Interaction between an α-Helical Polypeptide Containing Side-Chain Pyridyl Groups and Poly(4-vinylpyridine) in the Ternary Cu^{2+} Ion Complex

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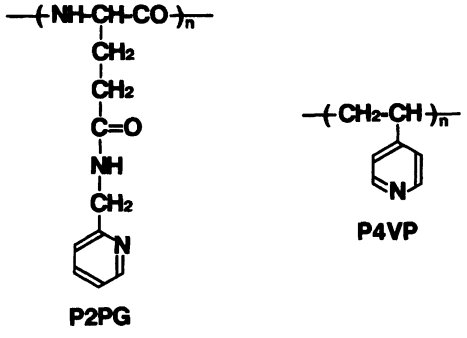
Interaction between a Cu^{2+}-α-helical polypeptide containing side-chain pyridyl groups, poly(N^{ω}-2-pyridylmethyl-L-glutamine) (P2PG), and poly(4-vinylpyridine) (P4VP) as a macromolecular guest was investigated by absorption and circular dichroism spectroscopies in 2,2,2-trifluoroethanol solutions. P4VP was added to the Cu^{2+}-one pyridyl side chain complex system prepared in advance. One or two P4VP nitrogens could bind to the remaining coordination sites of the Cu^{2+} ion in the host system, yielding ternary Cu^{2+} complexes. The circular dichroism spectra of Cu^{2+}-P2PG with P4VP indicated that P4VP might bind to the α-helix of P2PG in such a manner as to form a left-handed superhelix around the α-helical core resulting from the formation of ternary Cu^{2+} complexes. The structure of the interpolymer complex system was also investigated by a monolayer method. The surface pressure–area isotherm of a Cu^{2+}-P2PG-P4VP monolayer and its hysteresis supported the formation of the supramolecular structure suggested by the spectroscopic methods.

Macromolecule–macromolecule complexes such as protein–nucleic acid and enzyme-substrate complexes in biological systems play various important roles in the maintenance of life.[1—5] For example, a nucleosome, a subunit of chromatin, forms a supercoiled structure surrounded by DNA complexed with core histone proteins within the nucleosome, and closely relates to genetic information.[2] Model polypeptides, whose amino acid compositions and sequences were controllable, were also investigated in order to determine the molecular parameters regulating their interactions with natural or model macromolecules.[6—21] These complex formations were affected by specific interactions, such as electrostatic, hydrophobic, and hydrogen bonding interactions, between functional groups of the macromolecules paired. However, the resulting structures have not been clarified satisfactorily. There have been only a few reports[9—12] concerning supramolecular structure formation under limited pH conditions. Moreover, linear polymers were reported, in many cases, to destabilize the α-helix structure of polypeptides through their complexation processes.[15,19,20]

In the present study, interpolymer complex formation between an α-helical polypeptide containing side-chain pyridyl groups, poly(N^{ω}-2-pyridylmethyl-L-glutamine) (P2PG, Scheme 1), and poly(4-vinylpyridine) (P4VP, Scheme 1) as a linear polymer with the aid of a ternary Cu^{2+} complex, was investigated by absorption and circular dichroism spectroscopies in 2,2,2-trifluoroethanol (TFE) solutions. A supramolecular structure was expected to be formed between the host Cu^{2+}-P2PG system and the guest P4VP, since P2PG exhibited significant optically active structure on the periphery of the α-helix resulting from the ternary Cu^{2+} complexation with low-molecular weight guests, such as monomeric pyridine and tryptophan.[22,23] We also employed surface-pressure measurements of a Cu^{2+}-P2PG-P4VP monolayer to clarify its supramolecular structure, since the surface pressure–area isotherm is reflected by macromolecular structures such as extended or condensed structures.[24] It was demonstrated that the system consisted of rigid rod-like P2PG, flexible P4VP, and Cu^{2+} ion forming a supramolecular structure, i.e., α-helical P2PG might act as a useful tool for structural regulation of flexible polymers with the aid of Cu^{2+} ion.

Experimental

Materials. Poly(N^{ω}-2-pyridylmethyl-L-glutamine) (P2PG, $\overline{DP}$=1049, Scheme 1) was prepared by introduction of 2-(aminomethyl)pyridine (2-AmPy) to the side chains of poly(L-glutamic acid) (PGA).[22,23] PGA was obtained by saponification of poly(γ-methyl L-glutamate) ($\overline{DP}$=1049, kindly supplied by Ajinomoto, Co., Ltd.).[25] PGA (1.0 g) was dissolved in dimethylformamide (DMF, 50 mL) at 0 °C with 2-AmPy (2.5 g), 1-hydroxy-1*H*-benzotriazole (4.6 g), and dicyclohexylcarbodiimide (6.2 g). This reaction mixture was stirred for 72 h at 25 °C. The precipitated dicyclohexylurea was removed, and the DMF solution was poured into diethyl ether. The residues obtained were washed with ether 3 or 4 times until unreacted 2-AmPy reagents could not be detected spectroscopically. Near-complete conversion (ca. 98%) of PGA side chains to the pyridylmethyl derivative was confirmed by the high-resolution ^{1}H NMR spectrum (Varian XL-200 spectrometer) of the product in trifluoroacetic acid (10 mg mL^{-1}), comparing the overlapping peak



Scheme 1. Host and guest polymers.

area assigned to the pyridyl and amide groups (7.8—9.0 ppm) with that of the side chains (1.8—3.1 ppm) assigned to the CH_2 groups.

Poly(4-vinylpyridine) (P4VP, Scheme 1) was obtained by the polymerization of 4-vinylpyridine (guaranteed reagent, Nacalai Tesque, Co., Ltd.) using potassium peroxodisulfate (guaranteed reagent, Nacalai Tesgue, Co., Ltd.) as the initiator.[26] 4-Vinylpyridine (5 mL) was dissolved in an acetone–water (1 : 1 v/v) mixture (50 mL). Potassium peroxodisulfate was added and the mixture was kept at 50 °C for 6 h. The reaction mixture was then poured into a large amount of water (500 mL). The purification of the resulting polymer was carried out by repeated reprecipitation of its ethanol solution into water. The mean degree of polymerization, $\overline{DP}=447$, was estimated from the measurement of intrinsic viscosity, $[\eta]$, in ethanol, using the equation[27] $[\eta]=2.5\times10^{-4} M^{0.68}$ ($M=105\times\overline{DP}$).

$CuCl_2\cdot2H_2O$ was a guaranteed reagent from Nacalai Tesque, Co., Ltd. 2,2,2-trifluoroethanol (TFE, Nacalai Tesque, Co., Ltd.) was of spectroscopic grade.

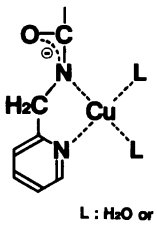
Measurements. Absorption and circular dichroism (CD) spectra of TFE solutions of P2PG were obtained with a spectrophotometer (JASCO, UVIDEC-670) and spectropolarimeter (JASCO, J-600), respectively. Solutions of 5.0×10^{-4} base molar P2PG in TFE were used.

The surface pressure–area (π–A) isotherm of a spread Cu^{2+}-P2PG-P4VP monolayer was obtained with an L–B moving wall-type trough (Nippon Laser & Electronics Lab., NL-LB240-MWA) at 25 °C. The Cu^{2+}-P2PG-P4VP complex in TFE solution (50 μL) used in the spectroscopic measurements was placed on a pure water surface (240 cm^2) in the trough. The solution spread over the surface and the solvent evaporated leaving a Cu^{2+}-P2PG-P4VP monolayer. P2PG and P4VP monolayers were formed in a similar manner and their π–A isotherms were also obtained.

Absorption spectra of the Cu^{2+}-P2PG-P4VP monolayer were obtained with a Spectro Multi-Channel Photo Detector, Luminescence Spectroscopy (Otsuka Electronics Co., Ltd., MCPD-1000).

Results and Discussion

Ternary Cu^{2+} Complex Formation of P2PG with P4VP. The host Cu^{2+}-P2PG system was prepared by addition of 1.0×10^{-3} mol dm^{-3} Cu^{2+} ion to P2PG in a TFE solution, resulting in the formation of Cu^{2+}-one pyridyl side chain complexes (1 : 1 Cu^{2+}-P2PG, Scheme 2) on the periphery of the α-helix of P2PG.[22,23] The absorption spectra of the Cu^{2+}-P2PG system with and without P4VP are shown in Fig. 1. The 1 : 1 Cu^{2+}-P2PG complex exhibited a d–d band of Cu^{2+} near 680 nm owing to the coordination of two nitrogens of the pyridyl and amide groups of the pyridyl side chain and a shoulder band near 330 nm assigned to charge transfer from the deprotonated amido nitrogen of P2PG to Cu^{2+} (Fig. 1, broken line).[22,23] In the presence of P4VP (2.0×10^{-3} residue mol dm^{-3}), the parent 680 nm band was blue-shifted to 650 nm and the intensity increased. These changes may correspond to those previously reported due to the coordination of monomeric pyridines to 1 : 1 Cu^{2+}-P2PG complexes[22]



Scheme 2. Anticipated complex structure of 1 : 1 Cu^{2+}-P2PG.

and coordination of three or four nitrogens of P4VP to free Cu^{2+}.[28] In addition, the charge transfer band near 330 nm changed little in the presence of P4VP (Fig. 1, solid line), indicating that the host complex structure, 1 : 1 Cu^{2+}-P2PG, was preserved.[23] Therefore, the blue-shifted band at 650 nm indicated that one or two nitrogens of P4VP bound to the remaining coordination sites of Cu^{2+} in the host system. It was also observed that the pyridyl ring of P4VP caused an increase in the absorption band near 245 nm by overlapping with the side-chain pyridyl band of P2PG.

Conformation of the P2PG and P4VP Ternary Cu^{2+} Complex. P2PG exhibited significant optically active structure on the periphery of the α-helix resulting from ternary Cu^{2+} complexation with low-molecular-weight guests, such as monomeric pyridine and tryptophan, in TFE solutions.[22,23] Therefore, an optically active structure was expected to form between the host Cu^{2+}-P2PG system and the guest P4VP. Changes in the CD spectra of Cu^{2+}-P2PG with and without P4VP are shown in Fig. 2. In the absence of P4VP no optical activity was observed, except for the peptide region ($\lambda<250$ nm). In contrast, addition of P4VP to 1 : 1 Cu^{2+}-P2PG complexes induced new CD bands in the wavelength range 240—400 nm and an enhanced CD band in the peptide region. Above 250 nm, the Cu^{2+}-P2PG-P4VP system displayed two asymmetric positive couplets, with peaks at 351 and 294 nm and at 270 and 255 nm, respectively. The former couplet centered at 330 nm corresponded to the charge transfer band from the deprotonated side-chain amido nitrogen of P2PG to Cu^{2+} (Fig. 1). The latter couplet centered at 260 nm corresponded to the π–π^* transition band based on the pyridyl chromophore of P2PG (Fig. 1).[22,23] Furthermore, it was noted that a weak positive band appeared near 240 nm, which was not observed even with the 2 : 1 Cu^{2+}-P2PG complex involving regularly arranged pyridyl side chains.[22,23] This CD band, therefore, may be assigned to the pyridyl groups of P4VP having an absorption band near 245 nm (Fig. 1). That is to say, the rotational freedom of the pyridyl rings of P4VP was reduced by ternary complexation, and an interaction between the transition dipole moment of the pyridyl chromophore of P4VP and that of the α-helical backbone of P2PG former.[29] The positive sign of the band was attributed to the fact that the pyridyl chromophores of P4VP preferentially bore

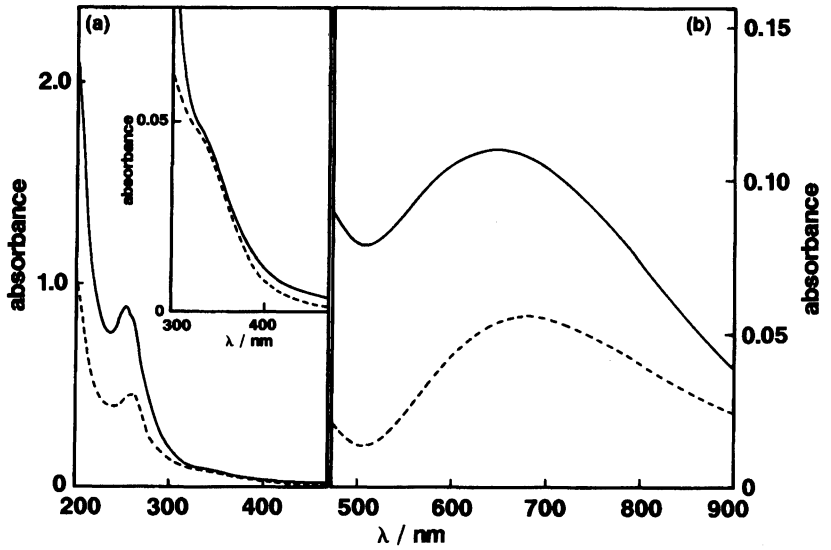


Fig. 1. Absorption spectra of Cu^{2+}-P2PG with (—) and without (---) P4VP in TFE solutions. The light-path lengths of the cells were (a) 0.1 cm and (b) 1.0 cm. [P2PG]$=5.0\times10^{-4}$ residue mol dm^{-3}. [Cu^{2+}]$=1.0\times10^{-3}$ mol dm^{-3}. [P4VP]$=2.0\times10^{-3}$ residue mol dm^{-3}.

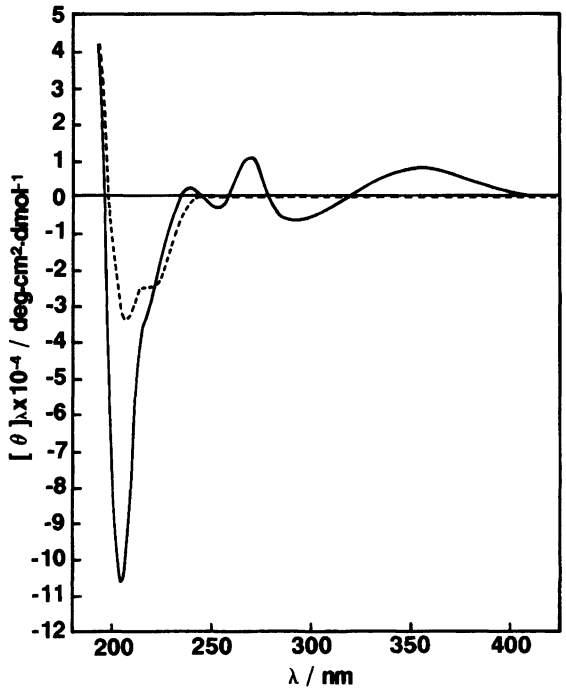


Fig. 2. Circular dichroism spectra of Cu^{2+}-P2PG with (—) and without (---) P4VP in TFE solutions. [P2PG]$=5.0\times10^{-4}$ residue mol dm^{-3}. [Cu^{2+}]$=1.0\times10^{-3}$ mol dm^{-3}. [P4VP]$=2.0\times10^{-3}$ residue mol dm^{-3}.

a left-handed regularity, since the α-helical poly(L-glutamic acid)–acridine orange system showed a positive CD band arising from the transition polarised perpendicular to the α-helix axis.[30] That is, this indicated that P4VP tended to make the side chain of P2PG rigid and P4VP itself gained a structural regularity around the α-helix. In the peptide region, two negative bands were observed near 222 nm characteristic of the α-helix structure and near 205 nm assigned to the π–π^* peptide transition band due to the helical conformation of the backbone and the spiral arrangement of the side-chain amide bonds around the helical backbone.[22,23] Monomeric pyridine merely produced small changes in the CD spectra.[22] These facts supported the hypothesis that P4VP, whose pyridyl ligands were connected by the backbone, tended to make the side chains of P2PG rigid. It took about 8 h to complete the CD spectral changes, whereas the changes in the d–d band in the absorption spectra, i.e., the formation of the unit complex structure, occured within a few minutes. These results indicated that the higher-order structure of the Cu^{2+}-P2PG-P4VP ternary complex was gradually formed by conformational changes of P4VP with intramolecular ligand exchanges. As a result, P4VP might bind to the α-helix of P2PG in such a manner as to form a left-handed superhelix around the α-helical core with the aid of the formation of ternary Cu^{2+} complexes.

Monolayer Characteristics of the Cu^{2+}-P2PG-P4VP Ternary Complex. To clarify the supramoleculer structure of the Cu^{2+}-P2PG-P4VP interpolymer complex, a surface pressure–area isotherm of its monolayer was depicted and compared with those of P2PG and P4VP monolayers. Figure 3 shows the surface pressure–area isotherms for Cu^{2+}-P2PG-P4VP, P2PG, and P4VP. P4VP showed an expanded isotherm, whereas the isotherms of Cu^{2+}-P2PG-P4VP and P2PG were typical condensed types and had small plateaus when the monolayer was compressed strongly.[31] The second steep rise above the plateau was associated with the lamination of the monolayers.[32] Similar behavior was also observed with other α-helical polypeptides such as poly (γ-benzyl L-glutamate)

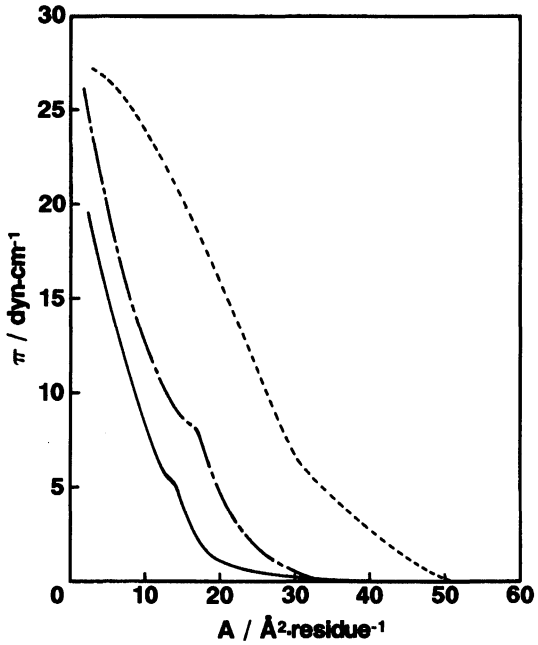


Fig. 3. Surface pressure–area isotherms for Cu^{2+}-P2PG-P4VP (—), P2PG (---), and P4VP (---) at 25 °C. [P2PG]/[P4VP]=0.25.

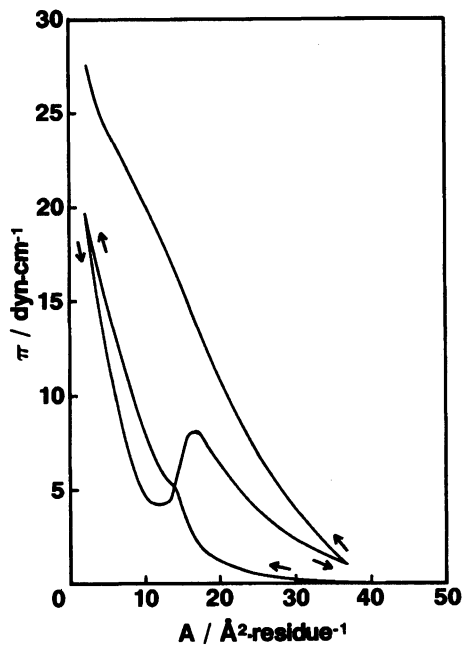


Fig. 4. Hysteresis of surface pressure–area isotherm for Cu^{2+}-P2PG-P4VP at 25 °C. Rates of compression and expansion=2 mm min^{-1}. [P2PG]/[P4VP]=0.25.

(PBG) and PMG.[33—35] It was considered, therefore, that the ternary complexation forced the coordinated P4VP to condense around α-helical P2PG, resulting in the formation of a pseudo-rod-like structure. The limiting area derived from extrapolating the first steep rise of the π–A curve to zero pressure for Cu^{2+}-P2PG-P4VP was about 18.6 $Å^2$ $residue^{-1}$, which was smaller than the value for P2PG (23.7 $Å^2$ $residue^{-1}$, a value comparable to that of PBG reported by G. I. Loeb,[33] 23.3 $Å^2$). This implies that the coordinated residues of P4VP were forced to exist partly in the air phase without contributing to the apparent area.[36] Therefore, these results suggested two structural models for the interpolymer complex; (i) P4VP may bind preferentially above or below the α-helix of P2PG at the air/water interface owing to the polarity difference between them, and (ii) P4VP may bind to the α-helix of P2PG in such manner as to form a superhelix around the core. These models may be consistent with the condensed isotherm and the smaller limiting area.

Figure 4 shows a hysteresis of the π–A isotherm for the Cu^{2+}-P2PG-P4VP monolayer. The first compression process exhibited a condensed isotherm as mentioned above, however, an unexpected increase in surface pressure was found during the following expansion process. The second compression process exhibited an expanded isotherm which was clearly different from the isotherm of the first compression process. To clarify the unexpected behavior of the Cu^{2+}-P2PG-P4VP monolayer, absorption spectra of the monolayer were measured at various areas (Fig. 5). At a larger area per residue of polymers than that of the plateau region,

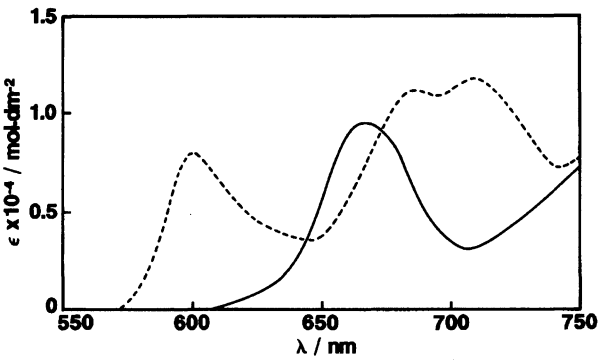


Fig. 5. Absorption spectra of Cu^{2+}-P2PG-P4VP monolayer at 20 $Å^2$· $residue^{-1}$ (—) and 10 $Å^2$· $residue^{-1}$ (---) during first compression. The molar absorption coefficient, ε, was normalized by mean residual concentration of polymers. [P2PG]/[P4VP]=0.25.

this monolayer exhibited a d–d band of Cu^{2+} near 665 nm corresponding to the ternary complex (solid line). Further compression led to significant spectral changes, that is, three bands near 600, 685, and 710 nm appeared along with the disappearance of the band near 665 nm (broken line). The band near 600 nm corresponded to the d–d band of Cu^{2+} coordinated by four pyridyl nitrogens of P4VP.[28] The band near 710 nm was assigned to the d–d bands of Cu^{2+} coordinated by one nitrogen from P4VP.[28] The band near 685 nm might be composed of the d–d bands of Cu^{2+} coordinated to two nitrogens of P4VP[28] and two side-chain nitrogens of P2PG.[34,35] It was considered, therefore, that Cu^{2+}-P2PG-P4VP ternary complexes were converted to Cu^{2+}-P4VP com-

plexes and 1 : 1 Cu^{2+}-P2PG complexes in the monolayer phase by the monolayer compression process. Further spectral changes were not found during the expansion and second compression process. Thus the interpolymer complex was destroyed and dissociated during the expansion process. As a result, the monolayer was converted to a mixed one consisting of Cu^{2+}-P2PG and Cu^{2+}-P4VP resulting in the expanded isotherm of the second compression process.[37] The reasons why the operation of compression and expansion of the monolayer made the ternary complex unstable are not clear at present. These results support the superhelix model (ii), since the monolayer based on model (i) might exhibit the condensed isotherm again during the second compression process.

In conclusion, it was demonstrated by absorption and CD spectroscopies that α-helical P2PG gave the guest linear polymer, P4VP, structural regularity, resulting from ternary Cu^{2+} complexation. Thus, P2PG may act as a useful tool for structural regulation of linear polymers. The present results also suggest that the monolayer method is useful for clarifying the supramolecular structure of an interpolymer complex.

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References

1) W. N. Lipscomb, J. A. Hartsuck, F. A. Quiocho, and G. N. Reeke, Jr., *Proc. Natl. Acad. Sci. U. S. A.*, **64**, 28 (1969).
2) J. P. Baldwin, P. G. Boseley, E. M. Bradbury, and K. Ibel, *Nature*, **253**, 245 (1975).
3) F. A. Cotton, E. E. Hazen, Jr., and M. J. Legg, *Proc. Natl. Aced. Sci. U. S. A.*, **76**, 2551 (1979).
4) U. Heinemann and W. Saenger, *Nature*, **299**, 27 (1982).
5) J. A. Cox, *Biochem. J.*, **249**, 621 (1988).
6) C. Chang, M. Weiskopf, and H. J. Li, *Biochemistry*, **12**, 3028 (1973).
7) R. A. Gelman and J. Blackwell, *Biopolymers*, **13**, 139 (1974).
8) R. E. Williams and S. L. Kielland, *Can. J. Chem.*, **53**, 542 (1975).
9) A. Nakajima, K. Shinoda, T. Hayashi, and H. Sato, *Polym. J.*, **7**, 550 (1975).
10) K. Shinoda, T. Hayashi, T. Yoshida, K. Sasaki, and A. Nakajima, *Polym. J.*, **8**, 202 (1976).
11) K. Shinoda, K. Sasaki, T. Hayashi, and A. Nakajima, *Polym. J.*, **8**, 208 (1976).
12) K. Shinoda, T. Hayashi, and A. Nakajima, *Polym. J.*, **8**, 216 (1976).
13) H. Sato, T. Hayashi, and A. Nakajima, *Polym. J.*, **8**, 517 (1976).
14) M. F. Pinkston, A. H. Ritter, and H. J. Li, *Biochemistry*, **15**, 1676 (1976).
15) K. Abe, M. Koide, and E. Tsuchida, *Macromolecules*, **10**, 1259 (1977).
16) C. S. Cho, A. Nakagami, T. Komoto, and T. Kawai, *Makromol. Chem.*, **179**, 1345 (1978).
17) A. M. Schwarts and G. D. Fasman, *Biopolymers*, **18**, 1045 (1979).
18) A. Domard and M. Rinaudo, *Macromolecules*, **13**, 898 (1980).
19) S. M. Park, S. H. Jeon, and T. Ree, *J. Polym, Sci., Part A : Polym. Chem.*, **27**, 4109 (1989).
20) S. H. Jeon, S. M. Park, and T. Ree, *J. Polym. Sci., Part C : Polym. Lett.*, **27**, 161 (1989).
21) H. Sato and H. Seki, *Polym. J.*, **25**, 529 (1993).
22) Y. Nagata, S. Kanuka, T. Kinoshita, A. Takizawa, Y. Tsujita, and H. Yoshimizu, *Biopolymers*, in press.
23) Y. Nagata, S. Kanuka, T. Kinoshita, A. Takizawa, Y. Tsujita, and H. Yoshimizu, *Bull. Chem. Soc. Jpn.*, **66**, 2972 (1993).
24) M. Kawaguchi, M. Tohyama, Y. Mutoh, and A. Takahashi, *Langmuir*, **4**, 407 (1988).
25) M. Sato, T. Kinoshita, A. Takizawa, and Y. Tsujita, *Macromolecules*, **21**, 3419 (1988).
26) R. Ramaraj and P. Natarajan, *J. Polym. Sci., Part A : Polym. Chem.*, **29**, 1339 (1991).
27) J. B. Berkowitz, M. Yamin, and R. M. Fuoss, *J. Polym. Sci.*, **28**, 69 (1958).
28) Y. E. Kirsch, V. Y. Kovner, A. I. Kokorin, K. I. Zamaraev, V. Y. Chernyak, and V. A. Kabanov, *Eur. Polym. J.*, **10**, 671 (1974).
29) H. Nomori, N. Tsuchihashi, S. Takagi, and M. Hatano, *Bull. Chem. Soc. Jpn.*, **48**, 2522 (1975).
30) Y. Sato and M. Hatano, *Bull. Chem. Soc. Jpn.*, **46**, 3339 (1973).
31) C. -S. Cho, A. Yagawa, S. Takahashi, M. Kunou, and T. Akaike, *J. Polym. Sci., Part A : Polym. Lett.*, **28**, 89 (1990).
32) B. R. Malcolm, *Proc. R. Soc. Ser., A*, **A305**, 363 (1968).
33) G. I. Loeb, *J. Colloid Interface Sci.*, **26**, 236 (1968).
34) G. I. Loeb and R. E. Baier, *J. Colloid Interface Sci.*, **27**, 38 (1968).
35) M. Higuchi, A. Takizawa, T. Kinoshita, Y. Tsujita, and K. Okochi, *Macromolecules*, **23**, 361 (1990).
36) T. Isemura and T. Yamashita, *Bull. Chem. Soc. Jpn.*, **32**, 1 (1959).
37) H. E. Ries, Jr., R. A. Ahlbeck, and J. Gabor, *J. Colloid Sci.*, **14**, 354 (1959).