THE COLLOIDAL BEHAVIOUR OF SERICIN. VII.*

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Sec. V. The Distribution of the Various Forms of Nitrogen in Sericin A and Sericin B.

The very close resemblance in properties between naturally occurring proteins and the higher synthetic polypeptides suggests that proteins are essentially formed by the condensation of varying numbers of groups of diamino- and monoamino-acids mainly by means of the peptide linkage.

R. Inoue, Abderhalden, and others have thoroughly investigated the nitrogen distribution in the true and the wild silks, and R. Inoue showed that fibroin and sericin are similar in the distribution of various forms of nitrogen, but their amino-acid compositions are greatly different. In order to compare the nitrogen distribution in sericin A and sericin B I have studied the same problem.

As for the structure of proteins, the molecule is found to consist essentially of a main-axis constructed with repeated three-atom-units in a zigzag chain. The middle carbon atom of the three-atom-unit joins an organic

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* Continued from p. 474 of this volume, and concluded.

radical forming a long side-chain. If the side-chain be such a group as hydrogen, methyl, $CH_2(OH)-$, etc., that is, if the chain is formed of glycocoll, alanine, serine, etc., the molecule is permitted to pack together sufficiently closely to form fibers. In the case of silk fibroin, no less than 40 per cent. of the weight of the protein appears as glycocoll and a further 25 per cent. as alanine, so that most part of side-chain components in this protein is hydrogen-atom and methyl-group.

The contents of glycocoll and alanine in sericin are much less than in silk fibroin, so we can presume that in sericin molecules the main-axis components are smaller, but the side-chain components are greater than in fibroin. If a protein molecule has long, bulky side-chains, its main-axis cannot be brought into close approximation to that of another molecule of the same kind, but there will be a space left between them, and this space, in an aqueous solution, will obviously be filled up mainly with water and thus will be hydrated. The difference in the proportion of their components leads to easy separation into two proteins, fibroin and sericin, on boiling in water.

If the quantity of the nitrogen dissolved in water observed by R. Inoue and K. Kitazawa,(60) is compared with the lysine contents of silk, both are closely related as given in Table 105.

Table 105.

	N (%) dissolved in water by an hour boiling	Lysine content (%)
True silk	1.789	0.86
Antheraea Yamamai silk	1.339	0.19
Antheraea pernyi silk	0.428	0.03
Attacus cynthia silk	0.110	0.04

Amino-acid composition of sericin has been reported as shown in Table 106.

Table 106.

	Amino-acids	Turk(61)	Abderhalden	Inoue
Main-axis components	Glycocoll	—	1.1	4.38
	Alanine	—	10.10	8.86
	Serine	6.81	11.25	4.45

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(60) R. Inoue and K. Kitazawa, *Bull. Seri. and Silk Ind. Japan*, **5** (1932), No. 1.

(61) Turk, *Z. physiol. Chem.*, **111** (1932), 69.

Table 106. (*Concluded*)

	Amino-acids	Turk	Abderhalden	Inoue
Side-chain components	Tyrosine	—	3.50	—
	Arginine	4.56	3.75	5.67
	Lysine	1.69	1.90	3.51
	Histidine	1.02	1.00	1.99

G. Yamanouchi(62) reported that in the silk gland of silkworms fibroin is surrounded by three sericin layers which are different in the degree of dying with acid fuchsin. The sericin in the outer layer, S_3, is dyed the most easily with this colour and the sericin in the middle layer, S_2, is difficult to be dyed.

I have shown that the cocoon layer having good "kaijo" possesses a greater capacity of adsorbing dyes than that having bad "kaijo", and that sericin A can adsorb many dyes like acid fuchsin, congo red, methylviolet, etc. stronger than sericin B. From these results it seems to me that these two components of sericin is separately secreted from different parts of the silk gland.

Six grams of sericin A or sericin B was dissolved in 120 c.c. of 30 per cent. hydrochloric acid and the solution was boiled in a tared flask under a reflex condenser until it gave no longer the biuret reaction. After 6 hours the hydrolysis of sericin was stopped and the solution was filtered, and diluted to 500 c.c. Various forms of nitrogen in sericin were determined by van Slyke's method. The results are given in Table 107.

Table 107.

	Sericin A (%)	Sericin B (%)	Sericin (%) (Inoue)	Fibroin (%) (Inoue)
Amide N	2.63	2.40	2.34	1.26
Humine N	2.51	2.47	1.93	2.05
Cystine N	0.70	0.83	0.70	0.40
Arginine N	8.43	5.14	5.67	4.85
Histidine N	0.69	2.48	0.49	6.74
Lysine N	4.34	4.83	4.88	0.13
Mono-amino N	76.74	80.53	81.52	75.19
Non-amino N	2.60	2.02	2.34	7.42
Hexon base N	14.16	13.28	11.74	12.12
Lys.+arg. N	12.77	9.97	10.55	4.98

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(62) G. Yamanouchi, *J. Coll. Agri. Hokkaido Imp. Univ.*, **10** (1922), Part 4, 1–41.

As shown in Table 107, sericin A and sericin B are similar in the distribution of various forms of nitrogen. Sericin A is poorer in mono-amino nitrogen and richer in non-amino and hexon base nitrogen than sericin B. This difference may lead to different physico-chemical behaviours in viscosity, swelling, and gelatinization.

Block and Vickery(63) found that the basic amino components in keratins obtained from various sources have the following proportion :

Histidine : Lysine : Arginine = 1 : 4 : 12.

The results for sericin are shown in Table 108.

Table 108.

	Histidine : Lysine : Arginine
Sericin A	0.69 : 4.34 : 8.42 = 1 : 6.3 : 12.2
Sericin B	2.48 : 4.83 : 5.14 = 1 : 1.9 : 2.7
Fibroin	(Vickery and Block) 1 : 3.6 : 10.6

The molecular weights calculated on the assumption that the molecule of sericin contains one molecular equivalent of arginine, or lysine, are shown in Table 109.

The number obtained are to be regarded as the minimum values, and the molecular weight must be multiplied by a factor n, where n may be any integer.

Table 109.

	Sericin A	Sericin B
Arginine	17835	—
Lysine	20423	18718

Sec. VI. The Relation between Fibroin and Sericin.

Shelton and Johnson(64) showed that fibroin is probably a mixture of proteins loosely combined with one another. From the microscopical and chemical investigations we can presume that most of amino-acid components of silk fibroin consist of the main-axis components like glycocoll,

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(63) Block and Vickery, *J. Biol. Chem.*, **93** (1931), 105, 113.

(64) Shelton and Johnson, *J. Ind. Eng.*, **22** (1930), 387.

alanine, etc. and a part of it consists of the side-chain components which are similar to those of sericin. The following orders hold good: fibroin $>$ sericin A $>$ sericin B, in main-axis components, fibroin $<$ sericin B $<$ sericin A, in side-chain components. The first order may be parallel with the easiness of precipitation with $(NH_4)_2SO_4$, and the second with their solubilities.

The solubility of sericin may depend for the most part on its basic components. And on boiling the sericin solution, the basic side-chain components may gradually separate off and the degree of hydration may be diminished. Therefore, the viscosity of the sericin solution may gradually diminish on boiling, and gelatinization may become imperfect. Then, the solubility of sericin in water may depend on the quantity of sericin, especially of sericin A, existing on the cocoon fibre and also on the quantity of the basic components.

Though the solubility of sericin increases on boiling under pressure, sericin B and a part of amorphous components of silk fibroin which are similar to those of sericin B may be dispersed into the solution. This may lead to decrease in the strength, elongation, and luster of silk fiber. For the same reason the ferment which decomposes the sericin B especially may also be able to attack on the amorphous components of fibroin, and the similar bad effects may be caused in the silk fiber.

Tyrosine contents of sericins and fibroin. Sericin was hydrolysed by six hours boiling with 25 per cent. sulphuric acid six times as much as the quantity of the sericin, the solution, after filtration, was diluted with twice its volume of water and neutralized with a strong solution of baryta, the excess of which was then quantitatively removed by dilute sulphuric acid. The solution, together with the water used for thoroughly washing the precipitate of barium sulphate, was then evaporated, until tyrosine crystallized out. It was collected; the filtrate was concentrated, and further crops of crystals were obtained until the mother liquor gave no longer Millon's reaction for tyrosine. It was purified by recrystallization from water, decolourization of the solution being effected by charcoal.

The tyrosine content of fibroin is on an average about 10 per cent., and it is a few times as great as that of sericin.

Table 110.

	Tyrosine	Gly./Tyr.
Sericin A	2.62%	2.38
Sericin B	5.53	0.84
Sericin (Abderhalden)	3.50	—
Fibroin (,,)	11.00	3.59

Sericin B contains much more tyrosine component than sericin A. The value of glycine : tyrosine is the greatest in fibroin, next in sericin A, and the least in sericin B. The above results lead to the conclusion that sericin is characteristically rich in side-chain components which have the great affinity with water, that fibroin is richer, on the contrary, in the main-axis components, and that in the distribution of nitrogen in the side-chain components sericin B is similar to the amorphous components of fibroin.

As is well known the high temperature and high relative humidity influence greatly the spinning velocity of liquid silk by silk worms. Y. Tanzawa(65) showed that the spinning velocity was proportional to the temperature under the same conditions. The greater the spinning velocity becomes, the more sericin B which is considered to exist closely near the fibroin layer is spinned out. Then the quantity of sericin B contained in the outer layer of cocoons increases with rising temperature, and this causes the retardation of the gelatinization of silk owing to the nature of sericin B, especially at high relative humidity. These unfavourable conditions may cause firm adherence of fibers and make them thinner.

PART III. CONCLUSION.

Sec. I. Varieties of Sericin.

The varieties of sericin which have been reported are summarized in Table 111.

Table 111.

Author	Varieties of sericin
K. Kodama	Sericin, Sericin pepton
K. Kondo	Sericin I, Sericin II.
Shelton and Johnson	Sericin A, Sericin B.
H. Kaneko	Sericin A, Sericin B.
T. Watanabe	Alcohol soluble and insoluble sericins.

K. Kondo prepared sericin for examination by soaking silk in water and treating it under 2.5–3 atmospheric pressures of steam for three hours. After three such treatments the water-soluble fraction was concentrated and the sericin was precipitated by adding two volumes of 80 per cent.

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(65) Y. Tanzawa, *Sangyo Shimpô*, 1930, No. 448.

alcohol. This sericin was washed thoroughly with absolute alcohol and ether, and designated as sericin I. Sericin II was obtained by further concentrating the alcoholic filtrate and treating the concentrate with 80 per cent. alcohol. The resulting precipitate, washed with absolute alcohol and ether, gave a yellow powder.

Shelton and Johnson extracted sericin from silk with a small amount of water under a pressure of 50 cm. of mercury. They found that the whole protein was precipitated by means of alcohol and that after precipitation only a portion would again dissolve in cold water. They also found, as in the case of fibroin, that varying concentrations of $(NH_4)_2SO_4$ gave two kinds of precipitates, the first being a gelatinous substance insoluble in cold water.

T. Watanabe obtained two sericins from the sericin solution with alcohol. One was insoluble in 50 per cent. alcohol, and the other soluble in it. The results lead to the conclusion that sericin is not a simple compound, but it is a mixture of at least two different substances.

When I investigated the physico-chemical properties of the sericin solutions obtained from the cocoons having various " kaijo ", I noticed many differences in their behaviours, though these differences may be partly due to the impurities contained in the cocoon layers. Then I obtained two different sericins from the sericin solution by the fractional precipitation with $(NH_4)_2SO_4$ as done by Shelton and Johnson. But I further found that the separation into these two components can be effected also by other electrolytes, by freezing, and by the electric current. The one which was easily salted out from solution by electrolytes was named " sericin A " and the other " sericin B." These two sericins were similar in the distribution of various forms of nitrogen, but very different in their physical properties.

Sec. II. The Distribution of Two Sericins on the Cocoon Fibers.

The distribution of sericin A and sericin B on the cocoon fibers has an intimate relation with the " kaijo " of cocoons, because they have different physico-chemical properties. The relation between the proportion of two sericins and " kaijo " are given in Table 112.

The better " kaijo " a cocoon has, the more sericin A is contained. H. H. Mosher(66) estimated two sericins in several raw silks by boiling cocoons in water at 115°C. under pressure.

In the same cocoon the outer layer is rich in sericin A and the inner layer in sericin B. So when we intend to reel cocoons at low temperature,

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(66) H. H. Mosher, *American Dyestuff Reporter*, No. 11, 1932.

we must bear it in mind that the inner layer containing more sericin B should be thoroughly cooked.

Table 112.

" Kaijo "	Sericin A	Sericin B
Good	61.23%	38.76%
Middle	55.22	44.78
Bad	43.32	56.68

These two sericins seem to exist already in the liquid silk of the silkworm, and these may probably be secreted from different parts of the silk gland. Therefore, the "kaijo" of cocoons may primarily depend on the varieties of the cocoons and secondarily on the external conditions during the spinning of liquid silk and physical treatments.

The temperature influences the speed of spinning of liquid silk and may cause a change in the proportion of two sericins. The high relative humidity during the spinning retards the evaporation of water and consequently the gelatinization. Especially, heat may cause the transformation of sericin A into sericin B, so we must take care when we dry cocoons.

In gelatinization the inner and middle layers of cocoons are placed in worse conditions than the outer and further they may easily absorb the fatty matter from the pupa. These may lead to worse " kaijo."

Sec. III. The Micellar Structure of Sericins and Their Physico-Chemical Properties.

It has been considered that sericin is an amorphous substance. Although sericin and fibroin have remarkably different appearance, a micellar structure can be presumed for sericin, especially sericin A, as for the fibroin. The difference in the characteristic behaviours of these two proteins may be due to the difference in the proportion of the main-axis and side-chain components. Naturally fibroin is richer in the former component and sericin in the latter which has great affinity with water. As already described sericin A is coagulated in a fibrous form by many electrolytes, by freezing, and by applying the electric field in the sericin gel. The coagulate is considered to have a micellar structure.

The experiment concerning the distribution of various forms of nitrogen in sericin A and sericin B has shown that in the former more basic amino-acids are contained than in the latter. For this reason sericin A may be stronger hydrated in aqueous solution and in fact it shows higher viscosity, stronger swelling capacity, easier gelatinization, and heavier apparent specific gravity than sericin B.

Sericin A is apt to denature on drying, to turn slightly gray, and to shrink easily to a hard mass. Hence the solubility of sericin A in water is greatly reduced by drying though it seems that it ought to be more soluble than sericin B.

By the X-ray analysis of sericin powder it is shown that sericin has no micellar structure. But, it seems to me that this is the result of the shrinking property of sericin A as in the case of not stretched caouchouc. Recently K. Obara also has advanced the same opinion for the existence of a micellar structure in sericin on the ground of his microscopical investigation. Electrically charged sericin which tends to form molecular aggregates in solution, exists in solution as comparatively large particles associated with very large quantities of water. This aggregation of the sericin micellæ in aqueous solution varies with concentration and also with time. The physico-chemical properties of the sericin solution change, therefore, rapidly near the concentration of 0.3 per cent. as shown in Table 113. Effects of time of standing at the same concentration are given in Table 114.

Table 113.

	With increasing concentration, near 0.3%
Relative viscosity	rapidly increases
Surface tension	rapidly decreases
Diffusion coefficient	,, ,,
Specific rotation	,, ,,
Electric spec. conductivity	rapidly increases
Flocculation value	,, ,,
Gelatinization capacity	,, ,,
Depression of freezing point	,, ,,

Table 114.

	Maximum	Conc. (%)
Relative viscosity	about 90 min. later	0.32
Lowering of surface tension	,, 35 ,, ,,	0.40
Electric spec. conductivity	,, 30 ,, ,,	0.53
Gelatinization capacity	,, 120 ,, ,,	1.18
Protection against the decomposition of H_2O_2 by Pt sol	,, 90 ,, ,,	0.4–0.3

The aggregation of sericin micellæ depends also greatly on the temperature at the same concentration. From other experiments I can presume that the temperature of transformation of sol into gel corresponds to about 50°C.

Sec. IV. The Isoelectric Point of Sericin.

At the isoelectric point the dissolved protein is all electrically neutral. Such a solution is characterized by the tendency to aggregate and to take a high degree of turbidity and by the maximum ease in precipitation with alcohol or other lyophilic reagents. It has a greater surface activity. Dissolved protein always lowers the surface tension of water, but the effect is the greatest at the isoelectric point and is, therefore, due more to the electrically neutral protein than to the electrically charged. Hardy showed that the position of the maximum precipitation is influenced by the salt content. The isoelectric points obtained by various experiments are shown in Table 115 and 116.

Table 115. The isoelectric point of the natural sericin (mixed sericin).

	Good	Middle	Bad	Conc. (%)
Minimum relative viscosity	*p*H 3.95	4.12	4.18	0.2
Minimum surface tension of water	4.25	4.30	4.40	0.08
Minim. spec. rotation	4.24	4.30	—	0.2
Minim. emulsifying power	4.0	4.5	4.8	0.13
Maxim. precipitability with				
Methyl alcohol	4.0	—	—	0.2
Ethyl alcohol	3.8	—	—	,,
Acids	3.9	—	—	,,
$FeCl_3$, $AlCl_3$, etc.	4.2	—	—	,,
Nitrates	4.6	—	—	,,
Acetone	4.0	—	—	0.12
Minim. cataphoretic velocity	4.35	4.45	4.45	

Table 116. The isoelectric point of sericin A and sericin B.

	Sericin A	Sericin B
Minim. swelling	*p*H 3.80	4.50
Maxim. degree of turbidity	4.05	4.20
Minim. cataphoretic velocity	4.10	4.25
Minim. solubility	4.50	4.70

The isoelectric point of the natural sericin in which sericin A and B are mixed depends on the concentration and also on the "kaijo" of cocoons. In low concentrations the isoelectric point corresponds to about *p*H 4.0 in the sericin solution obtained from cocoons of good "kaijo", and it is shifted to the alkaline side in the solution obtained from cocoons of bad "kaijo".

The isoelectric points of sericin A and sericin B are respectively about pH 3.8 and pH 4.50. So we can understand that the isoelectric point of the sericin solution varies with the "kaijo" of cocoons, and that the sericin solution shows usually the isoelectric range in which sericin is capable of being precipitated by the addition of electrolytes or alcohol. If these two sericins are contained in equal amounts in the sericin solution, its isoelectric point may be probably pH 4.15.

Sec. V. The Molecular Weight of Sericin.

The probable molecular weight of sericin micellæ calculated from various data are given in Table 117. From these results the molecular weight of the sericin micellæ is considered to have an order of ten thousand.

Table 117. Molecular weight of sericin $= 403 \times n$.

Calculated from	Mol. wt.	n
Viscosity	4026 (0.35%)	10
Diffusion coefficient	29441 (0.38%)	73
Depression of f. p.	8936 (0.34%)	22
Chemical analysis	19129 (sericin A)	47

Sec. VI. The Fibroin and Sericin, Two Proteins of Silk.

As shown by R. Inoue, fibroin and sericin are similar in the distribution of various forms of amino-acids, but very different in the proportion of the main-axis and the side-chain components. So the difference between fibroin and sericin exists in the proportion of components, their arrangement, and the manner in which they are linked.

The amorphous side-chain components of fibroin are especially similar to those of sericin B. The complete separation of sericin B from fibroin without doing damage to fibroin components may be theoretically difficult.

The formation of a film which protects the fibroin fiber must be necessary for the existence of sericin A which has probably a micellar structure.

In conclusion the author wishes to express his cordial thanks to Prof. Y. Shibata for his kind guidance and encouragement.

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