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On the Effect of Sodium Dodecyl Sulfate on the Structure of β-Galactosidase from *Escherichia coli*. A Fluorescence Study[1]

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An understanding of the structure–function relationship of proteins under different chemical-physical conditions is of fundamental importance for an understanding of their structure and function in cells. In this paper we report the effects of sodium dodecyl sulfate and temperature on the structure of β-galactosidase from *Escherichia coli*, as monitored by fluorescence spectroscopy. The structure of the protein was studied in the temperature range of 10–60°C in the absence and presence of sodium dodecyl sulfate by frequency-domain measurement of the intrinsic fluorescence intensity and anisotropy decays. The time-resolved fluorescence data in the absence of SDS indicated that at 10°C the tryptophanyl emission decays were well described by a three exponential decays model, and that the temperature increase resulted in shortening of the long-lived component with little change in the short- and middle-lived components. The addition of SDS to the protein solution also affected the long-lived component. The effects of the detergent and temperature on the enzyme structure were also investigated by means of quenching experiments and anisotropy decays. The obtained results showed that the presence of SDS confers more flexibility to the protein structure, and suggest a strict relation between enzyme activity and protein flexibility.

Key words: β-galactosidase, frequency-domain fluorometry, protein stability, SDS.

The three-dimensional structure of a native protein is determined by the amino acid sequence of the protein, but only when the physicochemical properties of the physiological environment are appropriate. Interactions of proteins with surfactants constitute a widely used tool for characterization of the structural features of soluble and membrane proteins (*1*). For example, the anionic detergent sodium dodecyl sulfate (SDS) is helpful in maintaining the hydrophobic milieu when the lipid is removed from a membrane protein, or in estimating polypeptide size by gel electrophoresis. In addition, it has also been shown that SDS stimulates the activity of certain enzymes (*2–5*), and it is often used as an additive to facilitate proteolytic cleavage experiments. It is apparent that the study of SDS–protein interactions can be useful for understanding the structure–function relationships in proteins and enzymes.

β-Galactosidase from *Escherichia coli* (*Ecβ Gal*) is an oligomer composed of four identical subunits with a monomer molecular mass, M_r, of 116,350. *Ecβ Gal* catalyzes the hydrolysis of a wide variety of β-galactosides, including natural substrates such as lactose and artificial substrates such as *o*-nitrophenyl-β-galactoside (ONPG) (*6*). In a recent study, Muga and coworkers (*7*) showed that the β-galactosidase activity was stimulated in the presence of SDS. They studied the effect of SDS on the enzyme structure by means of Fourier transform infrared spectroscopy (FT-IR), and concluded that the SDS-induced enzyme activation was related to "loosening" of the tightly packed tetrameric protein structure on the addition of the detergent (*7*). They also showed that the addition of SDS did not affect the secondary structure of the protein. Similar results were obtained for a thermostable archaebacterial β-glycosidase that is considered to be homologous to the bacterial β-galactosidase (*8–10*). These observations prompted us to investigate the effect of SDS on the conformational dynamics of *Ecβ Gal* by means of steady state and time-resolved fluorescence. Time-resolved fluorescence is one of the most common spectroscopic methods used to elucidate the dynamic aspects of protein structures, and tryptophan residues in proteins are used as probes to monitor the structural changes of macromolecules in solution.

In this paper we show the effects of temperature and SDS on the fluorescence emission of *Ecβ Gal*. The tryptophanyl emission decays of *Ecβ Gal* can be interpreted as arising from two classes of indolic residues, each of which is affected to a different extent by SDS addition. Moreover, the addition of the detergent also results in loosening of the three-dimensional organization of the enzyme, as shown by

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Abbreviations: SDS, sodium dodecyl sulfate; *Ecβ Gal*, β-galactosidase from *E. coli*; ONPG, *o*-nitrophenyl-β-galactoside; NATA, *N*-acetyl-L-tryptophanamide.

acrylamide quenching experiments.

MATERIALS AND METHODS

Reagents—$NaH_2PO_4 \cdot H_2O$ was obtained from J.T. Baker Chemicals (New York, NY, USA). All other chemicals were commercial samples of the purest quality. Pure β-galactosidase from *E. coli* [EC 3.2.4.7] and sodium dodecyl sulfate (99% pure) were purchased from Sigma.

Protein Assay—The protein concentration was determined by the method of Bradford (*11*), with bovine serum albumin as the standard.

Preparation of Samples for Spectroscopic Analysis—For fluorescence measurements protein samples were washed several times in 10 mM Na phosphate buffer, pH 7.0, using an ultrafiltration apparatus equipped with PM-10 membranes (Amicon).

Fluorescence Spectroscopy—Emission spectra were obtained with a SLM spectrofluorometer, at a protein concentration of 0.05 mg/ml in 10 mM Na phosphate buffer, pH 7.0, plus the specified amounts of SDS. The excitation was set at 295 nm in order to exclude the tyrosine contribution to the overall fluorescence emission.

Frequency domain data were obtained with a frequency domain fluorometer operating between 2 and 200 MHz (*12–16*). The modulated excitation was provided by the harmonic content of a laser pulse train with a repetition rate of 3.75 MHz and a pulse width of 5 ps, from a synchronously pumped and cavity dumped rhodamine 6G dye laser. The dye laser was pumped with a mode-locked argon ion laser (Coherent, Innova 100). The dye laser output was frequency doubled to 295 nm for tryptophan excitation. For intensity decay measurements, magic angle polarizer orientations were used. The emitted light was observed through an interference filter at 340 nm. The frequency-domain intensity data fitted the time-resolved expression

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i} \quad (1)$$

where α_i is the pre-exponential factor, t, the decay time, and $\Sigma\alpha_i = 1.0$. The frequency-domain anisotropy data fitted

$$r(t) = r_0 \sum_i g_i e^{-t/\theta_i} \quad (2)$$

where $r_i = r_0 g_i$ is the amplitude of the component with rotational correlation time θ_i. The parameters were recovered by non-linear least squares using the theory and software described elsewhere (*17–19*).

The standard deviation errors for phase and modulation were 0.3 and 0.005 degrees, respectively. The mean lifetime values were calculated according to the equation:

$$\tau = \Sigma f_i \tau_i \quad (3)$$

where f_i is the fractional intensity of each lifetime component. The values of f_i were given by

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j} \quad (4)$$

RESULTS AND DISCUSSION

In Fig. 1 is shown the three-dimensional structure of *Ecβ Gal* solved at 3.5 Å, in which the indolic residues are shown in yellow (*6*). The enzyme, a very large protein with a tetrameric molecular mass of about 465 kDa, is active in the tetrameric form. Each monomer folds into five compact sequential domains, plus an extended segment of about 50 residues at the N-terminus, and contains 23 tryptophan residues. The domains are packed very tightly to give a compact monomer. The residues at the N-terminus of each monomer are required for formation of the active tetrameric enzyme.

In an interesting study, Muga *et al.* (*7*) investigated the effects of SDS and temperature on the functional and structural features of *Ecβ Gal*. They showed that the addition of 1.0% SDS to the protein solution resulted in an about 30% increase in the enzyme activity at room temperature. They also found that the enzyme is active in the presence of 1.0% SDS in the temperature range of 10 to 46°C. The authors also investigated the effects of the detergent and temperature on the protein structure by means of Fourier transform infrared spectroscopy (FT-IR). They observed that the hydrogen/deuterium exchange was faster in the SDS-treated enzyme than in the absence of the detergent, and suggested that the addition of 1.0% SDS resulted in loosening of the tightly packed protein structure. Similar results were obtained on studying the effect of the detergent on a thermostable β-glycosidase from the archaeon *Sulfolobus solfataricus*, which is homologous to *Ecβ Gal* (*8–10*). In particular, it was shown that the thermophilic enzymes in the presence of SDS was also active at low temperatures (*e.g.* 10°C), an unusual feature for a thermophilic protein, and that the presence of SDS conferred more flexibility to the macromolecule structure. Taken together these observations prompted us to investigate the effect of SDS on the conformational dynamics of *Ecβ Gal* by means of fluorescence spectroscopy.

Figure 2 shows the intensity normalized fluorescence

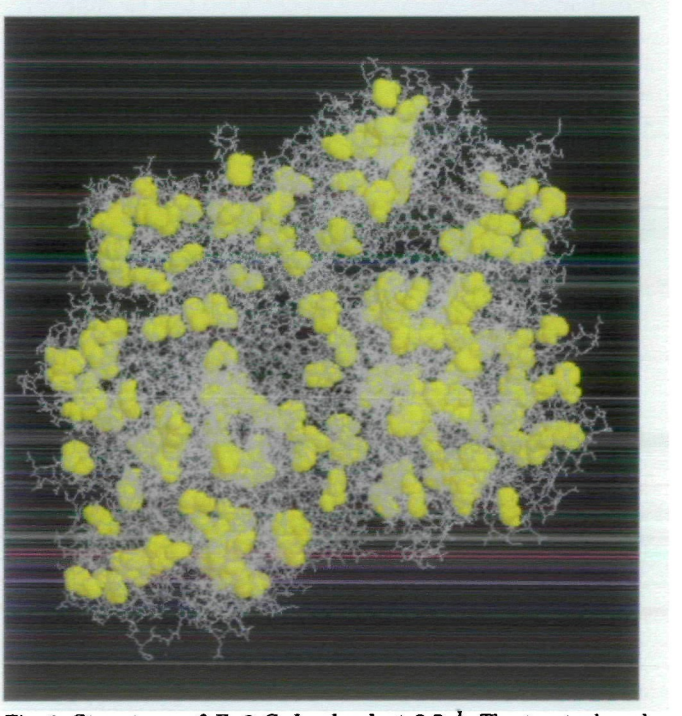

Fig. 1. **Structure of *Ecβ Gal* solved at 3.5 Å.** The tryptophanyl residues are shown in yellow.

spectra of *Ecβ Gal* at 10°C and 40°C in both the absence and presence of 1.0% SDS, as well as the spectrum of *N*-acetyl-L-tryptophanamide (NATA). The emission spectra of the protein in the absence and presence of the detergent at 10°C show a maximum at 340 nm. The position of the emission maximum is blue-shifted with respect to the emission maximum of NATA, which is centered at 352 nm, indicating that the protein fluorescence emission arises from tryptophan residues that are embedded in the buried and/or unrelaxed microenvironment. Figure 2 also shows *Ecβ Gal* spectra at 40°C in both the absence and presence of 1.0% SDS. Both spectra are 4 nm red-shifted with respect to the emission maximum exhibited at 10°C. The temperature-dependence of the *Ecβ Gal* emission maximum suggests that the protein in both the absence and presence of the detergent does not undergo dramatic conformational changes as the temperature increases. At 40°C and in the presence of 1% SDS, the emission spectrum of *Ecβ Gal* shows a wider emission band due to the increase of the emission in the long-wavelength region. This change could be due to greater exposure of indolic residues to the solvent and/or to a modest increase in the extent of the spectral relaxation for more of the tryptophanyl residues.

Figure 3 shows the effect of acrylamide on the fluorescence emission of *Ecβ Gal* at 10°C in both the absence and presence of 1.0% SDS. The addition of acrylamide to the enzyme solution in the absence of the detergent results in a linear Stern-Volmer plot. The calculated K_{SV} and k_q are 1.8 M^{-1} and 0.49 × 10^9 M^{-1} s^{-1} (see Table I for the lifetime values used for the calculations), indicating that the *Ecβ Gal* indolic residues are about 10 % accessible to the solvent. On the other hand, acrylamide addition to the protein solution in the presence of 1.0 % SDS still results in a linear of Stern-Volmer plot, but the calculated K_{SV} and k_q are 4.4 M^{-1} and 1.1 × 10^9 M^{-1} s^{-1}. This indicates that in the absence of the detergent the protein tryptophan residues are shielded from the solvent, and that in the presence of 1.0% SDS *Ecβ Gal* has a structure that is more flexible and/or solvent exposed. These results are in agreement with the FT-IR data reported by Muga *et al.* (*7*), and corroborate the conclusion of their study.

In order to gain information on *Ecβ Gal* conformational

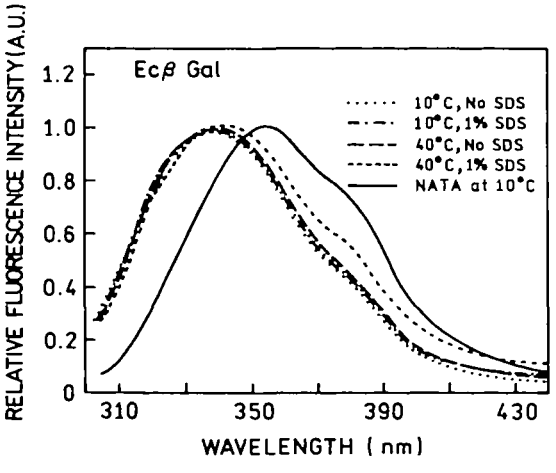

Fig. 2. **Steady-state fluorescence emission spectra of *Ecβ Gal* in the absence and presence of 1.0% SDS at 10 and 40°C.** The excitation was at 295 nm and a 1.0-cm path length quartz cuvette was used. The absorbance of the protein solution was below 0.1 at the excitation wavelength. The protein concentration was 0.05 mg/ml.

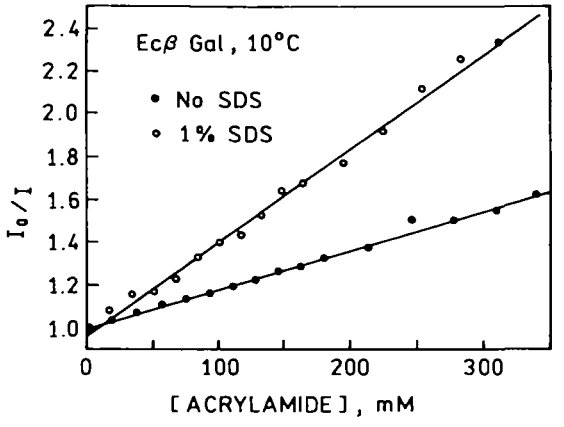

Fig. 3. **Effect of acrylamide on the tryptophanyl emission of *Ecβ Gal* in the absence and presence of 1.0 % SDS.** The temperature was 10°C. The protein concentration was 0 05 mg/ml.

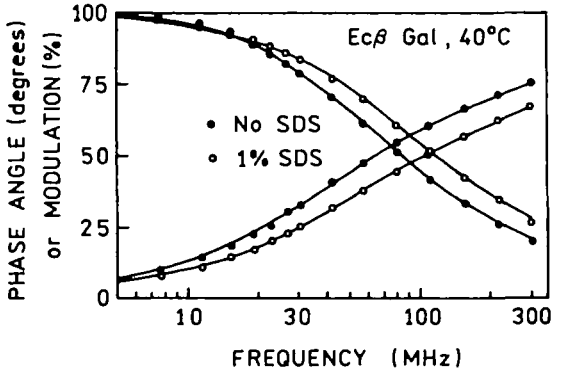

Fig. 4. **Frequency-dependence of the phase shift and demodulation factors of *Ecβ Gal* fluorescence in the absence and presence of 1.0% SDS at 40°C.** Excitation was at 295 nm and the absorbance of the protein solution was below 0.1. The protein concentration was 0.05 mg/ml.

TABLE I. **Multi-exponential analysis of the fluorescence intensity decays of Ecβ Gal in the absence and presence of 1.0% SDS.**

EcβGal	Temp. (°C)	τ (ns)*	α_1	τ_1 (ns)	α_2	τ_2 (ns)	α_3	τ_3 (ns)	χR^2
No SDS	10	3.7	0.03	9.2	0.54	3.2	0.43	0.66	2.3
	40	3.6	0.07	7.3	0.48	2.9	0.45	0.59	1.8
	60	3.2	0.06	6.9	0.45	2.6	0.06	0.59	2.5
1% SDS	10	4.0	0.03	10.4	0.43	3.0	0.54	0.59	2.3
	40	3.2	0.01	12.6	0.39	2.5	0.61	0.52	2.5
	60	2.5	0.01	10.6	0.36	1.9	0.62	0.45	1.8

*τ=mean lifetime calculated according to Eq. 3.

dynamics, we have investigated the protein fluorescence properties by means of frequency-domain fluorometry and anisotropy decays. The intensity decays of the intrinsic fluorescence of *Ecβ Gal* in both the presence and absence of the detergent were measured by means of the frequency-domain method. The data were analyzed in terms of the multi-exponential models and are shown in Fig. 4. In all cases, the best fits were obtained using the three exponential model, characterized by reduced χR^2 values that were much lower than those obtained with simpler models (data not shown). Table I shows the results of multi-exponential analysis of *Ecβ Gal* in the absence and presence of 1.0% SDS, in the temperature range of 10–60°C. Figure 5 shows the effect of temperature on the mean lifetime values. The mean lifetimes of *Ecβ Gal* in the absence of SDS at 10, 40, and 60°C are 3.7, 3.6, and 3.2 ns, respectively. On the other hand, the *Ecβ Gal* means lifetime at 10, 40, and 60°C in the presence of 1.0% SDS and 4.0, 3.2, and 2.5 ns, respectively. Both in the absence and presence of 1.0% SDS the protein's mean lifetimes are, at the investigated temperatures, longer than those exhibited by the aqueous tryptophan solution, indicating that the indolic residues in both enzymes are shielded from the solvent in the entire range of the investigated temperatures.

Table I shows the temperature dependence of *Ecβ Gal* fluorescence decay parameters in both the absence and presence of 1.0% SDS. At 10°C, *Ecβ Gal* in the absence of SDS exhibits three lifetime components at 0.66 (τ_3), 3.23 (τ_2), and 9.2 ns (τ_1), respectively. The middle-lived component (τ_2) is almost coincident with the typical lifetime value observed for NATA, while the long-lived component (τ_1) can be ascribed to tryptophan residues located in compact homogeneous, partly buried and/or unrelaxed interiors of the protein matrix. The addition of 1.0% SDS to the protein at 10°C affects the long-lived component which increases from 9.2 to 10.4 ns, the short-, and middle lived components remaining almost unchanged. At 40°C, the protein in the absence of the detergent shows three lifetimes centered at 0.59, 2.9, and 7.3 ns. When 1.0% SDS is added at 40°C to the protein solution the long-lived component moves from 7.3 to 12.6 ns, and the short- and middle-components become 0.52, and 2.5 ns, respectively. Finally, at 60°C *Ecβ Gal* in the absence of the detergent shows a mean lifetime of 3.2 ns. On the other hand, the protein in the presence of 1.0% SDS and at 60°C exhibits a mean lifetime of 2.5 ns. This marked decrease in the mean lifetime values suggests that the protein structure in the presence of the detergent undergoes denaturation to a greater extent than in the absence of the detergent.

In an attempt to visualize the conformational dynamics of *Ecβ Gal* in the absence and presence of SDS we analyzed protein fluorescence lifetimes by means of distribution analysis (*20*). The best fits were obtained from a bimodal distribution with a Lorentian shape. It is our opinion that interpretation of emission decays in terms of continuous distribution is more informative than by means of discrete components. The use of a lifetime distribution allows one to visualize the various decay time components and their amplitudes more readily than examination of the α_i and τ_i values obtained with Eq. 1.

Figure 6 shows the *Ecβ Ga1* lifetime distributions at 40°C in both the absence and presence of 1% SDS. Table II shows the *Ecβ Gal* fluorescence values obtained on lifetime distribution analysis. The fluorescence lifetimes in the absence of SDS appear to be distributed in two well distinct peaks, suggesting that the emissive properties of *Ecβ Gal* arise from different tryptophanyl microenvironments during the lifetime of the excited state (*22*), which are subject to different degrees of tertiary constraints. At 40°C and in the absence of the detergent (Fig. 6), two components appear in the lifetime distribution: one centered at 0.65 ns and the other at 3.58 ns. The short component (0.65 ns) is very broad, showing a width of 0.56 ns. The long component (3.58 ns) is sharper, with a width of 0.05 ns. The pres-

TABLE II. **Fluorescence lifetime distribution of *Ecβ Gal* in the absence and presence of 1% SDS at 40°C.**

Ecβ Gal	c_1 (ns)	c_2 (ns)	hw_1	hw_2	χR^2
NO SDS	0.65	3.58	0.56	0.05	1.7
+ 1% SDS	0.48	2.67	0.45	0.08	1.3

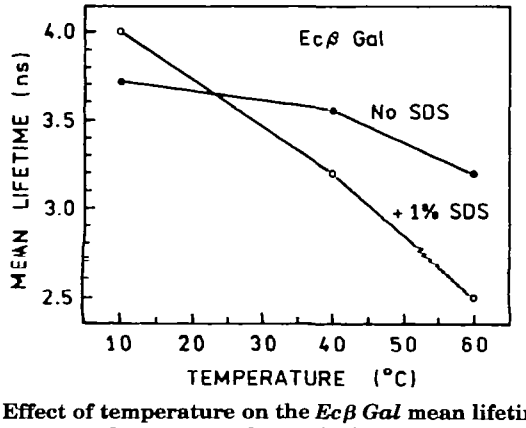

Fig. 5. **Effect of temperature on the *Ecβ Gal* mean lifetime in the absence and presence of 1.0% SDS.** The temperature was 40°C.

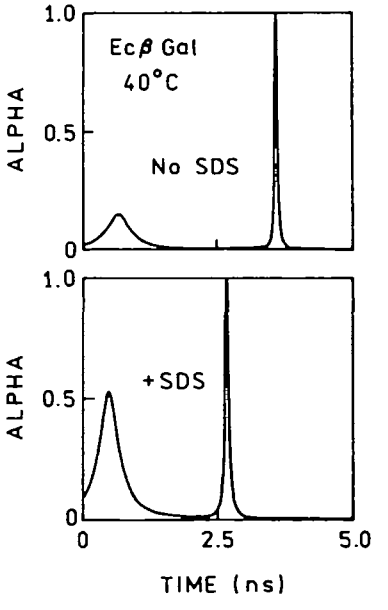

Fig. 6. **The bimodal Lorentian lifetime distribution of *Ecβ Gal* in the absence and presence of 1.0% SDS.** Temperature was at 40°C.

TABLE III. **Anisotropy decay analysis of *Ecβ Gal* in the absence and presence of 1.0 % SDS.**

T (°C)	*Ecβ Gal*					*Ecβ Gal* + 1% SDS				
	r_0g_1	r_0g_2	θ_1 (ns)	θ_2 (ns)	χR^2	r_0g_1	r_0g_2	θ_1 (ns)	θ_2 (ns)	χR^2
40	0.194	0.101	0.33	16.4	1.1	0.277	0.02	0.31	8.6	1.4

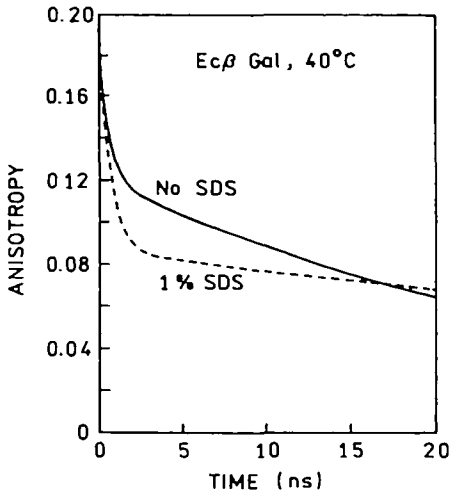

Fig. 7. **Anisotropy decays of *Ecβ Gal* in the absence and presence of 1.0% SDS at 40°C.**

ence of 1.0% SDS results in shortening of the short- and long-component distribution centers to 0.48 and 2.6 ns, respectively. Moreover, the width of the long component becomes almost two times, broader (0.05 to 0.08 ns), suggesting that the tryptophanyl microenvironments are more homogeneous and/or that the interconversion between different protein sub-conformational states is faster in the presence of the detergent than in its absence (*21–23*).

The frequency-domain anisotropy decays of *Ecβ Gal* in both the absence and presence of 1.0% SDS are shown in Table III. The best fit was obtained with the two-correlation time model. In Fig. 7 are depicted the time-domain anisotropy decays of *Ecβ Gal* at 40°C. The two correlation times indicate both local motions of *Ecβ Gal* tryptophanyl residues and flexibility of the entire protein structure. The short correlation time is associated with the local freedom of the tryptophanyl residues, as found in several studies on the anisotropy decays of proteins (*17–19*). The longer correlation time can be associated with both the overall rotation of the protein and the segmental motions of the side chains of *Ecβ Gal*. When the detergent is added to the protein solution the long correlation times component becomes shorter (16.4 to 8.6 ns), suggesting a gradual increase in the overall flexibility of the protein structure. This conclusion is supported by the gradual increase in the fraction of the shortest correlation time (r_0g_i = 0.194 without SDS and 0.277 with SDS). In our opinion this is conceivable since, at least in part, the flexibility of the protein structure, upon the addition of SDS, may be due to the effect of the detergent molecules binding to the highly hydrophobic domains of the protein.

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