後部絹糸腺フィブロインより調製した結晶部ペプチドの性質について

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Characterization of the Crystalline-Region Peptides Prepared from the Posterior Silk Gland Fibroin

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Crystalline-region peptides (Cp) of silk fibroin were prepared by α-chymotryptic digestion from the large subunit of fibroin, which was prepared from the posterior silk glands of *Bombyx mori* with cold 30% ethanol treatment. The ratio of crystalline to amorphous regions in the large subunit was about 55 to 45 in terms of amino acid residues. Sephadex G-50 gel filtration gave a single peak with a slight shoulder, indicating the molecular size of Cp lies within a fairly limited range. On the other hand, elution profile of Cp from a column of DEAE-Sephadex A-50 exhibited one main peak with a shoulder and three minor peaks. This result shows the heterogeneity of Cp peptides in respect to their electrostatic charges. Amino acid analysis of these peptide fractions also supports this opinion. Molecular weight of Cp was estimated to be about 3900~4100 from terminal analyses and amino acid composition.

When an aqueous solution of fibroin is hydrolyzed by α-chymotrypsin, a massive precipitate is formed amounting to about 60% of the fibroin (Drucker and Smith, 1950; Drucker *et al.*, 1953; Lucas *et al.*, 1957). This peptide fraction has been considered to be derived from the crystalline-region of fibroin molecules. On the other hand, the peptide fraction remained in the supernatant of a chymotryptic digest is regarded as being formed from the amorphous region of fibroin. Although a number of studies on the peptide components contained in both fractions have been carried out (Drucker *et al.*, 1953; Narita, 1954 a, b; Lucas *et al.*, 1957; Waldschmidt-Leitz and Kirchmeier, 1958; Ziegler and Spoor, 1959; Ziegler and La France, 1960; Lucas *et al.*, 1962; Shaw, 1964 a, b; Geddes *et al.*, 1969; Robson *et al.*, 1970; Earland and Robins, 1973; Strydom *et al.*, 1977), we have still insufficient informations on the chymotryptic peptides to construct a primary structure of fibroin molecule.

Almost all previous works on the chemical structure of fibroin were carried out with raw silk. It has, however, a disadvantage that the procedures of desericinization cause varying degree of molecular degradation, consequently, it gives additional troubles to perform structural analysis of fibroin. In a previous paper (Shimura *et al.*, 1976), we have presented the method to prepare fibroin from the posterior silk glands of *Bombyx mori* and purify the large and small subunits of fibroin. This paper describes the preparation, purification and characterization of the Cp of large subunit of fibroin. The data obtain-

This is the second paper in a series of "Studies on silk fibroin of *Bombyx mori*". The previous paper is Ref. Shimura *et al.* (1976).

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Abbreviations: DFP, diisopropylfulorophosphate; TLCK, N-tosyl-L-lysine chloromethylketone; TCA, trichloroacetic acid.

ed here show a heterogeneity of the Cp, mainly depending on the differences of minor amino acid contents, particularly of acidic amino acid ones.

Materials and Methods

Materials : α-Chymotrypsin (treated with TLCK) was obtained from Worthington Biochemical Corp., Freehold, New Jersey. Carboxypeptidase A (treated with DFP) was purchased from Sigma Chemical Co., St. Louis, Mo. All other reagents were of analytical grade.

Preparation of Silk Fibroin from the Posterior Silk Gland : The posterior silk glands of silkworms, *Bombyx mori*, on the fifth day of the fifth instar were excised, washed briefly in ice-cold 1.15 % KCl, immersed immediately in ice-cold 30 % ethanol, and stood overnight at 4°C. The coagulated fibroin was separated with a small forceps from the gland tissue which surrounded the coagulated fibroin. The fibroin obtained was washed several times with deionized water, and then with 50 % ethanol, absolute alcohol, and ether.

Purification of Silk Fibroin : The silk fibroin was dissolved in 10-15 volumes (v/w) of 60 % LiSCN (pH 7.0) and carboxymethylated as described in a previous report (Shimura *et al.*, 1976). The solution of carboxymethylated fibroin (CM-fibroin, about 15 mg/ml) in 5 M urea-20 mM Tris-HCl, pH 8.0 (buffer A), was made to 19 % saturation with powdered ammonium sulfate. After standing overnight at room temperature, the resulting precipitate was collected by centrifugation at 17500×*g* for 40 min at 4°C. To the supernatant ammonium sulfate was further added to obtain precipitable fractions at 30 % and 50 % saturation, respectively. Each fraction obtained was dissolved in 60 % LiSCN and applied to a Sephadex G-200 column (4.7×44 cm) previously equilibrated with buffer A, and eluted with the same buffer. Fractions from the Sephadex G-200 column were further purified on a DEAE-cellulose column previously equilibrated with 7 M urea-20 mM Tris-HCl, pH 8.5 (buffer B). Gradient elution was done with 0-0.2 M NaCl in buffer B. The 8 M urea solution used for preparing buffers A and B had been deionized by passing through a mixed bed ion exchange column.

Preparations of Cp : The purified fibroin large subunit was digested with α-chymotrypsin in the presence of 2 M urea-20 mM Tris-HCl, pH 8.0 (buffer C), for 6 h at 37°C (E/S=1/100). The hydrolysis was stopped by heating the mixture in a water bath at 90°C for 10 min. The resulting precipitate (Cp) was washed with 50% ice-cold ethanol, absolute ethanol, and ethanol-ether. For further purification, the Cp was dissolved in 1-2 ml of 60 % LiSCN and dialyzed against buffer A using a Spectrapor membrane (Por 6, molecular weight cutoff 1000). The dialyzed sample was chromatographed on a column (1.8×86 cm) of Sephadex G-50 equilibrated with buffer A. The column was run at a flow rate of 17 ml/h. The fraction from the Sephadex G-50 column was applied to a DEAE-Sephadex A-50 column (1.4×150 cm) and eluted with a linear gradient of 0-0.1 M NaCl in buffer A. Protein was determined by the method of Lowry *et al.* (1951) with serum albumin as a standard.

C-Terminal Analysis : Cp was dissolved in 60% LiSCN and dialyzed against buffer A and then against buffer C, using a Spectrapor membrane. The Cp dissolved in buffer C was digested with carboxypeptidase A (55 : 1～72 : 1, molar ratio of Cp to enzyme) at 25°C. Timed aliquots were removed from the digest and the reaction was stopped by addition of 0.02 N HCl (pH 2.2). Released amino acids were analyzed by a Hitachi 835 amino acid analyzer.

N-Terminal Analysis : Cp fraction employed for analysis of N-terminal residue was purified under the same condition as used for C-terminal analysis except that 9 M LiBr instead of 60 % LiSCN and 30 % formamide-20 mM Tris-HCl, pH

8.0, instead of buffers A and C, were used. The Cp fraction obtained was dissolved in 1 ml of 9 M LiBr, dialyzed against 6 M guanidine-HCl overnight and then against dimethylallylamine (DMAA) buffer overnight, which contained 15 ml of pyridine, 10 ml of water, and 1.18 ml of DMAA, and was adjusted to pH 9.5 with 10 % trifluoroacetic acid. Phenylthiocyanation of Cp was performed essentially according to the procedure described by Iwanaga *et al.* (1969). The phenylthiohydantoin (PTH)-Cp formed was hydrolyzed with 6 N HCl containing 0.1 % $SnCl_2$ for 4 h at 150°C (Mendez and Lai, 1975). The released amino acids were analyzed by the amino acid analyzer.

Amino Acid Composition : Amino acid composition of the peptide was determined by the amino acid analyzer after hydrolysis with 6 N HCl at 110°C for 24 h in evacuated tubes.

Gel Electrophoresis : Polyacrylamide tube gels (7.5 %) were run in the presence of 0.1 % SDS according to the method of Weber and Osborn (1969). Gels were stained with Coomassie Brilliant Blue R-250.

Results

Ammonium Sulfate Fractionation of CM-fibroin : CM-fibroin was fractionated into three protein fractions by ammonium sulfate precipitation (19, 30, and 50 % saturation) as described in "Materials and Methods". They were designated fractions I, II, and III, respectively, in order of precipitation. In a typical experiment, fractions I, II, and III at this step occupied 16, 82, and 2 % of a sum of each fraction. The recovery of total protein was about 82 %.

Gel Filtration of Ammonium Sulfate Fractions : Each of above fractions was applied to a Sephadex G-200 column equilibrated with buffer A. The elution profiles are shown in Fig. 1. Fraction I consisted of large and small molecular size proteins, whereas fraction II contained mainly large molecular size proteins. The fractions

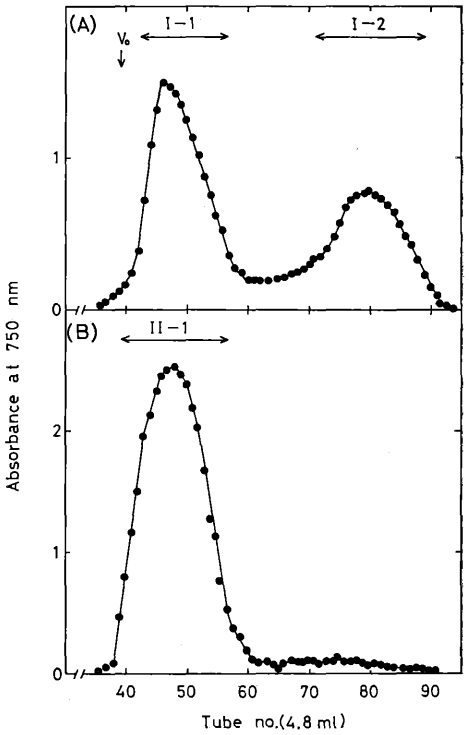

Fig. 1. Elution pattern of ammonium sulfate fractions of CM-fibroin on Sephadex G-200.
Samples were applied to a column (4.7×44 cm) and eluted with 5 M urea-20 mM Tris-HCl, pH 8.0 (buffer A). (A) Fraction obtained at 19 % ammonium sulfate saturation. (B) Fraction obtained at 19-30 % ammonium sulfate saturation. Each fraction marked by a horizontal arrow was pooled as designated in the figures. The flow rate was 33 ml/h.

marked by horizontal arrows in Figs. 1 (A) and (B) were pooled, dialyzed against water and lyophilized. As only a little amount of fraction III was obtained, no further fractionation procedure was applied to this fraction. Polyacrylamide gel electrophoretic analysis of fraction III (Fig. 3-d) showed that this fraction comprised proteins with quite heterogeneous molecular sizes, ranging from about 25000 to nearly 200000, just corresponding to the fraction III reported in the previous paper (Shimura *et al.*, 1976).

DEAE-Cellulose Chromatography : Each fraction obtained in Fig. 1, fractions I-1, I-2, and II-1, was applied to a DEAE-cellulose column

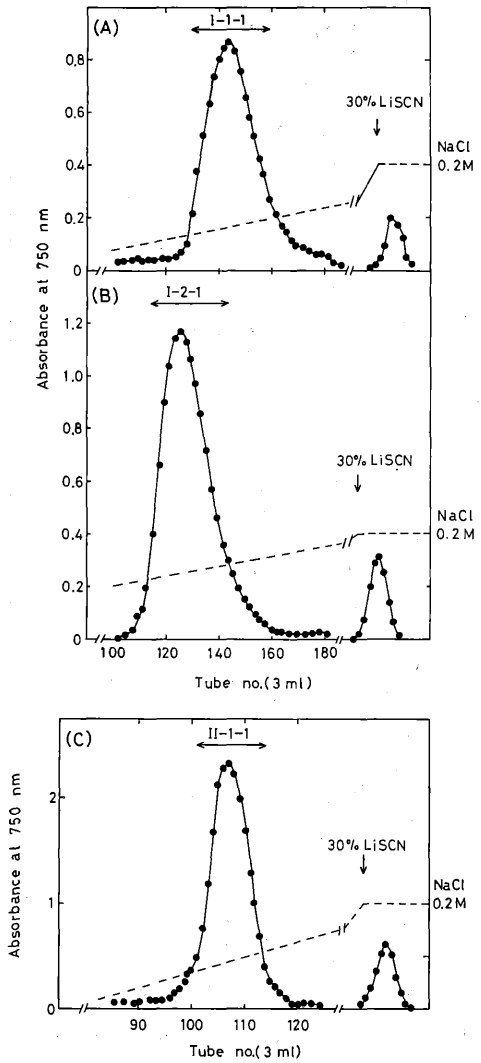

Fig. 2. DEAE-Cellulose chromatography of the Sephadex G-200 fractions. Fractions from the Sephadex G-200 column were applied to a DEAE-cellulose column and eluted with a linear gradient of 0-0.2 M NaCl in 7 M urea-20 mM Tris-HCl, pH 8.5 (buffer B). (A) fraction I-1; (B) fraction I-2; (C) fraction II-1. Each fraction marked by a horizontal arrow was pooled as designated in the figures.

and eluted with buffer B. The chromatographic elution profiles are shown in Figs. 2-(A), -(B), and -(C). Fraction II-1, a main fraction of fibroin, was eluted at about 0.08 M NaCl. The srtrongly adsorbed component (less than 2% of

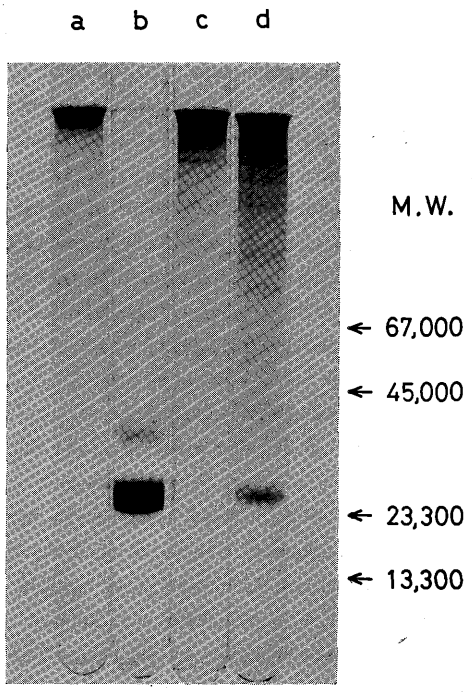

Fig. 3. SDS gel electrophoresis of DEAE-cellulose fractions. About 30 μg of each sample obtained from a DEAE-cellulose column was placed on 7.5% polyacrylamide gel. a) fraction I-1-1; b) fraction I-2-1; c) fraction II-1-1; d) fraction III. The molecular weights indicated in the figure were estimated from the migration distance of standard proteins, *i.e.* cytochrome c (13300), chymotrypsinogen (23300), egg albumin (45000), and bovine serum albumin (67000). The direction of migration was from top to bottom.

the total protein recovered) was eluted with 30% LiSCN (Fig. 2-(C)). Fraction I-1 gave a similar elution pattern to that of fraction II-1 (Figs. 2-(A) and -(C)). Elution pattern of fraction I-2, a small subunit fraction, gave one major peak and a small peak of strongly adsorbed proteins (Fig. 2-(B)). The former fraction comprised about 7.5% of the silk fibroin and exhibited a molecular weight of about 25000 on SDS-polyacrylamide electrophoresis as shown in Fig. 3. This value agreed with those of Tokutake (1980) and Shimura *et al.* (1982).

Preparation of Cp from "Large Subunit": The fraction II-1-1' was digested with α-chymotryp-

Table 1. Ratio of Cp to Cs in the large subunit of fibroin*

Exp. 1	Exp. 2	Average M. W. of amino acid residues	% (in amino acid residue)
Cp 47.6 mg (51.8%)	49.0 mg (52.3%)	69	55
Cs 44.6 mg (48.2%)	44.6 mg (47.7%)	78	45

* fraction II-1-1

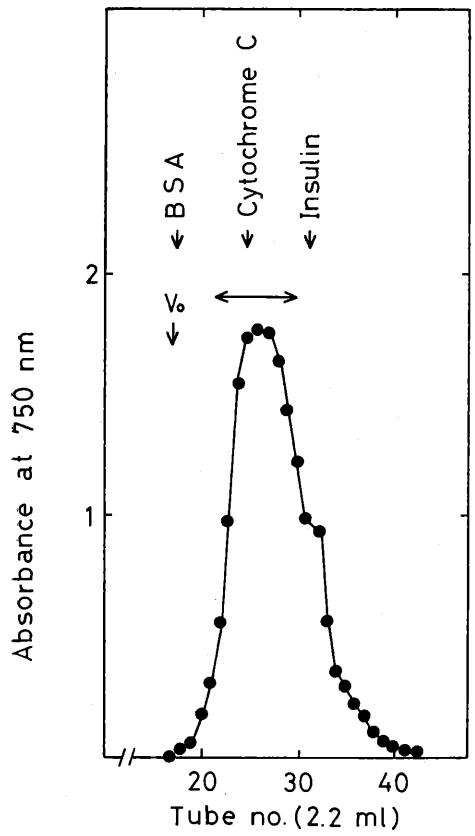

Fig. 4. Gel filtration of Cp on a Sephadex G-50 column.
The sample was dissolved in 1 ml of 60% LiSCN and dialyzed against buffer B. The dialyzed sample was applied to a Sephadex G-50 column (1.8×86 cm) and eluted with 2 M urea-20 mM Tris-HCl, pH 8.0 (buffer C). Void volume was determined using dextran blue 2000 (Pharmacia Fine Chemicals, Inc.).

sin in the presence of buffer C as described in "Materials and Methods". The resulting precipitate was collected by centrifugation at 450×*g* for 10 min. The precipitate (Cp) and the supernatant (Cs) were analyzed by the amino acid analyzer to determine the ratio of Cp to Cs. As shown in Table 1, large subunit was composed of 55% Cp and 45% Cs in terms of amino acid residue.

Purification of Cp : The Cp obtained above was dissolved in 1-2 ml of 60% LiSCN, dialyzed against buffer C and applied to a Sephadex G-50 column. As shown in Fig. 4, the Cp was eluted with a profile having a slight shoulder, and the fractions marked by a horizontal arrow were collected and combined. This fraction was further applied to a DEAE-Sephadex A-50 column equilibrated with buffer A. As shown in Fig. 5-(A), the fraction was separated into five fractions and each fraction was designated fractions Cp I～V. The fractions Cp II and III were rechromatographed on DEAE-Sephadex A-50, respectively, under the same column condition as used in Fig. 5-(A). Their elution profiles are shown in Figs. 5-(B) and -(C). The Cp (II-2, III-1, see in Figs. 5-(B), (C)) were eluted slightly slower than Cp (II-1), suggesting the existence of a slight difference in electrostatic charge between them. The sum of proteins in fractions Cp II-1, -2, and III-1 accounted for about 88% of the fraction Cp (see in Table 2).

C-Terminal Amino Acid Determination of Cp : Digestion of fractions Cp II-1, -2, and III-1 with carboxypeptidase A released tyrosine and

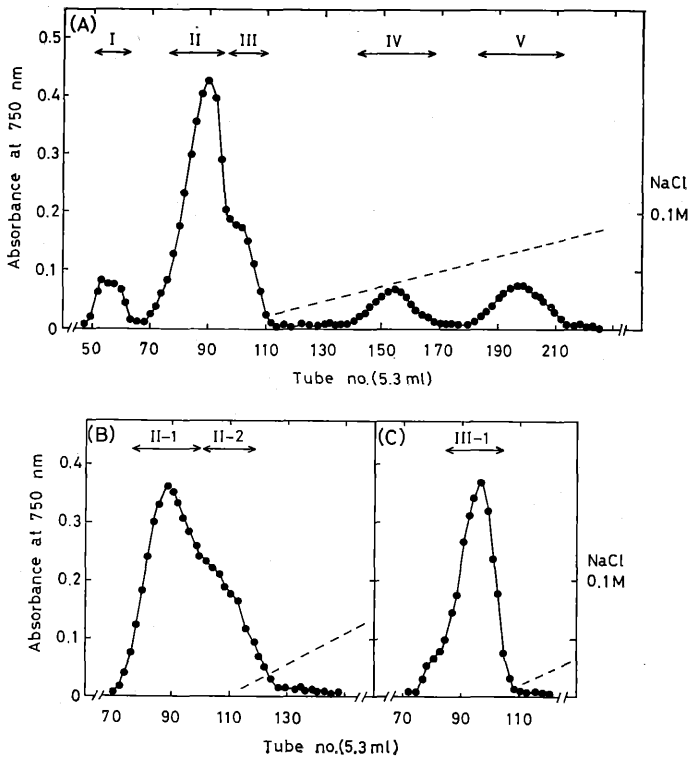

Fig. 5. DEAE-Sephadex A-50 chromatography of Cp fractions from Fig. 4. A) the first chromatography,(B) and(C) rechromatography of fractions Cp II and III. Each fraction marked by a horizontal arrow was pooled to give fractions Cp I, II-1, II-2, III-1, IV, and V, as designated in the figures.

Table 2. Estimation of molecular weight of Cp by C-terminal analysis with carboxypeptidase A

	DEAE-Sephadex A-50 fraction		
	II-1	II-2	III-1
Cp used (μg)	147.2	96.6	111.8
Amino acid released (nmole)			
Tyrosine	36.7	21.7	21.2
Phenylalanine	1.9	2.4	6.5
M. W. calculated	3800	4000	4000

Table 3. Estimation of molecular weight of Cp by PTH-method

Exp.	Cp* used (μg)	Amino acid released Gly	Ala (nmole)	M. W. calculated
1	267	50.9	8.2	4500
2	106	25.8	1.5	3900
3	228	49.7	4.8	4200

* Cp was prepared by DEAE-Sephadex A-50 chromatography specifically for N-terminal analysis as described in "Materials and Methods".

phenlyalanine ; no other amino acids were liberated. Tyrosine was released rapidly during 5 min, whereas only a trace amount of phenylalanine was released throughout the reaction time. The ratios of tyrosine/phenylalanine at 60 min for the three fractions were about 10 : 1, 15 : 1, and 18 : 1, respectively. From the amounts of tyrosine and phenylalanine released, molecular weight of fractions Cp II-1, -2, and III-1 were estimated to be 3800, 4000, and 4000, respectively, as shown in Table 2 (three determinations each).

N-Terminal Amino Acid Determination of Cp : For the assay of N-terminal amino acids of Cp, purification of Cp on DEAE-Sephadex A-50 chromatography was carried out by using a 30 mM formamide-20 mM Tris-HCl buffer (pH 8.0) instead of buffer A in Fig. 5-(A) to avoid artificial blocking of N-terminal amino group of Cp by cyanate derived from urea in buffer A. The elution profile of Cp from a DEAE-Sephadex A-50 column was almost the same as that shown in Figs. 5-(A) and -(B) except that the peaks corresponding to fractions Cp II-2 and III were relatively low (data not shown). The fraction corresponding to Cp II-1 was used for N-terminal analysis. N-terminal amino acids detected were glycine and a small amount of alanine. From the amounts of glycine and alanine released, the mean molecular weight of Cp was estimated to be about 4200 in three experiments as shown in Table 3.

Amino Acid Composition of Cp : Amino acid compositions of the Cp fractions are shown in Table 4. Fractions II-1, -2, and III-1—the main components of Cp—were essentially the same in glycine, alanine, and serine contents, but appreciably different in phenylalanine, threonine, and acidic amino acids. The other fractions (I, IV, and V) contained less glycine and alanine, and more serine, phenylalanine, threonine, aspartic acid and glutamic acid, comparing with the main fractions (II-1, -2, and III-1). The molecular weights of Cp II-1, -2, and III-1 were calculated to be about 3900, 4000, and 4300 respectively, from the sum of tyrosine and phenylalanine contents. These results indicate a heterogeneity of the crystalline region in fibroin with respect to its amino acid composition.

Discussion

In our previous paper (Shimura *et al.*, 1976), it was reported that the silk fibroin prepared from the posterior silk glands of *Bombyx mori* with cold 5 % TCA treatment consists of at least two protein groups of large-molecular size, three or four small-molecular size proteins and, in addition, a group of proteins with molecular sizes ranging from 25000 to 100000. As there was the possibility that the last group of proteins might be some degradation products of fibroin with cold 5 % TCA, we treated the posterior silk glands with cold 30 % ethanol instead of 5 % TCA in the present work. It was revealed that the 30 % ethanol treatment reduced markedly

Table 4. Amino acid composition[a] of fractions Cp I to V obtained from DEAE-Sephadex A-50 chromatography of Cp

	Fibroin[b] large subunit	DEAE-Sephadex A-50 fraction					
		I	II-1	II-2	III-1	IV	V
Gly	47.7	45.7	46.8	46.4	47.3	41.0	39.7
Ala	32.0	33.1	35.0	35.3	34.6	29.4	25.8
Ser[c]	10.0	16.0	15.3	15.4	15.3	17.3	19.2
Tyr[d]	4.35	0.59	1.72	1.71	1.57	1.81	2.05
Val	1.83	0.82	0.39	0.31	0.57	1.04	0.64
Phe	0.46	1.17	0.19	0.31	0.48	1.46	2.77
Thr[e]	0.70	3.33	0.25	0.40	0.55	1.61	2.28
Asp	0.92	0.61	0.10	0.14	0.15	1.99	2.90
Glu	0.77	0.88	0.12	0.14	0	1.91	3.21
Ile	0.27	0.53	0.06	0	0	0	0
Leu	0.15	0.30	0	0	0	0	0
Pro	0.19	0	0	0	0	0	0
Met	0.04	0	0	0	0	0	0
CM-Cys	0.05	0	0	0	0	0	0
Lys	0.14	0	0	0	0	0	0
His	0.09	0	0	0	0	0	0
Arg	0.21	0	0	0	0	0	0
%[f]		1.7	47.9	25.8	13.9	4.0	6.7
molecular weight calculated from amino acid composition			3920	3990	4310		

a) Data are expressed as mol percent. b) Fraction II-1-1. c) The 24-, 48-, and 72-h values for serine have been extrapolated to zero time to correct for decomposition during hydrolysis except fibroin (I-1-1). d) and e) No corrections were made for losses during acid hydrolysis. f) A sum of the Cp fractions recovered from DEAE-Sephadex A-50 was made as 100.

the amount of the protein group in question, which corresponds to the fraction III in the present work. Therefore, it is likely that silk fibroin contains a linkage (s) fairly unstable in an acidic condition, even in cold 5% TCA, as already suggested by Earland and Robins (1973). Thus, the 30% ethanol treatment is preferred as an improved method of the preparation of fibroin from the posterior silk glands.

As shown in Table 1, the ratio of Cp to Cs was 55 : 45 in terms of amino acid residue. This ratio is slightly lower than that reported by Lucas *et al.* (1957), who presumed that Cp comprises about 58% of the total weight of fibroin (61% in terms of amino acid residue) in the case of the fibroin prepared from raw silk. This may be partly attributable to the difference of the buffers used in the formation of Cp, that is, a buffer containing 2 M urea was used in the present experiment in place of about 0.03 M phosphate buffer used by Lucas *et al.* (1957). As will be reported in the succeeding paper, Cp-like proteins, not identical with Cp in respect to their molecular size, were found in the soluble

fraction in our experiment. These Cp-like proteins, seem to go into either Cp or Cs fraction depending on the buffer conditions at the formation of Cp.

It was unexpected results that the Cp was separated into five fractions by DEAE-Sephadex A-50 chromatography (Fig. 5-(A)). The amino acid analysis of these fractions (Table 4) revealed that the major component, fraction Cp II-1, and its shoulder component, fractions Cp II-2 and III-1, were almost the same, although they showed slight differences in the content of minor amino acids such as phenylalanine, valine, and threonine. Fractions Cp IV and V can be distinguished from fractions Cp II-1, -2, and III-1 by lower content in glycine and alanine, and higher content in phenylalanine, threonine, aspartic acid, and glutamic acid. Fraction Cp I—a minor component—seems to possess an intermediate character between the two groups mentioned above except the lowest content in tyrosine mentioamong the Cp peptides obtained.

The molecular weight of Cp fractions (II-1, -2, and III-1) was estimated to be about 3900～4100 from C-terminal and N-terminal analyses (Tables 2 and 3). From the gel filtration profile on Sephadex G-50 (Fig. 4), however, the average molecular weight of Cp was estimated to be about 12000 using bovine serum albumin, cytochrome c, and insulin as markers. Electrophoretic studies on a SDS-polyacrylamide gel gave also a molecular weight of about 11000 for Cp (data not shown). These values are greatly different from that estimated by terminal analyses. A possible explanation for this disagreement may be that Cp possesses a tendency to associate into dimer or trimer even in the presence of strong dissociating agents such as 5 M urea, 6 M guanidine-HCl, 10 M LiBr and SDS. In brief, we have considered that the value of molecular weight of Cp from terminal analyses was more reliable than that of SDS-polyacrylamide gel electrophoresis and gel filtration.

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References

DRUCKER, B., and SMITH, S.G. (1950) : Nature, **165**, 196-197.

DRUCKER, B., HAINSWORTH, R., and SMITH, S. G. (1953) : Shirley Inst. Mem., **26**, 191-206.

EARLAND, C., and ROBINS, S.P. (1973) : Int. J. Peptide Protein Res., **5**, 327-335.

GEDDES, A.J., GRAHAM, G.N., MORRIS, H.R., LUCAS, F., BARBER, M., and WOLSTENHOLME, W.A. (1969) : Biochem. J., **114**, 695-702.

IWANAGA, S., WALLEN, P., GRÖNDAHL, N.J., HENSCHEN, A., and BLOMBÄCK, B. (1969) : Eur. J. Biochem., **8**, 189-199.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L., and RANDALL, R.J. (1951) : J. Biol. Chem., **193**, 265-275.

LUCAS, F., SHAW, J.T.B., and SMITH, S.G. (1957) : Biochem. J., **66**, 468-479.

LUCAS, F., SHAW, J.T.B., and SMITH, S. G. (1962) : Biochem. J., **83**, 164-171.

MENDEZ, E., and LAI, C.Y. (1975) : Anal. Biochem., **68**, 47-53.

NARITA, K. (1954 a) : J. Chem. Soc., (in Japanese) **75**, 487-494.

NARITA, K. (1954 b) : J. Chem. Soc., (in Japanese) **75**, 1005-1016.

ROBSON, A., WOODHOUSE, J.M., and ZAIDI, Z.H. (1970) : Int. J. Protein Res., **II**, 181-189.

SHAW, J.T.B. (1964 a) : Biochem. J., **93**, 45-54.

SHAW, J.T.B. (1964 b) : Biochem. J., **93**, 54-61.

SHIMURA, K., KIKUCHI, A., OHTOMO, K. KATAGATA, Y., and HYODO, A. (1976) : J. Biochem., **80**, 693-702.

SHIMURA, K., KIKUCHI, A., KATAGATA, Y., and OHOTOMO, K. (1982) : J. Seric. Sci. J., **51**, 20-26.

STRYDOM, D.J., HAYLETT, T., and STEAD, R.H. (1977):Biochem. Biophys. Res. Commun., **79**, 932-938.

TOKUTAKE, S. (1980) : Biochem. J., **187**, 413-417.

WALDSCHMIDT-LEITZ, E., and KIRCHMEIER, O. (1958) : Z. Physiol. Chem., **312**, 127-139.

WEBER, K., and OSBORN, M. (1969) : J. Biol. Chem.,

244, 4406-4412.

ZIEGLER, K., and SPOOR, H. (1959) : Biochim. Biophys. Acta, **33**, 138-142.

ZIEGLER, K., and LA FRANCE, N. H. (1960) : Z. Physiol. Chem., **322**, 21-27.

片方陽太郎・菊地愛子・志村憲助：後部絹糸腺フィブロインより調製した結晶部ペプチドの性質について

5令期5日目の家蚕より後部絹糸腺を摘出し冷30%エタノール処理後，組織蛋白を除去しフィブロインを得た。このフィブロインに対しカルボキシメチル化を行い，フィブロイン large subunit を調製した。これをα-キモトリプシン (1/100=E/S, 37°C, 6^{hrs}) 分解し，沈殿 (結晶部ペプチド，Cp) と上清 (非結晶部ペプチド，Cs) を得た。両者の割合はアミノ酸残基数にして約55：45であった。Cp を Sephadex G-50 および DEAE-Sephadex A-50 を用いて精製した結果，分子サイズはかなり限られた範囲内にあるが，主成分の他に3画分に分離された。アミノ酸分析の結果から，これらの画分はいずれも4種の主要アミノ酸 (Gly, Ala, Ser, Tyr) からなることは共通であるが，その他に少量の Val, Phe, Thr, Asp, Glu 等を含み，これらのアミノ酸の含量に各画分間で差異の存在することが明らかになった。また Cp の分子量は N- および C- 末端分析ならびにアミノ酸組成から約3900～4100と推定された。