THE EFFECT OF SODIUM DODECYLSULFATE ON THE STRUCTURE OF YEAST ENOLASE

John M. Brewer and Terry E. Spencer

Department of Biochemistry
University of Georgia
Athens, Georgia 30601

Received May 23, 1968

The work of Steiner and Edelhoch (1), and Edelhoch and Lippoldt (2) with calf thyroglobulin showed that sodium dodecylsulfate (SDS) converted the protein into a form which had many of the properties of a random coil. We have found that yeast enolase (2-phospho-D-glycerate hydrolyase, E.C.4.2.1.11), which behaves as a compact sphere in the native state, behaves in excess SDS as an asymmetric, rigid complex with a partial specific volume of 0.825, an intrinsic viscosity of 25 dl/g and a $S^{\circ}_{20,w}$ of 3.3S.

MATERIALS AND METHODS

The magnesium form of yeast enolase (3) was prepared by the method of Westhead and McLain (4). It gave a single symmetrical peak in the ultracentrifuge and a major band and minor band on disc electrophoresis at pH 2.3 and 9.5. The specific activity was equal to that obtained by Westhead and McLain (4). The dimethylamino naphthalene sulfonyl (DNS) - conjugate of enolase was prepared as described in Reference 3.

Tris was purchased from Eastman Kodak and recrystallized twice from aqueous ethanol. SDS was purchased from Mann and Sigma. It was recrystallized once from water before use. Other chemicals were A.R.grade. Doubly glass-distilled water was used throughout.

Sedimentation studies were made with a Beckman-Spinco Model E analytical ultracentrifuge using interference and schlieren optics. Viscosity measurements were performed using a Cannon-Ubbelohde dilution viscometer. It was kept in a chromatography tank filled with water whose temperature was held constant to within ±0.05° C with a Forma refrigerated constant temperature bath. All solutions used in the viscometer were filtered through Millipore filters (0.22 μ pore size). Fluorescence emission spectra were measured with an instrument built for Dr. M. J. Cormier of this Department by Lebaco, Inc. The design of this instrument is based on that of Weber and Young (5). Measurements of polarization of fluorescence were made with an instrument similar to that of Weber (6), built for J. M. B. by Lebaco, Inc.

RESULTS AND DISCUSSION

The extent of binding of detergent to enolase was measured at several ratios of SDS to protein by comparing the refractive index increments, using interference optics, produced in sedimentation velocity studies of enolase in the presence and absence of SDS (the "fringe drop" method)(Table I). About 1.5 - 1.7 mg. of detergent is bound per mg. of protein, depending on whether it is bound as the tris cation or sodium salt, at a protein concentration of 3 mg./ml. While more can be absorbed (Table I), the fact that at least half the SDS is unbound at 3 mg. enzyme/ml. and 1% SDS is taken as a convenient point of "saturation". At ratios of SDS to protein of 1.7 mg./mg. or less, apparently all the detergent is bound (some data not shown), and this is independent of protein concentration at least over the range of 1 - 3 mg./ml.

The binding of so much detergent should affect the partial specific volume of the complex. Knowing the partial specific volume of the enzyme (0.735)(7)

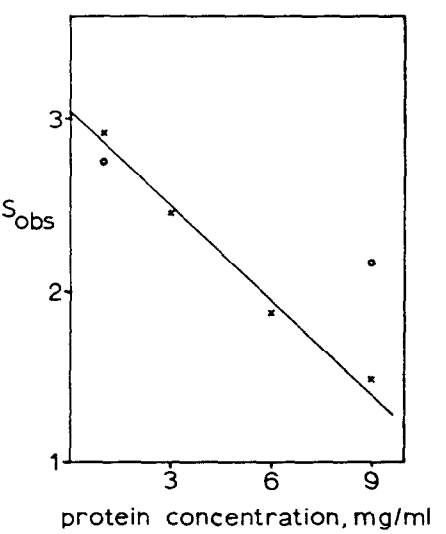

Figure 1. Concentration dependence of the sedimentation constant of the SDS-protein complex. The solutions were $\frac{\Gamma}{2}$ = 0.1 in tris-HCl, protein as indicated and either 1% SDS (open circles)or 3.3 mg. SDS per mg. protein (crosses). The temperature was 20.0° C and the rotor speed was 60,000 rpm. All experiments were done using a standard and a wedge cell, and the protein concentration in one cell was 3 mg/ml., with 1% SDS. The relative sedimentation constants obtained for other protein concentrations were normalized to 2.46S, the average observed sedimentation constant obtained with 1% SDS and 3 mg. protein per ml. The observed sedimentation constants were not corrected for changes in density and viscosity due to the tris-dodecylsulfate micelles, as these are small (2% or less) in all cases.

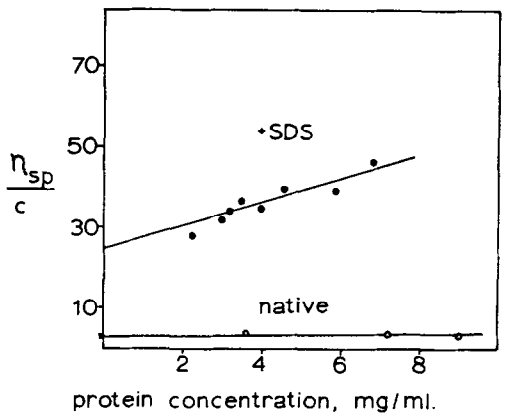

Figure 2. Reduced viscosity of enolase versus protein concentration in the presence and absence of SDS. 1.0 to 1.1 ml. of a solution which was $\frac{\Gamma}{2} = 0.1$ in tris-HCl, pH 8.1, and protein as indicated was equilibrated to 22.75° (±0.05°) C for 15 minutes in the viscometer. After the flow times for buffer were determined, that for the buffer plus enzyme but without SDS were measured (in quadruplicate)(open circles), 3.3 mg. SDS/mg. protein was added, equilibrated 15 minutes, and the new flow time determined. The SDS-protein solutions were diluted by addition of buffer to obtain viscosities at lower protein concentrations (filled circles). The triangle refers to the intrinsic viscosity of native enolase reported by Westhead (11). Flow times were corrected for the change in viscosity produced by free SDS, assuming 1.7 mg. bound/mg. enzyme (which salt is bound makes little difference), and for density changes produced by SDS and protein.

---

and of SDS (0.885), and assuming that the sodium salt is bound, the partial specific volume of such a complex is calculated (8) to be 0.828. An examination of the effect of 50, 67, 75 and 90% D_2O (8) on the relative sedimentation velocities of the enzyme in the presence and absence of saturating SDS was made, and the data showed that the complex has a partial specific volume of 0.825 ±0.013.

We observed a considerable self-sharpening of the boundary in sedimentations of the complex. This appears to be due to a high concentration dependence of the sedimentation constant (Figure 1). A similar effect was observed with thyroglobulin (2).

The figure shows relative observed S values obtained at a constant SDS/protein ratio of 3.3 mg./mg., and at 1% SDS and varying protein concentrations. Assuming a linear relation between S and concentration, a limiting value of 3.04S was obtained using a constant SDS/protein ratio. This corresponds to a value of $S^\circ_{20,w}$ of 3.3S. $S^\circ_{20,w}$ for the native enzyme is 5.90S(3).

While it must be remembered that the concentrations dealt with here should be multiplied by 2.5 - 2.7 to give the actual concentration of material sedimenting, the concentration dependence is still at least five-fold higher

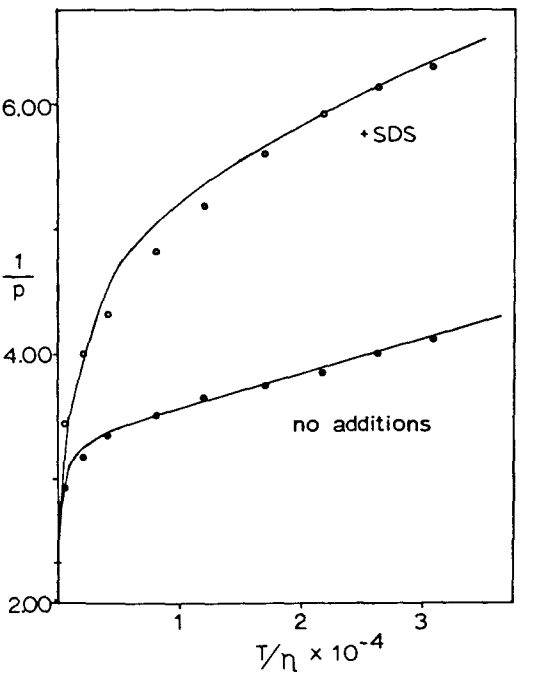

Figure 3. Polarization of fluorescence of DNS-enolase in the presence and absence of SDS. 2.0 ml. of a solution which was $\frac{\Gamma}{2}$ = 0.05 in tris-HCl, pH 8.1, and 1.0 mg/ml. in a preparation of DNS-enolase (0.7 moles DNS/mole enzyme), with (open circles) or without (closed circles) 1% SDS, was equilibrated to 22.0° C for 10 minutes in the dark. The solution was illuminated with the 366 mμ line from a mercury arc lamp and the polarization of the fluorescence emission measured. Corning 3-72 glass filters were used to reject scattered exciting light. Weighed amounts of sucrose were added to the solution, and the polarization of fluorescence measured after the sucrose was dissolved by stirring with a glass rod. Viscosities were estimated using the tables of Swindells et al. (13). The solid lines are theoretical curves, obtained from relaxation times determined by the method of Wahl and Weber (12). The parameters used in calculating the theoretical curves were: (no SDS): f = 0.687, ρ_F = 2.2 x 10^{-10} sec., ρ_h = 105 x 10^{-9} sec.; (with SDS): f = 0.422, ρ_F = 7 x 10^{-10} sec., ρ_h = 119 x 10^{-9} sec. The lifetime of excited state of the DNS group was assumed to be 12 x 10^{-9} sec. in both cases (see ref. 14).

than that found for the native enzyme (3). This indicates that the complex may be rigid and asymmetric or a flexible, random coil (9,10).

Figure 2 shows the intrinsic viscosity of yeast enolase, measured in the presence and absence of 3.3 mg. SDS/mg. enzyme.

In the presence of excess detergent, the intrinsic viscosity of the enzyme increases by a factor of 8, from 2.9 dl/g (11) to 25 dl/g. Note the

TABLE I

Protein concentration, mg/ml.	mg. detergent bound/mg. enzyme	
	as the sodium salt:	as the tris salt:
0.4	2.5	2.2
0.6	1.9	1.7
1.0 (0.33% SDS)	2.1	1.8
1.0 (0.16% SDS)	1.9	1.7
1.5	1.8	1.6
3.0	1.7	1.5

The first and last determinations were from a different enzyme preparation. In all cases, the rotor temperature was 20.0° C, the solvent was $\frac{\Gamma}{2} = 0.1$ in tris-HCl, pH 8.1. Unless otherwise stated, the SDS concentration was 1%. The amount of detergent sedimenting along with the protein was calculated from the difference in fringe number between the top of the boundary and plateau region, produced by the sedimenting protein in the presence and absence of SDS. The molar refractive indices of the sodium and tris salts of the detergent were determined from the numbers of fringes produced in synthetic boundary experiments by SDS, tris-HCl and NaCl.

increase in concentration dependence of the reduced viscosity seen in the presence of SDS.

The formation of the detergent-protein complex is accompanied by an increase in total volume of about a factor of 3, as determined from the increase in partial specific volume and stoichiometry of binding. If the complex is rigid, this would not account for the 8-fold increase in intrinsic viscosity, which suggests the effective hydrodynamic volume is even larger, that is, the complex is also asymmetric.

The fact that the dependence of reduced viscosity and sedimentation constant on concentration is high relative to the native enzyme, is consistent with this suggestion. On the other hand, the increase in intrinsic viscosity and high concentration dependences of reduced viscosity and sedimentation constant could be due to increased solvent-protein interactions, if the enzyme is a flexible, random coil in SDS. We have examined the polarization of fluorescence of a DNS-labeled preparation of the enzyme in the presence and absence of SDS to distinguish between these two possibilities (Figure 3).

An analysis of the curves by method of Wahl and Weber (12) indicates that in the presence of 1% SDS the DNS moiety now has much greater freedom of rotation. The solid lines are theoretical ones, calculated using the parameters in the legend to Figure 3. The relatively poor fit of the theoretical curve to the experimental points in the presence of SDS is probably

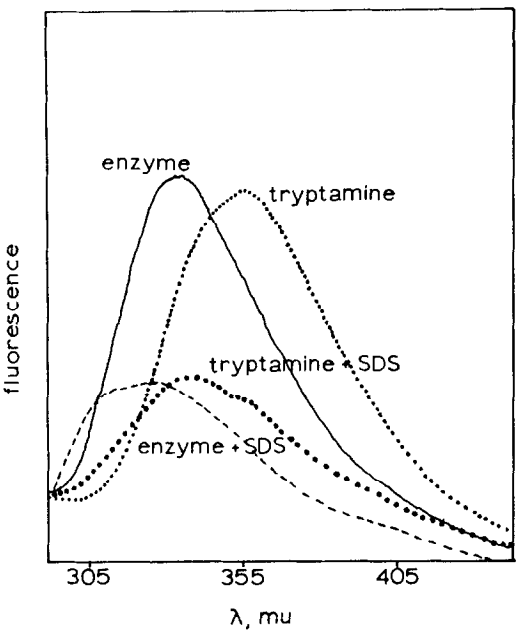

Figure 4. Fluorescence emission spectra of enolase and tryptamine in the presence and absence of SDS. 2.0 ml. of a solution which was $\frac{\Gamma}{2}$ = 0.1 in tris-HCl, pH 8.1, 0.5 mg/ml. in yeast enolase (solid line) or approximately 6 x 10^{-5} M in tryptamine (dotted line) was excited at 275 mμ using front-face illumination. The fluorescence emission spectra of similar solutions, containing 1% SDS as well, were also determined (broken line for enolase and open circles for tryptamine). All solutions were at room temperature (about 23° C). Band widths of excitation and emission were 3.3 mμ. The spectra are not corrected for photomultiplier or monochromator efficiency, as these corrections are small.

due to difficulty in determining the asymptotic slope (12). The rotational relaxation time of the enzyme in the absence of SDS is 105 x 10^{-9} sec., and 119 x 10^{-9} sec. in the presence of SDS. The data do not indicate a greater flexibility of the molecule in the presence of SDS, such as one obtains with urea or guanidine hydrochloride (14). If the molecule is rigid and asymmetric, the relaxation time obtained would be the harmonic mean of the two principal relaxation times of the ellipsoid of revolution describing the protein, and would consequently be dominated by the smaller relaxation time (15). Similar results were obtained with two other preparations of DNS-enolase, one of fluorescein-enolase, and two of DNS-bovine serum albumin (14).

Steiner and Edelhoch (1) interpreted their polarization of fluorescence data as indicating that DNS-thyroglobulin in SDS lost rigidity. Our interpretations are in disagreement with theirs. Since they varied temperature/viscosity by changing the temperature while we kept the temperature constant,

it is likely that their results were influenced by a temperature-dependent side chain effect. It is known that the barrier to rotation about the chain between the DNS moiety and the protein is temperature dependent (12). At the time when their work was done (1961), the effect on polarization measurements of rotation of the DNS group relative to the protein was not known.

In order to determine whether the detergent actually penetrates the protein or merely attaches to the surface, we examined the effect of SDS on the fluorescence emission spectrum of yeast enolase (Figure 4).

The detergent produces a pronounced blue shift and quenching of the fluorescence of indole in both enzyme and tryptamine. The tyrosine fluorescence of enolase, which is very low in the native enzyme (3), markedly increases. The changes observed in enzyme fluorescence indicate a profound alteration in the structure of the enzyme. Detergent binding only to the surface of the enzyme would not be expected to produce the effects observed.

ACKNOWLEDGEMENTS

The authors are indebted to Carling's, Inc. of Atlanta for a generous gift of yeast, and to Drs. W. F. Harrington and A. Rawitch, for some stimulating comments and suggestions. This work was supported by a N. S. F. grant (GB 5918) to J. M. B.

REFERENCES

1. Steiner, R. F. and Edelhoch, H., J. Am. Chem. Soc. 83 1435 (1961).
2. Edelhoch, H. and Lippoldt, R. E., J. Biol. Chem. 235 1335 (1960).
3. Brewer, J. M. and Weber, G., J. Biol. Chem. 241 2550 (1966).
4. Westhead, E. W. and McLain, G., J. Biol. Chem. 239 2464 (1964).
5. Weber, G. and Young, L. B., J. Biol. Chem. 239 1424 (1964).
6. Weber, G., J. Opt. Soc. America 46 962 (1956).
7. Bergold, G., Z. Naturforsch. 1 100 (1946).
8. Schachman, H. K. in S. P. Colowick and N. O. Kaplan (Editors), Methods in Enzymology, Vol. IV, Academic Press, New York, p. 32 (1957).
9. Tanford, C., Physical Chemistry of Macromolecules, J. Wiley and Sons, New York (1961).
10. Schachman, H. K., Ultracentrifugation in Biochemistry, Academic Press, New York (1959).
11. Westhead, E. W., Biochemistry 3 1062 (1964).
12. Wahl, P. and Weber, G., J. Mol. Biol. 30 371 (1967).
13. Swindells, J. F., Snyder, C. F., Hardy, R. C. and Golden, P. E. Supplement to National Bureau of Standards Circular 440, Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. (1958).
14. Brewer, J. M. and Spencer, T. E., to be published.
15. Weber, G., Adv. Prot. Chem. VIII, 415 (1953).