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THE AMINO ACIDS OF THE SERICIN FRACTIONS OF SILK

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Raw silk consists of two proteins, inner fibrous core "fibroin" and sheath "sericin". The sericin is easily removed from the fibroin with boiling water. Recent literature (1) (2) (3) (4) (5) states that the sericin extracted from raw silk is a mixture of, at least, two simpler proteins showing different behaviors, such as solubilities, their resistance to proteolytic enzymes, and their action in electric dialysis. Although it is probable that the greater part of these different properties is produced by the drustic action at the extraction of sericin and during the aging of raw silk, whether the sericin in the raw silk, after spun by silk worm, is simple homogeneous protein or a mixture of simpler proteins has not been accertained. For example, Rutherford and Harris (5) stated that sericin B is gradually converted into sericin A during the extraction of raw silk in an autoclave at 114–115°, and the contents of tyrosin in sericin A and sericin B prepared from the raw silk extracted for a short time with hot water are apparently different: Mosher (2) stated that sericin B is slowly converted into sericin A by aging.

Many studies (6) (7) (8) (9) concerning the amino acids of the hydrolysate of the sericin were reported, but most of the previous studies were carried out by the old ester method or with several limited amino acids, and, thus far, only 50% of the sericin has been accounted for in the yields of amino acids isolated.

Since it was our purpose to determine the amino acid content in the sericin, and, if possible, to compare the difference of

chemical composition of sericin fractions, we exployed recently devcloped methods for the isolation of amino acids. We recovered about 65% of the total nitrogen from sericin B and sericin A respectively as crystalline amino acids or their derivatives except ammonia and triptophane, and found no appreciable difference of amino acids content between these fractions which had different physical properties. Besides our experiment, Abderhalden and Zumstein (8) found norvaline, chitosamine and glucuronic acid, and Alders (10) found cystine from the unfractionated sericin.

The figures of amino acids nitrogen of the sericin summed up in the above experiments does not exceed 70%. We cannot understand the causes of this low value, since one of our writers, applying almost the same procedures, recovered about 80% amino acids from the proteins of soybean (*Soja hisoida*) (13), and ryokuto (*Phaseolus Mungo* L. var *radiatus* Bak) (14) respectively.

Experimental

The Preparation of Sericin Fractions from Cocoons

Because of the differences in the nature of silk from different varieties of worms and in the age of the silk, we prepared our sericin from cocoons known as "Gunze Blue" recently spun in laboratory.

200 g. of sericin was extracted two times successively with 2l of water for one half hour at a pressure of 15 lbs. in an autoclave. The extract was acidified with acetic acid to pH 4.0. The precipitate, sericin B, was filtered, and washed well with water, alcohol and ether successively. The aqueous filtrate and the washing from sericin B were poured into 4 volumes of 95% alcohol. The precipitate, sericin A, was filtered, and washed with alcohol and ether. The yield of sericin B was 13.3%, and that of sericin A was 8.9% respectively from the cocoons as moisture free bases.

Table 1.

	Moisture	Ash	Nitrogen (ash and moisture free bases)
Sericin B	5.5	0.5	16.3
Sericin A	6.0	1.8	16.5

Sericin B precipitated in the bottom of a beaker as a white powder and sericin A aggregated in the liquid as a somewhat fibrous flocculence. The ash content of sericin A was reduced to 1.1% when this precipitate had been dissolved in 0.2% NaOH solution, dialyzed, and reprecipitated with alcohol.

Isolation of Amino Acids from the Hydrolysate of Sericin

The sericin was hydrolyzed for 25 hours with 20% HCl or 25% H_2SO_4. We selected either HCl or H_2SO_4 in accordance with the method of isolation of individual amino acid. When monoamino monocarboxylic acids were isolated by the ester method, basic amino acids had been previously removed with phosphatungstic acid from the hydrolysate of the sericin, dicarboxylic acids removed as barium salts according to the method of Jones and Moeller (12), and then the esters of monoamino monocarboxylic acids were prepared according to the method of Forman (15).

Ammonia.——Ammonia was volumetrically estimated by the usual method from the distillate of the weak alkaline solution of the hydrolysate of the sericin.

Glycine.——Two methods of isolation were used. (A). The hydrolysate of the sericin was esterified as usual. The distillate of lower boiling point (temperature of vapor up to 70° at 16 mm pressure) of esters was hydrolyzed with boiling water and converted into cupper salts. From the water soluble and methanol insoluble cupper salts (16) glycine was isolated as picrate according to the method of Levene and van Slyke (17). (B). Glycine was directly crystallized as glycine potassium trioxalatochromate according to the method of Bergmann and Fox (18).

Alanine.——Two methods of isolation were used. (A). Alanine was isolated from the filtrate of the glycine picrate (17). (B). Alanine was directly crystallized as alanine dioxypyridate according to the method of Bergmann (19).

Valine.——Valine was isolated from the combined hydrolysates of esters of lower and higher boiling point (temperatures of vapor up to 70° at 16 mm pressure and up to 70° at 3 mm pressure) purifying as methanol soluble cupper salt (16) and ammoniacal water soluble lead salt (20) successively.

Leucine.——Leucine was isolated from the hydrolysate of esters

of higher boiling point purifying as water insoluble cupper salt (16) and ammoniacal water insoluble lead salt (20) successively.

Isoleucine.——Isoleucin was not detected. If it was present, it would be isolated from the leucine fraction as its methanol soluble cupper salt and ammoniacal water insoluble lead salt.

Phenylalanine.——Phenylalanine was not detected. If it was present, it would be isolated as its hydrochloride from the distillation residue of the esters.

Serine.——Serine was isolated as water soluble cupper salt from the unesterified residue according to the ester method of Foreman (15).

Proline and Oxyproline.——These amino acids were detected neither by the method of Town (21) nor by the method of Bergmann (22).

Arginine.——Arginine was isolated as flavianate according to the micromethod of Vickery (23), of which we has proved to obtain most accurate result.

Histidine and Lysine.——These amino acids were estimated from the filtrate of the arginine flavianate according to the micromethod of Tristram (24).

Tyrosine.——Tyrosine was recovered in a free state from the several fractions of Foreman's ester method (15).

Aspartic Acid and Glutamic Acid.——These amino acids were isolated according to the method of Jones and Moeller (12).

Oxyglutamic acid.——Oxyglutamic acid was not detected according to the method of Dakin (25).

Tryptophane.——Tryptophane was colorimetrically determined according to the method of Folin and Marenzi (28).

Table 2.

	% of weight of protein		% of total nitrogen	
	Sericin B	Sericin A	Sericin B	Sericin A
Ammonia	2.2	2.0	11.1	10.0
Glycine	4.1 (3.2)*	3.8 (3.0)*	4.7	4.3
Alanine	11.9 (10.6)*	12.0 (9.4)*	11.5	11.4
Valine	1.3	0.9	1.0	0.7
Leucine	1.7	1.4	1.1	0.9
Isoleucine	none	none	none	none
Phenylalanine	none	none	none	none
Serine	13.5	12.6	11.1	10.2
Proline	none	none	none	none

Oxyproline	none	none	none	none
Arginine	5.1	5.1	10.0	10.0
Histidine	1.1	1.3	1.8	2.1
Lysine	1.2	1.1	1.4	1.3
Tyrosine	5.2	4.9	2.5	2.3
Aspartic acid	13.8	13.0	8.9	8.3
Glutamic acid	2.9	2.4	1.7	1.4
Oxyglutamic acid	none	none	none	none
Tryptophane	1.0	1.1	0.8	0.9
Total	65.0	61.6	67.6	63.8

* These figures are the results of ester method.

In addition to the above figures sericin contains 1.25% of norvalin (8), 0.5% of chitosamine (8), 0.6% of glucuronic acid (8) and 1.04% of cystine (10).

SUMMARY

Sericin, one of the proteineous constituents of raw silk, was extracted with water in an autoclave at a pressure of 15 lbs., and divided into two fractions, sericin B and Sericin A. The former was insoluble in water at pH 4.0; the latter was soluble in water at pH 4.0, but insoluble in concentrated alcohol. Although these two fractions were different in appearance and physical properties, they seemed to the identical with amino acids content within the limit of experimental errors.

Recovery of total nitrogen as amino acid was about 70%.

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