EFFECT OF SODIUM DODECYL SULFATE ON GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

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Treatment of native glyceraldehyde-3-phosphate dehydrogenase with sodium dodecyl sulfate (SDS) results in a sequential change in the circular dichroism spectrum from one characteristic of native β-structure to one characterisitc of increasing amounts of α-helix. Computer calculations of protein secondary structures also indicates increasing α-helix and random structure with concomitant decrease in β-structure. The enzyme is dissociated to inactive monomers in the presence of SDS. No intermediate dimers were apparent. An increase in pH at the same SDS concentration facilitates enzyme dissociation.

The interaction of proteins with amphiphiles containing hydrocarbon tails of twelve or more carbon atoms usually leads to a conformational change in the protein moiety (1). Jirgensons and co-workers (2, 3), using optical rotatory dispersion and circular dichroism, and Visser and Blout (4), using infrared absorption, have shown that the effect of sodium dodecyl sulfate (SDS) on protein structure is dependent upon the structure of the native protein.

Previous studies of the action of sodium dodecyl sulfate on glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.2.12) have indicated that the enzyme is conformationally altered (5-8), dissociated (5-9), and inactivated (5-9) in the presence of SDS. Bolotina *et al.* (5) reported that swine glyceraldehyde-3-phosphate dehydrogenase (GPD) was dissociated to 5.5s or 2.8s particles depending upon the weight ratio of SDS to protein. However, Elodi *et al.* (6) reported that swine GPD was dissociated to a 2s particle (molecular weight, 47,000), but no other intermediate particles. A similar situation persists for the rabbit enzyme; Marti and White (9) reported the dissociation of the rabbit enzyme to a particle of molecular weight 72,000 (dimer), but Magar (8) reported dissociation to a 2.0s monomer.

Preliminary studies (7) in our laboratory indicated that SDS produced sequential changes in the protein structure. The data reported here indicates that increasing concentrations of SDS cause a sequential loss in β-structure with concomitant increases in α-helix and random coil. In addition, enzyme activity loss is directly correlated with production of 2.0s monomers.

MATERIALS AND METHODS

Crystalline rabbit muscle glyceraldehyde-3-phosphate dehydrogenase was purchased as an ammonium sulfate suspension from Worthington Biochemical Corporation, Freehold, N.J. Enzyme concentration was determined by absorbance measurements at 280 nm using $E^{1\%}_{1cm} = 10.02$ (10). Sodium dodecyl sulfate was purchased from Sigma Chemical Company, St. Louis, Mo. Protein and sodium dodecyl sulfate solutions were prepared at twice the desired final concentrations. Equal volumes of these solutions were then mixed gently to give the reported concentrations of protein and SDS. Enzyme activity was measured by the method of Allison and Kaplan (10). Aliquots of the SDS-protein solutions were added to the assay substrates until 1-5 μg of protein was present in the assay.

Circular dichroism measurements were performed on a Cary 60 recording spectropolarimeter equipped with a Model 6002 circular dichroism attachment and programmed to a band width of 15 A°. A mean residue weight of 109 (11) was used in calculations of a mean residue ellipticity.

Sedimentation velocity studies were performed in a Beckman Model E analytical ultracentrifuge. The schlieren optics system was used at phase angles of 50-70°. The areas under schlieren peaks (hence particle concentration) were calculated by projecting the image of the schlieren photograph onto a piece of paper and tracing the peak outline. The tracing was then cut out and weighed; the weight of the paper thus became a measure of the concentration of the particle giving rise to the peak. In most cases, at some time during ultracentrifuga-

tion, the peaks were separated completely, with peaks returning to the baseline. When the peaks could not be separated by prolonged centrifugation, the peaks were assumed to be symmetrical and extrapolated to the baseline. Peak concentrations are reported relative to the total area under all peaks present (usually only 2 peaks). No effort was made to correct for Johnston-Ogston effects (12).

Sedimentation coefficient s_{obs}, were calculated from log r *vs.* t plots (r = distance of peak maximum from center of rotation, t = time) and corrected to s_{20}.

Protein structure fractions were calculated by the method of Greenfield and Fasman (13). Poly-l-lysine circular dichroism spectra reported by Greenfield and Fasman (13) were used as the secondary structure standards. The ellipticity values chosen for analysis were 205, 208, 211, 214, 215, 217, 220, 222, 225, 230, 234, 238 and 240 nm only. Structure fractions were calculated by the non-weighted least squares method described by Magar (14) with the aid of an IBM 360-65 computer. Standard deviations for the calculated structure fractions were less than 5%.

RESULTS

The circular dichroism spectra of glyceraldehyde-3-phosphate dehydrogenase as a function of SDS concentration is given in Figure 1. The protein (3.90 mg/ml) was dissolved in 50 mM tris-chloride buffer (pH, 8.4), 1 mM EDTA, 0.1 mM dithiothreitol at 23°. These spectra are similar to those previously reported by us (7). As the concentration of SDS increased, the circular dichroism spectrum changed from a spectrum characterized by a single negative band at 217 nm (native) to a spectrum characterized by two negative bands at 222 and 209 nm. At a protein concentration of 3.90 mg/ml, the change in conformation was fully manifested at 0.50% SDS and underwent no further spectral changes up to 4.0% SDS. The spectral change was complete after 2 hours and was not altered further up to one week later.

Since the qualitative examination of the circular dichroism spectrum in the presence of SDS indicated that considerable α-helix was formed, it was of interest to examine which secondary structures were being altered. To examine the individual secondary structure changes, we calculated the secondary structure fractions for each of the circular dichroism spectra given in Figure 1

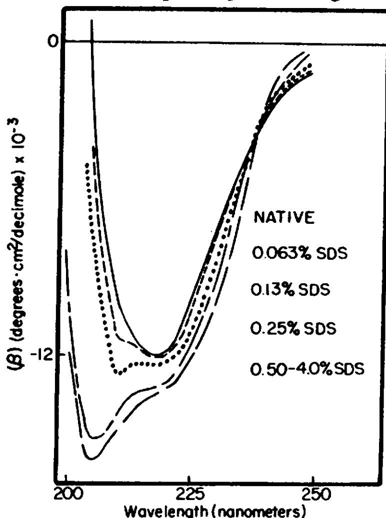

FIGURE 1. Circular dichroism spectra of glyceraldehyde-3-phosphate dehydrogenase in SDS.

using the method of Greenfield and Fasman (13). These secondary structures are plotted against SDS concentration in Figure 2. As

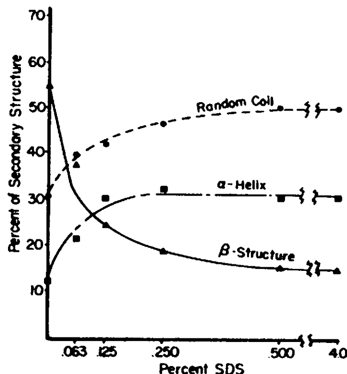

FIGURE 2. Computed secondary structure fractions for glyceraldehyde-3-phosphate dehydrogenase in SDS.

the SDS concentration increases, β-structure is destroyed and both α-helix and random coil structures are formed.

The enzyme activities and sedimentation velocity data for the solutions depicted in Figure 1 are given in Table 1. Enzyme

TABLE 1. *Enzyme activity and sedimentation coefficients for glyceraldehyde-3-phosphate dehydrogenase in the presence of SDS.*

Sample[a] (% SDS)	Activity (% of control)	Sedimentation[b] coefficient S_{20}
0.000	100	7.5
0.063	35	7.5, 1.9
0.084	22	7.5, 1.9
0.13	2	7.5, 1.9
0.25	0	2.0
0.50	0	2.0

[a] Protein-SDS solutions were prepared in 50 mM tris-chloride buffer, pH 8.4, 1 mM EDTA, and 0.1 mM dithiothreitol at 23 C.
[b] Ultracentrifuge rotor speed was 52,000 rpm except for 0% and 0.063% SDS samples where the speed was 60,000 rpm. Temperature was 25 C.

activity loss leveled off within 5 min after mixing and remained constant for at least 5 hr. Table 1 shows that enzyme activity loss can be related to the change in the circular dichroism spectra. At 0.50% SDS, a concentration at which the circular dichroism change was fully manifested, the enzyme was inactive.

Sedimentation velocity ultracentrifugation studies in Table 1 were completed 2 hr later than were the enzyme activity and circular dichroism experiments. However, since the SDS-induced changes in both circular dichroism spectra and enzyme activity were fully manifested before ultracentrifugation commenced, and were not further altered for the duration of the studies, the ultracentrifugation studies can be compared favorably. In the absence of SDS, the sedimentation pattern described a single peak with a sedimentation coefficient of 7.5s. In the presence of increasing concentrations of SDS, glyceraldehyde-3-phosphate dehydrogenase was progressively dissociated to a 1.9-2.0s particle. This particle was assumed to be an unfolded monomer (see Discussion).

The sequential increase in monomer concentration is apparent from the shift in peak areas given by the schlieren diagram in Figure 3. In 0.063% SDS, the 7.5s tetramer

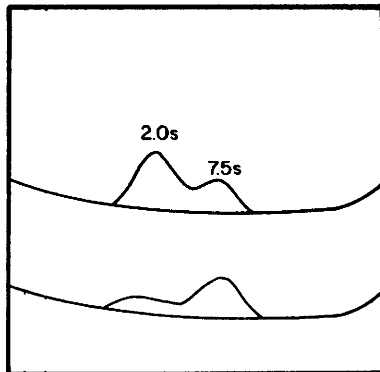

FIGURE 3. Schlieren diagram of glyceraldehyde-3-phosphate dehydrogenase in 0.063% and 0.13% SDS. (Protein-SDS preparations used were aliquots of those described in Figure 1. Speed, 52,000 rpm; temperature 25 C. Picture taken 24 min after operating speed was attained. Sedimentation is left to right. Top: GPD in 0.13% SDS; bottom: GPD in 0.063% SDS.)

was the predominant particle, but when the SDS concentration was increased to 0.13%, the 2.0s monomer was the predominant species. No intermediate peaks (hence, dissociated species) were visible or hinted, although this does not exclude the possibility of their existence.

Since increasing the SDS concentration at constant protein concentration resulted in a loss of enzyme activity and an increase in 2.0s monomer concentration, we determined the relationship between enzyme activity loss and production of the 2.0s monomer. To examine this, we incubated various concentrations of glyceraldehyde-3-phosphate dehydrogenase (0.97 mg/ml-4.84 mg/ml) in 0.063% SDS. At 0.97 mg/ml, the only species apparent was the 2.0s monomer, while at 4.84 mg/ml, the enzyme was only slightly dissociated. At intermediate protein concentrations, the relative monomer concentration increased and relative tetramer concentration decreased as the protein concentration decreased. In all cases, peaks were nearly symmetrical and did not show peak distortion that would be expected from rapidly equilibrating additional intermediate species (15). The relative areas of monomer and tetramer at each protein concentration are plotted as a function of pro-

tein concentration in Figure 4. The total area under the monomer and tetramer peaks at each protein concentration was assumed to be 100%. The concentration of monomer increased linearly as the concentration of protein decreased.

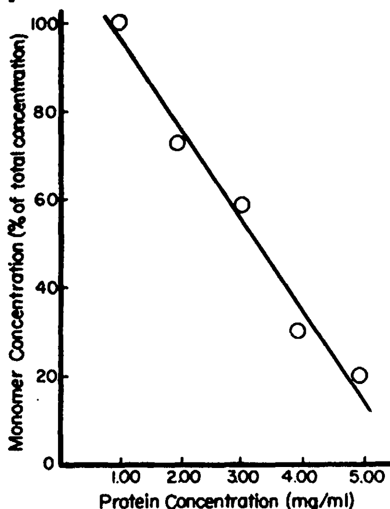

FIGURE 4. Glyceraldehyde-3-phosphate dehydrogenase monomer concentration in 0.063% SDS as a function of protein concentration.

To determine whether enzyme activity was directly related to the relative concentrations of tetramer and monomer, aliquots of the same solutions used for ultracentrifugation (Fig. 4) were assayed for enzyme activity. Enzyme activity is plotted against monomer concentration (from Fig. 4) in Figure 5. Enzyme activity loss is directly related to the concentration of 2.0s monomer. Thus, in the presence of SDS, 2.0s monomers of glyceraldehyde-3-phosphate dehydrogenase are inactive.

The effect of pH on the inactivation of glyceraldehyde-3-phosphate dehydrogenase by SDS was also investigated (Table 2). Protein-SDS solutions were prepared in Tris buffers at pH 8.9 and 8.3 as previously described. The solutions were incubated 2 hr at 23 C before enzyme activity was determined. Enzyme activity dropped more rapidly in the solutions at pH 8.9 than those at pH 8.3. In 0.063% SDS, the enzyme

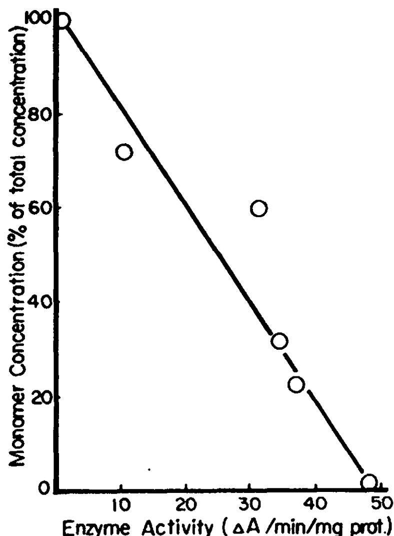

FIGURE 5. Enzyme activity in relation to monomer concentration.

TABLE 2. *SDS inactivation of glyceraldehyde-3-phosphate dehydrogenase as a function of pH.*

Sample[a] (% SDS)	Activity (% of control)[b] pH 8.3	pH 8.9
0.000	100	100
0.042	57	15
0.063	47	0
0.083	31	0
0.13	0	0

[a] Protein (3.9 mg/ml)-SDS solutions were prepared in 50 mM tris-chloride buffer, pH 8.3 or 8.9; 1 mM EDTA and 0.1 mM dithiothreitol at 23 C. Solutions were incubated 2 hr at the pH indicated before enzyme activity was determined.

[b] Enzyme activity was determined at the respective pH.

retained 47% activity at pH 8.3, while at pH 8.9 the enzyme was completely inactive. Instability of the enzyme at the higher pH can not be the sole reason for the increased rate of inactivation since the specific activities of the controls (no SDS) were almost identical at both pH's. Schlieren diagrams for enzyme in 0.063% SDS showed only a monomer at pH 8.9, but both 2.0s monomer and tetramer at pH 8.3. Furthermore, the relative area under the monomer peak at

pH 8.3 was almost identical (52%) to the amount of inactivation, 53% (line 3, Table 2).

DISCUSSION

By using relatively low concentrations of SDS, we have been able to induce sequential changes in the circular dichroism spectrum, sedimentation pattern, and enzyme activity of glyceraldehyde-3-phosphate dehydrogenase. When protein secondary structures were calculated from the circular dichroism spectra in Figure 1, sequential changes in the individual secondary structures were also apparent. The calculations indicate that treatment of glyceraldehyde-3-phosphate dehydrogenase with SDS results in a sequential decrease in β-structure with concomitant increases in α-helix and random coil structures. The absolute numerical values of the protein secondary structures, calculated by the circular dichroism method proposed by Greenfield and Fasman (13) and used here, must be accepted only with caution since several laboratories have indicated that the choice of poly-l-lysine circular dichroism spectra as secondary structure standards is questionable (13, 16-20).

Our use of calculated protein secondary structures to examine the SDS induced secondary structure changes induced in glyceraldehyde-3-phosphate dehydrogenase represents the first attempt to use calculated secondary structures for purposes other than comparison to x-ray determined values. Thus, although the absolute numerical values of the structure fractions calculated in Figure 2 may be in error, the sequential changes in the secondary structures may be real. Jirgensons (2) reported that proteins possessing considerable β-structure in the native conformation acquire considerable α-helix in the presence of SDS. He also indicated that proteins which are mostly disordered in the native state (histones or soybean trypsin inhibitor) are partially ordered by detergents. The sequential structure changes described in Figure 2 indicate that the β-structure is both re-ordered and disordered by SDS. Although the effects of SDS on proteins have been reported to be non-specific (21, 22), it is not unreasonable to assume that local interactions have an important role in determining the interactions of SDS. A slight change in pH greatly changes the effects of SDS (Table 2). A similar pH change in the absence of SDS does not produce any changes in the circular dichroism spectrum, sedimentation properties, or enzyme activity.

Sodium dodecyl sulfate induces dissociation of native, tetrameric glyceraldehyde-3-phosphate dehydrogenase to an enzymatically inactive 1.9-2.0s particle. The unfolded monomer of glyceraldehyde-3-phosphate dehydrogenase was reported to be a 1.3-2.0s particle under the following conditions: 10 M urea, pH 12.5, and performic acid oxidized (23). The compact monomer produced in 0.15 M KCl (24) and in the presence of ATP (25) has a sedimentation coefficient of 2.8-3.2s. The compact dimer produced in 0.15 M KCl (24) and in the presence of ATP (25) has a sedimentation coefficient of 4.5-4.6s. To the authors' knowledge there have been no reports of dimers with sedimentation coefficients below 4.0s. Therefore, it is reasonable to assume that the 1.9-2.0s produced in SDS is an unfolded monomer.

Throughout the dissociation studies, intermediate dimers were not apparent. Monomer and tetramer sedimentation profiles were not distorted in any manner (15) that would suggest the presence of additional species. In addition, sedimentation coefficients were not protein concentration dependent over the concentration range 0.5 mg/ml to 5 mg/ml. Thus, our results are consistent with those reported by Magar (8), but are contrary to the dimer reported by Marti and White (9). The discrepancy with the latter work is unexplained.

As evidenced by the direct relationship between monomer concentration and loss of enzyme activity (Fig. 5), the monomer of glyceraldehyde-3-phosphate dehydrogenase is inactive in the presence of SDS. Magar (8) suggests that SDS could affect the binding or environment of the bound coenzyme, NAD^+, which is required for enzyme activity. However, our results and those of others (5, 6) indicate that a secondary structure change may also be important. These studies do not indicate whether the native structured monomer is active.

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