Original Articles

Transactions
of the
Chemical Society of Japan

(Received October 2, 1981)

STRUCTURE OF POLYPEPTIDES MODEL RELATED TO SILK FIBROIN

By Masuhiro Tsukada^{*1}, Guy Bertholon^{*2} and Kiyoshi Hirabayashi^{*3}

^{*1} (Sericultural Experiment Station, Yatabe, Ibaraki, Japan)

^{*2} (Laboratoire de Chimie Industrielle, Lyon 1, France)

^{*3} (Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan)

In order to elucidate the polypeptide model, which is closely related to a modification of silk of *bombyx mori*, the molecular structure of poly(Gly-L-Ala) and copoly(L-Ala-Gly, L-Ser-Gly) (2:1) have been studied by x-ray powder diffraction, infrared spectra and circular dichroism. The main interests are focused on the effect of introducing the seril residue into the polypeptides chains. A marked similarity between x-ray powder patterns, and CD spectra in the DCA-TFE mixture suggest that poly(Gly-L-Ala) bears a marked structural resemblance to copoly(L-Ala-Gly, L-Ser-Gly) (2:1). However, in the amide I region, a strong infrared band comparing with that of poly(Gly-L-Ala) at 1695 cm^{-1} in copoly(L-Ala-Gly, L-Ser-Gly) (2:1) assigned to the antiparallel beta structure are observed. The principal advantage of the effect on introducing the seril residues into the polypeptide backbone's chain can be summarized as follows. The backbone chains of copoly(L-Ala-Gly L-Ser-Gly) (2:1) holding at the position of C^α atoms of seril residues crystallize into cross beta structure or antiparallel beta form, while that of poly(Gly-L-Ala) possesses a slight amount of antiparallel beta structure.

1. INTRODUCTION

Three conformations, i.e. silk I structure, silk II structure and random coil, are known to exist in the molecular form of silk fibroin. Although the structure of silk fibroin and their model polypeptides has been investigated from very early ages¹⁾, the structural analysis of silk I has not been developed²⁾. The detailed studies on the model polypeptides, which have been carried out by Lotz et al.³⁾, Hayakawa⁴⁾, Brack et al.⁵⁾, and Hirabayashi et al.⁶⁾ revealed that the chain of the silk I has an overall crankshaft geometry in which the structural repeat unit consisted from dipeptides. The conformation of the alanine and glycine residues are widely different and close those of the β structure and α helix, respectively.

Careful analysis has been proved that the princi-

pal amino acid composition which constitute the crystalline region of the silk fibroin are glycine, alanine and serine, contained in the proportional ratio of 3:2:1. Principally, it may be reasonable to use the sequential polypeptides of three amino acid residues to study the influence of chemical conformation on molecular conformation in proteins. The research groups have been trying to elucidate the molecular structure of copolypeptides such like Gly-X-Gly-X, in which X represents for example alanine residue. Although there are a few study^{4),15)} on discussing the effect of introducing the seril residue to the poly(Gly-Ala), the molecular conformation of the polymer having identified each residue has not been detailedly characterized both in the solution and solid state.

In this paper, we discuss the change in molecular conformation of the model polypeptides, poly(Gly-

L-Ala) and copoly(L-Ala-Gly, L-Ser-Gly) (2:1) in the mixed solution of dichloroacetic acid (DCA) and trifluoro ethanol (TFE), by the introducing of seril residue into the polypeptide chains of poly(Gly-L-Ala).

2. EXPERIMENTAL

2.1 Materials

Poly(Gly-L-Ala) and copoly(L-Ala-Gly, L-Ser-Gly) (2:1) were procured from Pilot Chemicals.

2.2 Apparatus and Procedure

Infrared spectra of the silk model polymers, using the KBr method were recorded using a JASCO DS-701 spectrophotometer. X-ray patterns were recorded using an X-ray source with $\text{CuK}\alpha$ radiation ($\lambda=1.54 \text{ \AA}$). The voltage and current of the X-ray source were about 40 KV and 20 mA, respectively.

Circular dichroism (CD) spectra of the silk fibroin and its related polypeptide models in the liquid phase were measured at room temperature in a quartz cell having a path length of 0.1 mm, using a JASCO-20 Recording spectropolarimeter. CD spectra of poly(Gly-L-Ala) and copoly(L-Ala-Gly, L-Ser-Gly) (2:1) were also measured in the mixed solution of dichloroacetic acid and trifluoro ethanol in the ratio of 1:9 (volume).

3. RESULTS

X-ray diffraction pattern

The X-ray diffractograms of amino acid polymers related to the silk fibroin are shown in Figure 1. Poly(Gly-L-Ala) (Figure 1 (A)) showed diffraction peaks corresponding to the spacing of 9.00, 6.40,

Fig. 1 X-ray diffraction patterns of the poly(Gly-L-Ala) (A), and copoly(L-Ala-Gly, L-Ser-Gly) (2:1) (B).

4.70, 4.25 and 3.60 $\text{\AA}$, while the copoly(L-Ala-Gly, L-Ser-Gly) (2:1) (Figure 1 (B)) showed the diffraction patterns corresponding to the spacing of 6.43, 4.75, 4.20, and 3.50 $\text{\AA}$. There is close resemblance between the specimens. The diffraction patterns of poly(Gly-L-Ala) and copoly(L-Ala-Gly, L-Ser-Gly) (2:1) have good accordance with data reported by Hayakawa et al.^{4),10)} From the results of X-ray diffractions, distinguished difference in the crystalline structure could not be found between these two samples.

Infrared spectroscopy

Figures 2 and 3 show infrared spectra of poly(Gly-L-Ala), copoly(L-Ala-Gly, L-Ser-Gly) (2:1), powder samples (A) and those dissolved in 9 M LiBr aqueous solution for 2 hrs, dialyzed thoroughly against pure water (B) which abbreviated as LiBr poly(Gly-L-Ala) and LiBr copoly(L-Ala-Gly, L-Ser-Gly) (2:1). The absorption bands of 1630 cm^{-1} (Amide I), and 1526 cm^{-1} (Amide II) which are attributed to the β structure⁹⁾ is observed in poly(Gly-L-Ala) (Figure 2 (A)). Amide I and amide II bands at around 1651 cm^{-1} and 1540 cm^{-1} found in LiBr poly(Gly-L-Ala) (Figure 2 (B)) are due to the silk I form¹⁰⁾. The absorption bands of 1695 cm^{-1} , 1621 cm^{-1} (Amide I) and 1525 cm^{-1} (Amide II) which assigned to anti-parallel β structure⁹⁾ is observed in copoly(L-Ala-Gly, L-Ser-Gly) (2:1) (Figure 3 (A)). Amide I and amide II bands at around 1650 cm^{-1} , 1522 cm^{-1}

Fig. 2 Infrared spectra of the powder sample of poly(Gly-L-Ala) (A), and the specimen treated of poly(Gly-L-Ala) in 9 M aqueous lithium bromide solution (B).

Fig. 3 Infrared spectra of the powder sample of copoly(L-Ala-Gly, L-Ser-Gly) (2:1) (A), and the specimen treated of (A) in 9 M aqueous lithium bromide solution (B).

due to the silk I form¹⁰⁾ are also found in LiBr copoly(L-Ala-Gly, L-Ser-Gly) (2:1) (Figure 3 (B)). It should be noted that the amide I band of specimen (Figure 2 (B)) showed very small absorption at around 1695 cm^{-1} which is attributed to the antiparallel β structure⁹⁾ However, it may be acceptable that copoly(L-Ala-Gly, L-Ser-Gly) (2:1) possesses a certain amount of antiparallel beta structure.

Circular Dichroism

The measurement of circular dichroism was carried out within the wave length of 213–240 nm. It is impossible to obtain the circular dichroism in wave length below 213 nm, because of the strong absorption caused by the solvent. Poly(Gly-L-Ala) (Figure 4) shows weak absorption at about 222–223 nm. Quite similar absorption is observed in copoly(L-Gly-Ala, L-Ser-Gly) (2:1) (Figure 5). Measured circular dichroism was compared with that of calculated from the molecular conformation of the samples in the liquid state according to the method proposed by Greenfield et al.⁷⁾ and Komoto et al.⁸⁾ As is seen in figures 4 and 5, the calculated CD curves approximately agree with the measured curves. The conformations of poly(Gly-L-Ala) and copoly(L-Ala-Gly, L-Ser-Gly) (2:1) were similar to each other, as obviously seen in

Fig. 4 Observed (—) and calculated (---) circular dichroism of poly(Gly-L-Ala) in the mixed solution of DCA and TFE.

Fig. 5 Observed (—) and calculated (---) circular dichroism of copoly(L-Ala-Gly, L-Ser-Gly) (2:1) in the mixed solution of DCA and TFE.

Figures 4 and 5, proportion of the β structure and random coil being 53 and 46 mole%, respectively (Table 1). The regenerated silk fibroin was prepared by dissolving in aqueous LiBr solution and the circular dichroism was compared with these of polymers (Figure 6). The best fit of the experimental value with calculated one are observed in the wide range of the wave length of 205–240 nm,

Table 1 Calculated conformation (in mol %) of Poly-(Gly-L-Ala), Copoly(L-Ala-Gly, L-Ser-Gly) (2:1) in dichloroacetic acid-trifluoro ethanol mixtures and regenerated silk fibroin in aqueous solution.

	α -Helix	β -Structure	Random coil
Poly(Gly-L-Ala)	1	53	46
Copoly(L-Ala-Gly, L-Ser-Gly) (2:1)	1	53	46
Regenerated Silk Fibroin	2	32	66

Fig. 6 Observed (—) and calculated (---) circular dichroism of regenerated silk fibroin, silk fibroin thread is solved into the solution of LiBr.

the beta form and random coil were 32 and 66 mole%, respectively. Contents of the β structure in the regenerated silk fibroin is smaller than that of polymers related to polyamino acids. However, it should be noted that it is rather difficult to obtain the quantitative estimation from these data, owing to the approximation involved in the calculation in which the molar ellipticity of a copolypeptides is treated as a linear combination of those corresponding to α helix, β structure and random coil of poly(L-lysine)⁸⁾.

4. DISCUSSION

We have tried to elucidate the structural and conformational characteristics of the polymers introducing the seril residues into the poly(Gly-L-Ala) by X-ray diffractometry, infrared spectroscopy and circular dichroism measurement. From the results of the IR spectra, the structure of the copoly(L-Ala-Gly, L-Ser-Gly) (2:1) is found to be different from that of the poly(Gly-L-Ala). A strong bands at 1965 cm^{-1} in amide I reflects antiparallel β structure⁹⁾. The conformation of the copoly-amino acid in the solid state and aqueous state was not appreciably different with that of the poly(Gly-L-Ala) from the results of X-ray diffraction pattern and circular dichroism. However, the content of the α helix was negligibly for both polymers in liquid state. This finding should be in accord with the result of recent publication¹⁵⁾, describing that poly(L-alanyl-glycyl-L-alanyl-glycyl-L-alanyl-glycyl-L-seryl-glycine), silk fibroin model, takes only random coil structure in dichloroacetic acid. Hayakawa et al.⁴⁾ have analysed the structural characteristics using many model polypeptides, for example, poly(Gly-L-Ala) and copoly(L-Ala-Gly, L-Ser-Gly) (3:1) by IR and X-ray diffractometry and they concluded that the structure of copolymers having the seril residue was not markedly differ from that of corresponding model polypeptides. However, our experimental results showed that the copoly(L-Ala-Gly, L-Ser-Gly) (2:1) has antiparallel β structure. This fact may be explained on the basis of the results of infrared spectroscopy. The copoly(L-Ala-Gly, L-Ser-Gly) (2:1) formed a clear antiparallel β structure, as shown in the absorption band of

1695 cm^{-1} attributed to the antiparallel β structure, while the band owing to the antiparallel β structure was not certain in poly(Gly-L-Ala). These observation for poly(Gly-L-Ala) are in accord with the prediction from the results of the X-ray diffraction¹¹⁾. If the arrow ($\rightarrow$) may be assumed the direction from $\text{N}-\text{C}^\alpha$ to $\text{C}^\alpha-\text{C}$, we can represent this pleated sheet structure of these polyamino acid in Figure 7. Moreover if we assume the molecular weight of these two samples are about 2000–3000, there exists 10–15 amino acid residues in one arrow. Poly(Gly-L-Ala) forms partially antiparallel β structure at most. Copoly-(L-Ala-Gly, L-Ser-Gly) (2:1) in which the seril residue is introduced, crystallizes into antiparallel β structure or cross β structure. There should be noted that the molecular chain folds at the part of the C^α position of the seril residues (Figure 7).

Fig. 7 Schematic structure in the molecular form of poly(Gly-L-Ala) (A) and copoly(L-Ala-Gly, L-Ser-Gly) (2:1) (B).

Aqueous silk fibroin solution, in which the 93% ethanol is titrated slowly, gives the cross β structure^{12),13)}, which also gives the circular dichroism band centered at 197 nm (positive band), and at 217 nm (negative band). The silk fibroin hydrolyzed by aqueous HCl solution gives antiparallel β structure¹⁴⁾, which is identified from the IR

absorption at 1695 cm^{-1} . In this reason, we can conclude that the structure of the copoly(L-Ala-Gly, L-Ser-Gly) (2:1) is much more similar to that of the silk fibroin in the molecular conformation.

REFERENCES

- 1) Y. Go, J. Noguchi, M. Asai and T. Hayakawa, *J. Polym. Sci.*, **21**, 147 (1956)
- 2) Y. Go, "Structure of Silk Fibers", N. Hojo ed., Shinshu University, Ueda, p1 (1980)
- 3) B. Lotz and C. Cesari, *Biochimie*, **61**, 205 (1979)
- 4) T. Hayakawa, Y. Kondo, H. Yamamoto and J. Noguchi, "Structure of Silk Fibers", N. Hojo ed., Shinshu University, Ueda, p 417 (1980)
- 5) A. Brack and G. Spack, *Biopolymers*, **11**, 563 (1972)
- 6) K. Hirabayashi, K. Uchiyama, H. Ishikawa and Y. Go, *Sen-i Gakkaishi*, (*Journal of the Society of Fiber Science and Technology, Japan*), **23**, 538 (1967)
- 7) N. Greenfield and G. D. Fasman, *Biochemistry*, **8**, 4108 (1969)
- 8) T. Komoto, Y. Kojima and T. Kawai, *Makromol. Chem.*, **179**, 1861 (1978)
- 9) T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961)
- 10) T. Hayakawa, Y. Kondo, S. Yamamoto and J. Noguchi, *Kobunshi Kagaku*, **27**, 229 (1970)
- 11) B. Lotz, Personal Communication
- 12) E. Iizuka and J. T. Yang, *Biochemistry*, **7**, 2218 (1968)
- 13) K. Hirabayashi, K. Uchiyama, H. Ishikawa and Y. Go, *Sen-i Gakkaishi*, **23**, 538 (1967)
- 14) M. Tsukada, K. Hirabayashi, T. Komoto and Y. Kawai, *Sen-i Gakkaishi*, **34**, 349 (1978)
- 15) B. Nakajima, K. Hirata, N. Nishi and J. Noguchi, *Int. J. Biol. Macromol.*, **3**, 46 (1981)

絹フィブロインのポリペプチドモデルの構造

農林水産省蚕糸試験場
リヨン大学工業化学研究室
東京農工大学工学部

塚田 益 裕
Guy Bertholon
平 林 潔

絹フィブロインの合成アミノ酸モデルである poly(Gly-L-Ala) および copoly(L-Ala-Gly, L-Ser-Gly) (2:1) の構造を究明するため, X線回折, 赤外吸収スペク

トルおよび円二色性の測定結果を通して, ポリペプチド主鎖にセリン残基が導入されることによる構造の変化を検討した。X線回折および円二色性による分子形態測

定の結果には、試料間の構造上の差異が見られない。赤外吸収スペクトル測定によると、copoly(L-Ala-Gly, L-Ser-Gly)(2:1)はpoly(Gly-L-Ala)より明瞭な逆平行 β 構造をとっていることが明らかとなった。実験結果をもとにセリン残基がポリペプチド主鎖に導入されることによる構造上の効果を検討し次の結論を得た。

copoly(L-Ala-Gly, L-Ser-Gly)(2:1)のポリペプチド主鎖はセリン残基の C^α の位置を中心にしておりたまれ、分子鎖はcross β あるいは逆平行 β 構造に結晶化する。これに対し、poly(Gly-L-Ala)の分子鎖は部分的に逆平行 β 構造をとるものと推論した。