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Note

SILK PROTEIN PRODUCTION BY THE IMMOBILIZED SILK GLAND

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The posterior silk glands of the silkworm, *Bombyx mori*, were immobilized in acrylamide gel. This immobilized organ produced silk protein in the presence of amino acids and energy sources. Amino acids began to be incorporated into the immobilized silk glands 2.5 h after incubation, and the incorporation continued for more than 20 h. The apparent lifetime of the posterior silk gland was remarkably elongated by the immobilization.

INTRODUCTION

Enzyme immobilization techniques have been successfully applied not only to biologically active molecules, but also to subcellular organelles and microbial whole cells as well (1–4). Such remarkable progress has encouraged potential users of biocatalysts to consider these systems in their processes. We have extended this technology further by making use of an immobilized organ.

It is well known that the silk glands of the silkworm, *Bombyx mori*, are capable of synthesizing fibroin and sericin in their posterior and middle glands, respectively. These glands consist of monolayer cells which can take up amino acids and other nutrients from the exterior and excrete the product to the interior. A schematic profile is presented in Fig. 1. Silk-fibroin DNA is specifically synthesized on the third and fourth days of the last larval instar (5). Termination of DNA production triggers the synthesis of fibroin and sericin. Since considerable amounts of polyribosomes are orderly arranged in the cells of these silk glands, they are considered to be an ideal process for producing a specific protein. Silk glands at an appropriate larval instar are immobilized to a matrix; a continuous process for producing a specific protein is then possible. The present communication describes preliminary results on fibroin production by the immobilized posterior silk gland.

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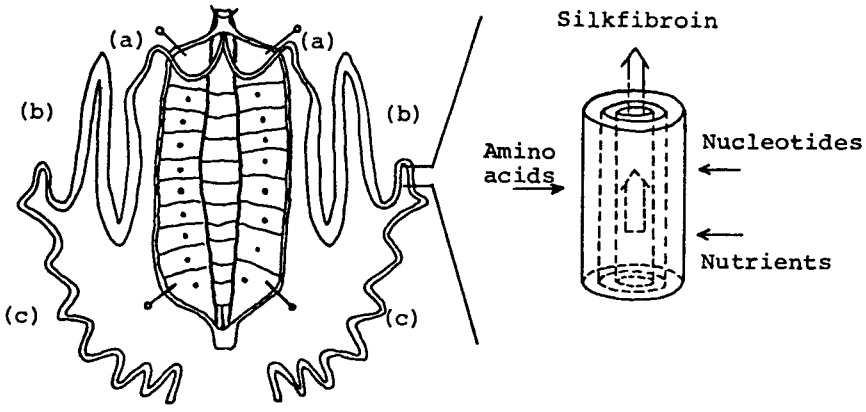

FIG. 1. Schematic representation of the silk gland of *Bombyx mori*. (a) Anterior silk gland; (b) middle silk gland; (c) posterior silk gland.

EXPERIMENTAL

Materials

Hybrids of *Bombyx mori* strains *Shunrei* and *Shogetsu* were provided by the courtesy of Drs. Yoshitake and Kobayashi (Department of Agriculture, University of Tokyo). These hybrids were reared on fresh mulberry leaves at room temperature in the laboratory, and sacrificed on the fifth or sixth day of the fifth instar. A pair of posterior silk glands were dissected from each larva in the cold as shown in Fig. 1. To remove the adherent fat and the tracheae, the gland was thoroughly rinsed with 1.15% KCl solution.

Immobilization of the Posterior Silk Gland

Acrylamide and *N,N'*-methylene bisacrylamide were dissolved in distilled water at a concentration of 5% and 1%, respectively. After addition of 0.1 ml of *N,N,N',N'*-tetramethylethylenediamine (TEMED) and 1 ml of 1% ammonium persulfate, 5 g of the posterior silk gland was added to 20 ml of the resulting solution. It was allowed to stand until gelation was complete. The entire procedure was carried out in an ice bath. The immobilized silk glands were cut into 5-mm cubes.

Protein Synthesis by the Immobilized Posterior Silk Gland

The immobilized silk glands were incubated in an appropriate culture medium (medium specified in Table 1) at 26°C with aeration for 25 h.

TABLE 1. **Protein Synthesis by Immobilized Posterior Silk Glands**[a]

Conditions	
Immobilized silk glands: 8.0 g in wet base	
Medium I	
2% NaCl, 0.2% KCl, 0.08% $CaCl_2$, 0.04% $MgCl_2$	9.0 ml
0.2 M KH_2PO_4-Na_2HPO_4 (pH 7.4)	3.0 ml
1.9 mg/ml glucose, 3.4 mg/ml G-6-P	1.0 ml
H_2O	17 ml
Protein secreted (mg/g immobilized PG)	0.26 ± 0.05
Immobilized silk glands: 7.2 g in wet base	
Medium II	
2% NaCl, 0.2% KCl, 0.08% $CaCl_2$, 0.04% $MgCl_2$	9.0 ml
0.2 M KH_2PO_4-Na_2HPO_4 (pH 7.4)	3.0 ml
1.5 mg/ml L-glu, 0.4 mg/ml gly, 0.7 mg/ml DL-ala, 0.2 mg/mg L-ser, 0.2 mg/ml L-tyr	15 ml
1.9 mg/ml glucose, 3.4 mg/ml G-6-P	1.0 ml
H_2O	2.0 ml
Protein secreted (mg/g immobilized PG)	1.83 ± 0.05

[a]Each medium containing the immobilized silk glands was aerated at 26°C for 21 h after the initial 4-h preincubation. Protein was determined by the method of Lowry et al. (6). The quantity of protein secreted during the 4-h preincubation was subtracted from the total.

Medium II contained glycine, alanine, serine, and tyrosine, which are major amino acid components of fibroin; glucose, glucose-6-phosphate, and L-glutamic acid as energy sources; and inorganic salts. Medium I was prepared in the same composition as Medium II but contained no amino acids. Experiments were performed with both mediums. An aliquot of each culture medium was assayed at an appropriate time for its protein content by the method of Lowry et al. (6). A calibration curve for the protein analysis was prepared with bovine serum albumin.

RESULTS AND DISCUSSION

The immobilized posterior glands (8.0 g in wet weight) were immersed in Medium I and aerated at 26°C. Since Medium I contains no amino acid, no protein synthesis by the silk gland was expected. However, the amount of excreted protein drastically increased, reaching a steady level within 4 h. The increase in protein secretion may be ascribed to the excreted silk fibroin that had been accumulated in the gland before the dissection of larva. No appreciable protein secretion resulted from further incubation up to 25 h.

These results indicate that 4-h preincubation would be necessary for the experiment.

In a similar way, 7.2 g of the immobilized posterior silk glands were aerated in Medium II, which contained amino acids, glucose, and glucose-6-phosphate. A drastic increase in protein secretion was observed in the initial 4 h in a manner similar to the control. In sharp contrast, however, protein secretion continued to increase even after the 4-h preincubation. Incubation was continued for 21 h after an additional 4 h. The considerable increase in protein produced after preincubation is believed to result from protein synthesis by the immobilized silk glands, into which amino acids were incorporated. The net quantities of secreted protein after preincubation are given for both media in Table 1. The marked difference in protein secretion between both media shows that the amino acids contained in Medium II were incorporated into the posterior silk glands to synthesize silk fibroin. As was expected, the immobilized posterior silk glands thus retained their capability for protein synthesis under these conditions.

According to the time course of protein secretion observed during the preincubation, it appears that amino acids begin to be incorporated into the immobilized silk gland approximately 2.5 h after incubation. On the other hand, we found that free posterior silk glands incorporated amino acids of Medium II after only 20–30 min incubation. The immobilized posterior silk glands thus required a little longer time for amino acid uptake as compared with the free ones, which might be due to the diffusional resistance of the polyacrylamide gel layer around the posterior silk glands.

It was found that an intact posterior silk gland continued protein synthesis in Medium II for about 2 h. The immobilized posterior silk gland, however, kept synthesizing protein for more than 20 h. The apparent lifetime of the posterior silk gland was considerably elongated by the immobilization.

Tissue culture has become applicable for the production of biologically important products (7). Organ culture also has the potential capability of producing bioactive substances. We have reported here silk protein production by the immobilized silk gland of *Bombyx mori* as a model system incorporating immobilized organs to elucidate the future perspective of organ culture.

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