A FLUORESCENT PROBE, TOLUIDINYL-NAPHTHALENE-SULFONATE, SPECIFIC FOR THE β STRUCTURE OF POLY-L-LYSINE*

Jackson Lynn and Gerald D. Fasman

From the Graduate Department of Biochemistry,
Brandeis University, Waltham, Massachusetts 02154
(Publication No. 594)

Received September 10, 1968

The use of fluorescent probes to assess the conformation and conformational changes of proteins has shown considerable promise (Edelman and McClure, 1968). Fluorescent probes have been shown to fluoresce with greater efficiencies in an hydrophobic environment, e.g., when bound to proteins, and with very low efficiencies in highly polar solvents like water (McClure and Edelman, 1967).

We wish to report that toluidinyl-naphthalene-sulfonate (TNS)[1] was found to be an excellent fluorescent probe for the β structure of poly-L-lysine. Neither the α-helical nor the random coil conformation caused enhanced fluorescence of the probe, as was found with the β structure. The β conformation of poly-L-lysine, which can be produced by either (1) heating at pH 11.5, (2) addition of LiCl at pH 11.1, or (3) at pH 5.4 in the presence of SDS, was found to interact with the probe. The hydrophobic regions stabilizing the β structure (Davidson and Fasman, 1967) must be responsible for the binding interaction with TNS.

* This work was supported by grants from the National Science Foundation (GB-5576) and the National Institutes of Health (AM-05852) and a research contract from the U.S. Army Medical Research and Development Command (DA-49193-MD-2933).

[1] Abbreviations used: TNS, 2-p-toluidinyl-naphthalene-6-sulfonate; ORD, optical rotatory dispersion; SDS, sodium dodecyl sulfate.

Polarization of fluorescence studies showed that the β structure had a much higher polarization than either the helical or random conformation.

ORD and circular dichroism studies have shown that poly-L-lysine can be converted from the random coil to the β conformation and from α helix to β (Sarkar and Doty, 1966; Davidson et al., 1966; Townend et al., 1966; Davidson and Fasman, 1967). Side-chain interactions between lysine residues through hydrophobic bonding have been proposed to account for the conversion of the α helix to the β conformation by means of heating poly-L-lysine at pH 11 (Rosenheck and Doty, 1961; Davidson and Fasman, 1967).

Experimental. Poly-L-lysine hydrochloride, GF-16-249-24, $[\eta]^{1.0\ M\ NaCl}_{pH\ 4.5} = 0.97$, mol wt = 85,000, was synthesized as previously described (Fasman et al., 1961). TNS was a gift from Dr. W. O. McClure of Rockefeller University. SDS, 95% purity, from Matheson Coleman and Bell, was recrystallized according to the procedure of Kay et al. (1952). LiCl was reagent grade from the Fisher Chemical Company. Fluorescence measurements were performed with a Zeiss spectrofluorometer model ZFM 4C. The excitation wave-length was 350 mμ. The emission spectra are uncorrected. Polarization spectra were obtained on a polarization spectrofluorometer of the design of Weber and Bablouzian (1966). A pair of Corning glass filters, CS 3-72, and sodium nitrite solution filters were used to isolate the emission from the excitation radiation for the polarization measurements. Fluorescence cells used in all measurements were 1 cm path length. The concentration of TNS used in the experiments was 1.2×10^{-6} M and that of poly-L-lysine 0.03 mg/ml.

Fluorescence Intensity of TNS with the β structure of Poly-L-lysine. The fluorescence of TNS in the presence of poly-L-lysine

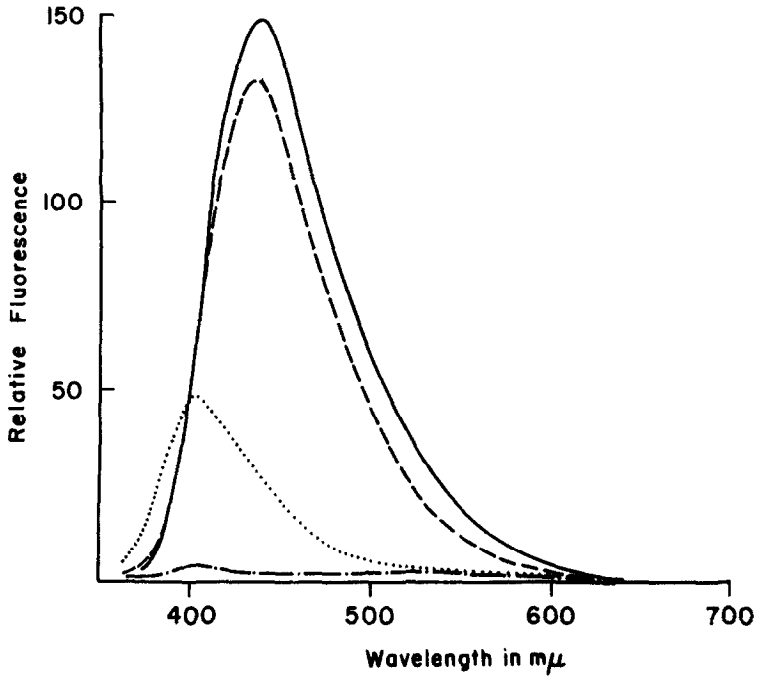

Fig. 1. Fluorescence of TNS in the presence of poly-L-lysine. -·-·-, at pH 6 to pH 11.5, or acidification of the heated solution to pH 2.5; -----, solution at pH 11.5 heated to 55° for 3 min, followed by cooling to room temperature for measurement; _____, in 2.0 M LiCl, at pH 11.1; ·····, in 2.0 M LiCl at pH 2.1.

is negligible at pH 6 to 7 where the polypeptide is in the random conformation (Fig. 1). At pH 11.5, where the conformation is helical, the fluorescent intensity remains unchanged. When this alkaline solution of poly-L-lysine was heated to 55° for 3 min followed by cooling to room temperature, the conditions for β formation, the fluorescence increased immensely with an emission maximum at about 440 mμ. The fluorescent intensity and the emission maximum remained constant upon allowing the solution to stand at room temperature for an hour. However, a drop of about 50% of the original fluorescent intensity was recorded when the solution was cooled in an ice bath for an hour, conditions known to partially destroy the β structure (Davidson and Fasman, 1967). Acidification (pH 2.5) of heated poly-L-lysine solutions caused

the fluorescent intensity to return to that of the solution before heating (Fig. 1). When TNS was treated alone in a fashion similar to that used with poly-L-lysine, there was very low fluorescence.

Poly-L-lysine at pH 11 can be converted to the β structure upon the addition of LiCl (Davidson and Fasman, 1967). The fluorescence of TNS and poly-L-lysine in 2.0 M LiCl is shown in Fig. 1. Upon adding LiCl to a poly-L-lysine solution in the presence of TNS at pH 11.5, which showed a low fluorescent intensity, the fluorescence intensity increased 150-fold with an emission maximum at 440 mμ. The controls using only TNS with or without 2.0 M LiCl at pH 11.1 showed low fluorescence. When the solution of poly-L-lysine, 2.0 M LiCl, and TNS, pH 11.1, was acidified to pH 2.0, the fluorescent intensity decreased with a concomitant shift of the emission maximum to 400 mμ (Fig. 1), but did not return to the original random coil value.

Poly-L-lysine, pH 7, can be converted from the random form to the β structure in the presence of SDS (Sarkar and Doty, 1966). The results of the study of poly-L lysine with SDS at pH 5.4 in the presence of TNS are illustrated in Fig. 2. A poly-L-lysine solution (pH 5.4) in the presence of TNS, enhanced the fluorescence intensity of the probe about 150-fold over the control of TNS alone in SDS. The emission maximum was now found at 460 mμ. The fluorescence intensity, however, was sensitive to the ionic strength; an NaCl solution (0.2 M) decreased the fluorescence to about 10% of the salt-free value.

Polarization of Fluorescence Study of the Binding of the Fluorescent Probe to the β Structure. The technique of polarization of fluorescence has been used to assess the degree of binding of fluorescent probes to proteins (Weber and Young, 1964; Stryer,

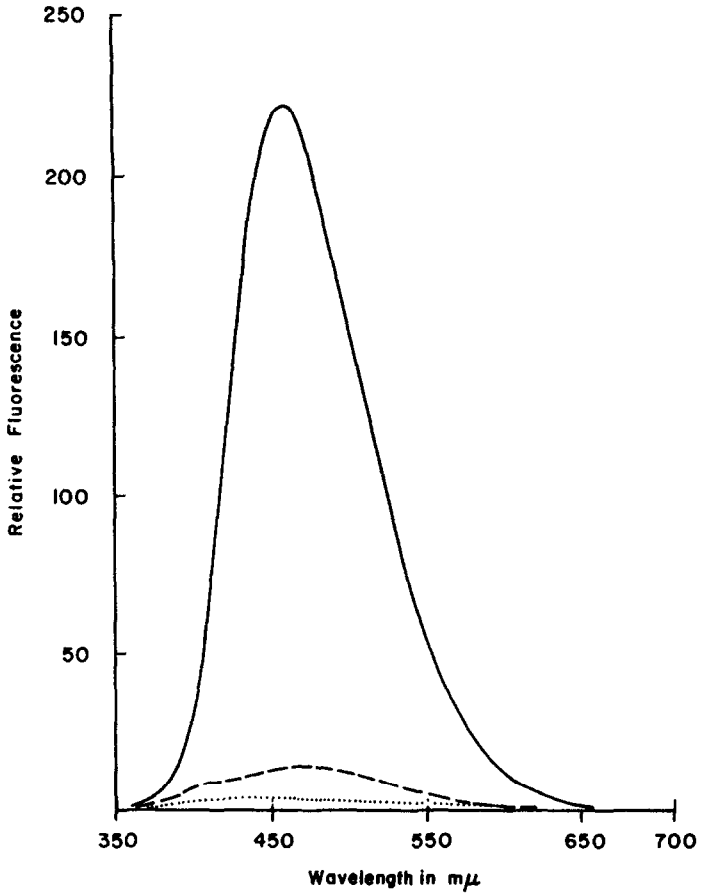

Fig. 2. Fluorescence of TNS in the mixture of SDS and poly-L-lysine. ———, TNS in SDS and poly-L-lysine at pH 5.4 without NaCl; ----, TNS in SDS and poly-L-lysine with 0.2 M NaCl; ·····, TNS in SDS. SDS, 0.006 mg/ml.

1965; Daniel and Weber, 1966) and to indicate the formation of rigid structures. The polarization of fluorescence of poly-L-lysine with TNS is illustrated in Fig. 3. A random coil poly-L-lysine solution, pH 6.5, in 0.2 M NaCl with TNS had significant polarization values throughout the polarization spectrum with a peak at around 325 mμ, p = 0.165. The same solution at pH 11.5, now in the α-helix conformation, showed still higher polarization values, with a peak at 325 mμ, p = 0.206. But when the same alkaline solution was heated and cooled as previously described,

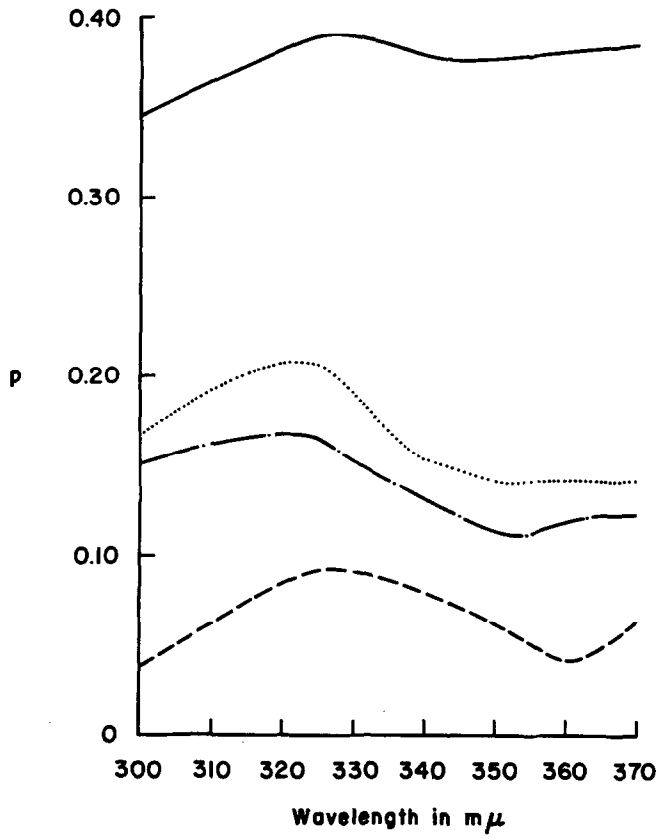

Fig. 3. Polarization fluorescence spectra of TNS in poly-L-lysine, 0.2 M NaCl. ———, solution at pH 11.5 heated to 55° for 3 min and then cooled to room temperature for measurement; ·····, solution at pH 11.5, α-helix form; -·-·-, solution at pH 6.5, random form; ----, acidification of the heated solution to pH 2.5.

the polarization spectrum clearly demonstrated much higher polarization values throughout the 300 to 370 mμ region with a peak at 325 mμ, p= 0.380. However, after the solution had been acidified to pH 2.5, destroying the β structure, the polarization spectrum showed low polarization values throughout, even smaller than at pH 6.5. The peak at 325 mμ was observed, p_{325} = 0.080. TNS in water at pH 6.5 showed a p_{325} = 0.22, which may be due to association, as the polarization was reduced to zero in absolute ethanol.

Discussion. It has been shown that an increase of fluorescence of TNS occurs only in the presence of the β conformation of poly-L-lysine, which has been generated by three different methods. The increase of fluorescence as illustrated in Figs. 1 and 2 indicates that TNS is located in a hydrophobic environment, in a manner analogous to that previously described by McClure and Edelman (1967). As the polarization spectrum of the β conformation (Fig. 3) is seen to be much higher than that of the α-helix or the random form, this indicates that the β form has more rigid and larger overall dimensions, causing slower rotational tumbling in solution.

The forces responsible for binding TNS to the β structure at pH 11 after heating are independent of the ionic strength, as might be expected for hydrophobic interactions. It is likely that hydrophobic interactions between the β structure and the aromatic moiety of the TNS probe are solely responsible for the binding of the probe. This necessary hydrophobic region is found only in the β structure of poly-L-lysine and is produced by the interaction of the lysine side chains, which must be absent in the helical and random coil forms. The production of the β structure of poly-L-lysine from the charged random form by mixing with SDS must, in part, be due to charge neutralization. This salt linkage depends on the ionic strength, as fluorescence can be diminished by an increase in the ionic strength (Fig. 2).

Thus, TNS has been found to be a fluorescent probe for the β structure of poly-L-lysine.

References

Daniel, E. and Weber, G. (1966). Biochemistry 5, 1960.
Davidson, B., Tooney, N. and Fasman, G. D. (1966). Biochem. Biophys. Res. Commun. 23, 156.
Davidson, B. and Fasman, G. D. (1967). Biochemistry 6, 1616.

Edelman, G. M. and McClure, W. O. (1968). Accounts of Chemical Research 1, 65.
Fasman, G. D., Idelson, M. and Blout, E. R. (1961). J. Am. Chem. Soc. 83, 709.
Kay, E. R. M., Simmons, N. S., and Alexander, L. D. (1952). J. Am. Chem. Soc. 74, 1724.
McClure, W. O. and Edelman, G. M. (1967). Biochemistry 6, 567.
Rosenheck, K. and Doty, P. (1961). Proc. Natl. Acad. Sci. U. S. 47, 1775.
Sarkar, P. K. and Doty, P. (1966). Proc. Natl. Acad. Sci. U. S. 55, 981.
Stryer, L. (1965). J. Mol. Biol. 13, 482.
Townend, R., Kumosinski, T. F., Timasheff, S. N., Fasman, G. D. and Davidson, B. (1966). Biochem. Biophys. Res. Commun. 23, 163.
Weber, G. and Bablouzian, B. (1966). J. Biol. Chem. 241, 2558.
Weber, G. and Young, L. (1964). J. Biol. Chem. 239, 1424.