The Gross Conformation of Protein-Sodium Dodecyl Sulfate Complexes*

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SUMMARY

The interaction of sodium dodecyl sulfate with a wide variety of proteins is characterized by a high binding ratio when the monomer concentration of amphiphile exceeds $5 \times 10^{-4} \text{M}$. This binding ratio on a gram to gram basis is identical for all proteins investigated. The protein portion of the complex contains a high degree of order, and hydrodynamic studies suggest that the complex is a rodlike particle, the length of which varies uniquely with the molecular weight of the protein moiety. These results explain the empirical observation that proteins dissolved in aqueous solutions containing high concentrations of sodium dodecyl sulfate have electrophoretic mobilities on polyacrylamide gels which are a unique function of their molecular weights. In addition, the data suggest a possible model for the conformation of membrane proteins and their interactions with phospholipid.

The interaction of proteins with amphiphiles containing hydrocarbon tails with 12 or more carbon atoms usually leads to a conformational change in the protein moiety. Specifically, the amphiphile, sodium dodecyl sulfate, induces alterations in protein structure at monomer concentrations of $1 \times 10^{-4} \text{M}$. Recently, we have demonstrated that a wide variety of reduced proteins bind identical amounts of SDS on a gram to gram basis when the equilibrium monomer concentration of amphiphile is greater than $5 \times 10^{-4} \text{M}$ (2). A saturated complex with a stoichiometry of 0.4 g of SDS per g of protein is formed between 5 and $8 \times 10^{-4} \text{M}$ SDS monomer, and a second complex which is saturated at 1.4 g of SDS per g of protein is observed above $8 \times 10^{-4} \text{M}$ SDS monomer. This nonspecificity of SDS toward proteins derived from a multitude of sources and having very different "native" states suggests a binding-induced conformational change in the polypeptide chain which leads to a uniform structure in the complexed state. We have studied the hydrodynamic shape and size of these protein-SDS complexes by means of intrinsic viscosity and compared the conformational states of the protein in its altered form using optical rotatory dispersion.

Since all amphiphilic compounds have properties in common which are the result of their containing both a polar head group and a hydrophobic tail on the same molecule, it may be possible to extend the results of these studies to other amphiphile-protein systems. In particular, the SDS-protein complex may be a reasonable model system for biological membranes in which the amphiphilic species is primarily phospholipid. The interaction of lipids with some membrane proteins has been shown to be primarily hydrophobic (3, 4) just as the SDS-protein complex is the result of primarily hydrophobic interactions (2). However, the conformational state of the membrane "structural" protein is still a highly controversial subject, and the exact location of the protein within the biological membrane is not known (5). The studies reported here may be used as a basis for tentative suggestions on this subject.

EXPERIMENTAL PROCEDURE

Materials—The proteins used and their sources are shown in Table I. Phosphate buffers were prepared from Baker analytical grade Na$_2$HPO$_4$·7H$_2$O and NaH$_2$PO$_4$·H$_2$O. Sodium dodecyl sulfate was a highly pure grade obtained from Mann. Guanidine hydrochloride from Huco, Delaware Water Gap, Pennsylvania was used without further purification.

Preparation of Protein-SDS Complexes—Protein was dissolved in 6 M GuHCl and 0.1% β-mercaptoethanol to obtain polypeptide chains in the random coil conformation (9). The GuHCl was then removed by dialysis against H$_2$O containing a reducing agent, SDS, β-mercaptoethanol, and the appropriate buffer were dialyzed into the bag containing the protein solution. The amount of bound SDS and the concentration of protein were determined as described previously (2). It has been demonstrated that the same final binding ratio is reached when the complex is obtained by the above procedure as when it is formed by treating the native protein directly with SDS and β-mercaptoethanol. Protein-SDS complexes were formed by both procedures and compared in the experiments reported here.

Methods—Intrinsic viscosities were determined in Cannon-Manning semimicro viscometers immersed in a water bath thermostated to ±0.005°C. Solvent flow times ranged from 250 to 300 sec. Optical rotatory dispersion measurements were carried
### Table I

<table>
<thead>
<tr>
<th>Protein</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome c</td>
<td>Sigma</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Pentex</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Gift of Dr. J. Steinhardt, Georgetown</td>
</tr>
<tr>
<td></td>
<td>University, Washington, D.C.</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>Gift of Dr. R. Townend, Brandeis University</td>
</tr>
<tr>
<td></td>
<td>Waltham, Massachusetts</td>
</tr>
<tr>
<td>F1-histone, F2alpha-histone</td>
<td>Gift of Dr. K. McCarty, Duke University</td>
</tr>
<tr>
<td></td>
<td>Durham, North Carolina</td>
</tr>
<tr>
<td>Chymotrypsinogen</td>
<td>Sigma</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Worthington</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>Sigma</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Gift of Dr. M. Schlesinger, Washington</td>
</tr>
<tr>
<td></td>
<td>University, St. Louis, Missouri</td>
</tr>
<tr>
<td>Rabbit γ-G, heavy chain</td>
<td>Prepared by method of Levy and Sober (6)</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>Nutritional Biochemicals</td>
</tr>
<tr>
<td>Myosin, rabbit muscle</td>
<td>Prepared by the method of Perry (7)</td>
</tr>
<tr>
<td>Erythrocyte ghost proteins</td>
<td>Prepared by the method of Dodge, Mitchell,</td>
</tr>
<tr>
<td></td>
<td>and Hanahan (8)</td>
</tr>
</tbody>
</table>

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**RESULTS**

**Hydrodynamic Properties of Protein-SDS Complexes**—The interaction of SDS with 17 different reduced proteins of molecular weight 12,400 to 200,000 has been reported elsewhere (2). The degree of binding is dependent only on the SDS monomer concentration and is independent of the number or size of the micelles present. At monomer concentrations above $5 \times 10^{-4}$ M, two saturation levels of binding are observed, one at 0.4 g of SDS per g of protein, and a second at 1.4 g of SDS per g of protein.

The intrinsic viscosities, $[\eta]$, of seven of these protein-SDS complexes have been measured at both saturation levels of binding. Fig. 1 shows representative data for three proteins from which $[\eta]$ is obtained by extrapolation of the reduced viscosity, $\eta_{sp}/c$, to $c = 0$. We have no immediate explanation for the negative slopes that were found with most of the complexes except the possibility of aggregation to a more symmetrical particle at increased concentrations. This hypothesis is currently being investigated by methods of low angle x-ray scattering, the results of which will be published elsewhere.

For perfect, unsolvated spheres the intrinsic viscosity is independent of the molecular weight or chain length. However, for more extended homopolymers (10)

$$[\eta] = KM^n = K'n^x$$

where $K$, $K'$, and $x$ are constants, $M$ is the molecular weight, and $n$ is the number of residues per chain. The exponent, $x$, in Equation 1 varies between 0.5 and 0.8 for random coils. Values of $x$ above 1.0 indicate rodlike particles of roughly constant diameter with the length proportional to the molecular weight. The simplest model is a prolate ellipsoid, with constant minor axis, $b$, and major axis, $a$, proportional to $M$. For this model, $x$ is a slowly increasing function of the ratio $a:b$, from 1.4 for short, thick ellipsoids to about 1.8 for long thin ellipsoids. Similar values of $x$ are observed for other models used to represent rodlike particles (11). The value of $x$ decreases for rods that are not entirely rigid. For example, DNA of relatively low molecular weight (up to $2 \times 10^6$) has an $x$ value of 1.32 (12), although the ratio $a:b$ is very large.

The log of the intrinsic viscosity of protein-SDS complexes is an approximately linear function of log $n$ (Fig. 2). The slope for both sizes of complex is 1.2, and it can be concluded, therefore, that all protein polypeptide chains assume a similar, rodlike shape when complexed with this amphiphile. The function relating hydrodynamic properties to chain length must be the same for all these complexes. Studies of the protein-SDS complex by electron microscopy are underway, and preliminary results confirm the rodlike model.
To obtain some idea of the possible dimensions, we have interpreted the results in terms of a prolate ellipsoidal model, without assuming a constant value for the minor axis, \( b \).

The intrinsic viscosity is related to the hydrodynamic volume of a particle through the following equation (10):

\[
[s] = \frac{\pi}{6} (\bar{v}_{1} + \bar{v}_{2} + \bar{v}_{3})
\]

where \( \bar{v} \) is the Simha shape factor, \( \bar{v}_{i} = \frac{\text{specific volume of protein}}{\text{specific volume of SDS per g of protein}} \).

Assuming a prolate ellipsoid, it is now possible to obtain the axial ratio using the Simha relationship (10) between \( v \) and \( a \):

\[
[\text{SDS}]/[\text{H}_{2}O] = \frac{82}{81} = \frac{1.4 \text{ g of SDS per g of protein}}{1.34 \text{ g of } \text{H}_{2}O \text{ per g of protein}}
\]

The following values of these parameters were used together with the experimentally determined intrinsic viscosity to calculate \( v \):

\[
\bar{v}_{2} = 0.725
\]

\[
\bar{v}_{1} = 0.9 \text{ at } 1.4 \text{ g of SDS per g of protein} \text{ and } 0.2 \text{ at } 0.4 \text{ g of SDS per g of protein}^2
\]

\[
\bar{v}_{3}^0 = 1
\]

\[
\bar{v}_{3} = 1.4 \text{ g of SDS per g of protein} \text{ or } 0.4 \text{ g of SDS per g of protein}^2
\]

The calculated values of \( v, a, \) and \( \bar{b} \) are given in Tables II and III for the complexes containing 0.1 and 1.4 g of SDS per g of protein. The minor axis, \( b \), is seen to be approximately constant, about 14 A and 18 A, respectively, for the two classes of complex. When the major axis, \( a \), is plotted against molecular weight, an essentially linear relation is obtained. The rod length (2\( a \)) is about 0.61 A per amino acid residue for the 0.4 g of SDS per g of protein complex, and about 0.74 A for the 1.4 g of SDS per g of protein complex.

The hydration of globular proteins is frequently chosen in a rather arbitrary fashion as 0.2 g of \text{H}_{2}O per g of protein. However, the hydration of SDS micelles at ionic strengths 0.03 to 0.50 has been measured directly by intrinsic viscosity (J. A. Reynolds, unpublished results).

### Table II

<table>
<thead>
<tr>
<th>Protein</th>
<th>SDS content per g of protein</th>
<th>( [\text{SDS}] / [\text{H}_{2}O] )</th>
<th>Shape factor, ( v )</th>
<th>Ellipsoid axes ( a/\bar{b} )</th>
<th>Stokes radius, ( R_s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome c</td>
<td>11,700</td>
<td>11.0</td>
<td>7.2</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>14,400</td>
<td>9.7</td>
<td>3.3</td>
<td>0.6</td>
<td>1.4</td>
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<tr>
<td>Hemoglobin</td>
<td>15,950</td>
<td>8.5</td>
<td>7.2</td>
<td>0.5</td>
<td>1.4</td>
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<tr>
<td>( \beta )-Lactoglobulin</td>
<td>18,400</td>
<td>10.7</td>
<td>6.1</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Myosin light</td>
<td>20,000</td>
<td>14.0</td>
<td>6.1</td>
<td>0.5</td>
<td>1.4</td>
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<tr>
<td>( F_1 ) histone</td>
<td>23,000</td>
<td>14.5</td>
<td>6.1</td>
<td>0.5</td>
<td>1.4</td>
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<tr>
<td>Chymotrypsinogen</td>
<td>25,700</td>
<td>15.8</td>
<td>6.1</td>
<td>0.5</td>
<td>1.4</td>
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<tr>
<td>Lactate dehydrogenase</td>
<td>35,000</td>
<td>28.4</td>
<td>6.1</td>
<td>0.5</td>
<td>1.4</td>
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<td>Ovalbumin</td>
<td>43,000</td>
<td>33.5</td>
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<td>0.5</td>
<td>1.4</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>43,000</td>
<td>33.5</td>
<td>6.1</td>
<td>0.5</td>
<td>1.4</td>
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<tr>
<td>( \gamma )-G heavy</td>
<td>49,500</td>
<td>37.5</td>
<td>6.1</td>
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<td>Bovine serum albumin</td>
<td>69,000</td>
<td>54.2</td>
<td>6.1</td>
<td>0.5</td>
<td>1.4</td>
</tr>
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</table>

### Table III

<table>
<thead>
<tr>
<th>Protein</th>
<th>SDS content per g of protein</th>
<th>( [\text{SDS}] / [\text{H}_{2}O] )</th>
<th>Shape factor, ( v )</th>
<th>Ellipsoid axes ( a/\bar{b} )</th>
<th>Stokes radius, ( R_s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>14,400</td>
<td>4.2</td>
<td>3.2</td>
<td>0.5</td>
<td>1.4</td>
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<tr>
<td>Chymotrypsinogen</td>
<td>25,700</td>
<td>7.2</td>
<td>5.0</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>43,000</td>
<td>13.7</td>
<td>7.0</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>69,000</td>
<td>30.0</td>
<td>23.4</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Myosin</td>
<td>205,000</td>
<td>94.8</td>
<td>74.0</td>
<td>0.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

### Footnotes

1. Based on an assumed hydration of 0.9 g of \text{H}_{2}O per g of protein. Changes in assumed hydration would alter \( b \) (it would be about 4 A less for zero hydration, for example) but have no significant effect on \( a \).
2. Values in parentheses were determined indirectly from gel chromatography data described in the following paper (14). All viscosity values are in terms of cubic centimeter per g of dry protein.
3. The value of \( R_s \) applicable to viscosity measurements would not be exactly the same as the value applicable to frictional coefficients. An equivalent sphere model is not adequate for the detailed treatment of hydrodynamic properties.

The intrinsic viscosity of nonspherical particles can also be treated with an "equivalent sphere" model.

\[
[s] = 2.5 N/M(2\pi R_s^2)
\]
Conformation of Protein-SDS Complexes

Vol. 245, No. 19

parameter, \( R_v \), is sometimes called the Stokes radius.) The use of Equation 4 makes it possible to compare the results of viscosity and gel chromatography. All existing theories of gel chromatography are expressed in terms of \( R_v \), and we have used this experimental technique to obtain \( R_v \) values for several protein-SDS complexes for which viscosity data were not directly determined. \( R_v \) from gel chromatography has been converted to intrinsic viscosities using Equation 4, and \( v \), \( a \), and \( b \) calculated as before. These results are also given in Table II and are seen to be entirely consistent with the results obtained by direct intrinsic viscosity measurements. (Experimental and theoretical details of the gel chromatography results are given in the following paper (14).)

It is of interest to compare the rod lengths obtained for SDS-protein complexes with the lengths of native tropomyosin and paramyosin. These proteins consist of two \( \alpha \) helices, side by side, and twisted slightly about one another. Molecular weights and intrinsic viscosities have been measured by Lowey, Kucera, and Holtzer (15), Holtzer, Clark, and Lowey (16), and Olander, Emerson, and Holtzer (17). If we treat their data in terms of a prolate ellipsoid model, assuming hydration of 0.2 g per g of protein (although again hydration has little effect on the result) we obtain \( b = 11.4 \) A and \( a = 219 \) A for paramyosin and \( b = 11.3 \) A and \( a = 634 \) A for tropomyosin. These dimensions are very close to those calculated in the original papers by quite different procedures. The lengths correspond to 0.66 A per residue and 0.72 A per residue for tropomyosin and paramyosin, respectively. This comparison shows that a model consisting of a helical polypeptide chain, folded back upon itself near its middle to give a double helical rod, with the SDS forming a shell about the rod, would be consistent with the hydrodynamic data. Of course, there is no reason to believe that this is the actual structure of the complexes. In fact, the ORD data cited below suggest that the helix content of the complexes is less than 100%. Regardless of what the actual structure may be, the overall particle length is established as being about half the length of a fully extended \( \alpha \) helix.

Optical Rotatory Dispersion---Fig. 3 shows ORD spectra of protein-SDS complexes in the wave length range 2250 to 2600 A at binding levels of 1.4 g of SDS per g of protein. The protein is clearly not in a random coil conformation, since in that case the spectra would be a smooth curve of much lower magnitude, such as is observed when proteins are dissolved in 6 M GuHCl (9). The qualitative similarity between all the complexes is striking, especially when one keeps in mind that the "native" ORD spectra of these proteins are quite different, both from each other and from that of the corresponding SDS complex.

The effect of the amount of bound SDS on the ORD of myosin is shown in Fig. 4. The overall shape of the spectra is the same, but the magnitude of levorotation decreases at the higher level of SDS binding. This same phenomenon has been observed for lysozyme, \( \beta \)-lactoglobulin, and chymotrypsinogen in SDS.

All of the spectra have troughs near 2380 A and, in general, resemble spectra for polypeptide chains in the helical conformation. However, the magnitude of rotation is considerably less than the reported values for polypeptides or proteins that have been examined under conditions where they are known to be 100% helical. This could be accounted for in part by the fact that the helices in protein-SDS complexes might exist within a hydrophobic shell formed by the SDS; a medium of low dielectric constant is known to depress the magnitude of rotation somewhat (18), but the difference in magnitude between different proteins could not be accounted for in this way.

4 Both proteins were treated as cylindrical rods of 10 A radius. The corresponding rod lengths \( (2a) \) are given as 490 and 1330 A, respectively, for tropomyosin and paramyosin.
DISCUSSION

Application to Ultrastructure of Biological Membranes—Some functionally defined proteins, such as cytochrome c and ATPase from Streptococcus faecalis, are bound tightly to biological membranes but are apparently external to the bimolecular lipid leaflet (19, 20). The interaction between these proteins and the lipid is thought to be ionic in the case of cytochrome c and through a ternary Mg+2 complex in the case of the bacterial ATPase. However, there are polypeptide chains which are extremely difficult to dissociate from membrane lipid and are probably intimately associated with the lipid through primarily hydrophobic forces (3, 5). It is this latter group of proteins for which the protein-SDS complex may be a reasonable model. If the interaction of amphiphilic lipid with some specific membrane proteins parallels that of SDS, one can postulate an extended, ordered polypeptide chain associated with the hydrophobic regions of the bimolecular leaflet. The charged or polar groups (or both) on this chain would be expected to interact with the lipid phosphate head groups and be exposed to the aqueous media which bathes the membrane in vivo. It should be noted particularly that the use of the SDS-protein complex as a model system for the membrane structure precludes the possibility of the protein being in either a random coil or globular form when it is associated with lipid.

SDS Gel Electrophoresis—The recently developed method of determining the molecular weight of polypeptide chains in SDS on polyacrylamide gels (21, 22) has been empirically successful, but has thus far been without theoretical basis. The electrophoretic mobility of polypeptide chains in these gels can be a unique function of molecular weight only when the following criteria are met. (a) The charge per unit mass must be approximately constant. (b) The hydrodynamic properties must be a function of molecular length only. The high level of binding of SDS to proteins and the constant binding ratio on a gram to gram basis under the experimental conditions used in polyacrylamide gel electrophoresis assure a constant charge per unit mass. In addition, the data in this paper show that the hydrodynamic properties of protein-SDS complexes are a unique function of the polypeptide chain length.

Acknowledgment—We wish to express our gratitude to Dr. Wayne W. Fish for many helpful discussions and suggestions during the course of this work.

REFERENCES

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