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Binding of Dodecyl Sulfate to Proteins at High Binding Ratios. Possible Implications for the State of Proteins in Biological Membranes*

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Abstract. A wide variety of proteins have been shown to bind identical amounts of an amphiphile, sodium dodecyl sulfate, on a gram per gram basis at monomer equilibrium concentrations above 0.5 mM. The binding is independent of ionic strength and primarily hydrophobic in nature. Only the monomeric form of the amphiphile binds to proteins, not the micellar form. The application of these results to models for biological membranes and to gel electrophoresis in sodium dodecyl sulfate is discussed.

The molecular structure of biological membranes is not known in detail, and, more specifically, the mode of interaction between the two major chemical components—lipid and protein—has been the subject of great controversy. Morphological investigations by electron microscopy and x-ray diffraction have led to a widely accepted model for the organization of lipid within the membrane in which the polar head groups are aligned in two parallel layers with the hydrophobic tails forming the inside of a "sandwich" (commonly referred to as a bimolecular leaflet[1]). A second, less widely accepted, model proposes that globular, lipid-protein complexes aggregate in a linear array to form the membrane.[2] Proponents of the bimolecular leaflet model propose an extended polypeptide chain strung out along the outside of the lipid bilayer and interacting through ionic groups with the phosphate moiety. The subunit model requires hydrophobic bonding between lipid and protein and suggests that the protein is interior to the phosphate head groups. Minor variations of these extreme models have been proposed.[3]

It is apparent that one of the most important pieces of information necessary for defining the molecular structure of biological membranes is the nature of the binding forces in the lipid-protein complex. Ji and Benson[4] report hydrophobic interactions between spinach chloroplast protein and a variety of phospholipids. Braun and Radin[5] suggest ionic interaction between myelin proteins and anionic lipids and hydrophobic interaction between myelin proteins and nonionic or zwitterionic lipids.

The fact that amphiphilic compounds other than lipids interact primarily through hydrophobic forces with "native" proteins from a variety of sources has been well substantiated.[6] However, the weight ratio of amphiphile to protein in

membranes is much larger than that reported for the complex systems previously investigated (e.g., serum albumin-sodium dodecyl sulfate).

It is the purpose of this investigation to determine the nature of the interaction forces between proteins and a model amphiphile at binding levels similar to those found in biological membranes, and to study the specificity of the amphiphile for widely differing protein species. Sodium dodecyl sulfate (SDS) is used as a model system since its properties in aqueous solution are well understood and the variation of monomer and aggregate concentrations with such external parameters as ionic strength and temperature is known precisely.

Materials and Methods. Proteins were obtained from the following sources: bovine serum albumin, Pentex Corp. and Nutritional Biochemicals; chymotrypsinogen and ovalbumin, Sigma Chemical; lysozyme, Boehringer Chemical Co.; myosin, prepared from rabbit muscle by the method of Perry;[7,8] F1 and F2al Histone, gift from Dr. K. McCarty; horse ferrihemoglobin, gift from Dr. J. Steinhardt; erythrocyte ghost proteins, prepared by the method of Dodge *et al.*[9] Phosphate buffers were prepared from Baker analytical grade $Na_2HPO_4 \cdot 7H_2O$ and $NaH_2PO_4 \cdot H_2O$. Sodium dodecyl sulfate was a highly pure grade obtained from Mann Research.

Equilibrium dialysis of proteins was performed at 20°C in phosphate buffer at a variety of ionic strengths and at pH values ranging from 5.6 to 7.2. In most experiments protein (0.5–12 mg/ml) was dissolved in 6 M guanidine hydrochloride (GuHCl) and 0.1% beta-mercaptoethanol to obtain the polypeptide chain in a random coil conformation.[10] The GuHCl was then removed by dialysis against H_2O with reducing agent present and SDS dialyzed into the bag containing the protein solution. Dialysis against SDS and beta-mercaptoethanol was continued until equilibrium was reached as determined by analysis of SDS concentration within the bag as a function of time. The dialysate was changed daily to minimize the effects of hydrolysis of the alkyl sulfate. Reversibility was determined with ovalbumin, myosin, lysozyme, and bovine serum albumin by the addition of an excess of SDS to the denatured protein and subsequent dialysis to equilibrium. Binding ratios for bovine serum albumin and hemoglobin were also obtained by dialyzing the native protein against SDS and beta-mercaptoethanol thus omitting the GuHCl step.

Sodium dodecyl sulfate concentrations in the dialysate and the protein solution were determined by extraction of an alkyl sulfate-methylene blue complex into chloroform and reading the optical density of the chloroform at 6550 Å.[11,12] The previously published procedure was modified in this work by using 20 ml $CHCl_3$, 5 ml methylene blue, and 0.05 ml sample. Calibration curves were run using standard solutions of sodium dodecyl sulfate of the appropriate concentrations. It was found that in our system the above experimental procedure quantitatively removed the bound SDS from the protein. The reproducibility of this analytical method at alkyl sulfate concentrations between 0.5 and 5 mM is ± 20 μM. For protein concentrations of 1.4 g/liter this represents a possible error of $\pm 5\%$ in a measurement of g SDS bound/g protein.

Protein-SDS complex concentrations were determined from dry weights of the dialysate and the protein solution. Since the solutions contain inorganic phosphates which are normally hydrated, it was necessary to ascertain whether or not the drying procedure quantitatively removed the water of hydration from the buffer salts. Monobasic and dibasic sodium phosphate hydrates and SDS were weighed accurately on an analytical balance and dissolved in an appropriate volume of H_2O. Dry weights were then determined on aliquots of this solution. The calculated dry weight based on formula weight in one representative control experiment was 28.55 g/liter, the experimental value was 28.64 g/liter.

Results. In aqueous solution SDS can exist as monomers and as micellar aggregates, the concentration of each depending upon the total SDS concen-

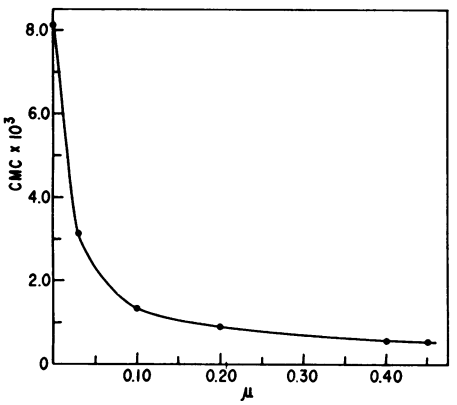

FIG. 1.—Ionic strength dependence of the critical micelle concentration (CMC).

tration, the ionic strength, and the temperature. An important property of this system is that increasing the total concentration at a given ionic strength will not result in a measurable increase in monomer concentration above a specific critical value, the critical micelle concentration. Above this concentration each new SDS molecule added to the system is incorporated into a micelle. The critical micelle concentration decreases with increasing ionic strength at constant temperature (Fig. 1).[13] In this system it is possible to vary the monomer and micellar composition by simply altering the ionic strength. Thus, the interaction of both SDS species with a protein can be studied.

Table 1 shows the binding data for SDS to a variety of proteins. The ionic

TABLE 1. *Binding of sodium dodecyl sulfate to proteins.*

		Equilibrium SDS Concentration (mM)					
Protein	μ	Total	Monomer	Monomers in micellar form	g SDS / g P	pH	Mol. wt.
Histone F2al	0.13	3.72	1.20	2.52	1.33	7.2	12,400
Lysozyme	0.26	3.51	0.80	2.71	1.39	7.2	14,400
	0.52	4.30	0.50	3.80	0.40*	7.2	...
Hb^+	0.13	0.50	0.50	0	0.37†	6.8	16,600
	0.13	2.67	1.20	1.47	1.4	7.2	...
Chymotrypsinogen	0.26	3.51	0.80	2.71	1.40	7.2	25,700
	0.52	4.09	0.50	3.59	0.40	7.2	...
Histone F1	0.13	3.72	1.20	2.52	1.42	7.2	23,500
Ovalbumin	0.26	3.27	0.80	2.47	1.46*	7.2	43,000
	0.52	4.60	0.50	4.10	0.37	7.2	...
Bovine serum albumin	0.005	3.58	3.58	0	1.35†	6.7	69,000
	0.033	0.632	0.632	0	0.44*	5.6	...
	0.52	4.48	0.50	3.98	0.36	7.2	...
Myosin	0.26	3.72	0.80	2.92	1.41*	7.2	220,000
Erythrocyte ghost proteins	0.13	3.40	1.20	2.20	1.40	7.2	

* Reversibility determined on these proteins.
† GuHCl step omitted.

strength has been varied from 0.005 to 0.52 and the total equilibrium concentration of SDS ranges from 0.5 to 4.6 mM. The equilibrium monomer concentration, based on the ionic strength dependence of the critical micelle concentration, has been varied between 0.5 and 3.58 mM. It is important to note that increasing the total concentration or the micellar concentration does not result in an increase in the binding ratio (bovine serum albumin, chymotrypsinogen, oval-

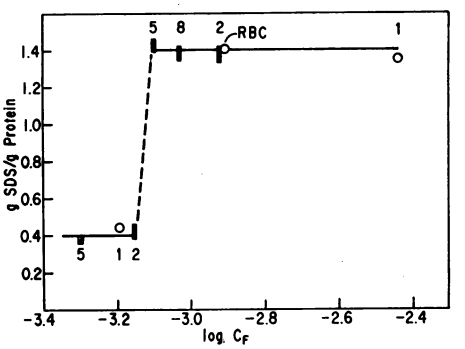

FIG. 2.—Binding of SDS to proteins. Open circles (○) represent results obtained with individual proteins under conditions where no micelles are present; i.e. the equilibrium monomer concentration C_f, is below the critical micelle concentration, CMC. The point labeled "RBC" represents a mixture of erythrocyte membrane proteins. Rectangles represent ranges of values for several different proteins at the same C_f. The number adjacent to each rectangle designates the number of different proteins. Filled rectangles (■) indicate that micelles of SDS were present in addition to free SDS (C_f = CMC). The half-filled rectangle (◪) represents some points with micelles present and some with only monomer present.

bumin, lysozyme). However, an increase in the equilibrium monomer concentration leads to a larger value of g SDS/g protein.

In Figure 2 the binding ratio is plotted as a function of the equilibrium monomer concentration (C_f). The resulting isotherm has two plateau regions, one at 0.4 g/g and a second at 1.4 g/g. All proteins investigated fall on the same curve. Binding data from another laboratory[14] are shown in Table 2 and are also plotted on Figure 2. The data in Table 2 were obtained by direct, prolonged exposure of native protein to SDS and beta-mercaptoethanol. The binding ratios are identical whether the protein is initially in the native state or is first converted to a random coil by treatment with 6 M GuHCl and reducing agent. Thus, the complex, SDS-protein, is the thermodynamically preferred state in this solvent system.

TABLE 2. *Binding of sodium dodecyl sulfate to proteins.**

Protein	μ	Equilibrium SDS Concentration (mM) Total	Monomer	Monomers in micellar form	g SDS / g P	pH	Mol. wt.
Ribonuclease	0.17	3.47	0.93	2.54	1.39	7.2	13,700
Apomyoglobin	0.17	3.47	0.93	2.54	1.34	7.2	17,200
Myoglobin	0.17	3.47	0.93	2.54	1.40	7.2	17,800
β-Lactoglobulin	0.17	3.47	0.93	2.54	1.19	7.2	18,400
Ovomucoid	0.17	3.47	0.93	2.54	1.42	7.2	30,000
Ovalbumin	0.17	3.47	0.93	2.54	1.40	7.2	43,000
Catalase	0.17	3.47	0.93	2.54	1.38	7.2	60,000
Hb^+	0.17	3.47	0.93	2.54	1.38	7.2	16,600
	0.32	3.47	0.70	2.77	0.46	7.2	16,600

* Pitt-Rivers and Impiombato (1968).

Phage coat protein from φX-174 was studied by Carusi and Sinsheimer.[15] These authors also find a binding ratio of 1.44 g SDS/g protein when their data are corrected to 20°C. The equilibrium monomer concentration in this experiment is ~7.20 mM based on the ionic strength dependence of the critical micelle concentration.

Discussion. It is significant that all proteins investigated bind identical

amounts of SDS at the same value of C_f, including the highly basic histones, the large helical myosin molecule, and the membrane proteins from erythrocyte ghosts. Previous studies[6] have indicated that SDS induces a conformational change in proteins at monomer concentrations higher than 0.1 mM. This is confirmed by studies in the authors' laboratory which will be published in the near future. Hydrodynamic and optical properties of the protein-SDS complexes in Table 1 clearly indicate a major conformational change as well as a uniformity in the hydrodynamic shape.

The binding of large amounts of SDS to protein is primarily hydrophobic in nature. The effect of ionic strength appears to be entirely on the concentration of free SDS monomer. Micelles do not bind to the proteins investigated. Thus, while the protein-SDS complex may bear some resemblance to a micellar system, the complex arises from the interaction of monomers with the macromolecule, not from incorporation of a protein into a micelle or the addition of micelles to the external surface of the protein.

The extension of these generalizations for SDS to protein-lipid interactions is reasonable in view of the general similarities of chemical behavior among all amphiphilic substances investigated to date. Very few quantitative data have been obtained for lipid-protein systems. However, it is significant that Ji and Benson[4] report identical binding ratios of monogalactosyl diglyceride, digalactosyl diglyceride, and phosphatidyl diglyceride to spinach chloroplast protein. These three lipids have identical hydrocarbon tails but different polar groups. Here is another example of the relatively small role played by the hydrophilic portion of an amphiphile in binding to proteins.

It should be noted that hydrophobic forces between protein and amphiphile do not necessitate the acceptance of either the bilayer or the subunit membrane model. Our findings do, however, eliminate the possibility of a long disordered polypeptide chain on the outside of the lipid bilayer interacting through purely coulombic forces with the phosphate head groups. This view of protein-lipid interaction has often been associated with the bilayer model and is not compatible with the results reported here.

It is probably possible to distribute portions of the protein moiety interior to the polar head of the lipid in a bimolecular leaflet without doing violence to the current interpretation of x-ray diffraction data. Unpublished results from this laboratory show clearly that the protein in a protein-SDS complex is not globular, but rather an extended polypeptide chain containing a significant degree of order. The electron density contributed by a protein molecule in this type of conformation would be confined to a relatively narrow portion of the membrane electron density profile, and would itself consists of subregions of variable density (e.g., aliphatic side chains of a protein would be indistinguishable from the hydrocarbon tail of a lipid molecule). Thus, some part of the electron density within the bimolecular leaflet that is commonly ascribed to hydrocarbon and/or cholesterol could be derived from protein molecules instead.

The predominance of hydrophobic interactions in the protein-SDS system does not exclude some ionic interactions, and in the extension of this work to membrane models we should keep in mind that charged groups on the protein

are probably exposed to the aqueous environment and may interact with charged groups on the lipid. The important point is that any model must include a major contribution from hydrophobic interactions.

Gel Electrophoresis in SDS. Apart from its relevance to membrane structure, this work is of interest in connection with the recent interest in determining polypeptide chain molecular weights in SDS by acrylamide gel electrophoresis.[16,17] No explanation has been advanced as to why electrophoretic mobilities are proportional to the polypeptide chain molecular weight of any protein dissolved in SDS. Observations that all proteins bind identical amounts of SDS on a g/g basis under the conditions used for electrophoresis are consistent with the observation that all protein specificity is lost and mobility in the gel is a measure of molecular size alone.

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