52. Purification of the Three Specific Soluble Chromoproteins from Chromogranules in Hypodermal Cells of the Silkworm Larva

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It was reported in our previous paper (Tsujita and Sakurai 1963, '64) that the chromogranules in hypodermal cells of silkworm larvae are an important factor participating in the manifestation of larval skin color or transparency. Those chromogranules are composed of proteins, lipids, RNA and sugar. Two kinds of proteins exist in the chromogranules, that is, soluble protein of the inner part of the granules, and insoluble protein of the granular pellicles. It was shown in our previous paper that the soluble protein combines with sepiapterin and can be separated from other proteins, such as those which combines with uric acid, or with isoxanthopterin. Therefore, we

![Graph showing the separation of the 3rd fraction containing the sepiapterin-protein complex from other soluble pterine-protein complexes.](image)

Fig. 1. Separation of the 3rd fraction containing the sepiapterin-protein complex from other soluble pterine-protein complexes (Material: larvae of the E-lem). A soluble protein of chromogranules dissolved in about 3 ml of 0.005 M phosphate buffer at pH 7.0 was absorbed on a DEAE cellulose column (1.4 x 30 cm). The compounds were eluted with the use of a linear gradient from 0.005 M phosphate buffer (200 ml) to 0.005 M phosphate buffer solution containing 0.3 M sodium chloride (270 ml) at pH 7.0.
continued our experiments in order to remove some impurities from the protein that combines with sepiapterin. Moreover, experiments to separate the protein combining with uric acid from that combining with isoxanthopterin were carried out. In this report these purification procedures are described.

Materials and method. Normal (C 124) and lem larvae at the stage from 4th to 5th day in 5th instar were used as materials.

The procedures of separation and purification of chromoproteins are described in the following experimental results.

Experimental results. 1. Separation of the protein combining with uric acid from that combining with isoxanthopterin.

As described in our previous paper (Tsujita and Sakurai 1963), the chromogranules (about 200 mg in wet weight) which were collected and purified from hypodermal cells of larvae were homogenized with 3 ml of 0.005 M phosphate buffer and centrifuged. The su-

Fig. 2. Separation of uric acid-protein and isoxanthopterin-protein complexes from other soluble proteins in the 4th fraction (Material: larvae of C 124). The contents of the 4th fraction were applied to a CM cellulose column (1.2×30 cm), and eluted with a linear gradient from 0.005 M phosphate buffer (200 ml) to 0.05 M phosphate buffer (80 ml) at pH 5.5.
permatant containing soluble proteins was absorbed on a DEAE cellulose column (1.4 x 30 cm) and separated into four major fractions, by gradient elution using 200 ml of 0.005 M phosphate buffer at pH 7.0 and 270 ml of 0.005 M phosphate buffer solution containing 0.3 M sodium chloride at pH 7.0 (Fig. 1).

When free amino acids were added and the elution process mentioned above was carried out, all samples were collected in 1st fraction. Therefore, it is clear that proteins which are composed of fairly complicated polypeptides are eluted in 2nd, 3rd, and 4th fractions.

For obtaining the sepiapterin-protein complex the 3rd fraction separated from the soluble protein of chromogranules of the E-lem larvae was used. However, the content of isoxanthopterin in hypodermal cells of this mutant larvae is so small (Tsujita 1963) that it is difficult to collect the isoxanthopterin-protein complex. Therefore, in order to purify the isoxanthopterin-protein complex and the uric acid-protein complex the 4th fraction separated from the soluble protein of chromogranules of the normal (C 124) larvae was used.

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**Fig. 3.** Separation of the uric acid-protein complex from the isoxanthopterin-protein complex in the 4th fraction. An aliquot of the sample was applied to a CM cellulose column (1.2 x 30 cm), and eluted with a linear gradient from 0.005 M phosphate buffer (200 ml) to 0.05 M phosphate buffer (80 ml) at pH 6.0.
Fig. 4. The 3rd fraction containing sepiapterin was applied to a Sephadex G 25 column (1.6×60 cm).

Fig. 5. After gel filtration on a Sephadex G 25, a sample of the sepiapterin-protein complex was eluted with a linear gradient from 0.005 M phosphate buffer (200 ml) to 0.05 M phosphate buffer (80 ml) at pH 4.8 on a CM cellulose column (1.2×30 cm).
The 4th fraction which contained uric acid-protein and isoxanthopterin-protein complexes was absorbed on a CM cellulose column (1.2 x 30 cm) and eluted with a linear gradient from 0.005 M (200 ml) to 0.05 M (80 ml) phosphate buffer at pH 5.5. The result is shown in Fig. 2. A small fraction containing some impurities produced at the left side of the principal fraction, was removed. The principal fraction was absorbed on a CM cellulose column (1.2 x 30 cm) and eluted with a linear gradient from 0.005 M (200 ml) to 0.05 M phosphate buffer (80 ml) at pH 6.0.

As shown in Fig. 3, the fraction containing the isoxanthopterin-protein complex was separated from the fraction containing the uric acid-protein complex.

2. Purification of the sepiapterin-protein complex

The 3rd fraction containing the sepiapterin-protein complex was purified by gel filtration on a Sephadex G 25 column (1.6 x 60 cm). Since a small fraction containing some impurities was produced at the left side of the principal fractions, as shown in Fig. 4, it was removed. After this gel filtration on a Sephadex G 25, the fraction was eluted by gradient elution through a CM cellulose column (1.2 x 30 cm) using 0.005 M phosphate buffer (200 ml) to 0.05 M (80 ml) phosphate buffer at pH 4.8. By means of these procedures a purified chromoprotein was obtained as shown in Fig. 5.

Summary. The three chromoproteins, sepiapterin-protein, isoxanthopterin-protein, and uric acid-protein complexes, were separated and purified from the soluble proteins occurring in the chromogranules in hypodermal cells of silkworm larvae. The procedure of purification is described.

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References