Spackman, D. H., Stein, W. H., and Moore, S. (1958), *Anal. Chem. 20*, 1190.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244*, 4406.
Woods, E. F. (1969), *Protein Res. 1*, 29.
Woods, E. F., Himmelfarb, S., and Harrington, W. F. (1963), *J. Biol. Chem. 238*, 2374.

Hemoprotein–Ionic Detergent Interaction. Volume Effects Produced by Reaction with Sodium Dodecyl Sulfate†

Sam Katz,* Jane E. Miller, and John A. Beall‡

ABSTRACT: The volume effects produced by the reaction of sodium dodecyl sulfate with sperm-whale ferrimyoglobin, human oxyhemoglobin, and methemoglobin are determined by the proteins' composition, three-dimensional structure, association state, the type and ligand state of the heme moiety. The reaction of proteins with sodium dodecyl sulfate produces volume changes which are the resultant of water–protein, water–detergent, and protein–detergent interactions. The volume changes were determined dilatometrically at 30.0 ± 0.001°. The data were converted to a δV function which is the measure of the volume effect resulting from protein–sodium dodecyl sulfate interaction (Katz, S., Shaw, M. E., Chillag, S., and Miller, J. E. (1972), *J. Biol. Chem. 247*, 5228). The δV isotherm for myoglobin is characterized by a sharp volume rise at low sodium dodecyl sulfate concentrations reaching a value of 200 ml/10^5 g of myoglobin at 0.005 M sodium dodecyl sulfate. This parameter decreased to a minimum of −450 ml/10^5 g of protein at 0.07 M sodium dodecyl sulfate and then exhibited a positive nonlinear functional dependence on sodium dodecyl sulfate reaching a value of 10 ml/10^5 g of myoglobin at 0.05 M sodium dodecyl sulfate concentration. The δV isotherms for oxyhemoglobin and methemoglobin were characterized by abrupt volume decrease at low sodium dodecyl sulfate concentrations, *i.e.*, 0.005–0.07 M, with values for the minima being −725 and −875 ml per 10^5 g of protein at about 0.09 M sodium dodecyl sulfate, respectively. As the sodium dodecyl sulfate concentration increased to 0.5 M there was a gradual increase of the δV isotherm with values of −550 and −750 ml per 10^5 g of protein found at 0.5 M sodium dodecyl sulfate. While kinetic effects were observed for myoglobin at a restricted range of concentrations, 0.005–0.02 M, the hemoglobins exhibited time-dependent volume changes over the entire range of detergent concentrations. These isotherms are the composite of detergent binding, conformational changes, disruption of quaternary structure, and the type and ligand state of the heme group.

It has been established that the reaction of sodium dodecyl sulfate with protein produces volume effects which are a function of the protein's composition, charge and three-dimensional structure (Katz *et al.*, 1972). This investigation was extended to hemoproteins because of their physiological significance and also since they provide useful models to examine the influence of protein's composition, quaternary structure, and prosthetic groups on this parameter. Myoglobin is a single-chain hemoprotein, mol wt 17,800, whereas hemoglobins, mol wt 64,500, differ not only with respect to size but also because they consist of four subunits maintained by noncovalent forces. The heme iron exists in the ferric state in metmyoglobin and methemoglobin but is in the ferrous state in oxyhemoglobin. Acrylamide gel electrophoresis reveals two categories of protein–dodecyl sulfate complexes: one type whose electrophoretic mobility increased with increasing sodium dodecyl sulfate concentration, while the other's mobility exhibited little dependence on the detergent's concentration. The first type predominated at detergent concentration <0.05 M and is considered responsible for the large negative volume effects. The second type which dominated at sodium dodecyl sulfate concentration ≥0.07 M is associated with the positive slope of the volume isotherm; the protein under these conditions undergoes drastic conformational change (Reynolds *et al.*, 1967; Reynolds and Tanford, 1970).

Experimental Section

Methods. The dilatometric procedure for studying the reaction of dodecyl sulfate with protein has been described (Katz *et al.*, 1972). The 8:2 mixing protocol involves the mixing of 8.00 ml of detergent with 2.00 ml of 10% protein. The detergent concentrations cited are the concentrations after mixing; these ranged from 0.001 to 0.5 *M*.[1] Oxyhemoglobin was reoxygenated for 0.5 hr and then evacuated for 5 min with a Cenco Hyvac vacuum pump before use. Water was saturated with oxygen for 0.5 hr and evacuated for 5 min; the P_{O_2} for both systems was 70–80 mm. The dilatometric experiments were performed at 30.0 ± 0.001° (Katz, 1963).

† From the Department of Biochemistry, School of Medicine, West Virginia University, Morgantown, West Virginia 26506. *Received August 22, 1972.* This research was supported in part by U. S. Public Health Service, National Heart and Lung Institute, Grant HE 12955. Portions of this study were presented at Extreme Environment Symposium sponsored by National Aeronautics and Space Administration at Ames Research Center, Moffet Field, Calif., June 26–28, 1972, and at the 164th National Meeting of the American Chemical Society, New York, N. Y., Aug 27–Sept 1, 1972.

‡ Present address: School of Dental Medicine, University of Pittsburgh, Pittsburgh, Pa. 15213.

[1] This convention is used in preference to estimating the amount of sodium dodecyl sulfate bound because the volume data were determined 5–60 min after mixing, whereas binding values are obtained from equilibrium data which generally are determined several days after the initial contact.

Protein concentrations were determined by drying *in vacuo* at 90° for 18–24 hr; on occasion these were checked with a Beckman spectrophotometer DU2. pH was determined with a Radiometer pH meter 26 using semimicro combination electrode GK2321C. Conductivity was measured with a Radiometer conductivity meter CDM3b. Oxygen contents were determined with a Corning Oxygen meter, Model 16. Acrylamide gel electrophoretic analyses were performed with Peacock buffer (Peacock *et al.*, 1965) used in conjunction with 7.5% acrylamide gels; the voltage was 250 V and running time was 2–2.5 hr (Katz and Denis, 1970).

Materials. Oxyhemoglobin was prepared from human blood, immediately after drawing, by conventional procedure (Geraci and Li, 1969). Methemoglobin was produced by adding a fivefold excess of $K_3Fe(CN)_6$ to hemoglobin (Gibson *et al.*, 1969); this was dialyzed against several changes of ten volumes of glass-distilled water until the conductivity of the retentate and diffusate was similar. The protein was concentrated to 10%, w/v, by pervaporation. Sperm-whale heart muscle metmyoglobin was purchased from Miles-Seravac. Tris(hydroxymethyl)aminomethane and sodium dodecyl sulfate were obtained from Sigma Chemical Co. The reagents for acrylamide electrophoresis were purchased from Fisher Supply Co.; the remainder of the reagent grade chemicals were from Mallinckrodt Co.

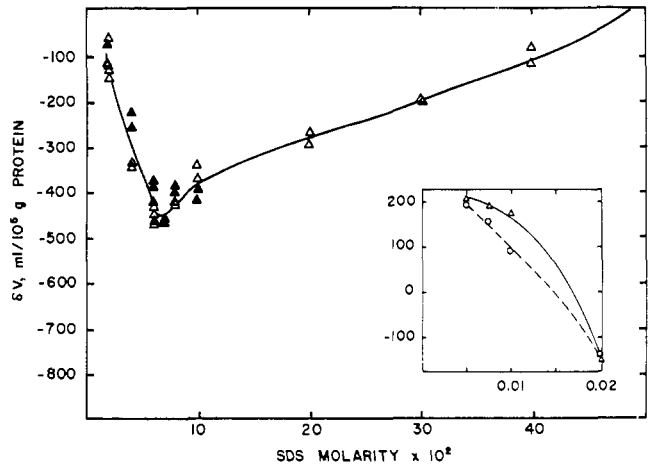

FIGURE 1: The δV isotherm produced by the reaction of sperm-whale myoglobin with sodium dodecyl sulfate. The sodium dodecyl sulfate concentration after mixing is indicated in the abscissa. The concentration of the protein after mixing is 2%. The insert contains δV values for this system exposed to sodium dodecyl sulfate concentrations which ranged from 0.005 to 0.02 M. The dotted line indicates the values 5 min after mixing while the solid line represents the steady-state values measured 60 min after mixing. Temperature is 30.0 ± 0.001°.

Results

The reaction of dodecyl sulfate with the several hemoproteins produces volume effects which are characteristic of the given protein. The volume changes are the sum of water–protein, water–sodium dodecyl sulfate, and protein–sodium dodecyl sulfate interactions; the data are expressed as a δV parameter which is an index of protein–sodium dodecyl sulfate interaction.[2] The δV parameter is defined as

$$\delta V = \frac{(\Delta V_{2d} - \Delta V_{1d})10^5}{w_2}$$

where ΔV_{2d} is the volume change produced when a given volume of sodium dedecyl sulfate is added to a defined volume of protein, ΔV_{1d} is the volume effect produced by the identical protein-free system, w_2 is the weight of protein, and the factor, 10^5, is used to convert the data to 10^5 g of protein.

At least four volume effects can be identified as a consequence of the reaction of myoglobin with sodium dodecyl sulfate (Figure 1). At low sodium dodecyl sulfate concentrations there was a sharp volume rise with a δV of 200 ml/10^5 g of proteins at 0.005 M sodium dodecyl sulfate. A kinetic effect was evident from 0.005 to 0.02 M sodium dodecyl sulfate, see insert Figure 1; steady-state values were obtained 45–60 min after mixing. Increasing the detergent concentration to 0.07 M caused a progressive volume decrease until a minimum of −450 ml/10^5 g of protein was reached at 0.07 M. At sodium dodecyl sulfate concentrations ≥ 0.08 M the isotherm described a positive nonlinear dependence on sodium dodecyl sulfate concentration, reaching a value of 10 ml/10^5 g of protein at 0.5 M sodium dodecyl sulfate.

The reaction of oxyhemoglobin with sodium dodecyl sulfate produced a δV isotherm which differed substantially from myoglobin. There was no volume rise, the kinetic effect spanned the entire range of sodium dodecyl sulfate concentrations, and the volume effect was larger than that of myoglobin (Figure 2). The volume changes were about 90% complete, 5 min after mixing; steady-state values required 45–60 min. As the sodium dodecyl sulfate concentration increased from 0.005 to 0.09 M sodium dodecyl sulfate there was an abrupt reduction of volume with a minimum of −725 ml/10^5 g of protein at 0.09 M sodium dodecyl sulfate. As the sodium dodecyl sulfate concentration exceeded 0.1 M there was a shallow rise of the δV isotherm reaching a value of −550 ml/10^5 g of protein at 0.05 M sodium dodecyl sulfate.

Methemoglobin produced volume effects qualitatively similar to oxyhemoglobin; however, the magnitude of the effects were about 20% larger (Figure 3). The kinetic effect was also larger; steady-state was reached about 60 min after mixing. As the sodium dodecyl sulfate concentration increased

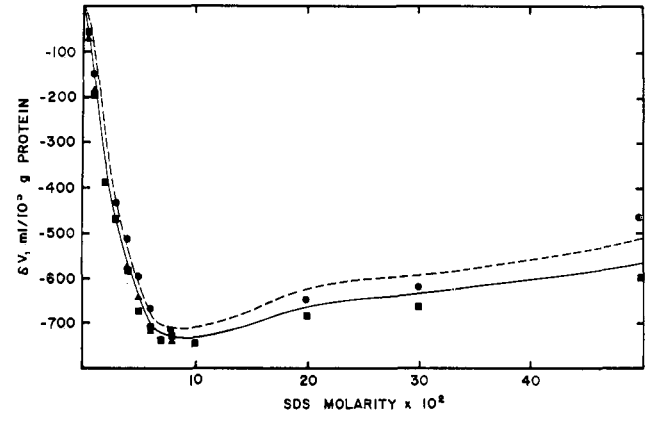

FIGURE 2: The δV isotherm produced by the reaction of human oxyhemoglobin with sodium dodecyl sulfate. The experimental details are identical with those of Figure 1. The experimental points are included for the steady-state readings but not for 5-min values for clarity purposes.

[2] The definition of the δV function, the basis for the choice of the ΔV_{12} term, and the rationale for selecting the 8:2 mixing protocol are in print (Katz *et al.*, 1972).

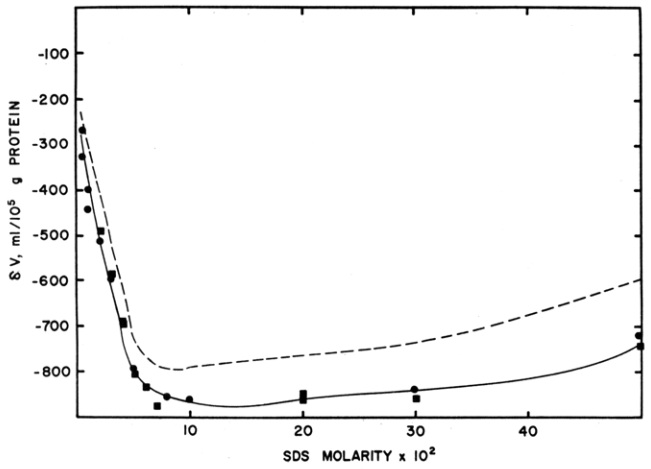

FIGURE 3: The δV isotherm produced by the reaction of human methemoglobin with sodium dodecyl sulfate. Experimental conditions similar to those in Figure 1.

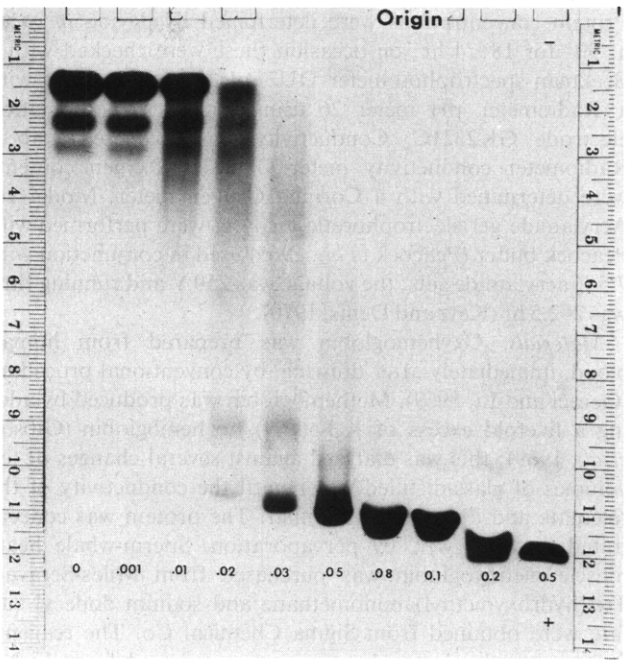

FIGURE 4: Acrylamide gel electrophoresis of sperm-whale myoglobin exposed to sodium dodecyl sulfate. The concentration of sodium dodecyl sulfate, expressed in molarity, is given on the bottom of the photograph. A 2% solution of protein was exposed to the sodium dodecyl sulfate for 5 hr at 30° before electrophoresis. Electrophoresis was performed in 7.5% acrylamide at 250 V for 3.5 hr at pH 9.1 (see text for specific details).

to 0.10 M sodium dodecyl sulfate there was a volume decrease reaching a minimum of -875 ml/10^5 g of protein at about 0.10 M sodium dodecyl sulfate. The isotherm increased at sodium dodecyl sulfate concentrations >0.1 M, reaching a value of -750 ml/10^5 g of protein at 0.5 M sodium dodecyl sulfate.

The systems studied by electrophoresis differed from the above since 5 μl of a protein–sodium dodecyl sulfate mixture was added to a large excess of sodium dodecyl sulfate free electrolyte before being subjected to 250 V for 2–3 hr. The complexes visualized by gel electrophoresis are those which are sufficiently stable to resist dissociation under these conditions. The 0.005 and 0.01 M sodium dodecyl sulfate systems were similar, *i.e.*, characterized by streaking of the pattern near the origin; with elevated sodium dodecyl sulfate concentrations, $\leq$0.05 M sodium dodecyl sulfate, there was a progressive increase of the mobility of the streaked components (Figure 4). When the sodium dodecyl sulfate concentration was $\geq$0.07 M there was a single well-defined fraction whose mobility exhibited a small dependency on sodium dodecyl sulfate concentration. Human oxy- and methemoglobin produced patterns which were qualitatively similar to myoglobin except that the high-mobility fraction did not exhibit any concentration dependence between 0.05 and 0.2 M sodium dodecyl sulfate. However, at $\geq$0.2 M sodium dodecyl sulfate there was a fourfold increase of the area occupied by this fraction; the midpoint of this zone coincided with the midpoint of the fast component present at lower sodium dodecyl sulfate concentrations.

Discussion

The composition, association state and the nature of the heme have a profound influence on the volume effects produced by protein–detergent interaction. The δV isotherm for myoglobin resembles that of isoinic albumin (Katz *et al.*, 1972) more than it does hemoglobins; *i.e.*, the sharp volume rise at low sodium dodecyl sulfate concentrations, and the large volume rise at sodium dodecyl sulfate concentrations >0.1 M. One can assume that similar mechanisms are operational but recognize that differences in detail exist because of compositional factors. The initial volume rise is explicable by the electrostatic interaction of the anionic detergent with the protein's cationic groups thereby displacing water of electrostriction (Gurney, 1953). Steinhardt's group (Reynolds *et al.*, 1967; Polet and Steinhardt, 1968) established that ten molecules of sodium dodecyl sulfate bind to bovine serum albumin with an affinity constant of 10^6; they postulate that this involves both a nonpolar and polar interaction. Estimates of a volume rise to 10–20 ml/mol of interacting anion–cation pair are based on ΔV of 10 ml/mol for the protonation of carboxylates (Kauzmann *et al.*, 1962; Katz and Miller, 1971), 17 ml/mol for the reaction of protonated amines with carboxylates (Linderstrøm-Lang and Jacobsen, 1941), and 25–45 ml for Mg^{2+} and Ba^{2+} with polycarboxylate copolymers (Begala and Strauss, 1972). The binding of 2–4 mol of sodium dodecyl sulfate/mol of myoglobin would suffice to produce this effect. Since this volume rise does not occur with hemoglobins, it appears that different mechanisms are involved; Van den Oord and Wesdorp (1969) report that myoglobin binds sodium dodecyl sulfate in numerous discrete steps, whereas sodium dodecyl sulfate is bound to methemoglobin in an all-or-none manner (Gersonde, 1969).

The volume decrease which occurs when sodium dodecyl sulfate concentrations increase from 0.02 to 0.07 M reflects the increased electrostriction of water produced by the augmented electrostatic charge of the protein–detergent complex (Ray *et al.*, 1966; Decker and Foster, 1967). This phenomenon is explicable by the Drude–Nernst (1894) hypothesis which predicts a geometric volume decrease when there is an increase of the electrostatic charge of a spherical particle immersed in water. Kauzmann *et al.* (1962) established that this relationship applied to polyfunctional organic acids providing the stereochemistry favored the overlapping and reinforcement of the electrostatic fields by a charge increase. When overlapping field effects did not occur there was no reinforcement of electrostriction effect. At these sodium dodecyl sulfate concentrations, conformational changes are absent in myoglobin

(Van den Oord and Wesdorp, 1969); thus we propose that the Drude–Nernst effect is operational. The occurrence of a minimum and the reversal of the isotherm's slope are consequences of the massive structural reorganization produced by exposing the protein to sodium dodecyl sulfate concentration ≥ 0.08 M (Tanford, 1972). This structural transition reduces the concentration of localized clusters of bound sodium dodecyl sulfate thereby diminishing the amount of overlapping electrostatic fields and thus minimizes the Drude–Nernst contribution. A more detailed discussion of this phenomenon has been reported recently (Katz *et al.*, 1972).

The kinetic effect noted between 0.005 and 0.02 M sodium dodecyl sulfate (see Figure 1) can be explained by the observation (Van den Oord and Wesdorp, 1969) that the binding of a mole of sodium dodecyl sulfate/mole of ferric myoglobin causes no spectral changes; the subsequent binding of three to four more molecules of detergent forms ferric myochrome. This conversion is completed when 15 mol of sodium dodecyl sulfate is bound at or about 0.02 M sodium dodecyl sulfate under our experimental conditions. These spectral data coincide with the time-dependent volume effects.

The quaternary structure and the state of heme have a profound influence on these volume effects (compare Figures 1–3). The values for minima for oxy- and methemoglobin were about 275 and 400 ml per 10^5 g of protein more negative than myoglobin; this additional contribution is due to the dissociation of the hemoglobins from the multisubunit state to individual chains. There is a resulting exposure of previously nonaccessible polar and nonpolar groups to the aqueous medium thereby producing additional electrostriction effects (Friedman and Scheraga, 1965; Katz, 1968). The kinetic effect which spans the entire range of sodium dodecyl sulfate concentration may reflect the operation of several processes: namely, the binding of sodium dodecyl sulfate, the dissociation of the protein's quaternary structure, the appearance of additional binding sites, and the structural reorganization of the ensuing single chain exposed to a different molecular environment. The kinetic effect noted for myoglobins at low sodium dodecyl sulfate concentration (Figure 1) occurs in hemoglobins (Gersonde, 1969); however, being of small magnitude it is masked by the dominant sodium dodecyl sulfate–hemoglobin molecular transition. The larger volume and kinetic effect manifested by methemoglobin compared to oxyhemoglobin must represent a more deep-seated disruption of the protein structure by the anionic detergent (Kellet and Schachman, 1971).

We conclude that the reaction of sodium dodecyl sulfate with heme proteins produces volume effects which result from protein–detergent binding, protein conformational changes and charge effects. The kinetics, magnitude and character of these isotherms are determined by the composition, conformation, state of heme group, and the disruption of the quaternary structure of the protein.

Acknowledgments

We thank Lawrence Zbach for checking the kinetics of the interaction of sodium dodecyl sulfate with myoglobin and Dr. H. Turndorf, Department of Anesthesiology, West Virginia University Medical Center, for providing the apparatus for oxygen determination.

References

Begala, A. J., and Strauss, U. P. (1972), *J. Phys. Chem. 76*, 254.

Decker, R. V., and Foster, J. F. (1967), *J. Biol. Chem. 242* 1526.

Drude, P., and Nernst, W. (1894), *Phyzik. Chem. 15*, 79.

Friedman, M. G., and Scheraga, H. A. (1965), *J. Phys. Chem. 69*, 3795.

Geraci, G., and Li, T. (1969), *Biochemistry 8*, 1848.

Gersonde, K. (1969), *J. Mol. Biol. 42*, 285.

Gibson, Q. H., Parkhurst, L. J., and Geraci, G. (1969), *J. Biol. Chem. 244*, 4668.

Gurney, R. W. (1953), Ionic Processes in Solution, New York, N. Y., McGraw-Hill Book Co., p 188.

Katz, S. (1963), *J. Chem. Educ. 40*, 266.

Katz, S. (1968), *Biochim. Biophys. Acta 154*, 468.

Katz, S., and Denis, J. (1970), *Biochim. Biophys. Acta 207*, 331.

Katz, S., and Miller, J. E. (1971), *J. Phys. Chem. 75*, 1120.

Katz, S., Shaw, M. E., Chillag, S., and Miller, J. E. (1972), *J. Biol. Chem. 247*, 5228.

Kauzmann, W., Bodanszky, A., Rasper, J. (1962), *J. Amer. Chem. Soc. 84*, 1777.

Kellet, G. L., and Schachman, H. K. (1971), *J. Mol. Biol. 59*, 387.

Linderstrøm-Lang, K., and Jacobsen, C. F. (1941), *C. R. Trav. Lab. Carlsberg 24*, 1.

Peacock, A. C., Bunting, S. L., and Queen, K. G. (1965), *Science 147*, 1451.

Polet, H., and Steinhardt, J. (1968), *Biochemistry 7*, 1348.

Ray, A., Reynolds, J. A., Polet, H., and Steinhardt, J. (1966), *Biochemistry 5*, 2606.

Reynolds, J. A., Herbert, S., Polet, H., and Steinhardt, J. (1967), *Biochemistry 6*, 937.

Reynolds, J. A., and Tanford, C. (1970), *J. Biol. Chem. 245*, 5161.

Tanford, C. (1972), *J. Mol. Biol. 67*, 59.

Van den Oord, A. H. A., and Wesdorp, J. J. (1969), *Eur. J. Biochem. 8*, 263.