On the Structures of Fibrous Proteins. II. New Polypeptide Structures of β- and Feather-Keratins, and Three Silk-Fibroins of *Bombyx mori, Saturnia pyri* and *Antheraea mylitta*

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(Received October 31, 1949)

I. Introduction.—In Part I of this series[1] the author presented a new structure for α-keratin by assuming reasonable values for bond lengths, bond angles and bond directions. In this paper we shall proceed to present new structures for the polypeptide chains in β-keratin, feather-keratin and three silk-fibroins of *Bombyx mori, Saturnia pyri* and *Antheraea mylitta*.

II. Polypeptide Structure of β-Keratin.—It was found by Astbury[2] that X-ray diffraction patterns of ordinary keratin is different from that of fully stretched one (elongated to about 29%). The stretched keratin is called β-keratin. It has a rectangular cell, the unit cell dimensions being: a=9.3 Å., b=6.64 Å., c=9.8 Å. Astbury called 6.64 Å. the identity distance, 9.8 Å. the side chain spacing, and 4.65 Å. (one half of 9.3 Å.) the backbone spacing.

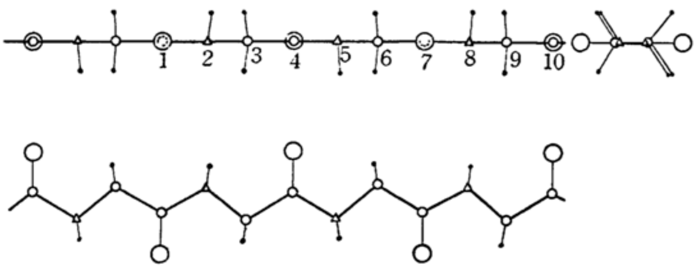

Fig. 1. —Polypeptide structure of polyglycine.

It is generally considered that in the polypeptide structure of β-keratin every bond in the chain lies in the *trans*-direction with respect to the next but one bond on either side.[3] It appears that this view has been accepted generally without any question, whilst the structure of α-keratin has very much been discussed about by many investigators. According to the X-ray diffraction results of glycine crystal by Albrecht and Corey,[4] the form of glycine molecule in the crystal is almost planar. From this fact we suggest a structure shown in Fig. 1 for the polyglycine chain formed of only glycine residues. Bond direction types used in this structure are given in Table 1, in which only the series $T_1^1—T_2—T_3^1$ repeats. All of these are *trans*. The calculated identity distance is 7.47 Å., somewhat larger than 6.64 Å. observed for β-keratin. On the other hand the X-ray diffraction experiment of DL-alanine crystal by Levy and Corey[5] shows that the alanine molecule is not planar. Leucine, one of the major constituents of keratin, has a radical $(CH_3)_2>CH-CH_2-$. 57 times heavier than a hydrogen atom. If we imagine the interactions among neighboring chains in β-keratin fibres, it seems very unlikely that all bonds in a chain strictly keep the *trans* orientations as stated above.

Table 1

← C (1)—— N (2) →	T_3^1
← N (2)—— C_α(3) →	T_1^1
← C_α(3)—— C (4) →	T_2
← C (4)—— N (5) →	T_3^1
← N (5)—— C_α(6) →	T_1^1
← C_α(6)—— C (7) →	T_2
← C (7)—— N (8) →	T_3^1
← N (8)—— C_α(9) →	T_1^1
← C_α(3)—— C (10) →	T_2

Now the polypeptide structure of β-keratin should be obtained from that of α-keratin. If we stretched our α-keratin chain (Fig. 4 of Part I) we would have the structure shown in Fig. 2. Bond types appearing in this structure are given in Table 2, in which the series

(1) Y. Kanda: *Bull. Chem. Soc. Japan*, **23**, 137 (1950).

(2) W. T. Astbury and A. Street, *Phil. Trans. Roy. Soc.*, **A 230**, 75 (1931); W. T. Astbury and H. T. Wood, *ibid.*, **A 232**, 333 (1933).

(3) K. H. Meyer and H. Mark, "Hochpolymere Chemie, II," 1940.

(4) G. Albrecht and R. B. Corey, *J. Am. Chem. Soc.*, **61**, 1087 (1939).

(5) H. A. Levy and R. B. Corey, *ibid.*, **63**, 2095 (1941).

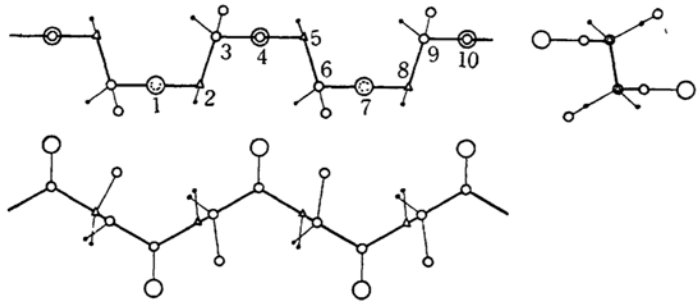

Fig. 2.—Polypeptide structure of feather-keratin.

Table 2

← $C^{(1)}$	— $N^{(2)}$	→ G_3^3
← $N^{(2)}$	— $C_\alpha^{(3)}$	→ T_1^1
← $C_\alpha^{(3)}$	— $C^{(4)}$	→ G_2^3
← $C^{(4)}$	— $N^{(5)}$	→ G_3^3
← $N^{(5)}$	— $C_\alpha^{(6)}$	→ T_1^1
← $C_\alpha^{(6)}$	— $C^{(7)}$	→ G_2^3
← $C^{(7)}$	— $N^{(8)}$	→ G_3^3
← $N^{(8)}$	— $C_\alpha^{(9)}$	→ T_1^1
← $C_\alpha^{(9)}$	— $C^{(10)}$	→ G_2^3

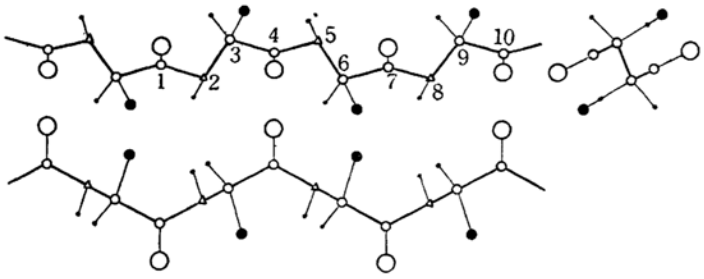

Fig. 3.—Polypeptide structure of β-keratin.

Table 3

← $C^{(1)}$	— $N^{(2)}$	→ G_3^5
← $N^{(2)}$	— $C_\alpha^{(3)}$	→ T_1^1
← $C_\alpha^{(3)}$	— $C^{(4)}$	→ G_2^5
← $C^{(4)}$	— $N^{(5)}$	→ G_3^5
← $N^{(5)}$	— $C_\alpha^{(6)}$	→ T_1^1
← $C_\alpha^{(5)}$	— $C^{(7)}$	→ G_2^5
← $C^{(7)}$	— $N^{(8)}$	→ G_3^5
← $N^{(8)}$	— $C_\alpha^{(9)}$	→ T_1^1
← $C_\alpha^{(9)}$	— $C^{(10)}$	→ G_2^5

T_1^1—G_2^3—G_3^3 repeats. The calculated identity distance is 6.06 Å., a little smaller than Astbury's 6,64 Å. for β-keratin. By stretching the chain still more we have the structure shown in Fig. 3 (bond direction types, Table 3), in which T_1^1—G_2^5—G_3^5 repeats. In this case the calculated identity distance becomes 6,67 Å., agreeing quite well with the observed value. We would therefore offer the structure of Fig. 3 as that of β-keratin.

III. Polypeptide Structure of Feather-Keratin.—Feather-keratin must have a similar structure to that of mammalian hair or wool keratin, but in the case of quill keratin the length of an amino acid residue was found to be 3.08 Å., different from 3.33 Å. for β-keratin.[6] The residue length of amino acid in feather-keratin (forol) appears to correspond to 3.07 Å. found by Corey and Wyckoff,[7] and twice this distance 6.14 Å. could be taken as the identity distance in this substance to which the structure of Fig. 2 corresponds quite well. The fact that feather-keratin can also be stretched into β-keratin as α-keratin can be understood if we consider the transition from Fig. 2 to Fig. 3.

IV. Polypeptide Structure of Silk-Fibroin of *Bombyx mori*.—Silk-fibroins, as well as α-amino acids constituting them, have extensively been investigated. Important constituents of silk-fibroins are glycine and alanine. Beside these there are tyrosine, arginine, serine, etc. A so-called periodic theory was offered to explain the structures of these substance by Bergmann and Niemann.[8] Meyer and Mark,[9] however, found that there are crystalline and non-crystalline parts in silk-fibroin and that the unit cell of the crystal is too small to contain a tyrosine residue, disagreeing with the theory of Bergmann and Niemann. Arginine and other amino acids are contained more often in the non-crystalline than in the crystalline parts. Meyer and Mark considered the chain structure of silk-fibroins in general as consisting of glycine and alanine (or serine etc.) residues alternating. The identity distance of silk-fibroin of *Bombyx mori* was found to be 6.95 ± 0.25 Å. by Kratky and Kuriyama.[10] Heertjes' reinvestigation[11] confirmed their results, and the unit cell dimensions were determined as a = 10.45 Å., b = 4.95 Å.,

(6) G. L. Clark, "Applied X-rays," p. 651 (1940).
(7) R. B. Corey and R. W. Wyckoff, *J. Biol. Chem.*, **114**, 407 (1936).
(8) M. Bergmann and C. Niemann, *J. Biol. Chem.*, **122**, 577 (1938).
(9) K. H. Meyer and H. Mark, *Ber.*, **61**, 1932 (1938).
(10) O. Kratky and S. Kuriyama, *Z. phys. Chem.*, **B 11**, 363 (1931).
(11) P. M. Heertjes, *Rec. Trav. Chim.*, **60**, 329 (1941).

c = 6.95 Å., $\hat{\gamma} = 61°10'$; its density $\rho = 1.341$ from the macroscopic density measurement.

The identity distance 6.95 Å. may be interpreted to correspond to the distance between two glycine residues. It is considered that in the polypeptide chain composed of only glycine residues (polyglycine chain) all chain-forming bonds stand mutually in *trans* relations as shown in Fig. 1 and Table 1. But in the residue of amino acid other than glycine, bond residue (1-2-3-4) of Fig. 1 and the amino acid residue (4-5-6-7) of Fig. 2 alternate, having bond series $T_1^1—T_2—G_3^3—T_1^1—G_2^3—T_3^1$ (Table 5). But as this arrangement is seen to be identical with the one given above, we need not treat it separately. The calculated identity distance for this structure is 7.02 Å., very near to the observed 6.95 Å.

V. Polypeptide Structure of Silk-Fibroin of *Saturnia pyri*.—Kratky and Kuriyama[10]

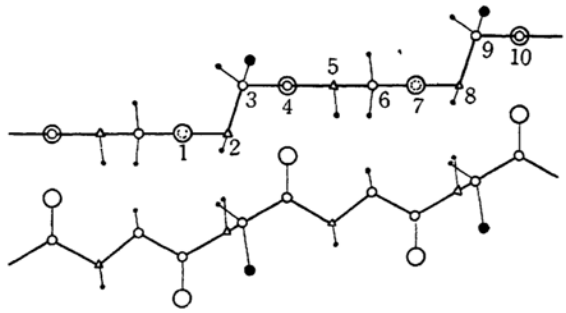

Fig. 4.—Polypeptide structure of silk-fibroin of *Bombyx mori*.

Table 4

← C (1)—N (2) →	G_3^3
← N (2)—C_α (3) →	T_1^1
← C_α (3)—C (4) →	G_2^3
← C (4)—N (5) →	T_3^1
← N (5)—C_α (6) →	T_1^1
← C_α (6)—C (7) →	T_2
← C (7)—N (8) →	G_3^3
← N (8)—C_α (9) →	T_1^1
← C_α (9)—C (10) →	G_2^3

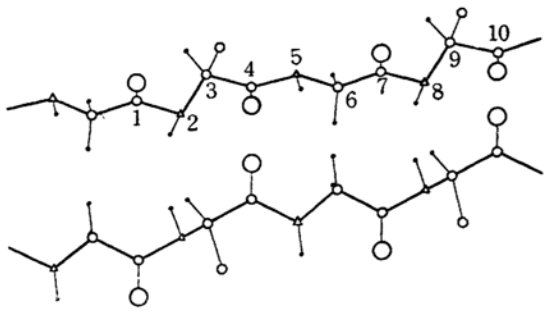

Fig. 5.—Polypeptide structure of silk-fibroin of *Saturnia pyri*.

Table 5

← C (1)—N (2) →	T_3^1
← N (2)—C_α (3) →	T_1^1
← C_α (3)—C (4) →	T_2
← C (4)—N (5) →	G_3^3
← N (5)—C_α (6) →	T_1^1
← C_α (6)—C (7) →	G_2^3
← C (7)—N (8) →	T_3^1
← N (8)—C_α (9) →	T_1^1
← C_α (9)—C (10) →	T_2

direction types are not all T but some are G. As the residue lengths of α-amino acids other than glycine are all smaller than that of glycine, it is not correct to take the identity distance for silk-fibroin simply as twice the length 3.5 Å. as we find in many literatures. The identity distance in β-keratin is smaller than that of silk-fibroin because it does not contain glycine residues. The polypeptide structure of silk-fibroin of *Bombyx mori* is shown in Fig. 4. In this structure we see the amino acid residue (1-2-3-4) which was used in the structure of feather-keratin of Fig. 2 and the glycine residue (4-5-6-7) used in the polyglycine structure of Fig. 1 are joined alternately. Bond direction types used in this structure are given in Table 4, in which the series $T_1^1—G_2^3—T_3^1—T_1^1—T_2—G_3^3$ repeats. For this fibroin we may construct the chain in another way, in which the glycine determined the unit cell dimensions of the fibroin "Satonia" as a = 6.46 Å., b = 15.43 Å., c = 7.2 Å. and $\hat{\gamma} = 61°10'$. According to Simizu[12] the wild silkworm "Satonia" studied by the above authors might have been *Saturnia pyri*. The structure of this silk-fibroin can be given as shown in Fig. 5, in which we see the amino acid residue (1-2-3-4) used in the β-keratin chain of Fig. 3 and polyglycine chain (4-5-6-7) of Fig. 1 are joined alternately. In this case, however, the plane of the glycine residue is slightly tilted. Bond series $T_1^1—G_2^5—T_3^1—T_1^1—T_2—G_3^5$ repeats in this structure, as given in Table 6. The identity dis-

(12) "The Fibre Structures by X-rays," (in Japanese), p. 99. Kōgaku-Syuppansya, Osaka (1944).

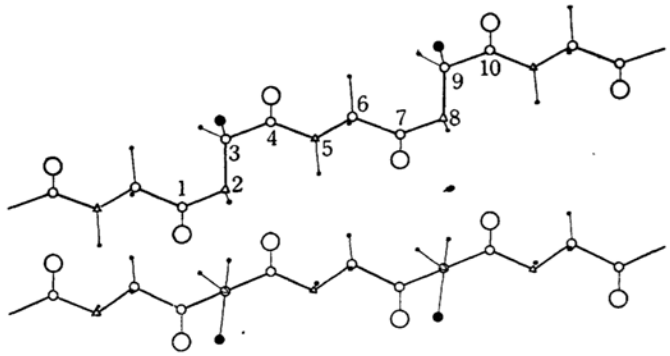

Fig. 6.—Polypeptide structure of silk-fibroin of *Antheraea mylitta*.

Table 6

← C (1)—N (2) →	G_3^5
← N (2)—C_α (3) →	T_1^1
← C_α (3)—C (4) →	G_2^5
← C (4)—N (5) →	T_3^1
← N (5)—C_α (6) →	T_1^1
← C_α (6)—C (7) →	T_2
← C (7)—N (8) →	G_3^5
← N (8)—C_α (9) →	T_1^1
← C_α (9)—C (10) →	G_2^5

Table 7

← C (1)—N (2) →	G_3^1
← N (2)—C_α (3) →	T_1^1
← C_α (3)—C (4) →	G_2^1
← C (4)—N (5) →	T_3^1
← N (5)—C_α (6) →	T_1^1
← C_α (6)—C (7) →	T_2
← C (7)—N (8) →	G_3^1
← N (8)—C_α (9) →	T_1^1
← C_α (9)—C (10) →	G_2^1

tance is calculated to be 7.25 Å., agreeing well with the observed value 7.20 Å. Fig. 5 shows the silk-fibroin chain of *Saturnia pyri*, while the structures (similar to β-keratin) of silk-fibroin discussed by previous workers were limited only to that of *Bombyx mori*.

VI. Polypeptide Structure of Silk-fibroin of *Antheraea mylitta*.—X-ray diffraction studies of silk-fibroin of "Tussahseide" or "*Antheraea mylitta*" were made by Trongs and Hess.[13] Beside the two characteristic identity distances 6.78 Å. and 6.71 Å., corresponding to the strongest reflections I_1 and I_2 some other values were also obtained. Taking the average of the values for I_1 and I_2 we get 6.75 Å., which is smaller than 6.95 Å. observed for *Bombyx mori* fibroin. The structure of *Antheraea* fibroin can be given as shown in Fig. 6, in which we see the amino acid residue (1-2-3-4) in the α-keratin structure of Fig. 4 of the previous paper (Part I) and that of glycine (4-5-6-7) of Fig. 1 joined alternately (repeating bond series T_1^1—G_2^1—T_3^1—T_1^1—T_2—G_3^1, Table 7). 6.72 Å. calculated for the identity distance agrees quite well with the above mentioned average value.

(13) C. Trongs and K. Hess, *Biochem. Z.* **260**, 376 (1933).

VII. Conclusion—We have determined the polypeptide structures of α-keratin (Part I) feather-keratin, β-keratin and silk-fibroins of *Antheraea mylitta*, of *Bombyx mori* and of *Saturnia pyri*, starting from the conditions stated in Part I together with experimentally obtained identity distances. These six substances seem to have the most typical polypeptide chain structures, but various intermediate type structures may also exist. For example, in the X-ray diffraction studies of collagen, the arc on the meridian at 2.8 Å. has not been clearly explained.[14] Assuming twice this distance 5.6 Å. as the identity distance, we may regard collagen as having a structure intermediate between α- and feather-keratins. Also, sericine[15] (identity distance 6.83 Å.) may be an intermediate type fibroin between those of *Antheraea mylitta* and *Bombyx mori*.

In the six typical structures given above of polypeptide chains, we see only nine bond types T_1^1, T_2, G_2^1, G_2^3, G_2^5, T_3^1, G_3^1, G_3^3 and G_3^5 occur. For intermediate structures we can assign intermediate bond types, for example, between G_2^1 and G_2^3. Heretofore types G_1^1, G_1^2, G_2^2, G_2^4, G_2^6, G_3^2, G_3^4 and G_3^6 among those shown in Figs. 1, 2 and 3 of Part I have not entirely been used. For the explanation of tangled chain structures, however, these types may play important rôles. Resumés for the six typical structures of polypeptide chains are given in Table 8.

Resumo.

(1) La polipeptidaj strukturoj de pluma keratino kaj β-keratino estas akiritaj kiam la α-keratinĉeno estas streĉita kaj uniĝtipa

(14) G. L. Clark and J. A. Schaad, *Radiolog.*, **271**, 339 (1936).

(15) M. Simizu, *Bull. Imperi. Sericult. Experi. Station*, (Tokyo), (in Japanese) **10**, 441 (1941a).

Table 8
Typical Structures of Polypeptide Chains

Fibrous Proteins	Identity Distance Obs., Å.	Identity Distance Cal., Å.	Bond Direction Types	Figure (Part 1)
α-Keratin	5.15 „	5.20 „	$T_1^1—G_2^1—G_3^1—T_1^1—G_2^1—G_3^1$	4
Feather-Keratin	6.14 „	6.06 „	$T_1^1—G_2^3—G_3^3—T_1^1—G_2^3—G_3^3$	2
β-Keratin	6.64 „	6.67 „	$T_1^1—G_2^5—G_3^5—T_1^1—G_2^5—G_3^5$	3
Silk-Fibroin of *Antheraea mylitta*	6.75 „	6.72 „	$T_1^1—G_2^1—T_3^1—T_1^1—T_2—G_3^1$	6
Silk-Fibroin of *Bombyx mori*	6.95 „	7.02 „	$T_1^1—G_2^3—T_3^1—T_1^1—T_2—G_3^3$	4
Silk-Fibroin of *Saturnia pyri*	7.20 „	7.25 „	$T_1^1—G_2^5—T_3^1—T_1^1—T_2—G_3^5$	5
Polyglycine*		7.47 „	$T_1^1—T_2—T_3^1—T_1^1—T_2—T_3^1$	1

* Added for reference, although not a natural fibrous protein.

serio fariĝas $T_1^1—G_2^3—G_3^3$ kaj $T_1^1—G_2^5—G_3^5$ respektive.

(2) La strukturo de silkfibro de *Antheraea mylitta, Bombyx mori* aŭ *Saturnia pyri* estas akirita kiam aminoacidaj restaĵo de poliglicino kaj tiu enhavita en α-keratino, pluma keratino aŭ β-keratino estas alterne kunligita.

(3) La strukturoj supre proponitaj povos esti konsideritaj kiel la tipaj strukturoj aldoniteblaj al polipeptidaj ĉenoj en naturaj fibraj proteinoj, pri kiuj ĉiu ripetdistanco estre proksima al observita valuo estas calculita. Rezultatoj estas resumitaj en Tabelo 8.

In conclusion, the author wishes to express his sincere thanks to Prof. S. Imanishi for the kind guidance and valuable discussions, and also to the Ministry of Education for grants in aid out of the Scientific Research Expenditure.

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