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Circular Dichroism and Optical Rotatory Dispersion Spectra of Poly[γ-(1-naphthylmethyl)-L-glutamate] and Its Charge-transfer Complex

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Poly-γ-(1-naphthylmethyl)-L-glutamate was synthesized and its structure analyzed by the measurements of circular dichroism and absorption spectra. When the circular dichroism band at 219 nm in the circular dichroism spectrum of the polymer can be assigned to the band due to n–π* transition in the peptide main chain of the polymer, the calculated content of the right hand helix in the polymer is about 54% in ethylene chlorohydrin solution. It was also found that the polypeptide can form a charge-transfer complex with an organic electron acceptor in ethylene chlorohydrin solution. In the charge-transfer complex, a circular dichroism band could be observed at the wavelength corresponding to that of the charge-transfer band in the absorption spectrum of the complex. From the results, it was confirmed that the charge-transfer complex contains α-helix structure of this polypeptide. The temperature dependence of the charge-transfer complex formation was discussed in terms of the formation constant and thermodynamic parameters. The polypeptides having various portions of the 1-naphthylmethyl-D-glutamate residue were synthesized from poly-γ-methyl-D-glutamate by a polymer reaction using *p*-toluenesulfonic acid. The values of b_0 for the polypeptides derived from poly-γ-methyl-D-glutamate were estimated by means of Moffitt-Yang's equation, and the correlation between the b_0 values and the contents of 1-naphthylmethyl residue in the polypeptides was discussed.

In 1952, Mulliken[1] suggested that charge-transfer complexes may play an important role in biological systems. Some possible implications have been discussed by Szent-Gyorgyi,[2] his major concern being with the proposal that charge-transfer complexes are involved in biochemical reactions. There are two procedures in studying the possibility firstly to show the existence of charge-transfer complex formation in the biological systems, and secondly to demonstrate that it is essential in biological reaction. In some cases, the existence of charge-transfer complex formation between components in a given biochemical system has been proposed on the most tenuous evidence. The mere production of a colour, when two biochemical (or other) reagents are mixed, has often been given as the sole evidence for the charge-transfer complex formation. Obviously, such an observation alone is insufficient to justify the conclusion. Recent discussion has mainly centred around particular groups of compounds, including model compounds containing what are thought to be essential structural features. In aqueous solution, where most biological reactions occur, the dissociation of the complex may be so high that any charge-transfer absorption can be detected only with difficulty. Quite apart from the experimental difficulties of detecting a possible charge-transfer absorption band, there remains the fact that for most charge-transfer complexes, charge-transfer forces are not the major factor contributing to the stability of the complex in the ground state. Consequently, correlation between electron-donating ability of one component (or electron-accepting ability of the second component) and the association constant, free energy, or enthalpy of formation, cannot in general be assumed to be a necessary feature of a group of charge-transfer complexes. This is especially important when large component molecules are involved; steric effects and other features of the molecule may completely overwhelm any trend in thermodynamic quantities resulting from charge-transfer forces. Thus, it is important to clarify the steric factors for the charge-transfer complex formation in biological systems. In view of this, we examined the formation of charge-transfer complexes of polypeptides having aromatic residues, which behave as electron donors, in order to clarify the correlation between the α-helix content in the polypeptide and formation of their complexes.

The polymer effect on the formation constant of the charge-transfer complex of poly-*N*-vinylcarbazole,

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1) R. S. Mulliken, *J. Amer. Chem. Soc.*, **74**, 811 (1952).

2) A. Szent-Gyorgyi, "Introduction to a Submolecular Biology," Academic Press, New York and London (1960).

which has a helical structure, with organic electron acceptors was recently studied.[3] The results indicate the polymer to be tactic in solution because of the steric hindrance resulting from interactions between the neighbouring carbazole units. Accordingly, the extraordinarily high photoconductivity of the charge-transfer complex of poly-*N*-vinylcarbazole with an organic electron acceptor may depend on the helical structure of poly-*N*-vinylcarbazole.

It is interesting to study various factors of the conformational stability of polypeptides in biological systems. The stability of the α-helix structure which is influenced by the sequence of the amino acid residues, solvents and side chain groups is of particular interest. Conformational analyses of various synthetic poly-α-amino acids have been carried out,[4] especially the effects of the side chain groups near the main chain on the conformational stability.[5,6] Fraser[7] studied the conformational stability attributed to van der Waals forces between the aromatic side chains in the polypeptides including poly-γ-(1-naphthylmethyl)-L-glutamate. We have synthesized poly-γ-(1-naphthylmethyl)-L-glutamate and analyzed the structures of its charge-transfer complex in ethylene chlorohydrin or tetrachloroethane solution by various spectral measurements.

Experimental

Preparation of the Polymers. *1-Naphthylmethyl-L-glutamate (I)*: 1-Hydroxymethyl-naphthalene (11.1 g; 0.07 mol) and L-glutamic acid (9.5 g; 0.065 mol) were dissolved in dioxane of 80 ml, and then *p*-toluenesulfonic acid (13.3 g) was added to the reaction mixture. The esterification reaction was carried out for 24 hrs. at 55 °C. After the reaction, the solution was neutralized with sodium bicarbonate, and the reaction product was separated as a white precipitate. The product was filtered on a glass filter, washed with 95% aqueous ethanol solution, and finally recrystallized from the mixture of acetic acid and acetone. Yield 33%. mp. 173—175 °C. Elemental analyses are as follows; Found: C, 65,93; H, 5.77; N, 4.88%. Calcd for $C_{16}H_{17}NO_4$, C, 64.65; H, 5.72; N, 4.71%. $[\alpha]_D^{22}=15.4°$ (in CH_3COOH).

The IR spectrum shows an ester band 1735 cm^{-1}.

1-Naphthylmethyl-L-glutamate NCA(II): Ester(I) (4.5 g, 0.016 mol) was suspended in anhydrous dioxane, and to this system was passed dry phosgene for about 30 min. At the end of the reaction, the suspended NCA(II) was dissolved in dioxane. NCA(II) obtained was recrystallized from ethyl acetate-cyclohexane system. mp. 72—73 °C. Elemental analyses:

Found: C, 63.81; H, 5.11; N, 5.84%. Calcd for $C_{17}H_{15}NO_5$, C, 64.15; H, 4.78; N, 4.47%.

The IR spectrum of NCA(II) shows two bands at 1850 and 1785 cm^{-1}, due to carboxyanhydride group, and an ester band at 1735 cm^{-1}.

Poly-γ-(1-naphthylmethyl)-L-glutamate(III): 1-Naphthylmethyl-L-glutamate NCA(II) was dissolved in 100 ml of anhydrous dioxane and the initiator (*n*-hexylamine) was added. Polymerization conditions and the intrinsic viscosities of the polymer are summarized in Table 1. The IR spectrum of (III) shows an ester band at 1735 cm^{-1} and an amide band at 1655 cm^{-1}. Elemental analyses:

Found: C, 70.74; H, 6.02; N, 5.20%. Calcd for $C_{16}H_{15}NO_3$, C, 71.37; H, 5.58; N, 5.20%.

In the measurements of various spectra, the polymer denoted by No. 1290—2 was used.

Ester Exchange Reaction of Poly-γ-methyl-D-glutamate with 1-Hydroxymethylnaphthalene: Poly-γ-methyl-D-glutamate, prepared by the conventional NCA method (Ajinomoto Co. Ltd.), was used. It has a 1.4 intrinsic viscosity in dichloroethane solution at 30 °C. The ester exchange reactions were carried out as follows. 0.5 g of *p*-toluenesulfonic acid and 2.0 g of 1-hydroxymethylnaphthalene were added to a solution (3% by weight) of poly-γ-methyl-D-glutamate in dichloroethane, and the reaction mixture was then stirred at 60 °C. The molar ratio of poly-γ-methyl-D-glutamate (based on the glutamyl residue) to 1-hydroxymethylnaphthalene in reaction system was varied as shown in Table 2. After the reaction, the reaction product was precipitated with methyl alcohol, and washed several times with methyl alcohol, and finally dried *in vacuo*. The extent of the ester exchange reactions was estimated by means of the nitrogen contents of the reaction products. The conditions and extent of reactions are given in Table 2. From viscosity measurements, it was confirmed that no or less decrease of the degree of the polymerization of the polymer was observed.

Spectral Measurements. Optical rotatory dispersion (ORD) and circular dichroism (CD) measurements were

Table 1. Polymerization of the NCA of γ-(1-naphthylmethyl)-L-glutamate

Sample No.	A/I[a]	Reaction Time	Reaction Temp.	$[\eta]$[b]
1146	100	5 day	Room Temp.	0.52
1290—1	75	24 hr	Room Temp.	0.45
1290—2	50	18 hr	Room Temp.	0.45
1290—3	50	18 hr	Room Temp.	0.30

a) The ratio of molar concentration of the monomer to that of initiator (*n*-hexylamine). b) Intrinsic viscosity of the polymer. The solution viscosity of the polymer was measured at 30 °C in dichloroethane.

Table 2. Relation between the values of b_0 and the extent of reaction in the ester exchange of poly-γ-methyl-D-glutamate with 1-hydroxymethyl-naphthalene

Sample[a]	Extent of the Ester Exchange Reaction (%)	Moffit's b_0
Polymer 1	20	+460
2	40	+440
3	77	+380
4	85	+370
5	100	+230
PMDG	—	+630
PNLG	—	−270

a) Polymers were prepared from poly-γ-methyl-D-glutamate (PMDG). PNLG denotes poly-γ-(1-naphthylmethyl)-L-glutamate synthesized according to the NCA procedure (see Text).

3) T. Enomoto and M. Hatano, *Makromol. Chem.*, in press.
4) G. D. Fasman, "Poly-α-amino Acids; Protein Models for conformational Studies," Marcel Dekker, New York (1967).
5) M. Hatano, M. Yoneyama, T. Nozawa, M. Nakai, and I. Ito, *J. Amer. Chem. Soc.*, **91**, 2165 (1969).
6) M. Hatano and M. Yoneyama, *ibid.*, **92**, 1392 (1970).
7) R. D. B. Fraser, B. S. Harrap, R. Ledger, T. P. Macrae, F.H.C. Stewart, and E. Suzuki, *Biopolymers*, **5**, 797 (1967).

carried out with JASCO ORD/UV-5 and J-20A polarimeters, respectively. Absorption (AB) spectrum was measured with a Hitachi EPS-3T spectrophotometer. In these measurements, spectral grade ethylene chlorohydrin and 1,1,2,2-tetrachloroethane were used as solvents. Ethylene chlorohydrin was dried with CaH_2, distilled over CaH_2 under reduced pressure, and stored with exclusion from moisture in air. Purified ethylene chlorohydrin has a cut-off near 205 nm and high solubility for this polymer and is the best solvent for its CD measurement. From the observed ORD curves, the so-called Moffitt-Yang plots were made by means of the equation

$$[m']\frac{\lambda^2-\lambda_0^2}{\lambda_0^2} = a_0 + b_0\frac{\lambda_0^2}{\lambda^2-\lambda_0^2} \quad (1)$$

where λ_0 was taken to be 212 nm, and the mean residue optical rotation, [m'], was estimated, assuming the refractive index of 1,1,2,2-tetrachroethane to be 1.49 and neglecting the wavelength dependence of the refractive index. Furthermore, the CD and AB spectra of the charge-transfer complex of this polymer with 2,4,7-trinitro-9-fluorenone or 2,4,5,7-tetranitro-9-fluorenone were observed.

Results and Discussion

Absorption Spectrum of Poly-γ-(1-naphthylmethyl)-L-glutamate. Hypochromism is a familiar phenomenon in nucleic acids. A similar situation prevails in α-helical polypeptides and proteins. The hypochromic effect with the α-helix formation was treated theoretically by Tinoco,[8] and can be qualitatively accounted for in terms of the interaction of the transition moment for the absorption band in question in one residue, with transition moments of other transitions of adjoining residues. When the chromophores are aligned side by side, lower energy transition will be hypochromic. Since this polymer includes an α-helical structure and the naphthyl residues in the amino acid units of the polymer may be aligned side by side to form a type of a stacking structure, it is expected that the absorption intensities of the transitions in naphthyl residue decrease appreciably.

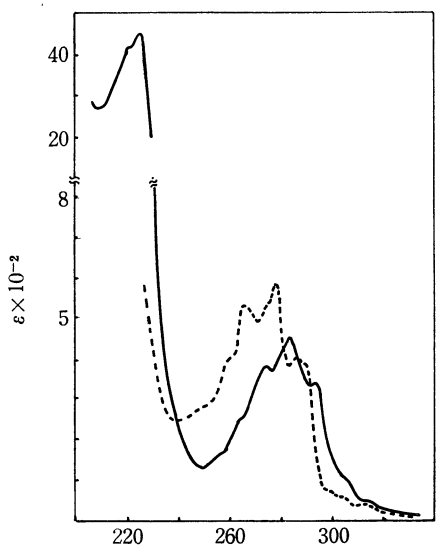

Fig. 1. Absorption spectra of naphthalene and poly-γ-(1-naphthylmethyl)-L-glutamate; dotted line, naphthalene; solid line, poly-γ-(1-naphthylmethyl)-L-glutamate.
Solvent: Ethylene chlorohydrin.

Figure 1 shows absorption spectra of both naphthalene and poly-γ-(1-naphthylmethyl)-L-glutamate in ethylene chlorohydrin. The ^1L_a(p) band including vibration modes of naphthyl residue in the polymer shifts to longer wavelength region than in the case of naphthalene. In this polymer, an appreciable hypochromicity can also be seen in the spectral region.

Circular dichroism spectrum of poly-γ-(1-naphthylmethyl)-L-glutamate. The CD spectrum in visible region of poly-γ-(1-naphthylmethyl)-L-glutamate in 1,1,2,2-tetrachoroethane is shown in Fig. 2. A very weak positive CD band is seen at 400 nm (25.0×10^3 cm^{-1}), and a weak band at 365 nm(27.4×10^3 cm^{-1}), which corresponds to the AB band at 365 nm in the AB spectrum of the polymer. The CD and AB bands at 365 nm may be assigned to an electronic transition, assumed to be as $^1L_b(\alpha)$, in naphthyl residue of the polymer. On the other hand, no CD band can be observed in the ^1L_a(p) band region. The CD spectra of poly-γ-methyl-L-glutamate and poly-γ-(1-naphthylmethyl)-L-glutamate in the spectral region near 222 nm are also shown in Fig. 2. The former exhibits a large negative CD band at 222 nm, and the latter a large negative CD band at 219 nm and a negative CD band at 230 nm. The CD band at 230 nm is assigned to $^1B_b(\beta)$ band in naphthyl residue of the latter polymer (Fig. 1).

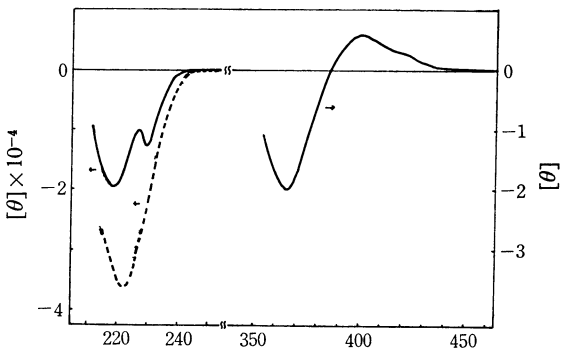

Fig. 2. Circular dichroism spectra of poly-γ-methyl-L-glutamate and poly-γ-(1-naphthylmethyl)-L-glutamate; dotted line, poly-γ-methyl-L-glutamate; solid line, poly-γ-(1-naphthylmethyl)-L-glutamate.
Solvent: Tetrachloroethane (in visible region).
Ethylene chlorohydrin (in ultraviolet region).

If the CD band at 219 nm in the CD spectrum of poly-γ-(1-naphthylmethyl)-L-glutamate can be assigned to the band due to n-π* transition in the peptide main chain of the polymer, the calculated content of the right-hand helix in the polymer is about 54% ethylene chlorohydrin solution. The reason for the CD band due to the n-π* transition shifting from 222 nm to 219 nm can be clarified theoretically.

Spectral-Analyses of Charge-transfer Phenomena between Poly-γ-(1-naphthylmethyl)-L-glutamate and an organic Elec-

8) I. Tinoco Jr., *J. Amer. Chem. Soc.*, **82**, 4785 (1960).

tron Acceptor. Figure 3 shows the spectral variation of the system of the peptide and 2,4,5,7-tetranitro-9-fluorenone when the molar ratio of the glutamyl redidue to the acceptor was varied in the ethylene chlorohydrin solution. A broad CD band can be observed at 483 nm in the CD spectra of the polymer-acceptor systems, increasing gradually with the increase of the molar ratio of acceptor to donor (Fig. 4). The wavelength of the charge-transfer band, as a shoulder, in the AB spectra is uncertain, but that of the CD trough at 483 nm can be determined exactly. Appearance of the CD band in the vicinity of the charge-transfer band, confirmed by the Benesi-Hildebrand plots for the absorbances at 483 nm in the AB spectra, indicates that the electric transition moments of the charge-transfer transition in the complex may orientate circularly around the polypeptide helix axis. This assumption can be easily elucidated by a simple exciton theory.[9] This is the first example for the origin of the optical activity to be discussed on the basis of the charge-transfer phenomena including polypeptide with electron acceptor.

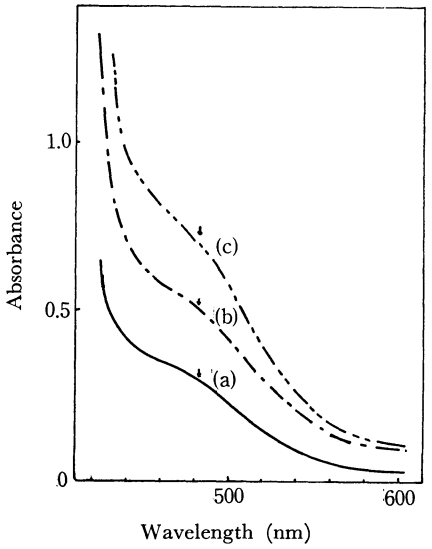

Fig. 3. Variation of the absorption spectra of the poly-γ-(1-naphthylmethyl)-L-glutamate-2,4,5,7-tetranitro-9-fluorenone system in ethylene chlorohydrin at 20 °C with the variation of the molar ratio of the glutamyl residue to the acceptor, D/A.
a) D/A=1/0.7, b) D/A=1/1, c) D/A=1/1.5.

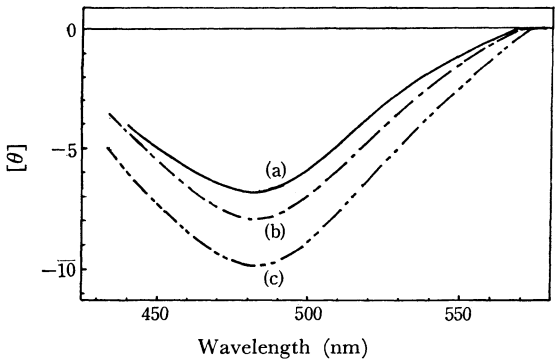

Fig. 4. Circular dichroism variation in the poly-γ-(1-naphthylmethyl)-L-glutamate-2,4,5,7-tetranitro-9-fluorenone system with the variation of the molar ratio of D/A (Refer to Fig. 3).
Solvent: Ethylene chlorohydrin.
a) D/A=1/0.7, b) D/A=1/1, c) D/A=1/1.5.

Table 3. Equilibrium constants and activation parameters in the charge-transfer complex formation of poly-γ-(1-naphthylmethyl)-L-glutamate with 2,4,5,7-tetranitro-9-fluorenone

	Temp. °C	λ_{CT} nm	ε $\times 10^3$	k M^{-1}	$-\Delta H$ kcal/mol	$-\Delta S$ e.u.
Naphthalene	15.1	481	2.0	8.9	6.0	16.5
	21.6			7.2		
	30.0			5.4		
PNLG[a)]	14.5	483	1.2	2.0	4.4	14.0
	25.5			1.5		
	31.1			1.3		

a) Poly-γ-(1-naphthylmethyl)-L-glutamate.

The Benesi-Hildebrand plots could be obtained in a conventional way from the variation of the absorbances at 483 nm in the AB spectra of the polypeptide-acceptor systems. The extinction coefficients and the equilibrium constants in the charge-transfer complex systems were then estimated from the plots, and several thermodynamic parameters in the charge-transfer complex formation were calculated. The calculated values are summarized in Table 3 together with those for the complex formation from naphthalene, which is a model compound for the polymer, and 2,4,5,7-tetranitro-9-fluorenone. The equilibrium constants in the complex formation between poly-γ-(1-naphthylmethyl)-L-glutamate and 2,4,5,7-tetranitro-9-fluorenone are appreciably smaller than those in the complex formation between naphthalene and the same acceptor. The decrease of the equilibrium constants in the polymer-acceptor system may result from a smaller enthalpy change. The results are in contrast to those for the complex formation from poly-*N*-vinylcarbazole and TCNQ, where the difficulty of complex formation was elucidated in terms of entropy change.[3]

Appreciable enhancement of helix stability in poly-γ-(1-naphthylmethyl)-L-glutamate by charge-transfer interaction with 2,4,5,7-tetranitro-9-fluorenone. In order to clarify the correlation between the helix content in the polypeptide and formation of the type of charge-transfer complex, the extent of helix in the polypeptide-acceptor complex was estimated at various ratios of the glutamyl residue to the acceptor. Figure 5 shows this variation of the CD spectra, particularly in the region of the n-π^* transition. We see that the formation of the charge-transfer complex stabilizes the helix structure in the polypeptide, and that some excess addition of the acceptor disturbs the formation of a helix structure in the polypeptide. At an equal molar ratio of the glutamyl residue to the acceptor, the complex exhibits the highest content of helix, which reaches about 100%. Thus, Szent-Gyorgyi's expectation could be confirmed experimentally. Although the enhancement of helix stability by the charge-

9) I. Tinoco Jr., *Advances in Chemical Physics*, **4**, 113 (1962).

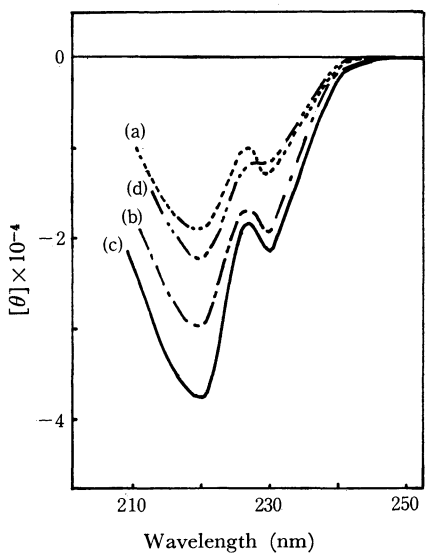

Fig. 5. Circular dichroism variation in the poly-γ-(1-naphthylmethyl)-L-glutamate-2,4,5,7-tetranitro-9-fluorenone system with the variation of the molar ratio of D/A (Refer to Fig. 3.).
a) D/A=1/0, b) D/A=1/0.35, c) D/A=1/1, d) D/A=1/2.

transfer interaction between the side chain groups of the polypeptide and the added acceptor molecules in ethylene chlorohydrin solution can not be directly correlated with most biological phenomena, it can be expected that some extent of looseness in the local motion of polypeptide main chains in enzymes are restricted by the charge-transfer interactions between the donor portions in enzymes and the added small acceptor molecules.

Correlation between Moffitt b_0 values and the contents of 1-naphthylmethyl residue in the polypeptides. Poly-γ-(1-naphthylmethyl)-L-glutamate has 54% helix content in ethylene chlorohydrin solution. This suggests that 1-naphthylmethyl groups in side chains of the polypeptide may disturb the formation of a helical structure in the polypeptide. The correlation between the Moffitt b_0 values and the contents of 1-naphthylmethyl residues in the polypeptides, synthesized by the ester exchange reaction between poly-γ-methyl-D-glutamate and 1-hydroxymethylnaphthalene with *p*-toluenesulfonic acid, is given in Table 2. The optical rotatory dispersion spectra were measured for the 1,1,2,2-tetrachloroethane solutions of the polypeptides. The observed value of b_0 for the polypeptide, in which the ester exchange reaction proceeded almost completely (polymer 5), was 230. This means that the polypeptide has 37% of helix content, assuming that b_0 is 630 in the completely helical polypeptide.[6] We see that the helix content of the polypeptides decreases gradually with the increase of 1-naphthylmethyl residues in the polypeptides. Thus, 1-naphthylmethyl groups in side chains of the polypeptides disturb the sterical formation of a helix structure in the polypeptide. However, the interference of 1-naphthylmethyl residue in the course of helix formation of the peptide may be so small that the interference can be removed by weak interactions such as charge-transfer interaction.

Side chain effect on the helix stability in the polypeptides. Introduction of nitro-group to the benzyl side chain in poly-γ-benzyl-L-glutamate or poly-β-benzyl-L-aspartate results in the lowering of helix content, and the polypeptides including nitroaromatic groups as side chains exhibit some CD bands which can be assigned to the transitions due to the nitroaromatic groups. This led us to assume that the nitroaromatic group in the polyglutamate or polyaspartate is an asymmetric environment forming a relatively rigid side chain helix of its own when the polypeptides are dissolved in helix solvents in a lower concentration.

Steric hindrance and/or electric dipole repulsion between the side chain groups may constrain the side chain groups to form a relatively rigid side chain helix around the axis of the main chain helix, and to disturb the helix formation of the polypeptide main chain. Steric considerations for poly-γ-(1-naphthylmethyl)-L-glutamate are similar, since the polypeptide exhibits some CD bands which can be assigned to the transitions due to the naphthyl residue in the side chain and has relatively low content of helix.

In the ethylene chlorohydrin solution of this polypeptide, the CD magnitude at 219 nm increased appreciably and the CD trough shifted to 222 nm, when the concentration of the polypeptide was increased to 0.1 M. This suggests that the interaction between the naphthyl groups in the side chains is too weak to increase the rigidity of the polypeptide main chain in a higher concentration.

Thus, an appreciable enhancement of helix stability by the charge-transfer interaction between the side chain group and the added acceptor molecule is possible, although the energetics in the helix formation should be analyzed in more detail in the correlation with other charge-transfer systems.

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