

Conformation of polyamino acids containing fluorine

Koichi Ikeda, Yoshiro Ogoma*, Toshihiro Fujii, Akira Hachimori, Yoshiyuki Kondo and Tadao Hayakawa

Department of Functional Polymer Science, Faculty of Textile Science and Technology, Shinshu University, Ueda 386, Japan

and Makoto Iwatsuki

New Business Development Department, Ajinomoto Inc., Kyobashi, Chuo-ku, Tokyo 104, Japan

and Toshihiro Akaike

Department of Material Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184, Japan

(Received 23 February 1989; revised 10 April 1989; accepted 24 April 1989)

The conformation of poly(γ -4-fluoro-benzyl-L-glutamate) (F-PBLG) and poly(γ -4-trifluoromethyl-benzyl-L-glutamate) (CF₃-PBLG) was investigated. Circular dichroism (c.d.), infra-red (i.r.) spectra and X-ray analysis indicated that the secondary structure of F-PBLG and CF₃-PBLG was α -helical both in the solid state and in chloroform solution, similar to PBLG, while it was random coil in trifluoroacetic acid (TFA) and dichloroacetic acid (DCA). In the chloroform-TFA solvent system, these polypeptides changed their conformation from helix to coil; the minimum TFA concentrations required for helix-coil transition were 10–12% in CF₃-PBLG, 14% in F-PBLG and 12–13% in PBLG. The results suggest that CF₃ groups have a pronounced effect on the stability of α -helical structure of these polypeptides. The helix-coil transition of the polypeptides was accompanied by a perturbation of ¹⁹F nuclear magnetic resonance chemical shift.

(Keywords: polyamino acid; fluorine; conformation; X-ray diffraction; infra-red spectroscopy; circular dichroism; nuclear magnetic resonance)

INTRODUCTION

The increased importance of fluorinated compounds in medicine and engineering applications, such as artificial blood, antipsychotics, anti-cancer drugs, insecticides and coolants, has stimulated the study of fluorine chemistry. Although the atomic size of fluorine (1.35 Å†) is nearly equal to that of hydrogen (1.20 Å), the introduction of fluorine to a molecule results in the endowment of electronic characteristics because of the greater electronegativity. ¹⁹F nuclear magnetic resonance (n.m.r.) spectroscopy has proved very useful for investigating the physical character and environment of fluorides¹. It has also been used in protein chemistry, e.g. for the analysis of the active site of fluorinated protein² and of the interaction of protein with drugs^{3–5}. Polyamino acids have good bio-compatibility, as the analogue of protein, and applications for bio-materials such as artificial skin⁶ have been reported. Polyamino acid with fluorine is expected to have some of the characteristics of both polyamino acid and fluorine.

In the present study, we synthesized the new polyamino acids, poly(γ -4-fluoro-benzyl-L-glutamate) (F-PBLG) and poly(γ -4-trifluoromethyl-benzyl-L-glutamate) (CF₃-PBLG), and examined the effect of fluorine on the secondary structure of these polymers in the solid state and in solution, by using spectroscopic measurements

such as infrared (i.r.) spectra, X-ray analysis, circular dichroism (c.d.) and ¹H n.m.r. spectra. The helix-coil transition of these polymers was also investigated by ¹⁹F n.m.r..

EXPERIMENTAL

Materials

F-PBLG and CF₃-PBLG were prepared by the NCA method. Poly(γ -benzyl-L-glutamate) (PBLG) used as a reference was obtained from Ajinomoto Inc. The degrees of polymerization (*DP*) of PBLG, F-PBLG and CF₃-PBLG determined by viscosity were 400, 400 and 200, respectively.

Measurement

I.r. spectra were measured with a Jasco IR A-302 at room temperature, using cast film from the chloroform solution.

X-ray diffraction photographs were taken with Rigakudenki Geigerflex (CuK α).

C.d. spectra were obtained with a Jasco J-40A automatic recording dichrograph. A cell of 1 mm path length was used for measurement in the far and near ultraviolet (u.v.) regions in the chloroform-trifluoroacetic acid (TFA) solvent system at 22°C. The concentration of the samples was 0.25 mg ml⁻¹.

¹H and ¹⁹F n.m.r. measurements were made with a

* To whom correspondence should be addressed
† 1 Å = 10⁻¹⁰ m

Table 1 Characteristic absorption bands of polyamino acids containing fluorine and PBLG

Assignment	Wavenumber (cm ⁻¹)		
	PBLG	F-PBLG	CF ₃ -PBLG
Amide A	3295	3310	3310
Amide I	1652	1650	1656
Amide II	1550	1548	1550
Amide V	612	600	610
C-F	-	1222	1328

Table 2 Spacings^a (Å) in X-ray photographs of polyamino acids containing fluorine and PBLG

PBLG	F-PBLG	CF ₃ -PBLG
	2.93 (w)	3.00 (w)
4.43 (v.s.)	4.29 (v.s.)	4.41 (v.s.)
5.24 (s)	5.22 (s)	5.20 (s)
9.73 (w)		
13.16 (v.s.)	13.28 (v.s.)	13.41 (v.s.)

^aIntensity: v.s., very strong; s, strong; w, weak

Bluker AC-250 FT n.m.r. spectrometer at frequencies of 250 and 235.3 MHz, respectively. The spectra were obtained in either *d*-chloroform-TFA or *d*-chloroform-dichloroacetic acid (DCA) mixed solvents. For ¹H n.m.r. chemical shifts (ppm) were measured from the internal standard of trimethylsilane (TMS), while for ¹⁹F n.m.r. chemical shifts were referred to signals of ¹⁹F nuclei of F-PBLG or CF₃-PBLG in *d*-chloroform. Resonances occurring in the downfield region were taken as positive.

RESULTS AND DISCUSSION

I.r. studies and X-ray analyses

The wavenumbers of absorptions characteristic of amide bands of PBLG, F-PBLG and CF₃-PBLG are listed in *Table 1*. The amide I and amide II of both F-PBLG and CF₃-PBLG were found around 1650 and 1550 cm⁻¹, respectively, similarly to those of PBLG. The C-F stretching vibrations of F-PBLG and CF₃-PBLG were observed at 1222 and 1328 cm⁻¹, respectively.

The spacings and intensities obtained from the X-ray diffraction patterns of the polymer films are listed in *Table 2*. Two strong spacings were found at 13.1–13.4 and ≈ 5.2 Å in all polymers. The results indicate that the polymers had α -helical structure in the solid state, and also that fluoride ions had very little influence on the backbone structure of the polymers.

C.d. spectra

Figure 1 shows the c.d. spectra of PBLG, F-PBLG and CF₃-PBLG in the far u.v. region. The polymers were dissolved in chloroform. A large negative c.d. band was observed at 222 nm in all samples, indicating that the polymers had right-handed α -helical structure in chloroform. Since TFA and DCA are known to convert the α -helical structure of PBLG to random coil, the helix-coil transitions of F-PBLG and CF₃-PBLG in chloroform-TFA mixed solvents were measured. The conformation of those peptides was also found to be affected by TFA. The TFA concentrations required for the helix-coil transition of PBLG, F-PBLG and

CF₃-PBLG were 12–13%, 14% and 10–12%, respectively (*Figure 2*). This suggests that, although these polypeptides have the same backbone structure, their stability seems to differ due to the fluorine (F-) or trifluoromethyl group (CF₃-) in the side chain.

¹H n.m.r. spectra

It has been reported that, when the α -helical structure of PBLG changes to random coil, the bandwidth of the single peak due to α -CH, which was observed around 4.6 ppm, becomes sharp, being shifted downfield⁷. Then ¹H n.m.r. spectra of F-PBLG and CF₃-PBLG were measured under two conditions: 2% TFA in solution in which the conformation of polymers was thought to be α -helical and 12% TFA in solution in which the polymers took random coil conformation (*Figure 3*). In the latter case, a single peak due to α -CH was observed at 4.8 ppm in both polymers. In the former case, the signal due to the phenyl proton of PBLG at 7 ppm was sharp, while the peaks of F-PBLG and CF₃-PBLG were broad, with that of CF₃-PBLG much broader than that of F-PBLG.

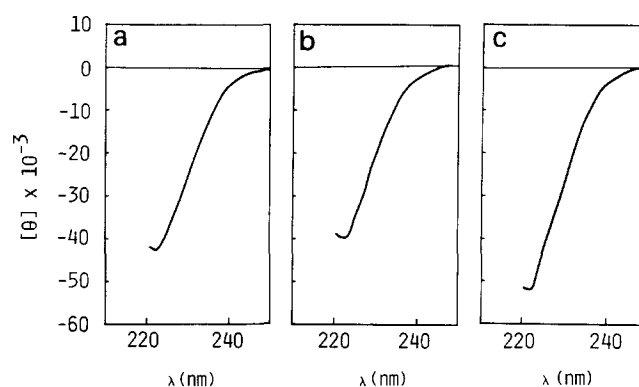


Figure 1 C.d. spectra of polyamino acids in chloroform at 22°C; (a) PBLG; (b) F-PBLG; (c) CF₃-PBLG

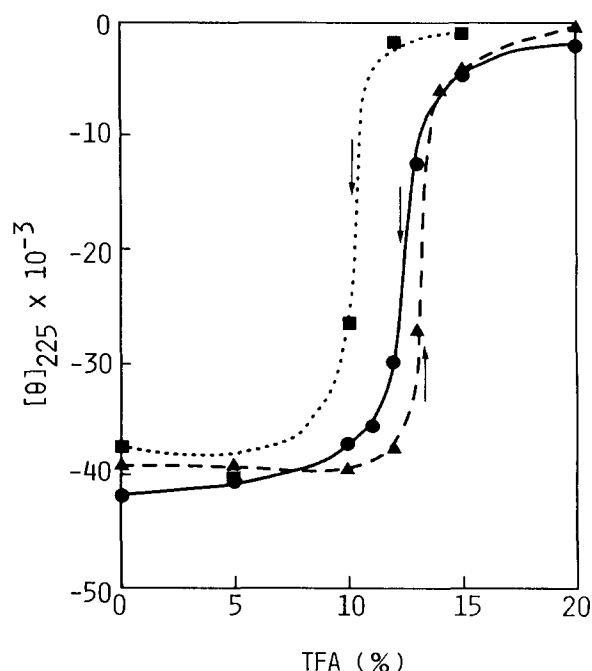


Figure 2 Helix-coil transition of PBLG (—), F-PBLG (---) and CF₃-PBLG (.....) in chloroform-TFA mixed solvents at 22°C

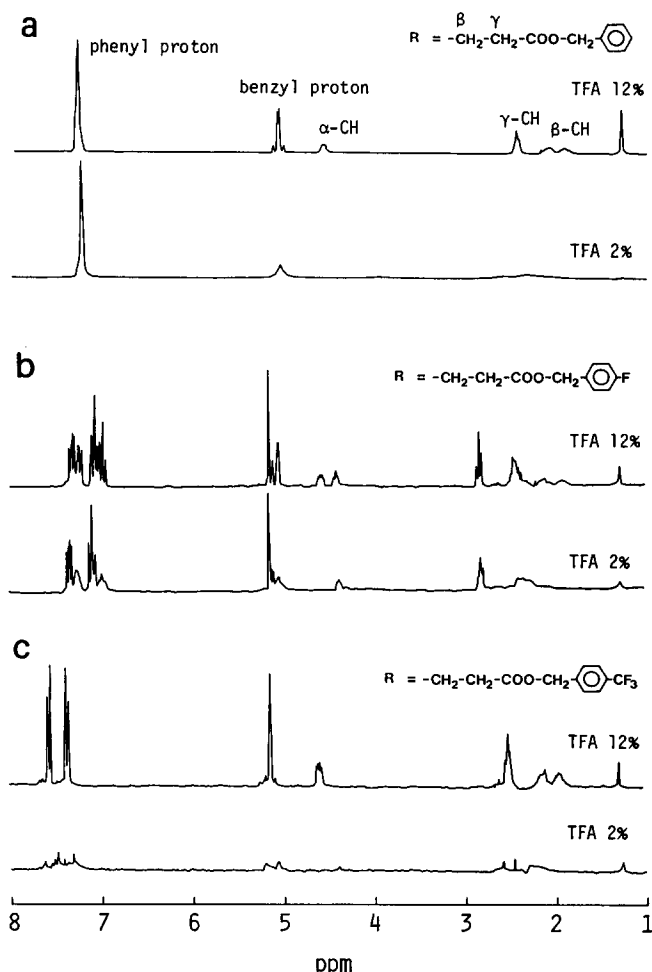


Figure 3 ^1H n.m.r. chemical shifts of polyamino acids in chloroform-TFA mixed solvents at 25°C: (a) PBLG; (b) F-PBLG; (c) CF_3 -PBLG

In general, when the molecular movement of hydrogen is restricted by hydrogen bonding or any other factor, the bandwidth of the n.m.r. signal becomes broad. Therefore, the present results suggest that the state of the side chain in CF_3 -PBLG may differ from that in PBLG, and the degree of freedom in the CF_3 -PBLG side chain is smaller than that in PBLG in the helical structure. This difference may be due to the hydrogen bonding between fluorine and hydrogen atoms in the backbone or other side chain, and many more bonds may be formed with trifluoromethyl groups.

^{19}F n.m.r. spectra

Since fluorine atoms are contained in F-PBLG and CF_3 -PBLG, ^{19}F n.m.r. is thought to be a good tool for investigating the polymer structure. We used ^{19}F n.m.r. to investigate the helix-coil transitions of these polypeptides in *d*-chloroform-DCA mixed solvents. Figures 4 and 5 show the ^{19}F n.m.r. chemical shift of F-PBLG in these solvents. The ^{19}F signal of F-PBLG in *d*-chloroform (DCA 0%) was observed at 0 ppm, and it shifted downfield in two steps with increasing DCA concentration. The chemical shift at 20% DCA was observed at ≈ 0.6 ppm and that at 80% DCA was at ≈ 0.1 ppm. When DCA concentration was $> 80\%$, the ^{19}F n.m.r. signal was split. However, this multiplet signal became singlet when the proton decoupling technique was used, suggesting that the split may be due to the

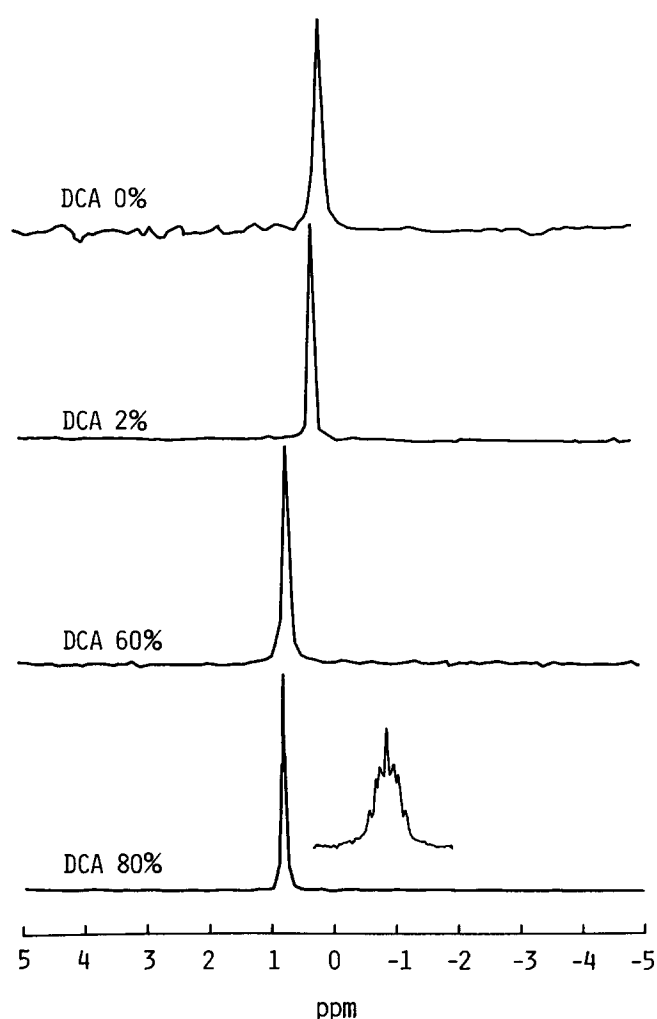


Figure 4 ^{19}F n.m.r. chemical shifts of F-PBLG in chloroform-DCA mixed solvents at 25°C

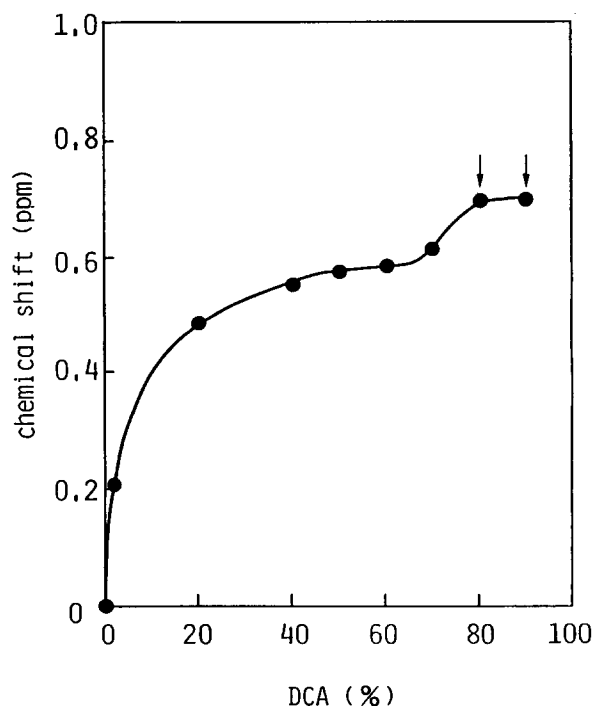


Figure 5 ^{19}F n.m.r. chemical shifts of F-PBLG in chloroform-DCA mixed solvents at 25°C

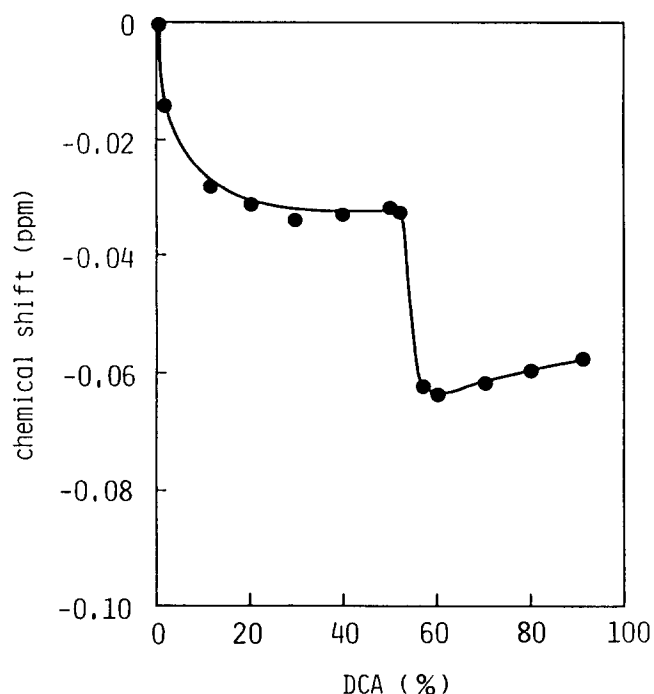


Figure 6 ^{19}F n.m.r. chemical shifts of CF_3 -PBLG in chloroform-DCA mixed solvents at 25°C

interaction between ^{19}F and ^1H nuclei. On the contrary, when the ^{19}F n.m.r. signal of fluorobenzene was measured, the change of chemical shift was proportional to the DCA concentration. Therefore, the inflectional point observed in the plot of change of chemical shift of F-PBLG against DCA concentration may be characteristic of fluorine involved in the polymer. The helix-coil transition of F-PBLG occurred at a DCA concentration between 70 and 80%, which was confirmed by optical rotary dispersion (o.r.d.), i.e. $b_0 = 600$ at 70% DCA, while it was $b_0 = 101$ at 80% DCA. Therefore, the change in chemical shift of F-PBLG at a DCA concentration between 70 and 80% seems to reflect the alteration of environment around fluorine due to the helix-coil transition. F-PBLG is thought to be in aggregate form in chloroform since PBLG in aggregate form dissolved in chloroform due to the large dipole moment. Thus the change in chemical shift at a DCA concentration between

0 and 20% seems to reflect the dissociation of the aggregate form of F-PBLG.

The ^{19}F n.m.r. chemical shift of CF_3 -PBLG in *d*-chloroform-DCA mixed solvent is shown in Figure 6. The chemical shifts at both 20 and 60% DCA were observed at ≈ 0.03 ppm. Unlike F-PBLG, the ^{19}F n.m.r. signal of CF_3 -PBLG shifted upfield with increasing DCA concentration. The opposite movements of chemical shifts of F-PBLG and CF_3 -PBLG with increasing DCA concentration may be attributed to the difference in distance of fluorine and benzene ring. However, the pattern of chemical shift-DCA concentration in CF_3 -PBLG was the same as in F-PBLG in that both had two inflection points (one observed at a DCA concentration between 0 and 20% and the other between 55 and 60% in CF_3 -PBLG). The inflection point at a DCA concentration between 55 and 60% may reflect the helix-coil transition since, from o.r.d. measurement, b_0 was 630 at 50% DCA, and 0 at 60% DCA.

As described above, we found that the conformational stability of the backbone as well as the side chain of these polymers was affected by fluorine, and the ^{19}F n.m.r. chemical shifts reflect the conformational transition of polyamino acids containing fluorine. It was also shown that the chemical shifts of ^{19}F n.m.r. of F-PBLG and CF_3 -PBLG differed, moving downfield or upfield with increasing concentration of DCA, suggesting that the electronic state of fluorine was controlled by the elements and groups around fluorine. Therefore, the environment of the side chain in F-PBLG is thought to differ from that of CF_3 -PBLG, and this difference is supposed to reflect their properties.

REFERENCES

- Gerig, J. T. *Biological Magnetic Resonance* Vol. 1 (Eds. L. J. Berliner and J. Reuben), Plenum Press, New York, 1978, pp. 139-203
- Cairi, M. and Gerig, J. T. *J. Am. Chem. Soc.* 1984, **106**, 3640
- Shimizu, T. and Hatano, M. *FEBS Lett.* 1983, **160**, 182
- Shimizu, T., Hatano, M., Muto, Y. and Nozawa, Y. *FEBS Lett.* 1984, **166**, 373
- Ogoma, Y., Miwa, T., Fujii, T., Kondo, Y., Hachimori, A., Shimizu, T. and Hatano, M. *Inorg. Chim. Acta* 1987, **138**, 145
- Lin, S. D. *et al. Trans. Am. Soc. Artif. Intern. Organs* 1981, **27**, 522-527
- Marlborough, D. I., Orrell, K. G. and Rydon, H. N. *J. Chem. Soc., Chem. Commun.* 1965, 518