FLUORESCENT LABELING OF PROTEINS IN SODIUM DODECYL SULFATE COMPLEXES WITH FLUORESCAMINE

Shu-I Tu and Leonard Grosso

Department of Chemistry, State University of New York

at Stony Brook, Stony Brook, New York 11794

Received June 29,1976

SUMMARY

The effects of sodium dodecyl sulfate on the fluorescent labeling of proteins were studied. Of 57 primary amine groups in bovine serum albumin, no more than 7 are titrable by fluorescamine. Fluorescamine labeling does not cause appreciable conformational changes of proteins. The extent of labeling of proteins decreases as the concentrations of sodium dodecyl sulfate increases. The fluorescence properties of labeled primary amine are only slightly affected by the polarities of the solvents. The inhibitory effects of sodium dodecyl sulfate upon labelings are interpreted as the low permeability of fluorescamine toward the highly charged envelopes of sodium dodecyl sulfate-protein micelles.

Recent studies on the topology of biological membranes revealed that fluorescamine which reacts specifically with primary amines (1,2) can be used as a surface labeling reagent. Nakaya, et al. (3) showed that fluorescamine complexed with Schardinger dextrin only labels the surface proteins of erythrocytes. They suggested that both the size and the hydroxyl groups of dextrin probably prevents the labeling reagent from passing through the lipid bilayer. Hawkes, et al. (4) used fluorescamine alone and showed that only the surface proteins of chick embryo fibroblasts are labeled. They explained this observation in terms of the high reactivity of fluorescamine toward primary amines and the rapid hydrolysis of excess reagent in aqueous medium. Reynolds and Tanford (5) showed that SDS[1]-protein complexes are formed predominantly by hydrophobic interactions between the hydrocarbon tail of SDS and proteins.

[1]Abbreviation: SDS - sodium dodecyl sulfate

The resulting micellar complexes with polar heads exposed to the aqueous environment were suggested as a model for biological membranes. In this report, the effects of SDS on the extent of fluorescamine labeling of proteins were studied. The results indicate that fluorescamine cannot pass through the highly charged envelopes of SDS-protein complexes.

MATERIALS AND METHODS

Fluorescamine was purchased from Pierce Chemical Co. Bovine serum albumin, ovalbumin, lysozyme and glycylglycine were obtained from Sigma Chemical Co. Electrophoresis grade SDS was the product of Bio-Rad Co. All other chemicals used were of the highest purity available.

Fluorescamine labeled n-butylamine was synthesized by mixing 2 ml of 5.0×10^{-4} M n-butylamine in benzene and 20 µl of 7.0×10^{-4} M fluorescamine in acetone. The solution immediately turned to yellow with a strong greenish fluorescence. The fluorescent product was separated on silica gel thin-layer-chromatographic plate using benzene as the developing solvent.

The fluorescence labeling was performed as fellows. A 10 µl of 4 mM fluorescamine in acetone was rapidly injected into a 3 ml of protein solution containing 3 mg protein, 10 mM phosphate pH 7.5, and SDS, and the whole solution was vortexed vigorously. The emission spectra of the labeled proteins were recorded with a Farrand Mark I Spectrofluorometer. The fluorescence intensities were measured by integrating the emission spectra. For all fluorescence experiments, the excitation wavelength was fixed at 390 nm.

Absorption and circular dichroism spectra were obtained with an American Instruments DW-2 Spectrophotometer and a JASCO J-20A Automatic Recording Spectropolarimeter, respectively.

RESULTS AND DISCUSSION

Labeling of bovine serum albumin: Bovine serum albumin is known to have 56 lysine residues and has an aspartic acid as the N-terminal group (6). Thus, in theory, one would expect that 57 amino groups could react with fluorescamine to form fluorescent groups. For primary amines, the efficiency of fluorescamine labeling has been determined (2) as 90% in aqueous medium at pH 8.0. Using the same method, we found that labeling efficiency is about 85% in phosphate buffer at pH 7.5. In Figure 1, the results of a titration of available primary amine groups in bovine serum albumin with fluorescamine are

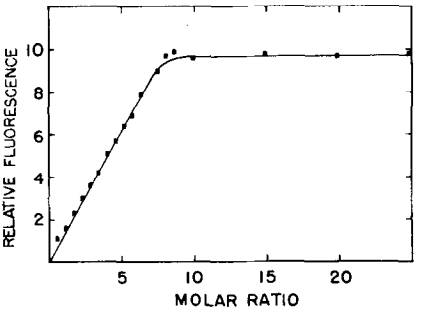

Figure 1. Titration of primary amine groups in bovine serum albumin with fluorescamine. Different concentrations of fluorescamine in 10 µl of acetone were added to 2 ml samples of 1.0 mM bovine serum albumin in 10 mM phosphate, pH 7.5. The abscissa indicates the molar ratio of fluorescamine to bovine serum albumin at the labeling stage.

shown. It was noted that the fluorescence intensity reached saturation when the molar ratio of fluorescamine added to bovine serum albumin was 8. This result indicates that only 7 out of 57 primary amine groups are available for the labeling. Those titrable amino groups are most likely located on the surface of the protein.

Effects of fluorescamine labeling on the conformation of proteins: With the labels mainly on the surface of proteins, one would expect that the conformations of the peptide chains of proteins would not be altered. The circular dichroism spectra in Figure-2 illustrates this point. As can be seen, fluorescamine labeling does not cause any detectable conformational changes with either bovine serum albumin or ovalbumin.

Effects of SDS on fluorescamine labeling of proteins: With both protein and fluorescamine concentrations maintained as constants, the fluorescence intensities resulting from the labeling decreased as the concentration of SDS increased in the labeling media. This inhibitory effect is depicted in Figure-3. We believe that the decrease of fluorescence intensities resulting from increased amounts of SDS may arise from the limited permeability of fluorescamine through highly charged sulfate groups at the envelopes of SDS-protein micelles. Alternative reasons include (i) quenching by micellar

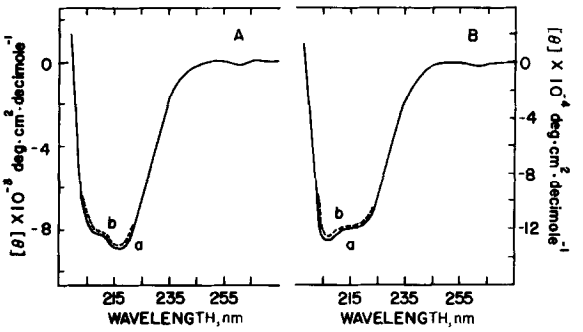

Figure 2. Circular dichroism spectra of proteins with and without fluorescamine labeling. In (A), ——— curve a: 9.8 nmoles of ovalbumin in 2.65 ml of 10 mM phosphate, pH 7.5, ----- curve b: same ovalbumin solution treated with 40 nmoles of fluorescamine. In (B), ——— curve a: 2.2 nmoles of bovine serum albumin in 2.55 ml of 10 mM phosphate, pH 7.5., ----- curve b: same bovine serum albumin solution treated with 40 nmoles of fluorescamine.

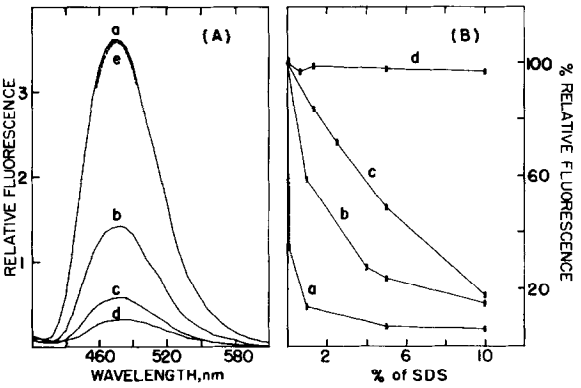

Figure 3. Inhibitory effects of SDS on fluorescamine labeling. In (A), 100 nmoles of fluorescamine in 10 µl of acetone was added to 3 ml of 1.5 x 10^{-5} M bovine serum albumin in 10 mM phosphate, pH 7.5, in the presence of SDS. ——— a, 0%, ——— b, 0.1%., ——— c, 1%., and ——— d, 10% of SDS. ----- e, labeling was performed in the absence of SDS as for ——— a, then SDS was added to 10% and pH readjusted to 7.5. In (B), 100 nmoles of fluorescamine in 10 µl of acetone was added to 3 ml of each of 1.5 x 10^{-5} M of bovine serum albumin (curve a), 2.2 x 10^{-5} M of ovalbumin (curve b), 7.0 x 10^{-5} M of lysozyme (curve c), and 7.6 x 10^{-4} M of glycylglycine (curve e), respectively, in 10 mM phosphate, pH 7.5 containing SDS with concentrations as indicated.

structure, (ii) an enhancement of the hydrolysis rate of fluorescamine in the presence of SDS, (iii) a lower reactivity of fluorescamine with amino groups in low dielectric hydrophobic environment, or (iv) lower quantum yields of

labeled fluorophores in nonpolar media. These alternatives were further investigated as described below.

It has been shown that the electrophoretic mobilities of proteins in SDS-acrylamide gel electrophoresis are not altered by fluorescamine treatment (2,3,7). These results indicate that the labeling does not change the nature of the binding of SDS to proteins. Curve a of Figure 3A is the emission spectrum of labeled bovine serum albumin and curve e is the spectrum of the same solution after 10% SDS was added. Since curves a and e are virtually the same, we conclude that micellar structure does not quench the emission from fluorophores. In Figure-1(B), curve d shows that the fluorescence resulting from labeling of glycylglycine in the presence of 0 to 10% SDS remained nearly constant. This indicates that the amount of fluorescamine available for the labeling of glycylglycine is not affected by SDS. Thus, SDS does not appear to affect the hydrolysis rate of fluorescamine.

If fluorescamine can penetrate the highly charged outer envelope of SDS-protein complex to label the amino groups situated in the hydrophobic environment, then the observed inhibitory effects by SDS could arise from either alternative reason (iii) or (iv), or both. It was noted that n-butylamine reacted with fluorescamine in benzene almost as rapidly as in the aqueous medium. As shown in Figure-4, the absorption maximum of labeled n-butylamine shifts from 393 nm in phosphate buffer to 391 nm in benzene. The emission maximum upon excitation at 390 nm, is at 488 nm in aqueous buffer and at 475 nm in benzene. From the absorbances at 390 nm and fluorescence intensities, we found that the relative quantum yield of labeled n-butylamine in benzene is 13% higher than in the buffer. These results suggest that the amino groups in the hydrophobic region of SDS-protein micelles contribute very little, if any, to the observed fluorescence in the labeling experiments.

In conclusion, the results indicate that fluorescamine labeling of proteins occurs mainly by reaction with free amino groups exposed to the hydrophilic environment, and that the polar groups on the membrane prevent the

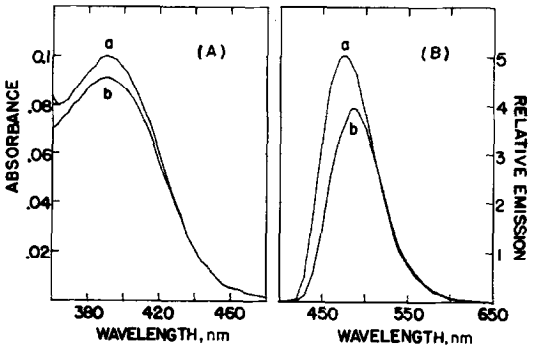

Figure 4. The absorption and fluorescence spectra of fluorescamine labeled n-butylamine. The concentration of labeled amine was 2.8 x 10^{-5} M. The absorption spectrum in benzene (——— a) and in 10 mM phosphate, pH 7.5 (——— b) are shown in (A). The fluorescence spectrum, upon excitation at 390 nm, in benzene (——— a) and in 10 mM phosphate, pH 7.5 (——— b) are shown in (B).

penetration of fluorescamine to the hydrophobic interior. Consequently, fluorescamine can be used as an ideal membrane and protein surface labeling reagent.

Acknowledgement: This work was supported by a Biomedical Sciences Grant from USPHS and a grant from the Research Foundation of the State University of New York.

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