lactate dehydrogenase, β-lactoglobulin, lysozyme, myoglobin, papain, ribonuclease A, and thermolysin in the presence of sodium dodecyl sulfate and dithiothreitol. While all spectra have the shape anticipated for a mixture of random coil and α helix, the intensities differ markedly ($[\theta]_{222}$ ranges from −1400 to −15 000 deg cm^2/dmol). The variation in the circular dichroism can be quantitatively explained by a model which assumes that the arginyl, histidyl, and lysyl residues have an enhanced probability of propagating a helical segment in the presence of the detergent. The model also permits the computation of dimensional properties (unperturbed end-to-end distance and radius of gyration) for polypeptides of known amino acid sequence. Such computations have been performed for 67 proteins. The computed dimensions are compatible with experimental values and with the molecular weight dependence of the transport properties of the complexes. Furthermore, the model can account for the abnormal transport properties of the sodium dodecyl sulfate complexes formed by ribonuclease A, collagen fragments, and histones f_2a_1, f_2a_2, f_2b, and f_3. Even though some of the protein—sodium dodecyl sulfate complexes have helical contents as high as 50%, their overall conformation more closely approximates that of a random coil than a rod.

A variety of proteins with reduced disulfide bonds form complexes containing approximately 1.4 g of sodium dodecyl sulfate/g of protein when the detergent monomer concentration exceeds 0.0008 M (Pitt-Rivers and Impiombato, 1968; Reynolds and Tanford, 1970; Takagi et al., 1975). The electrophoretic mobility in cross-linked polyacrylamide gels (Shapiro et al., 1967; Weber and Osborn, 1969; Dunker and Rueckerts, 1969), logarithm of the intrinsic viscosity (Reynolds and Tanford, 1970), logarithm of the Stokes radius (Fish et al., 1970), and slow birefringence relaxation time (Wright et al., 1975) are proportional to the logarithm of the molecular weight (or degree of polymerization) of reduced proteins under these conditions. Electrophoretic mobilities on free-boundary electrophoresis are independent of the molecular weight of the protein used to form the protein–sodium dodecyl sulfate complex (Shirahama et al., 1974). The optical activity of protein–sodium dodecyl sulfate complexes suggests the presence of appreciable amounts of α helix (Meyer and Kauzmann, 1962; Jirgensons, 1966, 1967; Reynolds and Tanford, 1970; Visser and Blout, 1971). However, the nature of the protein examined affects both the optical activity of the complex and the direction of the change in the rotatory strength associated with the peptide n–π* transition upon the formation of the complex from the native protein (Jirgensons, 1966; Visser and Blout, 1971).

The effect of sodium dodecyl sulfate on several synthetic homopolypeptides has been examined. Sodium dodecyl sulfate has little effect on the helix–coil transition of poly(L-glutamic acid) (Fasman et al., 1964). In marked contrast, cationic homopolypeptides readily adopt ordered conformations in the presence of sodium dodecyl sulfate. Poly(L-ornithine) (Grourke and Gibbs, 1967; Satake and Yang, 1973) and poly(L-arginine) (McCord, Blakeney, and Mattice, submitted) form an α helix, poly(L-histidine) forms a β structure (McCord, Blakeney, and Mattice, submitted), and poly(L-lysine) forms either a β structure (Sarkar and Doty, 1966; Satake and Yang, 1973, 1975; Mattice and Harrison, 1976) or α helix (Satake and Yang, 1975), depending on the pH and temperature. The crucial role played by the cationic side chains is evident from the insensitivity of the helix–coil transition of poly(N^5-ω-hydroxypropyl-L-glutamine) (Lotan et al., 1965; Igou et al., 1974) and poly(N^5,N^5-di(ω-hydroxyethyl)-L-glutamine) (Igou et al., 1974) to the detergent. This consideration is in accord with the pH dependence of the interaction of sodium dodecyl sulfate with pentalysine (Mattice and Harrison, 1976). The different behavior of proteins toward cationic and anionic detergents also suggests an interaction between the detergent head group and the protein (Nozaki et al., 1974).

The hydrodynamic behavior of the protein–sodium dodecyl sulfate complexes has led to the proposal of three models for the complex: a rod-like particle (Reynolds and Tanford, 1970), a "necklace" model (Shirahama et al., 1974), and a deformable prolate ellipsoid model (Wright et al., 1975). None of these models can account for the various effects produced by sodium dodecyl sulfate on the optical activity of the proteins, nor do they explain why anomalous hydrodynamic behavior is exhibited by certain proteins, such as ribonuclease A (Shapiro et al., 1967), various histones (Panyim and Chalkley, 1971), and peptides derived from collagen (Furthmayr and Timpl,

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TABLE I: Initial Values for σ and s.

Amino Acid Residues	σ	s	Reference[a]
Pro	0	0	Schimmel and Flory, 1967
Gly	0.00001	0.60	Ananthanarayanan et al., 1971
Ser, Cys, Thr	0.0001	0.76	Hughes et al., 1972
Val, Ilu	0.0001	0.95	Alter et al., 1973
Glu, Arg, Asn, Asp, Gln, Lys	0.0006	0.96	Maxfield et al., 1975
Ala	0.0008	1.06	Platzer et al., 1972
Phe, His, Try, Tyr	0.0018	1.07	Van Wart et al., 1973
Leu, Met	0.003	1.12	Alter et al., 1972

[a] Reference for the first named amino acid residue on each line.

1971). The present objective is to develop a model for the protein-sodium dodecyl sulfate complexes which is consistent with the observed hydrodynamic properties *and* optical activity, can account for the abnormal behavior of certain proteins, and is in harmony with the effects of sodium dodecyl sulfate on synthetic homopolypeptides.

Experimental Procedure

Materials. The proteins and dithiothreitol were obtained from Sigma Chemical Co. and the sodium dodecyl sulfate was obtained from Bio-Rad Laboratories. All other materials were reagent grade.

Protein Stock Solutions. Stock solutions for all proteins except collagen and histone f_2b were prepared by solution of the protein in 0.10 M sodium phosphate buffer, pH 6.86. Protein concentrations were obtained from the absorbance of an appropriately diluted aliquot, using the extinction coefficients summarized in the supplementary material. Water was used in place of the phosphate buffer for bovine histone f_2b. The concentration of this protein was determined using the spectral data reported by D'Anna and Isenberg (1972). Acid-soluble calf skin collagen was dried under vacuum overnight. The stock solution was prepared by the solution of a weighed amount of the collagen in 0.05 M sodium phosphate, pH 6.86, containing 0.018 M sodium dodecyl sulfate.

Absorbance. Absorption spectra were obtained at ambient temperature using a modified Durrum-Jasco J-20 recording spectropolarimeter. The absorbance mode was installed by Sproul Scientific Instruments.

Circular dichroism. Solutions for circular dichroism were obtained by dilution of the protein stock solution with water, 0.035 M sodium dodecyl sulfate, or 0.10 M sodium phosphate, pH 6.86. Dithiothreitol, when present, was the last component added to the solution. All spectra were measured at 25.0 °C using the Durrum-Jasco model J-20 recording spectropolarimeter. The instrument was calibrated with *d*-10-camphorsulfonic acid (Cassim and Yang, 1969). The light path was 1.00 mm in each case.

Computations

Configuration Partition Function. Equation 1 was used to compute the configuration partition function, Z, for a polypeptide chain containing n peptide bonds (Flory, 1969, 1974). The statistical weight matrix for amino acid residue i, $\mathbf{U}_i$, is defined in eq 2. Terminal matrices are defined by eq 3 and 4.

$$Z = \mathbf{U}_1{}^n \tag{1}$$

$$\mathbf{U}_i = \begin{bmatrix} 1 & \sigma s \\ 1 & s \end{bmatrix}_i \tag{2}$$

$$\mathbf{U}_{[1} = [1 \quad 0] \tag{3}$$

$$\mathbf{U}_{n]} = \text{col}\,(1, 1) \tag{4}$$

Symbolism of the type $\mathbf{U}_a{}^b$ denotes the product of b successive matrices, commercing with $\mathbf{U}_a$. The equilibrium constant for the propagation of a helical segment is denoted by s, while σs denotes the equilibrium constant for the initiation of a helical segment (Zimm and Bragg, 1959).

The initial values assigned to σ and s for the various amino acid residues are presented in Table I. Analysis of the composition and molecular weight dependence of the optical activity has yielded experimental values of σ and s for the glycyl (Ananthanarayanan et al., 1971), alanyl (Platzer et al., 1972), seryl (Hughes et al., 1973), leucyl (Alter et al., 1973), phenylalanyl (Van Wart et al., 1973), valyl (Alter et al., 1973), and glutamyl (Maxfield et al., 1975) residues in water at 25 °C. The prolyl residue merits an s of zero on steric grounds (Schimmel and Flory, 1967). The remaining amino acid residues were assigned initial values of σ and s through placement in one of the five following categories: aromatic side chain, heteroatom attached to the C^β atom, branching at the C^β atom, nonpolar CH_2R side chain, or polar CH_2R side chain. Methylated, acetylated, phosphorylated, hydroxylated, or glycosylated amino acid residues were also assigned initial σ and s values through placement in one of the preceding five categories.

Fraction Helix. Equation 5 was used to obtain the fraction, f, of the peptide bonds in the helical state (Flory, 1969). The supermatrix for internal peptide units is defined in eq 6, and terminal matrices are defined in eq 7 and 8.

$$f = Z^{-1}(n-2)^{-1}\hat{\mathbf{U}}_1{}^n \tag{5}$$

$$\hat{U}_i = \begin{bmatrix} 1 & \sigma s & 0 & \sigma s \\ 1 & s & 0 & s \\ 0 & 0 & 1 & \sigma s \\ 0 & 0 & 1 & s \end{bmatrix}_i \tag{6}$$

$$\hat{\mathbf{U}}_{[1} = [1 \quad 0 \quad 0 \quad 0] \tag{7}$$

$$\hat{\mathbf{U}}_{n]} = \text{col}\,(0, 0, 1, 1) \tag{8}$$

Mean Square Unperturbed End-to-End Distance. Dimensional properties were obtained using rotational isomeric state theory (Flory, 1974). The appropriate equation for the computation of the mean square unperturbed end-to-end distance, $\langle r^2\rangle_0$, is

$$\langle r^2\rangle_0 = Z^{-1}\mathbf{F}_1{}^n \tag{9}$$

The generator matrices are given by eq 10.

$$\mathbf{F}_i = (\mathbf{U}_i \otimes \mathbf{E}_s)\begin{bmatrix} \mathbf{G}_c & \mathbf{0} \\ \mathbf{0} & \mathbf{G}_h \end{bmatrix}_i \tag{10}$$

The identity matrix of order 5 is $\mathbf{E}_5$, $\mathbf{0}$ represents appropriate rectangular null matrices, and $\mathbf{G}_c$ and $\mathbf{G}_h$ are generator matrices for peptide unit i being in the coil and helix state, respectively. The virtual bond vector $\mathbf{1}$ and its transpose $\mathbf{1}^T$ have an initial element of 3.8 Å followed by two elements of zero

$$\mathbf{G}_{c,i} = \begin{bmatrix} 1 & 2\mathbf{1}^T\langle \mathbf{T}_c \rangle & l^2 \\ \mathbf{0} & \langle \mathbf{T}_c \rangle & \mathbf{1} \\ 0 & \mathbf{0} & 1 \end{bmatrix}_i \quad (11)$$

$$\mathbf{G}_{h,i} = \begin{bmatrix} 1 & 2\mathbf{1}^T\mathbf{T}_h & l^2 \\ \mathbf{0} & \mathbf{T}_h & \mathbf{1} \\ 0 & \mathbf{0} & 1 \end{bmatrix}_i \quad (12)$$

(Brant and Flory, 1965). The transformation matrix for the α helix is $\mathbf{T}_h$, and the average transformation matrices for residues in the random coil state are denoted by $\langle \mathbf{T}_c \rangle$. Terminal matrices are defined in eq 13 and 14.

$$\mathbf{F}_{[1} = [1 \quad 0 \quad \ldots\ldots\ldots \quad 0] \quad (13)$$

$$\mathbf{F}_{n]} = \text{col}\,(l^2, 1, 1, l^2, 1, 1) \quad (14)$$

Mean Square Unperturbed Radius of Gyration. The mean square unperturbed radius of gyration, $\langle R^2 \rangle_0$, was obtained using eq 15–20, which are analogous to eq 9–14 (Flory, 1974).

$$\langle R^2 \rangle_0 = Z^{-1}\,\mathbf{A}_1^{(n)} \quad (15)$$

$$\mathbf{A}_i = (\mathbf{U}_i \otimes \mathbf{E}_7) \begin{bmatrix} \mathbf{B}_c & \mathbf{0} \\ \mathbf{0} & \mathbf{B}_h \end{bmatrix}_i \quad (16)$$

$$\mathbf{B}_{c,i} = \begin{bmatrix} 1 & 1 & 2\mathbf{1}^T\langle \mathbf{T}_c \rangle & l^2 & l^2 \\ 0 & 1 & 2\mathbf{1}^T\langle \mathbf{T}_c \rangle & l^2 & l^2 \\ \mathbf{0} & \mathbf{0} & \langle \mathbf{T}_c \rangle & \mathbf{1} & \mathbf{1} \\ 0 & 0 & \mathbf{0} & 1 & 1 \\ 0 & 0 & \mathbf{0} & 0 & 1 \end{bmatrix}_i \quad (17)$$

$$\mathbf{B}_{h,i} = \begin{bmatrix} 1 & 1 & 2\mathbf{1}^T\mathbf{T}_h & l^2 & l^2 \\ 0 & 1 & 2\mathbf{1}^T\mathbf{T}_h & l^2 & l^2 \\ \mathbf{0} & \mathbf{0} & \mathbf{T}_h & \mathbf{1} & \mathbf{1} \\ 0 & 0 & \mathbf{0} & 1 & 1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}_i \quad (18)$$

$$\mathbf{A}_{[1} = [1 \quad 0 \quad \ldots\ldots\ldots \quad 0] \quad (19)$$

$$\mathbf{A}_{n]} = \text{col}\,(l^2, l^2, 1, 1, 1, l^2, l^2, 1, 1, 1) \quad (20)$$

Transformation Matrices. The transformation matrix for the α helix was calculated using $\varphi = 133°$, $\psi = 122.8°$, and 70.8° for the supplement of the N-C$^\alpha$-C′ bond angle (Ramachandran and Sasisekharan, 1968). The averaged transformation matrix appropriate for an amino acid residue in the coil state, and followed by a residue other than prolyl or hydroxyprolyl, is one of the following:

$$\mathbf{T}_h = \begin{bmatrix} 0.020 & -0.424 & -0.905 \\ -0.425 & 0.816 & -0.391 \\ 0.905 & 0.393 & -0.164 \end{bmatrix} \quad (21)$$

$$\langle \mathbf{T}_c \rangle \text{Ala} = \begin{bmatrix} 0.51 & 0.20 & 0.59 \\ -0.046 & -0.61 & 0.21 \\ 0.65 & -0.23 & -0.30 \end{bmatrix} \quad (22)$$

$$\langle \mathbf{T}_c \rangle \text{Gly} = \begin{bmatrix} 0.36 & -0.077 & 0 \\ -0.092 & -0.037 & 0 \\ 0 & 0 & -0.012 \end{bmatrix} \quad (23)$$

$$\langle \mathbf{T}_c \rangle \text{Pro} = \begin{bmatrix} 0.593 & 0.316 & -0.001 \\ 0.117 & -0.503 & -0.003 \\ 0.001 & 0.007 & -0.315 \end{bmatrix} \quad (24)$$

Results for the glycyl and alanyl residues are from Brant et al. (1967), while that for the prolyl residue is based on unpublished work by T. Ooi. The alanyl residue transformation matrix was used for all residues except prolyl, hydroxyprolyl, and glycyl. When a residue is followed by either a prolyl or hydroxyprolyl residue, the matrices shown below are used instead:

$$\langle \mathbf{T}_c \rangle \text{Ala–Pro} = \begin{bmatrix} 0.50 & 0.14 & 0.81 \\ -0.031 & -0.73 & 0.098 \\ 0.67 & -0.084 & -0.32 \end{bmatrix} \quad (25)$$

$$\langle \mathbf{T}_c \rangle \text{Gly–Pro} = \begin{bmatrix} 0.47 & 0.20 & 0 \\ 0.019 & -0.0021 & 0 \\ 0 & 0 & 0.038 \end{bmatrix} \quad (26)$$

$$\langle \mathbf{T}_c \rangle \text{Pro–Pro} = \begin{bmatrix} 0.423 & 0.474 & 0.548 \\ -0.589 & -0.221 & 0.582 \\ 0.640 & -0.667 & 0.186 \end{bmatrix} \quad (27)$$

The matrices in eq 25 and 26 were obtained by Schimmel and Flory (1968) and eq 27 from Mattice et al. (1973).

The transformation matrix in eq 22 is in accord with the unperturbed dimensions of several homopolypeptides containing CH_2R side chains (Brant and Flory, 1965; Mattice and Lo, 1972), the dipole moments of 14 small alanine peptides (Flory and Schimmel, 1967), and the NMR coupling constant between the NH and C$^\alpha$H protons of several homopolypeptides (Tonelli and Bovey, 1970). The matrix in eq 23 is in agreement with the dipole moments of small glycine peptides (Flory and Schimmel, 1967). When used in conjunction with eq 22, it produces reasonable agreement with the characteristic ratios of random copolypeptides containing glycine and glutamic acid (Miller et al., 1967). The matrices in eq 23–26 are consistent with the unperturbed dimensions of several sequential copolypeptides (Mattice and Mandelkern, 1971b; unpublished results), and eq 27 produces satisfactory agreement with the unperturbed dimensions of poly(L-proline) (Mattice and Mandelkern, 1971a; Mattice et al., 1973).

Results and Discussion

Circular Dichroism Spectra. Circular dichroism spectra for the proteins examined are shown in Figure 1. The spectra in the absence of sodium dodecyl sulfate are in reasonable agreement with results reported previously for albumin (Timasheff et al., 1967), α-chymotrypsinogen (Greenfield and Fasman, 1969), concanavalin A (McCubbin et al., 1971; Pflumm et al., 1971), elastase (Visser and Blout, 1971), hemoglobin (Visser and Blout, 1971), histone f_2b (D'Anna and Isenberg, 1972), α-lactalbumin (Cowburn et al., 1972), lactate dehydrogenase (Chen et al., 1972), β-lactoglobulin (Timasheff et al., 1967), lysozyme (Cowburn et al., 1972; Mulvey et al., 1973), myoglobin (Holzwarth and Doty, 1965; Greenfield and Fasman, 1969; Chen et al., 1972; Nicola et al., 1975), papain (Chen et al., 1972), and ribonuclease (Greenfield and Fasman, 1969). The result for subtilisin is about two-thirds as intense as that reported by Brown and Schleich (1975). Collagen was examined only in the presence of sodium dodecyl sulfate.

Addition of sodium dodecyl sulfate (0.017 M) modifies the circular dichroic spectra. In several instances (e.g., concanavalin A and histone f_2b) the spectral changes are drastic, while for certain other proteins (e.g., papain) the changes are comparatively minor. Sodium dodecyl sulfate changes the circular dichroism exhibited by concanavalin A from a single minimum near 222 nm to double minima near 204 and 220 nm, a result in harmony with previous work (Kay, 1970). These are the changes anticipated for the disruption of β structure and formation of α helix (Greenfield and Fasman, 1969; Kay, 1970). The circular dichroism of histone f_2b changes from a single minimum near 198 nm to double minima located near 205 and 220 nm, changes suggesting a partial coil–helix transition (Greenfield and Fasman, 1969). Seven other proteins (α-

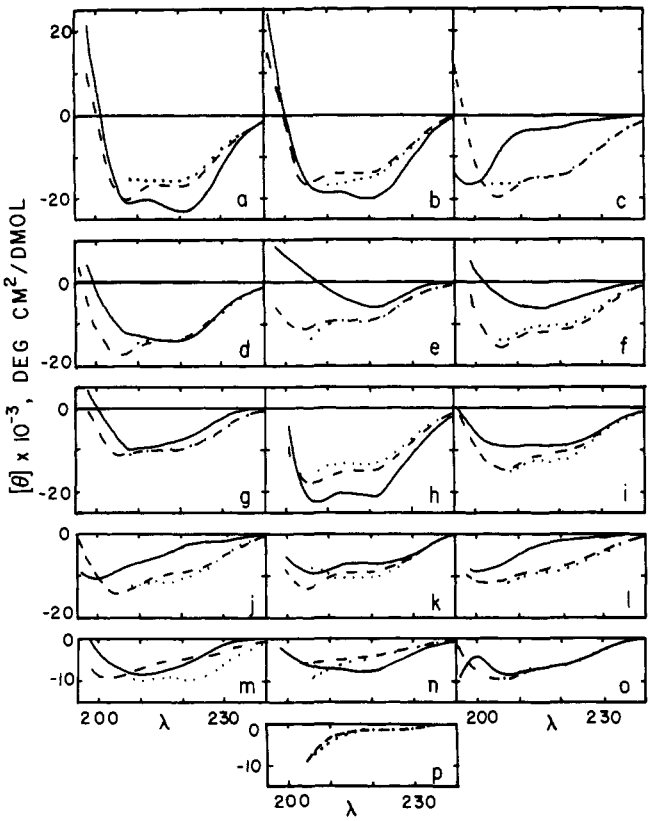

FIGURE 1: Circular dichroism of (a) myoglobin, (b) hemoglobin, (c) histone f_2b, (d) lactate dehydrogenase, (e) concanavalin A, (f) β-lactoglobulin, (g) thermolysin, (h) serum albumin, (i) α-lactalbumin, (j) α-chymotrypsinogen, (k) lysozyme, (l) elastase, (m) ribonuclease, (n) subtilisin, (o) papain, and (p) collagen at 25 °C. Tick marks along the abscissa represent 200, 210, 220, and 230 nm. The solvent for the solid line is 0.05 M sodium phosphate, pH 6.86, for all proteins except histone f_2b, in which case the solvent is water. The dashed line represents data obtained in 0.018 M sodium dodecyl sulfate, 0.05 M sodium phosphate, pH 6.86. The spectra represented by the dotted line were obtained in 0.03 M dithiothreitol, 0.018 M sodium dodecyl sulfate, 0.05 M sodium phosphate, pH 6.86; these solutions had been held at 70 °C for 30 min and then cooled to 25 °C. The protein concentration was generally in the range 0.1–0.2 mg/ml.

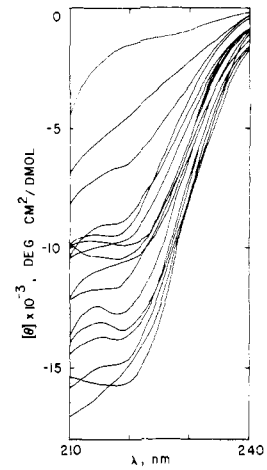

FIGURE 2: Circular dichroism of the 16 proteins in 0.03 M dithiothreitol, 0.018 M sodium dodecyl sulfate, 0.05 M sodium phosphate, pH 6.86, after heating for 30 min at 70 °C and subsequent cooling to 25 °C (from Figure 1).

chymotrypsinogen, elastase, α-lactalbumin, lactate dehydrogenase, β-lactoglobulin, lysozyme, and thermolysin) show an intensification of the circular dichroism in the 205–220 nm range in the presence of the detergent, while four proteins (albumin, hemoglobin, myoglobin, and subtilisin) exhibit a reduction in intensity in this spectral region. An intermediate situation is encountered with ribonuclease A. The variety of responses to sodium dodecyl sulfate is in accord with previous results reported for elastase, lysozyme, ribonuclease A, and hemoglobin (Visser and Blout, 1971).

Addition of dithiothreitol (0.03 M), followed by heating at 70 °C for 30 min and subsequent cooling to 25 °C, produces a major alteration in the circular dichroism obtained with ribonuclease A. With most other proteins the changes range from minor to nonexistent. The presence of dithiothreitol produces a deterioration in the signal to noise ratio near 210 nm, and prevents measurements appreciably below this wavelength.

Figure 2 presents the results obtained for all proteins above 210 nm in the 0.017 M sodium dodecyl sulfate–0.03 M dithiothreitol mixture after heating. The spectra differ markedly in intensity ($[\theta]_{222}$ ranges from −1400 to −15 000 deg cm^2/dmol). In all cases the circular dichroism patterns are those anticipated for a mixture of α helix and random coil (Greenfield and Fasman, 1969), with the amount of helix present ranging up to about 40%. Previous investigations have revealed that the optical activity of protein–sodium dodecyl sulfate complexes is suggestive of the presence of α helix (Meyer and Kauzmann, 1962; Jirgensons, 1966; Reynolds and Tanford, 1970; Visser and Blout, 1971).

Model. The alteration in the optical properties (Meyer and Kauzmann, 1962; Jirgensons, 1966; Reynolds and Tanford, 1970; Visser and Blout, 1971) of proteins that accompanies the formation of the complex containing approximately 1.4 g of sodium dodecyl sulfate/g of protein, as well as the effects of sodium dodecyl sulfate on synthetic homopolypeptides (Fasman et al., 1964; Lotan et al., 1965; Sarkar and Doty, 1966; Grourke and Gibbs, 1967; Satake and Yang, 1973, 1975; Igou et al., 1974; Mattice and Harrison, 1976; McCord et al., submitted), make it desirable to investigate a model that places emphasis on the enhanced tendency of lysyl, arginyl, and histidyl residues to become helical in the presence of the detergent. The important assumptions in the model are as follows.

(a) Disruption of the native tertiary and quaternary structure is accompanied by the exposure of additional binding sites for the detergent. Under the conditions used, the native conformations need not be considered.

(b) The conformational properties of the polypeptide chain in the presence of sodium dodecyl sulfate and a disulfide bond reducing agent are dominated by short-range interactions.

(c) Conformational energy maps for those amino acid residues that are not in helical segments are unaffected by the presence of the detergent.

(d) The parameters σ and s for a particular amino acid residue are unaffected by the detergent unless the amino acid side chain bears a positive charge. Residues with cationic side chains have a substantially greater tendency to become helical (have larger values of s) in the presence of the detergent.

Specification of the conformational energy maps and the σ and s values (see Computations) will now permit the computation of f (eq 5), $\langle r^2 \rangle_0$ (eq 9), and $\langle R^2 \rangle_0$ (eq 15) for proteins of known amino acid sequence.

Computed Helical Contents. Helical contents were computed for the proteins whose circular dichroism is shown in Figure 1 using the statistical weights shown in Table I and the amino acid sequences listed in the Supplementary Material. The helical contents ranged from 0.002 (collagen) to 0.087 (myoglobin), with an average of 0.046. These results are in-

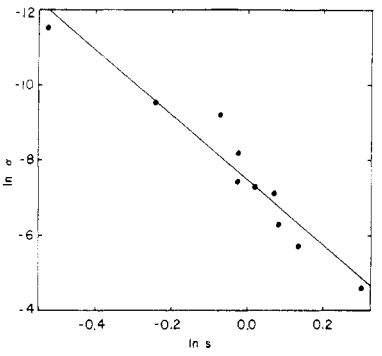

FIGURE 3: Correlation between σ and s for ten amino acid residues in water (see text).

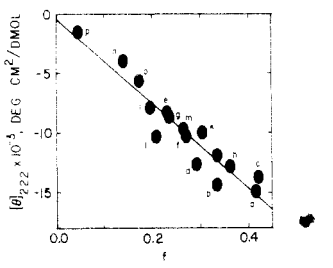

FIGURE 4: Correlation between $[\theta]_{222}$ from Figure 2 and helical content calculated using eq 5. The σ and s values for the arginyl, histidyl, and lysyl residues were 0.05 and 1.7, respectively. Statistical weights for all other amino acid residues were those shown in Table II. Proteins are denoted by the letters used in Figure 1.

compatible with the helical contents suggested by the circular dichroism of nearly all of the reduced proteins in sodium dodecyl sulfate.

In accord with assumption (d) in the description of the model, we now wish to examine the consequences of an increase in the value of s assigned to the arginyl, histidyl, and lysyl residues. The corresponding values of σ were obtained from the empirical correlation between σ and s for the N^5-ω-hydroxypropyl-L-glutaminyl (von Dreele et al., 1971), N^5-ω-hydroxybutyl-L-glutaminyl (von Dreele et al., 1971), glycyl (Ananthanarayanan et al., 1971), alanyl (Platzer et al., 1972), seryl (Hughes et al., 1972), leucyl (Alter et al., 1972), phenylalanyl (Van Wart et al., 1973), valyl (Alter et al., 1973), and the ionized and un-ionized glutamyl (Maxfield et al., 1975) residues in water (Table I, Figure 3, and eq 28).

$$\ln \sigma = -7.58 + 8.63 \ln s \tag{28}$$

Utilization of an s value in the vicinity of 1.7 for the arginyl, histidyl, and lysyl residues produces computed helical contents that range up to ~40%. Figure 4 shows the correlation between the f so obtained and the experimental $[\theta]_{222}$ from Figure 2. A linear relationship is obtained. The root mean square residual for $[\theta]_{222}$ is 1200 deg cm^2/dmol.

Typical values of $[\theta]_{222}$ for the helical forms of poly(L-glutamic acid) and poly(L-lysine) in aqueous solution are $-36\,000 \pm 3000$ deg cm^2/dmol (Adler et al., 1968; Greenfield and Fasman, 1969; Cassim and Yang, 1970). The result obtained from Figure 4 when $f = 1$ is $-36\,200$ deg cm^2/dmol, in excellent agreement with the result anticipated for a completely helical polypeptide in water. The $[\theta]_{222}$ typical of completely ionized poly(L-glutamic acid) and poly(-lysine) in water are in the range 3800 ± 800 deg cm^2/dmol (Adler et al., 1968; Greenfield and Fasman, 1969; Rao and Miller, 1973; Epand et al., 1974). Smaller values are obtained for random coil homopolypeptides that do not bear ionized side chains. The result for poly(N^5-ω-hydroxyethyl-L-glutamine) is about 1000 deg cm^2/dmol (Adler et al., 1968; Mattice et al., 1972), while a result of only about 100 deg cm^2/dmol has been estimated for random coil poly(L-alanine) in water (Mattice and Harrison, 1975). A negative value, -1000 deg cm^2/dmol, is obtained with poly(L-prolylglycine) (Rabenold et al., 1974). The result from Figure 4 when $f = 0$ is -300 deg cm^2/dmol, which is in excellent agreement with the $[\theta]_{222}$ obtained with poly(N^5-ω-hydroxyethyl-L-glutamine) and poly(L-prolylglycine), and the result estimated for poly(L-alanine). The line in Figure 4 provides a reasonable fit to the results obtained with 16 proteins *and* also extrapolates to reasonable values at $f = 0$ and $f = 1$.

The simple adjustment in the statistical weights assigned to the arginyl, histidyl, and lysyl residues permits a quantitative accounting for the circular dichroism of the protein–sodium dodecyl sulfate complexes. This is particularly impressive in view of the fact that results for proteins as diverse as collagen, myoglobin, concanavalin A, and histone f_2b are included in Figure 4. The wide variation in the observed $[\theta]_{222}$ (from -1400 to $-15\,000$ deg cm^2/dmol) is seen to be directly related to the amino acid sequences of the various polypeptide chains.

Utilization of $s = 1.7$, as opposed to the s assigned to the arginyl, histidyl, and lysyl residues in Table I, implies that the free energy change accompanying the propagation of a helical segment by one of these residues is made ~300 cal/mol more negative by the presence of the detergent. Consequently, the size of the interaction free energy required by the model is not unreasonable. The model can accommodate binding of sodium dodecyl sulfate by other amino acid residues provided such binding does not appreciably modify the Zimm–Bragg parameters.

Computed Values of $\langle r^2\rangle_0/\langle R^2\rangle_0$. The hydrodynamic properties of macromolecules are frequently interpreted in terms of a particular shape. Spheres, rods, ellipsoids, and random coils have all proven to be useful models for proteins under appropriate conditions (Tanford, 1961). It is apparent that the appropriate shape when $f = 0$ is a random coil, while a rod is required when $f = 1$. It is not immediately obvious whether either a rod or a random coil is an acceptable model when f assumes an intermediate value. The computed values of $\langle r^2\rangle_0/\langle R^2\rangle_0$ are pertinent at this point. This ratio will be 6 for random coils (Tanford, 1961; Flory, 1969) and 12 for rods (Tanford, 1961) of sufficiently high molecular weight.

Values of $\langle r^2\rangle_0/\langle R^2\rangle_0$ were computed (see Computations) for the 67 proteins listed in the supplementary material, using precisely the same statistical weights which were employed to obtain Figure 4. The computed values ranged from 5.98 (histone f_2a_2) to 6.94 (parvalbumin). The mean was 6.28, and the standard deviation was 0.19. The highest helix content computed (0.503) was for hemerythrin, yet its $\langle r^2\rangle_0/\langle R^2\rangle_0$ was only 6.67. It is apparent that the overall shape of these molecules approximates that of a random coil, as judged by $\langle r^2\rangle_0/\langle R^2\rangle_0$, even though certain of the molecules contain substantial amounts of helix. Consequently, we shall now inquire whether random coils of the computed dimensions are compatible with the results obtained experimentally with protein–sodium dodecyl sulfate complexes.

Relationship between R_s *and* $\langle R^2\rangle_0^{1/2}$. The relationship between the Stokes radius, R_s, and the root mean square radius of gyration, $\langle R^2\rangle^{1/2}$, for a random coil, can be written as

$$R_S = \xi \langle R^2\rangle^{1/2} \tag{29}$$

TABLE II: Ability of Amino Acid Composition to Predict Positioning in log $\langle R^2\rangle_0^{1/2}$ vs. log M.

	Mole Fraction Amino Acid Residue (± Standard Deviation)			
Protein Group	Gly	Pro	Arg, His, Lys	Gly, Arg, His, Lys
Error > 9%	0.067 ± 0.037	0.065 ± 0.049	0.089 ± 0.029	0.157 ± 0.036
9% > error > −9%	0.072 ± 0.030	0.043 ± 0.019	0.139 ± 0.044	0.211 ± 0.052
−9% > error	0.133 ± 0.084	0.062 ± 0.064	0.186 ± 0.049	0.319 ± 0.072

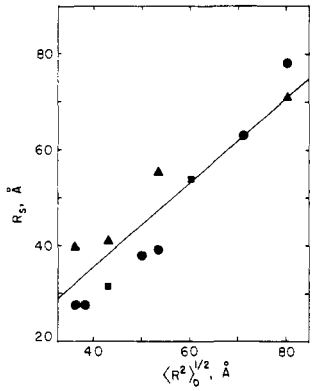

FIGURE 5: Correlation between the Stokes radii for the protein-sodium dodceyl sulfate complexes formed by lysozyme, chymotrypsinogen, hemoglobin, serum albumin, and the γG-immunoglobulin light and heavy chains (circles, from Tanford et al., 1974), β-lactoglobulin and lactate dehydrogenase (squares, from Fish et al., 1970), and lysozyme, β-lactoglobulin, chymotrypsinogen, and serum albumin (triangles, from Wright et al., 1975), and the computed root mean square unperturbed radius of gyration. See text for the details of the computation of $\langle R^2\rangle_0^{1/2}$.

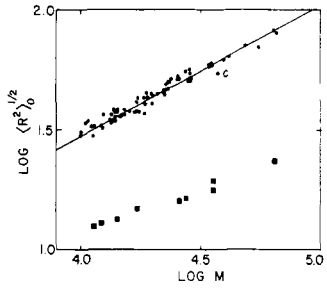

FIGURE 6: Correlation between the computed values of log $\langle R^2\rangle_0^{1/2}$ and log M for the 67 proteins shown in Table II of the Supplementary Material (circles). The straight line was drawn through the results from 45 of the 46 proteins (collagen fragment excepted) with $M > 15\,000$. The point for the collagen fragment is identified by "C". Squares represent results for the radius of gyration described by the shape of nine proteins in the crystalline state. See text for the details of the computation of $\langle R^2\rangle_0^{1/2}$

where ξ is anticipated to be in the range 0.78–0.875 for the viscosity increment (Tanford, 1961). The root mean square radius of gyration is related to its unperturbed value via the expansion factor, α (Flory, 1953). Combination of eq 29 and 30 yields 31.

$$\langle R^2\rangle^{1/2} = \alpha\langle R^2\rangle_0^{1/2} \quad (30)$$

$$R_S = \alpha\xi\langle R^2\rangle_0^{1/2} \quad (31)$$

The value of α for proteins in sodium dodecyl sulfate is not known, but a reasonable estimate can be made. Sodium dodecyl sulfate solutions act as "good solvents" for globular proteins because they induce these proteins to expand from their native structures. This consideration requires that α exceed unity. Since proteins retain some organized structure in sodium dodecyl sulfate, but are completely disorganized in 6 M guanidine hydrochloride (Tanford et al., 1967a,b; Nozaki and Tanford, 1967; Lapanje and Tanford, 1967), the expansion factors in sodium dodecyl sulfate should be smaller than those obtained for proteins in 6 M guanidine hydrochloride. The upper limit for the α obtained experimentally (Lapanje and Tanford, 1967) with ribonuclease A, β-lactoglobulin, chymotrypsinogen, aldolase, and serum albumin in 6 M guanidine hydrochloride containing mercaptoethanol is 1.32–1.35. On this basis, the probable range for the expansion factor appropriate for proteins in sodium dodecyl sulfate is $1.0 < \alpha < 1.3$, and $0.78 < \alpha\xi < 1.14$ is obtained for $\alpha\xi$.

Tanford and co-workers (Fish et al., 1970; Tanford et al., 1974) have tabulated Stokes radii for the sodium dodecyl sulfate complexes formed by 13 proteins. Eight of these proteins (lysozyme, hemoglobin, β-lactoglobulin, γG immunoglobulin light and heavy chains, chymotrypsinogen, lactate dehydrogenase, and serum albumin) are among those for which $\langle R^2\rangle_0$ was computed. Stokes radii can also be calculated from the axes reported for lysozyme, β-lactoglobulin, chymotrypsinogen, and serum albumin by Wright et al. (1975). Figure 5 shows these R_s as a function of the $\langle R^2\rangle_0^{1/2}$, computed using $s = 1.7$ for the arginyl, histidyl, and lysyl residues. The result for hemoglobin represents the average for the separate α and β chains. The straight line, drawn to pass through the origin, corresponds to the equation $R_s = 0.884\langle R^2\rangle_0^{1/2}$. A value of 0.884 for $\alpha\xi$ is within the range anticipated, and would suggest $1.0 < \alpha < 1.2$.

We conclude that the dimensions calculated from the model are in reasonable accord with the results obtained experimentally for the eight proteins shown in Figure 5

Correlation between $\langle R^2\rangle_0^{1/2}$ and Molecular Weight. The correlation between several transport properties (Shapiro et al., 1967; Weber and Osborn, 1969; Dunker and Rueckerts, 1969; Reynolds and Tanford, 1970; Fish et al., 1970; Tanford et al., 1974) and the molecular weight of proteins dissolved in sodium dodecyl sulfate requires that log $\langle R^2\rangle_0^{1/2}$ should be a linear function of log M when the molecular weight exceeds 15 000. Figure 6 shows the computed values of log $\langle R^2\rangle_0^{1/2}$ as a function of log M for the 67 proteins listed in Table II of the Supplementary Material. Computations were performed using $s = 1.7$ for the arginyl, histidyl, and lysyl residues. The straight line was drawn by least squares, using the results for 45 of the 46 proteins whose molecular weight exceeds 15 000. The point for the collagen fragment ($M = 38\,000$) was ignored for reasons which will be discussed below. Figure 6 also contains nine points that represent the dimensions anticipated for globular proteins in their *native* states. These points are the radii of gyration defined by the shape (Mattice and Carpenter, 1976) of parvalbumin (Kretsinger et al., 1972), cytochrome c_2 (Salemme et al., 1973), lysozyme (Blake et al., 1967),

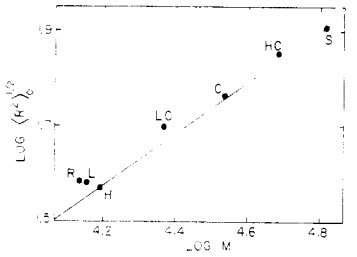

FIGURE 7: Comparison of log $\langle R^2\rangle_0^{1/2}$ and log M for ribonuclease A (R), hen lysozyme (L), hemoglobin (H), γG-immunoglobulin light (LC) and heavy (HC) chains, carboxypeptidase (C), and serum albumin (S). The point for hemoglobin represents the average of results computed for the α and β chains. The straight line is drawn through the results for hemoglobin and serum albumin.

myoglobin (Watson, 1969), elastase (Sawyer et al., 1973), subtilisin (Wright et al., 1969), carboxypeptidase A (Quiocho and Lipscomb, 1971), thermolysin (Matthews et al., 1974), and hemoglobin (Perutz et al., 1968) in the crystalline state. It is evident that the dimensions computed for the protein–sodium dodecyl sulfate complexes considerably exceed those anticipated for globular proteins in their native states.

Figure 6 shows that a linear correlation is obtained between log $\langle R^2\rangle_0^{1/2}$ and log M. A correlation is obtained in spite of the fact that the computed helical contents for the proteins used in this figure ranges from 0.05 to 0.50. This situation is not unexpected. Poly(L-alanine) chains with $f = 0.05$ may have unperturbed dimensions similar to those of completely disordered proteins (Miller and Goebel, 1968) if the helical sequences are short (Miller and Flory, 1966). The zero value of s for the prolyl and hydroxyprolyl residues, coupled with the small s value for the glycyl residue, discourages the formation of long helical segments in the proteins.

The straight line in Figure 6 provides a reasonable fit to the results for those proteins with $M > 30\,000$ (collagen expected). Scatter increases at lower molecular weight. The root mean square % error in the estimation of the molecular weight from the computed $\langle R^2\rangle_0^{1/2}$ and the straight line is 8.5% for the 45 non-collagen proteins with $M > 15\,000$. The corresponding figure for the 21 proteins for which $10\,000 < M < 15\,000$ is 9.8%. Uncertainties of this magnitude are not unusual for the estimation of the molecular weights of proteins from the transport properties of their sodium dodecyl sulfate complexes.

Protein–Sodium Dodecyl Sulfate Complexes with Abnormal Hydrodynamic Behavior. Seven of the proteins for which $\langle R^2\rangle_0$ was computed were among those studied by Shapiro et al. (1967). The computed log $\langle R^2\rangle_0^{1/2}$ vs. log M is shown for these seven proteins in Figure 7. (The point for hemoglobin represents the average of the results for the individual α and β chains.) The straight line drawn by Shapiro et al. (1967) in their Figure 2 passes through the points for serum albumin and hemoglobin. They observed that ribonuclease A and lysozyme deviated from this line, the larger deviation being obtained with ribonuclease A. Both ribonuclease A and lysozyme behaved as though their molecular weight exceeded the true molecular weight. These two proteins exhibit deviations in the same direction in Figure 7. Substantial deviation is also found for the γG immunoglobulin light chain in Figure 7; this protein behaved normally in the results reported by Shapiro et al. (1967).

Collagen peptides (Furthmayr and Timpl, 1971) are found experimentally to exhibit deviations in the opposite direction—their sodium dodecyl sulfate complexes act as though the molecular weight of the collagen peptide is lower than its true molecular weight. The result for the collagen fragment in Figure 6 deviates from the straight line in the appropriate direction, although the effect in Figure 6 is not as large as that seen experimentally.

Histones f_2a_1, f_2a_2, f_2b, and f_3 have been found to behave on polyacrylamide gel electrophoresis in sodium dodecyl sulfate as though their molecular weights are ~15–20% lower than their true molecular weight (Panyim and Chalkley, 1971). The model successfully predicts that the histones should be abnormal in this respect, although the degree of abnormality is somewhat underestimated. The molecular weight of these four histones is underestimated by 5.3–12.0% from their computed $\langle R^2\rangle_0^{1/2}$ and the straight line in Figure 6.

A search was conducted for possible correlations between amino acid composition and the position of a protein relative to the straight line in Figure 6. For this purpose the proteins were divided into three categories, according to the error in the estimation of their molecular weight from $\langle R^2\rangle_0^{1/2}$. The dividing lines for the categories were ±9%, the standard deviation. Results are shown in Table II for the amino acid residues of interest. A high content of either the glycyl residue or the cationic residues tends to cause the molecular weight to be underestimated. A slightly more reliable index is the combined total for the glycyl, arginyl, histidyl, and lysyl residues. This result is consistent with previous computational studies of copolypeptides and proteins. The presence of glycyl residues (Miller et al., 1967; Schimmel and Flory, 1968; Miller and Goebel, 1968; Mattice and Mandelkern, 1971b) and short helices (Miller and Flory, 1966) both reduce $\langle R^2\rangle_0$. While the combined glycyl, arginyl, histidyl, and lysyl content serves as a guide to the behavior of a protein, the size of the standard deviations listed in Table II shows that it is not infallible.

No correlation is found between the prolyl content and the behavior of the proteins.

Other Values for s. Computations were carried out using other values of s for the cationic residues. Values in the range 1.5–1.9 permit a reasonable interpretation of the properties of the protein–sodium dodecyl sulfate complexes. Utilization of identical Zimm–Bragg parameters for the arginyl, histidyl, and lysyl residues is not likely to be correct in detail. However, there seems to be little to be gained at this time by treating the parameters for these three residues as being independently adjustable.

Relationship of the Present Model to Other Models. Hydrodynamic properties of 13 protein–sodium dodecyl sulfate complexes have been interpreted using the Simha shape factor, ν (Reynolds and Tanford, 1970; Fish et al., 1970). The model obtained was a prolate ellipsoid of constant minor axis, with the major axis being 0.74 Å/residue. No known polypeptide helix has a translation per residue of 0.74 Å, but this result would be consistent with an α helix which folds back on itself (Reynolds and Tanford, 1970). The present model differs in that it treats the protein–sodium dodecyl sulfate complex as being flexible, with the overall shape of a random coil, and finds that it is constituted of two well-known structures, the α helix and random coil. Additional features of the present model, which are not shared by the prolate ellipsoid model, are the ability to explain the widely varying circular dichroism exhibited by the protein–sodium dodecyl sulfate complexes and the abnormal transport properties of complexes derived from collagen, ribonuclease A, and certain histones.

Free-boundary electrophoresis (Shirahama et al., 1974) and NMR experiments (Tsujii and Takagi, 1975) have suggested a "necklace" model for the protein–sodium dodecyl sulfate

complex. The polypeptide chain is viewed as flexible, with clusters of sodium dodecyl sulfate appearing along the chain. The "necklace" model shares the feature of chain flexibility with the present model. The complexes are treated as free-draining in the "necklace" model, while they are viewed as nondraining in the present model. Our treatment of the complexes as nondraining is based on two considerations: the computed $\langle r^2\rangle_0/\langle R^2\rangle_0$ are near six and $\langle r^2\rangle_0/M$ is not significantly larger than the result for completely disordered proteins (Miller and Goebel, 1968), which behave as nondraining random coils in concentrated guanidine solutions (Tanford et al., 1967a; Lapanje and Tanford, 1967). We note, however, that the electrophoretic mobilities are consistent with free draining, as has been pointed out by Shirahama et al. (1974). The "necklace" model cannot predict the widely varying circular dichroism exhibited by the protein-sodium dodecyl sulfate complexes nor the abnormal transport properties of certain of these complexes.

Flexibility is also a feature of a model based on transient electric birefringence (Wright et al., 1975). The fast and slow relaxation times were interpreted in terms of a prolate ellipsoid with a high axial ratio. This model cannot account for the variation in the circular dichroism of the protein-sodium dodecyl sulfate complexes, nor does it provide a basis for predicting the abnormal behavior of certain proteins.

None of the models (including the present one) provides a molecular explanation for the ability of diverse proteins to form complexes that contain approximately 1.4 g of sodium dodecyl sulfate/g of protein. The present model places emphasis on the change in conformational properties accompanying the strong interaction of sodium dodecyl sulfate with cationic amino acid residues, an effect which is supported by numerous investigations of synthetic polypeptides (Fasman et al., 1964; Lotan et al., 1965; Sarkar and Doty, 1966; Grourke and Gibbs, 1967; Satake and Yang, 1973, 1975; Igou et al., 1974; Mattice and Harrison, 1976; McCord et al., submitted). Binding of sodium dodecyl sulfate at noncationic sites on the protein is compatible with this model if such binding is not accompanied by changes in the Zimm-Bragg parameters for these noncationic residues.

Supplementary Material Available

Sources of the extinction coefficients and amino acid sequences used will appear following these pages in the microfilm edition of this volume of the journal (7 pages). Ordering information is given on any current masthead page.

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