BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 45, 2206—2207(1972)

A Fluorescence Study of a Random Coil-to-α-Helix Transition of a 1-Dimethylaminonaphthalene-5-sulfonyl Poly-L-lysine Conjugate

Takayoshi Iio, Yoshihiro Iwashita, and Hideharu Watanabe
Department of Physics, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya

(Received February 7, 1972)

In this communication, we wish to report on our studies of a 1-demethylamino-naphthalene-5-sulfonyl poly-L-lysine (DNS-PLy) conjugate by the fluorescence method.

Poly-L-lysine is reversibly transformed in its conformation from a random coil to an α-helix at room temperature when the pH value of the solution is changed from 8 to 11.[1] We intended to obtain information on the state of the L-lysyl residue in each conformation. For this purpose, we bound a fluorescent molecule, DNS, to the ε-N atom of the lysyl residue and observed the polarization of the fluorescence under various conditions.

We found that the L-lysyl residue got some flexibility in the random coiled conformation, while the flexibility of the L-lysyl residue was almost completely suppressed in the α-helical conformation.

Experimental

PLy was obtained according to the usual *N*-carboxyanhydride method.[2] The molecular weight of PLy was determined to be 30000 by the Archibald method;[3] the degree of polymerization was 230.

DNS was coupled to the L-lysyl residue according to the method reported by Weber.[4] The molar ratio of the bound DNS to the L-lysyl residue was 1 : 48.5.

All the fluorescence measurements were performed in polymer concentrations ranging from 0.75×10^{-7} to 3.7×10^{-7} mol residue/ml. In these concentrations, the relative fluorescence intensity was proportional to the polymer concentration.

The fluorescence spectra and the relative fluorescence intensity were measured with a Hitachi Fluorescence Spectrometer, MPF-2A.

The polarization of the fluorescence was measured with an improved apparatus which had originally been made for light scattering experiments by Shimadzu Seisakujo, Kyoto. The apparatus was improved in the same manner as is shown in Fig. 5 of ref. 5. A monochromatic filter for excitation light with a maximum of transmittance at 350 mμ, and a cut off filter for emitted light which transformed light only above 420 mμ, were used in this experiment. The size of a fused quartz cell was $6.5\times1.0\times1.0$ cm. The value of polarization (P) was calculated according to Eq. (1);

$$p=\frac{I_v-I_h}{I_v+I_h} \tag{1}$$

where I_v and I_h are the fluorescence intensity of vertically polarized light and that of horizontally polarized light when the solution is excited with vertically polarized light.

The optical rotatory dispersion curves were measured with a JASCO ORD/UV 5 apparatus.

Results and Discussion

The conformational change in PLy with the pH value is shown in Fig. 1, curve a.

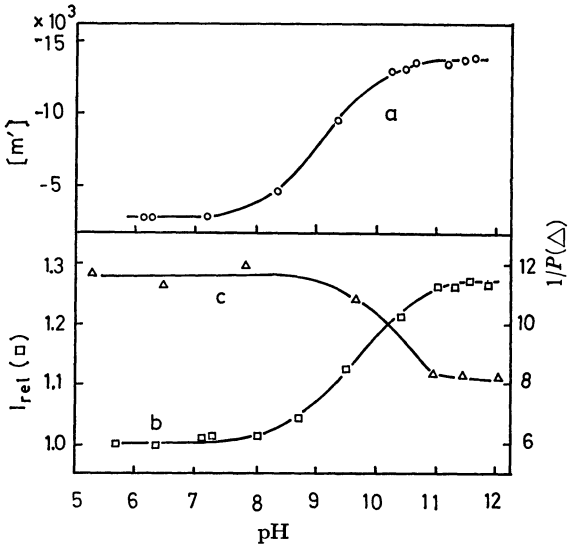

Fig. 1. pH Dependences of mean residue rotation [m'] at 233 mμ of PLy solution (curve a), relative fluorescence intensity (I_{rel}) of DNS-PLy conjugate (curve b), and of inverses value of polarization of the conjugate (curve c). These experiments were performed at room temperature (18—20°C)

DNS bound to PLy had a fluorescence maximum at 495 mμ when it was excited with a light of 350 mμ. The relative fluorescence intensity increased when the pH value of the solution was increased from 8 to 11, as shown in Fig. 1, curve b. In the following discussion, we will make use of the curve b for the purpose of estimating the lifetime of the DNS bound to α-helical PLy.

The inverse value of polarization indicates the degree of flexibility of the L-lysyl residue to which DNS is bound.[6] The pH dependence of this value is shown in curve c (Fig. 1). From an inspection of the curve

1) J. Applequist and P. Doty, "Polyamino acids, Polypeptides and Proteins" ed. by M. Stahmann, University of Wisconsin Press, (1962), p. 161.

2) J. Noguchi, "Poly condensation and Polyaddition Reaction" (in Japanese), Kyoritsu Shuppan, Tokyo (1952), p. 301.

3) K. Kakiuchi, *Seibutsubutsuri*, **7**, 38, (1967).

4) G. Weber, *Biochem. J.*, **51**, 155, (1952).

5) T. Takagi, "Atarashii Seibutsu Butsuri Kagaku Kenkyu-hou" ed. by T. Isemura, J. Tanaka, H. Noda, and T. Miyazawa, Kagakudojin, Kyoto (1967), p. 155.

6) G. Weber, Adv. in Protein Chem. VIII, 415 (1953).

c, we can conclude that the flexibility of the L-lysyl residue decreased as the conformation of PLy changed from the random coil to the α-helix.

Further information can be obtained from the Perrin plots for the DNS-PLy conjugate. In Fig. 2, we show the effects of the temperature and the viscosity on the polarization of the fluorescence. The data allow the conclusion that Perrin's law of depolarization (Eq. (2)) is closely followed in the cases of both the random coiled and the α-helical PLy.

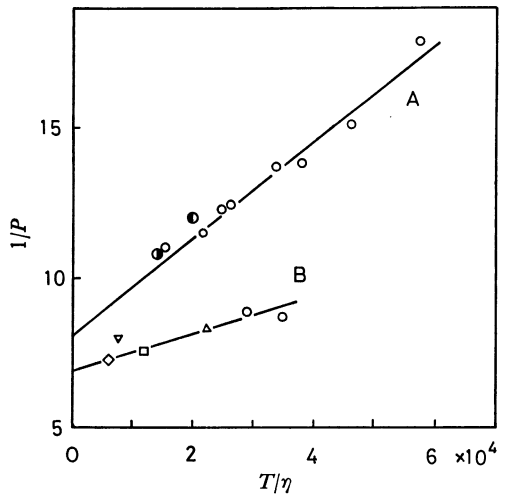

Fig. 2. Effects of temperature and viscosity on the polarization of the fluorescence of the conjugate. Plot A for random coiled conformation was obtained in pH 5.5—6.0 and plot B for α-helix was obtained in pH 11.3—11.9. Open circules were from aqueous solutions at various temperatures and the others were from conjugate-water-glycerine systems at 20°C; glycerine content were 15% (◐), 25% (◑), 10% (△), 30% (□), 40% (▽), and 50% (◇).

From the linear plots in Fig. 2, we can calculate the mean rotational relaxation time (ρ_h) of the DNS molecule bound to PLy according to Eq. (2);[6]

$$\left(\frac{1}{p}-\frac{1}{3}\right)=\left(\frac{1}{p_0}-\frac{1}{3}\right)\left(1+\frac{3\tau_0}{\rho_h}\right) \qquad (2)$$

where P_0 is an intrinsic polarization and where τ_0 is the lifetime of the excited state of the fluorescent molecule. The τ_0 value is proportional to the relative fluorescesce intensity.[7] In the neutral pH region, the τ_0 value has been reported as 1.2×10^{-8} sec,[4] so the τ_0 value in the high pH region (above 11.0) was estimated as 1.5×10^{-8} sec.

The ρ_h value was obtained as 5.6×10^{-8} sec at 20°C in the case of the random coil, and as 16.2×10^{-8} sec at 20°C in the case of the α-helix. The former value is equivalent to a rotational relaxation time of a sphere with a radius of 26 Å; this means that the DNS bound to the L-lysyl residue rotates with a few lysyl residues in the polypeptide chain. The latter value is approximately equal to a harmonic mean rotational relaxation time of an α-helical PLy molecule with a degree of polymerization of 230; that is, the flexibility of the L-lysyl residue is completely suppressed and the rotational motion of the DNS molecule coincide with that of one rigid α-helical PLy molecule.

These results are reasonable because the following situation may occur in the PLy molecule: in low-pH regions, where PLy takes the random coil, the main chain of PLy assumes a locally-extended form because of repulsive interaction between charged side chains, and both the main chain and the side chain get some flexibility. On the other hand, in the case of the α-helix, the main chain is regularly folded and the flexibility of the side chain may be completely suppressed.

Other evidence on this suppression of the flexibility of the L-lysyl residue in the α-helical conformation was obtained by a nuclear magnetic resonance study in D_2O solutions.[8]

As the temperature of an α-helical PLy solution (pH>11.0) is increased to 30°C or more, PLy is transformed in its conformation from the α-helix to the β-form.[9] An intermolecular β-form was also assumed in this case. The hydrophobic bonding between side chains is one of the main factors in stabilizing the β-form.[9] Therefore, we expected the $1/P$ value to decrease when PLy took the β-form. In fact, when the temperature of the PLy solution in pH 11.5 was raised to 34°C from 20°C, the $1/P$ value decreased to 6.0 from 8.7. The details of the fluorescence studies of the β-form will be published elsewhere.

The authors wish to thank to Dr. Sho Takahashi of the Institute for Chemical Research, Kyoto University, for his valuable discussions.

7) J. R. Laurence, "Physical Methods in Macromolecular Chemistry", ed. by B. Carroll, Marcel Dekker, New York (1969), p. 275.

8) E. M. Bradbury, C. C. Robinson, H. Goldman, and H. W. E. Rattle, *Biopolymers*, **6**, 851, (1968).

9) B. Davidson and G. D. Fasman, *Biochemistry*, **6**, 1616, (1967).