Fractionation of bombyx mori silk fibroin

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With 2 figures and 1 table

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Introduction

The question of whether silk fibroin (Bombyx mori) is composed of several protein chains has been repeatedly investigated. By acidifying a solution of fibroin in cupriethylenediamine, Coleman and Howitt (1) obtained a soluble and an insoluble fraction. However, Drucker and Smith (2) were able to show that the amino acid composition of these fractions is the same. Glanzmann (3) and Signer and Glanzmann (4) fractionated fibroin solutions in lithiumthiocyanate, by addition of water and shaking, into components with different viscosities and slightly different amino acid compositions [Glanzmann and Signer (5)]. Narita (6) fractionated by means of Rivanol (2-ethoxy-6,9-diaminocacidinlactate) which precipitates a small part of the fibroin as the Rivanolcomplex ("Plastin"), and left the major part (70-80%) in solution ("Fibrin"). The fractions were reported to have different amino acid compositions.

Zuber (7) investigated the tryptic digestion of fibroin and proposed, on the basis of his results, a single-chain and a multi-chain model of fibroin, respectively. A multi-chain molecule with cystine or ester cross-links seemed possible. Shaw (8), also investigating the tryptic digestion of fibroin, suggested that fibroin is a complex of several peptide chains linked by disulfide or ester links rather than by hydrogen bonds only. However, on repeating the experiments of Narita (6), Shaw (9) was unable to obtain any fractionation by Rivanol. The different results were attributed to the hydrolysis of peptide bonds caused by ammonia which Narita had applied for the degumming of raw silk. Shaw used aqueous soap solutions for degumming [Drucker et al. (10)] by which no peptide bond hydrolysis occurred.

Based on the presence of the four N-terminal endgroups glycine, alanine, serine, aspartic acid [Zahn and Würz (11); Narita (6); Braunitzer and Wolff (12)] Shaw (9) postulated a four-chain molecule. Lucas (13) came to the same conclusion when he found that fibroin, isolated from the gland, had four cystine residues per molecule and a molecular weight of \(4 \times 10^5\) as determined by ultracentrifugation.

The present work describes the fractionation of fibroin by three different methods.

Materials and methods

Disc electrophoresis

Separation by disc electrophoresis was carried out with the apparatus of Shandon Scientific Co., London.

Composition of buffers and gels:

a) 18.2 g tris-(hydroxymethyl)-aminomethane, 24 ml 1 M HCl, 0.23 ml tetramethyl-ethylenediamine, 36.0 g urea, made up to 100 ml with dist. water.

b) 1.7 g tris-(hydroxymethyl)-aminomethane, 1.5 g K_2HPO_4, 17.2 ml 1 N HCl, 36.0 g urea, made up to 100 ml.

c) 30.0 g acrylamide, 0.8 g N,N'-methylethylen-bisacrylamide, 15 mg K_3Fe(CN)_6, 36.0 g urea, made up to 100 ml.

d) 10.0 g acrylamide, 2.5 g N,N'-methylene-bisacrylamide, 36.0 g urea, made up to 100 ml.

e) 4 mg riboflavin, 36.0 g urea, made up to 100 ml.

Small pore gel A: 1 part a + 1 part c.

Small pore gel B: 0.14 g ammoniumperoxi-disulphate, 36.0 g urea, made up to 100 ml.

Small pore gel: 1 part A + 1 part B.

Large pore gel: 1 part b, 2 parts d, 1 part e, 4 parts 6 M urea.

Electrode-buffer: 28.8 g glycine, 6.0 g tris-(hydroxy-methyl)-aminomethane, 360 g urea, made up to 100 ml.

Staining-solution: 0.55 g amidoblock dissolved in 100 ml 7.5% acetic acid.

Destaining solution: 6.0 g acrylamide, 0.1 ml tetramethyl-ethylen-diamine, 0.1 g ammonium persulfate, filled up to 100 ml with water. The solution was allowed to polymerise for 10 min and diluted 1:1 with 14% acetic acid.

Preparation of tubes: After polymerization of small and large pore gels 0.1 ml of the fibroin solution in 6 M urea (fibroin content: 45 mg/ml) was placed on the gel layer. On the fibroin layer 0.1 ml small pore gel (without urea) was applied and polymerized. Electrophoresis was performed with 15 mA per tube for different times (15-60 min). Staining with amidoblock and electrophoretic destaining of excess dyestuff was carried out according to routine procedures.
Extraction of fibroin fibres by LiSCN solution

Japanese raw silk (26/30 den) was degummed with soap according to Drucker et al. (10). After conditioning over saturated NaCl solution (75% relative humidity) the fibroin contained 10.1% water.

A sample of 1 g fibroin was mechanically shaken with 100 ml of 50% (w/v) LiSCN solution (pH 6.9-7.1) in a polythene bottle. At intervals 100 ml of distilled water were added and the undissolved residue was collected on a sintered-glass suction filter, washed with distilled water, dried and weighed (fig. 2). Values below 1 min were obtained by stirring the fibroin with LiSCN solution with a glass rod in a beaker. The dissolved fibroin fraction was precipitated by (NH₄)₂SO₄, washed with water and then dried.

Fractionation of fibroin on DEAE cellulose

4.78 g (= 4.29 g of dry weight) of raw silk and 60 ml 50% (w/v) LiSCN solution (pH 6.9-7.1) were mechanically shaken for 8 hr. Undissolved sericin was filtered by suction, washed with 25% (w/v) LiSCN solution followed by distilled water and dried.

Weight balance was determined in a separate experiment, where the dissolved fibroin was precipitated, dried and weighed:

<table>
<thead>
<tr>
<th></th>
<th>dry weight (g)</th>
<th>% b.wt. of raw silk</th>
</tr>
</thead>
<tbody>
<tr>
<td>raw silk</td>
<td>4.29</td>
<td>100.0</td>
</tr>
<tr>
<td>fibroin</td>
<td>3.26</td>
<td>76.1</td>
</tr>
<tr>
<td>sericin</td>
<td>0.81</td>
<td>18.9</td>
</tr>
<tr>
<td>loss</td>
<td>0.22</td>
<td>5.0</td>
</tr>
</tbody>
</table>

The fibroin solution was transformed into a 6 M urea solution by dialysis according to Zahn et al. (14).

Determination of fibroin content of the fibroin solution in 6 M urea: 2.0 ml of fibroin solution in 6 M urea were mixed with 50 ml of saturated (NH₄)₂SO₄ solution. After 2 hr the precipitate was filtered through a sintered-glass-suction-filter (G 3), washed sulphate-free with water and dried for 2 hr at 100 °C. Fibroin content of the solution: 45 mg/ml.

Chromatography of fibroin solution on DEAE-cellulose: Buffers: A: 0.1 N acetic acid, 0.1 M ammonium acetate, 6 M urea. B: 1 N acetic acid, 1 M NaCl, 6 M urea.

DEAE-cellulose, (Serva, Heidelberg, Germany), suspended in buffer A was filled into a 15 × 2 cm column and equilibrated with buffer A. 3.0 ml of fibroin solution in 6 M urea were applied to the column and the elution started with buffer A. After elution of fraction F₁, elution was continued with buffer B. Fractions were precipitated with (NH₄)₂SO₄, washed with water and dried: F₁, DC: 123.0 mg (91% b. wt. of applied fibroin). F₂, DC: 2.8 mg (2% b. wt. of applied fibroin).

Amino acid analysis: Samples were hydrolysed in vacuo at 110° with 6 M HCl for 24 hr. The HCl was removed by evaporation and the contents of amino acids in the hydrolysate were determined by ion-exchange chromatography with an automatic amino acid analyser (Beckman-Spinco).

Results

1. Disc electrophoresis

By disc electrophoresis of fibroin in 6 M urea on acrylamide gel at pH 9.3 three main and several secondary zones were obtained (see fig. 1).

Fig. 1. Disc electrophoresis of fibroin solution in 6 M urea at pH 9.3

2. Fractionation by extraction with lithium-thiocyanate

On treating fibroin with 50% and also with 40% (w/v) lithium-thiocyanate the amount of residue decreased more or less linear with

Fig. 2. Velocity of dissolution of fibroin in 30, 40 and 50% (w/v) LiSCN

△ 30% (w/v) LiSCN
○ 40% (w/v) LiSCN
□ 50% (w/v) LiSCN