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PHOTO-INDUCED FORMATION OF α -HELICAL STRUCTURE OF POLY(L-GLUTAMIC ACID) CONTAINING PARAROSEANILINE IN THE SIDE CHAINS

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SUMMARY

A photoresponsive polypeptide has been prepared by condensation reaction of poly(L-glutamic acid) (PGA) with pararoseaniline (Rose) in dimethylformamide solution (Rose-modified PGA). The Rose side chains in the polymer exhibited the photo-ionization under uv light irradiation, yielding triphenylmethyl cations, which vanished completely in the dark. The helix content of Rose-modified PGA, 4.3% in the dark, was increased to 7.2% by uv light irradiation at pH 9.7, owing to the compensation for carboxylate anion (glutamic acid side chain) repulsions resulting from the production of the triphenylmethyl cation on the polymer chains by uv irradiation. The photo-induced changes of the conformation of Rose-modified PGA at pH 9.7 were reversible and in correlation with the change in absorbance at 560nm of Rose side chain groups. On the other hand, the Rose side chains in the polymer exhibited no photo-ionization under uv light at pH 5.7, owing to the neutralization effect in the low pH aqueous solution. As a result, uv irradiation did not induce any variations in conformation of Rose-modified PGA at pH 5.7.

INTRODUCTION

The photochromic dyes, which are covalently or non-covalently connected to polymers, have been used to control polymer conformation and to regulate photochemically the membrane functions.^{1)–4)} We have already shown that the membrane functions of poly(L-glutamic acid) containing azobenzene side chains, such as membrane potentials and transport properties, can be controlled by light irradiation.^{5)–7)}

Triphenyl methane leuco derivatives are well-known photochromic dyes, which dissociate into ion pairs under light irradiation with production of the corresponding dyes. Irie et al.⁸⁾ have shown the photo-induced conformational changes of vinyl polymers caused by a charge density change of the polymer resulting from the photoisomerization of photochromic side chains.

In this paper, using poly(L-glutamic acid) with a triphenyl methane leuco derivative, pararoseaniline, in the side chains, we report on the

photo-induced formation of α -helical structure of the polymer by light irradiation *via* the compensation effect for glutamic acid side chain repulsions resulting from the photo-induced production of triphenylmethyl cations on the polymer chains.

EXPERIMENTAL

Materials

Poly(L-glutamic acid) (PGA, 1.0g, $\bar{M}_v = 1.19 \times 10^5$) was dissolved in dimethylformamide (DMF, 50cc). Pararoseaniline (Rose, 0.68g), N-hydroxybenzotriazole (HOBT, 0.32g) and dicyclohexylcarbodiimide (DCCI, 0.48g) were added to the stirred mixture at 0°C. After 24h at room temperature, the bulk of the DMF solution was filtrated and the polymer was obtained by precipitation with methanol. Any unreacted Rose reagent was removed by extraction. The obtained PGA with Rose in the side chains (Rose-modified PGA) was dissolved in alkaline aqueous solution (8.7×10^{-4} g/cc) for UV and CD measurements. The Rose content determined on the basis of the molar

extinction coefficient of the triphenylmethyl cation ($\lambda_{\max} = 540\text{nm}$, in pH 5.7 water) was ca. 1.1 mol%.

Measurements

Absorption spectra and CD spectra of the solution were measured with a spectrophotometer (Shimadzu Co. Ltd. UV-200) and a spectropolarimeter (Jasco J-40), respectively. pH of the solution was measured by a pH meter (Horiba pH meter F7-ss). All the measurements were carried out at pH 9.7, 8.5, 5.7, respectively, at 25°C.

Irradiation was carried out with a 500W super-high-pressure mercury lamp (Ushio USH-500D) and the irradiation wave length was limited to the ultraviolet region using glass filter (Toshiba UV-D33S).

RESULTS AND DISCUSSION

Photo-isomerization

Rose dissociates into ion pairs under uv irradiation, yielding the colored triphenylmethyl cations, and the cations thermally recombine with counterions, as shown in Eq(1). This was confirmed by

the UV spectra resulting from the change in the absorption band at 540nm in Figure 1.

The Photo-isomerization (Photo-ionization) of Rose-modified PGA was also evaluated by UV

Fig. 1 Absorption spectra of paraseoaniline ($1.04 \times 10^{-5} \text{mol} \cdot \text{dm}^{-3}$) in aqueous solution of pH 9.5 containing distearyl-dimethylammonium chloride ($1.67 \times 10^{-3} \text{mol} \cdot \text{dm}^{-3}$) at 25°C (-----: in the dark, ———: after uv irradiation).

spectra. Figure 2 shows that UV spectra of Rose-modified PGA solution at pH 9.7. Before uv irradiation, no remarkable absorption bands between 700nm to 500nm were existence, but after uv irradiation the absorption band resulting from the triphenylmethyl cation appeared at 560nm. At lower pH, however, the ionization has already occurred without uv irradiation, which is owing to the neutralization effect in low pH aqueous solution (Figure 3). The pKa value of paraseoaniline was shown to be 7.6.⁹⁾ uv irradiation, therefore, did not induce any photo-ionization of Rose side chains at pH 5.7. Thus the ionization of side chain Rose moieties of Rose-modified PGA can be controlled by uv light at higher pH region.

Photo-induced formation of α -helical structure of Rose-modified PGA

The conformational changes of Rose-modified PGA upon uv irradiation were evaluated by CD measurement. Figure 4 shows the CD spectra of

Fig. 2 Absorption spectra of the Rose-modified PGA in aqueous solution of pH 9.7 at 25°C (-----: in the dark, ——— after uv irradiation).

Fig. 3 Absorption spectra of the Rose-modified PGA in aqueous solution of pH 5.7 at 25°C (-----: in the dark, ———: after uv irradiation).

Fig. 4 CD spectra of the Rose-modified PGA before (-----) and after (——) irradiation in aqueous solution of pH 9.7; (a), 8.5; (b), 5.7; (c) at 25°C.

Rose-modified PGA at various pH. CD measurement of the Rose-modified PGA could not be carried out below 200nm, since excess noise is produced in their CD spectra. The random coil state of poly(α -amino acid) is generally characterized by the negative band at 197nm ($[\theta]_{197} = -41.9 \times 10^3$) and positive band at 217nm ($[\theta]_{217} = 4.6 \times 10^3$) in the CD spectra. The positive values of the molar ellipticity, $[\theta]_{217}$, of the Rose-modified PGA in the dark (dashed curves in Figure 4), $3.00 \times 10^3 \text{ deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$ (pH 9.7), 2.70×10^3 (pH 8.5) and 2.10×10^3 (pH 5.7), indicate that the polymer is in random coil rich structure. Greenfield et al.¹⁰⁾ have shown the calculated CD curves which were obtained by theoretical combinations of α -helix and random forms poly(L-lysine in aqueous solution. The best fit of the experiment CD curves of Rose-modified PGA with calculated one from 210–240nm, which is proposed by Greenfield et al., gives a helical content of 4.3% (pH 9.7), 5.2% (pH 8.5) and 6.9% (pH 5.7), respectively. Similar procedure was applied to CD spectra after uv irradiation (solid curves in Figure 4), and gives a helical content of 7.2% (pH 9.7) and 6.5% (pH 8.5), respectively. That is, the formation of α -helical structure of the polymer was induced by uv irradiation at high pH region. It is noticeable that the degree of photo-induced increase in the helix content at pH 9.7, ca. 67%, is larger than that at pH 8.5, ca. 25%. As described above, Rose side chains are converted to the dye cations, yielding the free hydroxide anion by uv irradiation. Both ions have an opposite effect on the conformation of PGA polymer, i.e., the dye cations produce positive

fixed charges on the polymer chains among the negative charges of glutamic acid. On the other hand, the free hydroxide anions increase the degree of dissociation of the glutamic acid side chains. The former effect may result in the stabilization of the α -helix conformation of Rose-modified PGA owing to reduction of carboxylate anion repulsions, and the latter increases the anion-anion repulsions between glutamic acid side chains. At pH 9.7, the carboxylate anions of the Rose-modified PGA are fully ionized. Therefore, the former effect may be predominate at this pH. So that the photo-induced formation of α -helix structure of Rose-modified PGA at pH 9.7 can be explained in terms of the former effect, i.e., the compensation for carboxylate anion repulsions resulting from the production of the triphenylmethyl cations on the polymer chains by uv irradiation. On the other hand, the degree of ionization of the Rose-modified PGA side chains, carboxylate anions, at pH 8.5 is relatively lower than that at pH 9.7. Therefore, the effect of the yielding the free hydroxide anion on the conformation of the Rose-modified PGA (latter effect) may not be negligible at pH 8.5, i.e., the former effect which results in the stabilization of the α -helix structure is reduced by the latter. This is the reason why the degree of the photo-induced increase in the helix content of the polymer at pH 8.5 is lower than that at pH 9.7. At lower pH (pH 5.7) the value of $[\theta]_{217}$ was independent of uv irradiation (Figure 4(c)), i.e., the conformation of Rose-modified PGA was unchanged by uv irradiation at lower pH. This result is consistent with the fact that uv irradiation did not induce

Fig. 5 (a) Photo-induced changes in molar ellipticity, $[\theta]_{217}$, of the Rose-modified PGA in aqueous solution of pH 9.7 at 25°C.
(b) Photo-induced changes in absorbance at 560nm of the Rose-modified PGA in aqueous solution of pH 9.7 at 25°C.

any variations in ionization state of Rose side chains of PGA polymer as shown in Figure 3.

Figure 5(a) shows the decrease and recovery cycles of the value of $[\theta]_{217}$ of Rose-modified PGA solution at pH 9.7. The photo-induced changes of the value of $[\theta]_{217}$ are almost consistent with the absorption changes at 560nm of

Rose-modified PGA solution at pH 9.7. This result also suggests that the photo-induced formation of α -helix structure of Rose-modified PGA can be attributed to the ionization reaction of Rose side chains upon uv irradiation at higher pH region.

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REFERENCES

- 1) A. Ueno, J. Anzai and T. Osa, *J. Polym. Sci., Polym. Lett. Ed.*, **15**, 407 (1977).
- 2) M. Irie, Y. Hirano, S. Hashimoto and K. Hayashi, *Macromolecules*, **14**, 262 (1981).
- 3) S. Kato, M. Aizawa and S. Suzuki, *J. Membr. Sci.*, **2**, 39 (1977).
- 4) K. Ishihara and I. Shinohara, *J. Polym. Sci., Polym. Lett. Ed.*, **22**, 515 (1984).
- 5) A. Takizawa, M. Sato, T. Kinoshita and Y. Tsujita, *Chem. Lett.*, **1984**, 1963.
- 6) T. Kinoshita, M. Sato, A. Takizawa and Y. Tsujita, *J. Chem. Soc., Chem. Commun.*, **1984**, 929.
- 7) T. Kinoshita, M. Sato, A. Takizawa and Y. Tsujita, *Macromolecules*, in press.
- 8) M. Irie and D. Kungwachakun, *Makromol. Chem., Rapid Commun.*, **5**, 829 (1985).
- 9) R. J. Goldacre and J. N. Phillips, *J. Chem. Soc.*, **1949**, 1724.
- 10) N. Greenfield and G. D. Fasman, *Biochemistry*, **8**, 4108 (1969).

パラローズアニリンを側鎖に有するポリ(L-グルタミン酸)の光照射による α -ヘリックス構造の形成

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側鎖にトリフェニルメタン系染料の一種であるパラローズアニリン構造を有するポリ(L-グルタミン酸)(Rose-PGA)を合成し、UV照射に基づくこのポリマーの二次構造変化を解析した。pH=9.7の水溶液においてRose-PGAは、ランダムコイル構造をとるがUV照射により一部 α -ヘリックス構造が形成されることがわかった。また同系のpH=5.7で同様な測定を行なったが、二次構造には何ら変化は生じなかった。一方pH=9.7の水溶液におけるUV測定の結果、パラローズアニリン側鎖はUV照

射により光異性化しトリフェニルメタンカチオンを生成することが確認された。したがってUV照射によるポリマーの二次構造変化は側鎖パラローズアニリンの光異性化に基づいていることが裏付けられた。つまりUV照射に伴い側鎖パラローズアニリンが光異性化(トリフェニルメタンカチオン生成)し、グルタミン酸側鎖間の負電荷間の反発を抑制し、 α -ヘリックス構造が安定化されると結論づけられた。