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CONCENTRATION DEPENDENT CIRCULAR DICHROISM OF POLY-γ-(1-NAPHTHYLMETHYL)-L-GLUTAMATE

Takamichi ENOMOTO, Hiroyuki NOMORI, and Masahiro HATANO
Chemical Research Institute of Non-Aqueous Solutions,
Tohoku University, Katahira, Sendai 980

A marked concentration dependence was observed for the circular dichroism of poly-γ-(1-naphthylmethyl)-L-glutamate in ethylene chlorohydrin. This fact was explained in terms of the formation of the β-structure in the solution at high concentrations of the polyglutamate and the subsequent restriction of the side chain orientation relative to the dissymmetric field in the solution.

We have already shown that the α-helix content of the copolymer of γ-(1-naphthylmethyl)-D-glutamate and γ-methyl-D-glutamate decreases with the increasing molar ratio of the γ-(1-naphthylmethyl)-D-glutamate unit.1) We interpreted this phenomenon as the result of the increasing steric hindrance to the formation of the α-helical structure caused by the increasing interaction between the bulky side chain groups with the increasing concentration of the naphthyl group around the polyglutamate main chain. In that case, a circular dichroism (CD) with very small magnitude was found to appear in the wavelength region of the absorption of the naphthyl chromophore. We wish to report in this paper an additional result concerning the concentration dependence of the circular dichroism observed for the poly-γ-(1-naphthylmethyl)-L-glutamate (PNLG) solution in ethylene chlorohydrin in the concentration range of $1.2\times10^{-4} \sim 1.9\times10^{-1}$ M, and also to report a new mechanism of the induction of the circular dichroism to a side chain chromophore of a polypeptide molecule.

PNLG was synthesized by the conventional NCA method, as described in the previous paper,1) the intrinsic viscosity of which was measured as 0.45 dl/g at 25°C in ethylene chlorohydrin. The CD and the infrared absorption spectra were recorded on a JASCO J-20A polarimeter and a Hitachi EPI-G2 spectrometer, respectively. Ethylene chlorohydrin was used after the purification by the fractional distillation.

In Fig. 1 are given the CD spectra observed for the solutions of PNLG in ethylene chlorohydrin at various concentrations. The general features of the spectra at lower concentrations are such that the circular dichroism characteristic of the typical α-helical conformation of the polypeptide is superposed by the CD bands due to the naphthyl chromophore. The bands at 209 nm and at 222 nm, which have been attributed respectively to the π–π* transition and to the n–π* transition of the peptide group in the polypeptide main chain, and which have been taken as diagnostic of the existence of the α-helical structure, are quite predominant in magnitude, and the bands at 230 nm and around 280 nm which are regarded as due to the β-band and to the p-band, respectively, of the naphthyl chromophore have very small but definite magnitude. With the increase in the concentration, the CD peak at 209 nm decreases in magnitude and the crossover point at 202 nm moves to the longer wavelength side to 212 nm. In addition, the CD bands around 280 nm shows a drastic increase in magnitude with the increase in the concentration. Thus, the spectra at higher concentrations have the features of the superposition of the spectrum arising from the electronic transitions of the naphthyl chromophore on the spectrum typical of the β-conformation of the polypeptide. When the magnitude of a circular dichroism band corresponding to an electronic transition of the naphthyl chromophore, e.g. that at 283 nm, is plotted against the concentration of the solution, an inflection appears obviously at the concentration of about 0.06 M, where the CD magnitude begins to increase abruptly.

The viscosity of the solution increased in a monotonous way with the increasing concentration, until the concentration of about 0.06 M was attained where a sudden gelatinization of the solution took place. The critical concentration agrees well with the concentration of the beginning of the sharp increase in the size of the CD bands due to the electronic transitions of the naphthyl chromophore. Such is not the case for the polypeptide solution which forms a lyotropic liquid crystalline phase at higher concentrations, where a sharp decrease in the viscosity has been reported to take place at the concentration of the formation of the liquid crystalline phase.[2)] From this fact and the observation that no birefringence appears in the microscopic field of the solution with as high a concentration as the circular dichroism measurement was carried out for in this study, it follows that there does not seem to occur a liquid crystalline phase in the solution even with the highest concentration.

Infrared absorption spectra, which are shown in Fig. 2, indicate that the amide I band of the fluid solution at lower concentration is that characteristic of the α-helical conformation of the polypeptide giving an absorption peak at 1650 cm^{-1}, but that the band becomes superposed by the band of 1620 cm^{-1}, which is ascribed to the β-conformation of the polypeptide, when the solution is gelatinized by raising the concentration. Consequently, the gelatinized state is regarded as composed of the mixture of the α-helical conformation and the β-conformation of the polypeptide. The intermolecular hydrogen bonding constituting the β-structure presumably spreads over the solution including the polypeptide in the α-conformation and thus the whole system becomes gelatinized. We can find a similar situation in the result of Yang and Doty3) where the transformation of the random coil to the β-conformation of poly-γ-benzyl-L-glutamate was observed at a critical concentration of polyglutamate, but we believe that the present result is the first definite observation of the transition of the α-conformation to the β-conformation of a polypeptide accompanying the increase in the concentration.

As we showed in the previous papers,4,5,6) it is the prerequisite for a chromophore to exhibit a circular dichroism in a solution of an α-helical polypeptide that the chromophore is restricted in its rotational freedom through some mechanism or other. Our investigations on this subject have hitherto suggested two causes for the sufficient restriction of the freedom of the chromophore. First of them is the formation of a cholesteric helical structure by the α-helical polypeptide including the chromophore as the side chain residue of the solute polypeptide4) or as the additive component of the polypeptide solution.5) Another cause is the increase in the steric hindrance between the chromophores as the side chain residues of the α-helical polypeptide through the increase in the size of the chromophore or through the introduction of a polar substituent into the chromophore.4,5)

The present investigation indicates indeed that the bulkiness of the large naphthyl residue in the side chain of PNLG causes the restriction of the residue enough for the induction of the circular dichroism to the electronic transitions of the naphthyl chromophore, since a definite magnitude of circular dichroism was observed in the corresponding wavelength region for the solution of low concentration where no appreciable transition of the α-conformation of PNLG to the β-structure was observed. Thus, it must be true at least for the non-gelatinized

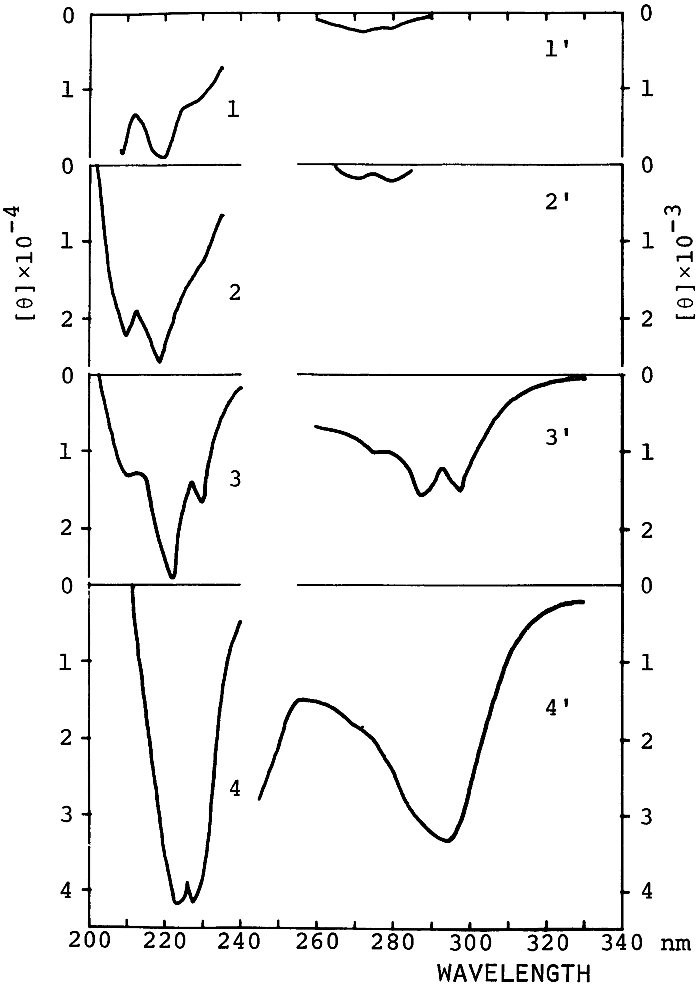

Fig. 1. CD spectra of the PNLG solutions in ethylene chlorohydrin in the fluid state at 1.20×10^{-4} (1), 1.85×10^{-3} (2 and 1') and 2.76×10^{-2} M (3 and 2') and in the gelatinized state at 9.35×10^{-2} (4 and 3') and 1.87×10^{-1} M (4').

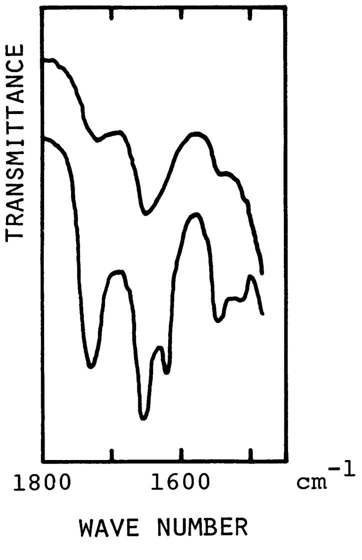

Fig. 2. IR absorption spectra of the PNLG solutions in ethylene chlorohydrin; upper: for the fluid solution at 5.07×10^{-2} M, lower: for the gelatinized solution at ca. 4×10^{-1} M (mixture of 251 mg of PNLG and 2 ml ethylene chlorohydrin).

dilute solution of PNLG that the circular dichroism due to the naphthyl chromophore is the consequence of the restricted orientation of the chromophore relative to the α-helical structure of the PNLG molecule caused by the bulkiness of the side chain residue. However, since the CD spectral features around 220 nm at higher concentrations suggest no increase of the α-helix content, but indicate the shift to the β-structure, and since no sign of the formation of a lyotropic liquid crystalline phase was detected in the solution, the observation of the sharp inflection of the magnitude of the molar ellipticity, at 283 nm for example, at the concentration of about 0.06 M necessarily impels us to invoke a third mechanism for the explanation of the circular dichroism corresponding to the electronic transitions of the naphthyl chromophore in the solution.

On the basis of the evidences mentioned above, we propose here that the formation of a network of the β-structure and the subsequent gelatinization of the solution is the cause for the restriction of the rotational freedom of the side chain naphthyl chromophore. This structural change can explain the abrupt loss of the rotational freedom of the chromophore with the increase in the concentration leading to the drastic enhancement of the molar ellipticity in the wavelength region of the absorption of the naphthyl chromophore. Considering the fact that the gelatinized state is composed of both α- and β-conformations, the origin of the dissymmetric perturbation exerted on the naphthyl chromophore can be both the helical structure of the α-conformation and the dissymmetric field of the large network of the β-structure. As is evident in the papers so far appeared,7) it is on the other hand certainly true that the formation of the β-structure by itself does not produce a sufficient restriction of a side chain chromophore of a polypeptide molecule resulting in the exhibition of a circular dichroism. The bulkiness of the side chain chromophore in concert with the formation of the β-structure is the necessary condition of the restriction enough for the induction of the circular dichroism to the chromophore.

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