Investigation of Amino Acid Composition in the Crystalline Region of Silk Fibroin

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Synopsis

Accurate estimation of amino acids composition has been carried out for hydrolysates of four varieties of Indian silks, viz., Mulberry, Tasar, Eri, and Muga. These studies have revealed that the hydrolysate (hydrofibroin or crystalline region) in the case of Mulberry consists of glycine, alanine, and serine, whereas in the case of Tasar, Eri, and Muga, it is found to be mainly alanine. Other amino acids were also found to be present in the hydrolysate of these silks. But, the quantities present in each case were found to be negligible when compared to those amino acids cited above. Furthermore, these results are in conformity with the structure elucidation made by infrared spectral studies and x-ray diffraction.

INTRODUCTION

Silk is one of the most important fibrous proteins. In the natural form, it consists of a waxy material called sericin which forms a cementing layer on the fibrous material called fibroin. Amino acid composition of different varieties of silk fibroin has been studied by Lucas et al.[1] The main four varieties of Indian silks viz., Mulberry, Tasar, Eri and Muga, have been investigated by Dhavalikar[2] with reference to the amino acid composition. Recently, Radha Pant and Unni[3] have estimated the amino acid composition of Tasar and Eri by paper chromatography. The values reported by these authors are, however, not in agreement with those reported by earlier workers.[1,2] It may be mentioned that the earlier workers[1,2] used the method of ion exchange chromatography, which is more accurate than paper chromatography. Comparison of all the data reveals that the values reported by Dhavalikar[2] are more accurate and have been accepted as reference values for control samples in the present studies.

Similarly, amino acid analysis of the chemically resistant fraction of the fibroin of a few varieties of silk obtained by partial acid or alkali hydrolysis have been reported by Shaw and Smith.[4] Further, it has been found that the chemically resistant fraction of the fibroin, "hydrofibroin" (hydrolysate), represents the crystalline region of the silk when experimental conditions are suitably selected. Therefore, in the present investigation the hydrolysates of four varieties of Indian Silk obtained by partial acid hydrolysis and

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representing mostly the crystalline region were studied to estimate the amino acid composition in them, using automatic amino acid analyzer.

EXPERIMENTAL

Four varieties of Indian silks were obtained from different sericultural research laboratories in India. Silk yarn was degummed by the method described earlier.[5] The partial acid hydrolysis was carried out using hydrochloric acid of different concentration, as reported earlier.[6] The residue obtained after partial acid hydrolysis has been called as "hydrofibroin or hydrolysate."

Samples for amino acid analysis were prepared by standard method.[7] The automatic Beckmann Unichrom amino acid analyzer, Model 4231, was used in conjunction with ion exchange columns. The ion exchange chromatograph showed the recovery of each amino acid quantitatively. However, since the major constituents were found to be glycine, alanine, and serine, the estimation has been restricted to these amino acids only.

RESULTS AND DISCUSSION

Table I shows the composition of amino acids found for different silk fibroins. Lucas et al.[1] have classified the silk according to the total composition of glycine, alanine, and serine. It can be seen from Table I that in the case of Mulberry the composition due to glycine, alanine, and serine was found to be about 80%. Further, it may be noted that the glycine content is more in the case of Mulberry whereas the alanine content is more for the wild silks.

Table II gives the composition of amino acids for the hydrolysate of different silk fibroins. In the case of Mulberry the amino acid composition did not change after 48 h of hydrolysis. However, for the hydrolysate obtained after (48 + 48) h indicated that glycine and alanine are major constituents having a detectable amount of serine. In contrast to this result, the amino acid composition in the case of Tasar, Eri, and Muga revealed alanine as the major constituent with small quantities of glycine and serine. Further hydrolysis of (48 + 48) h did not show any significant increase in alanine content.

It may be mentioned that the amino acid composition of the partially hydrolyzed silk for Tussah or Tasar as reported by Shaw and Smith[4] is in conformity with the results obtained presently. Furthermore, the crystalline structure of Tasar has been interpreted as the crystalline region possibly consisting of poly(L-alanine) type. On this basis, the comparison of high content of alanine in Eri and Muga indicates that these may also have a similar structure to Poly(L-alanine). However, further additional hydrolysis did not yield "hydrofibroin" completely free from glycine and serine which indicates that the polypeptide chains containing Gly Ala, Ser Gly, and Ser Ala may still be present in the "hydrofibroin" of wild silks. The results obtained by x-ray diffraction and infrared spectroscopy are in conformity with such a molecular structure.[6,8]

TABLE I
Amino Acid Composition of Different Varieties of Silk

Sample no.	Amino acid	Ref. no.:	Amino acid N/total N							
			Bombyx mori		*Antheraea mylitta*		*Philosamia cynthia*		*Antherae assamensi*	
			1	2	1	2	1[a]	2	1	2
1	Glycine		43.74	43.75	23.5	24.24	31.4	26.42		25.55
2	Alanine		28.78	29.05	36.0	39.00	47.9	35.35		34.34
3	Valine		2.16	1.85	0.8	0.67	0.57	0.54		0.53
4	Leucine		0.52	0.42	0.9	0.35	0.23	0.38		0.40
5	Isoleucine		0.65	0.53	—	0.36	0.34	0.52		0.39
6	Serine		11.88	10.00	9.8	8.82	5.10	4.96		7.88
7	Threonine		0.89	0.90	0.9	0.32	0.57	0.36		0.80
8	Aspartic acid		1.28	1.51	5.7	5.53	3.53	3.52		4.82
9	Glutamic acid		1.00	1.07	0.9	0.78	0.79	0.66		1.17
10	Phenylalanine		0.62	0.50	0.3	0.43	0.11	0.64		0.56
11	Tyrosine		5.07	5.42	4.8	4.71	5.56	5.37		4.61
12	Lysine		0.63	0.60	—	0.05	0.34	0.30		0.29
13	Histidine		0.53	0.36	—	1.68	1.40	1.53		1.06
14	Arginine		1.83	1.90	13.3	11.84	1.87	6.95		12.25
15	Proline		0.35	0.50	—	0.70	0.34	0.52		0.47
16	Tryptophan		0.33	0.39	3.1	2.04	—	0.52		2.80
17	Methionine		—	—	—	—	—	—		—
18	Cystine		—	0.08	—	0.29	—	—		—

[a] The values reported have been found to be for *Philosamia cynthia*. However, the values could be compared with those of Dhavalikar for *Philosamia cynthia ricini*.

TABLE II
Comparison of Amino Acid Composition of Hydrolysates (Residues or Hydrofibroins)

	Bombyx mori (Mulberry)				*Antheraea mylitta* (Tasar)			
	Earlier studies		Present investigations		Earlier studies		Present investigations	
Amino Acid	Control	6*N* HCl, 40°C, 48 h, residue wt 33%	6*N* HCl, 40°C, 48 h, residue wt 38%	6*N* HCl, 40°C, (48 + 48) h, residue wt 33%	Control	10*N* HCl, 40°C, 48 h, residue wt 39%	8*N* HCl, 40°C, 48 h, residue wt 38%	8*N* HCl, 40°C, (48 + 48) h, residue wt 32%
Glycine	44.3	45.0	45.9	40.0	24.50	9.4	12.7	7.4
Alanine	32.50	30.7	32.5	50.0	44.0	71.8	62.8	73.5
Serine	14.2	9.5	8.6	2.0	12.20	8.5	10.7	9.8
Others	18.7	8.5	15.2	15.0	40.00	8.2	15.00	10.00

	Philosamia cynthia (Eri)			*Antheraea assaminisa* (Muga)		
	Control	Present studies, 8*N* HCl, 40°C, 48 h, residue wt 39%	Present studies 8*N* HCl, 40°C, (48 + 48) h, residue wt 32%	Control	Present studies, 8*N* HCl, 40°C, 48 h, residue wt 40%	Present studies 8*N* HCl, 40°C, (48 + 48) h, residue wt 32%
Glycine	25.4	9.2	12.1	26.3	12.2	14.0
Alanine	40.6	70.5	65.0	41.80	71.1	65.80
Serine	11.00	8.7	11.00	12.9	8.2	8.90
Others	39.1	7.5	11.00	25.0	8.5	17.00

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