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A Fluorescence Study of a β-Structural 1-Dimethylamino-naphthalene-5-sulfonyl Poly-L-lysine Conjugate

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An α-helical poly-L-lysine (PLy) is irreversibly transformed in its conformation to a β-structure when the temperature of the solution is raised to 50°C.[1] It is agreed that the β-structure is stabilized by the hydrophobic bond between the side chains in addition to the hydrogen bond between the main-chain peptide groups. The intensity of the observed emission light of the 1-dimethylaminonaphthalene-5-sulfonate (DNS) bound to PLy is increased as the conformation of the DNS–PLy conjugate is changed from the α-helix to the β-structure (Fig. 1). On the other hand, the optical

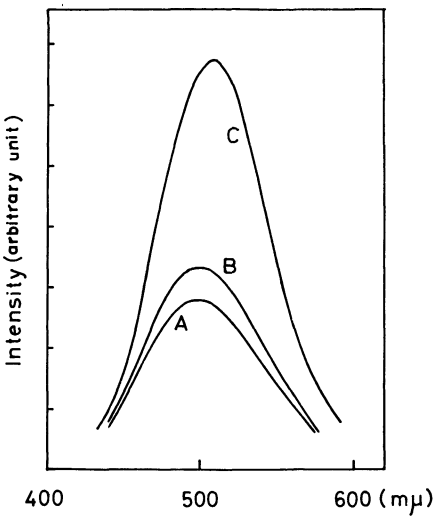

Fig. 1. Observed emission spectra of DNS-PLy conjugate in 0.01N KCl solutions at 20°C. Wavelength of excitation light was 350 mμ. A: Random coil (pH 5.8), B: α-Helix (pH 12.4), C: β-Structure (after heating the α-helical solution at 50°C for 30 min, then cooled to 20°C).

density of the conjugate at the wavelength of the excitation light scarcely changes during the conformational transition. The increase in the relative fluorescence intensity in the β-structural conjugate may be a reflection of the change in the environment around the DNS group. The DNS group may come in contact with the solvent molecules in the case of the α-helix; on the other hand, it may be incorporated in the hydrophobic region and surrounded by the hydrophobic residues when the conjugate takes the β-structure. As a support to this conclusion, we can offer the fact that the quantum yield of DNS is increased from 0.29 to 0.71 when the solvent is changed from water to *n*-butanol at 20°C.[2]

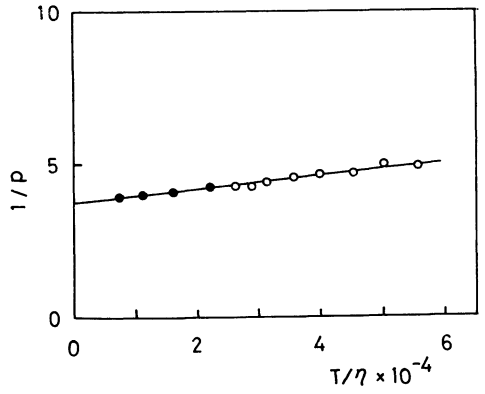

Fig. 2. Depolarization curve of β-structural DNS-PLy conjugate in 0.01N KCl (open circles) and in mixture of water and glycerine containing 0.01N KCl at 20°C (filled circles). Wavelength of excitation light and that of emission light were 350 mμ and 500 mμ, respectively.

We desire to obtain information on the rotational motion of the DNS group which is incorporated into the hydrophobic region. This information can be obtained from the depolarization curve[3,4] of the β-structural conjugate (Fig. 2). The isothermal plot (filled circle) and the heating plot (open circle) give the same straight line. From this plot, we can obtain the rotational relaxation time (ρ_h) at a particular temperature (T) of the DNS group bound to the β-structural PLy according to Eq. (1);

$$\rho_h = \left(\frac{1}{p_0} - \frac{1}{3}\right) \cdot \frac{3\tau}{\text{the slope}} \cdot \left(\frac{\eta}{T}\right)_T \qquad (1)$$

where p_0 is an intrinsic polarization, τ is the lifetime of the lowest excited state of the DNS group, and η is the viscosity of the solvent. The τ value is proportional to the relative fluorescence intensity,[5] so the τ value is obtained according to Eq. (2);

$$\tau_\beta = \tau_{coil} \cdot \frac{F_\beta}{F_{coil}} \cdot \frac{OD_{coil}}{OD_\beta} \qquad (2)$$

where F is the fluorescence intensity and OD is the optical density of the conjugate at the wavelength of the excitation light. The magnitude of F is calculated as follows: the corrected emission spectra which is obtained by calibrating the observed emission spectra (Fig. 1) for the detector system is plotted on graph paper, and the magnitude of F is determined by calculating the area beneath the curve. As the τ_{coil} value, we adopt 1.2×10^{-8} sec, this value is an average

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2) E. J. Bowen and D. Seaman, "Luminescence in Organic and Inorganic Materials," ed. by H. Kallmann and G. Spruch, John Wiley, New York (1962), p. 153.

3) F. Perrin, *J. Phys. Radium.*, **7**, 390 (1926).

4) G. Weber, *Adv. Protein Chem.*, **8**, 415 (1953).

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

of the measurements of three different DNS-protein conjugates in the neutral pH region.[6] The τ value for the β-structural conjugate is determined as 2.7×10^{-8} sec. Then we obtained $\rho_h=38.2\times10^{-8}$ sec for the β-structural conjugate at 20°C; this value is larger than that of the conjugate in the other conformations (see Ref. 7). The large value of ρ_h means that the rotational motion of the DNS group in the β-structural conjugate is extremely suppressed. The value of $\rho_h=38.2\times10^{-8}$ sec corresponds to the rotational relaxation time of a sphere with a radius of 50 Å, showing that the DNS group is incorporated in a very large β-structural region.

In the course of this experiment, we found a very interesting fact: the $1/p$ value of the β-structural conjugate do not depend on the wavelength of the excitation light (λ^{ex}). On the contrary, the $1/p$ values of the conjugate in the other conformations depend on the λ^{ex} value (Fig. 3). The DNS-protein conjugates show the same $1/p-\lambda^{ex}$ relationship as do the α-helical and random coiled conjugates.[8] The reason for these results is unknown at this stage; we wish to leave it for future investigation.

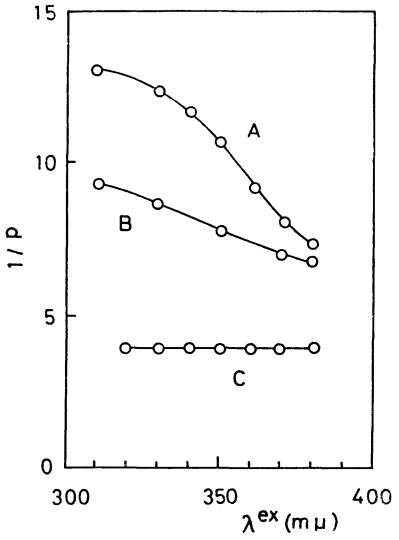

Fig. 3. Effect of wavelength of excitation light (λ^{ex}) on degree of polarization (p) of 500 mμ emission light in DNS-PLy conjugate. Measurements were performed in 0.01N KCl at 20°C. Slit width for excitation light was 6 mμ. Marks are the same as Fig. 1.

Experimental

DNS-Cl was bound to PLy (molecular weight, 30000) according to the method reported by Weber.[9] The molar ratio of the bound DNS to the L-lysyl residue was 1:48.5. All the fluorescence measurements were performed in a concentration of 3.5×10^{-6} mmol of DNS per ml. The fluorescence spectra and polarization of fluorescence were measured with a grating-type Hitachi Fluorescence Spectrophotometer MPF-2A. The degree of polarization (p) was calculated according to Eq. (3):

$$P=\frac{I_{vv}-GI_{hv}}{I_{vv}+GI_{hv}} \tag{3}$$

where I_{vv} and I_{hv} are, respectively the fluorescence intensity of vertically-polarized light and that of horizontally-polarized light when the solution is excited with vertically-polarized light, and where $G=I_{vh}/I_{hh}$ is a correction factor which is needed when the P value is measured using a grating-type fluorometer; here I_{vh} and I_{hh} are, respectively the fluorescence intensity of vertically-polarized light and that of horizontally-polarized light when the solution is excited with horizontally-polarized light. The depolarization curve was obtained by plotting the reciprocal of the polarization of the fluorescence ($1/p$) as a function of the temperature divided by viscosity (T/η in degree·poise^{-1}).

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