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Immunochemical cross-reactivity among proteins in male reproductive systems of a beetle and of wild and domestic silkmoths

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INTRODUCTION

Male accessory reproductive organs produce a variety of secretory products which nourish sperm, promote their final differentiation, and which package sperm in spermatophores. Knowledge of the structure and function of the male systems may help to develop better programs of insect husbandry.

The detailed morphology of the male accessory glands, seminal vesicles, vasa deferentia, and ejaculatory ducts vary greatly among different orders and species (Happ, 1984), yet it remains possible that there might be strong similarities at the biochemical level. For example, the proteins that facilitate sperm maturation or those that form the structural wall of the spermatophore might be conserved in evolution.

Recent work on the male reproductive system of the beetle, Tenebrio molitor, has made available a panel of polyclonal and monoclonal antibodies which recognize male secretory antigens. Some of these antigens form the wall of the spermatophore (Grimnes and Happ, 1985; Grimnes, Bricker and Happ, 1986; Shinbo, Yaginuma and Happ, 1987) while others mix with the sperm in the lumen of the spermatophore. In the present study, we used one of these monoclonal antibodies, designated PL 15.2, to

determine whether epitopes in moths might be recognized by this antibody against beetle secretions.

MATERIALS AND METHODS

Animals and Chemicals

Hybrid larvae obtained from crosses made between N140 and C145 strains of Bombyx mori were reared on an artificial diet (Yakult Co., Japan) at 25℃. The larvae of Antheraea pernyi and Antheraea yamamai were reared on oak leaves and those of Samia cynthia pryeri on ailanthus leaves at 25C. Adults of Rhodinia fugax and Dictyoploca japonica were collected in the field. Pupae of Bombyx mandarina reared on mulberry leaves were kindly supplied by Dr. O. Ninagi, National Institute of Sericultural and Entomological Science.

Acrylamide, ammonium persulfate, N,N'-ethylene-bis-acrylamide, Coomassie brilliant blue R-250, Tween-20, gelatine, goat anti-mouse IgG and horseradish peroxidase color development reagent (contains 4-chloro-1-naphtol) were purchased from Bio-Rad, U.S.A.; Tris (hydroxymethyl) aminomethane and SDS-molecular weight markers from Sigma, U.S.A.; durapore (GVHP) from Millipore. Other chemicals used were of reagent grade.

Protein Samples

Groups of three to ten animals were used each analysis. Dissections were performed in ice cold phosphate-buffered saline (PBS, 0.01M sodium phosphate buffer-0.14M NaCl, pH 7.0). Reproductive organs, such as testis, ductus deferens, accessory gland, vesicula seminalis and ejaculatory duct were each excised from unmated male moths just after the emergence. Organs were disrupted with Kontes homogenizer in 100-200 μl of ice cold PBS. Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Monoclonal antibody

The PL 15.2 monoclonal antibody which recognizes an antigen in the tubular accessory gland of T. molitor (Grimnes, Bricker and Happ, 1986) was used for the experiment.

Electrophoresis on SDS-polyacrylamide slab gel and Western blotting

Electrophoresis on SDS-polyacrylamide was carried out by the method of Laemmli (1970) with 3% stacking gel and 12% separating gel. Organ proteins separated on SDS-polyacrylamide slab gel were blotted onto a durapore filter electrophoretically for 4 hr at 15℃. The electrode solution consisted of 25mM Tris base, 192mM glycine, 20% methanol and 0.05% SDS. After blotting, the filter was washed with about 200 ml of distilled water. Then the filter was immersed in 50 ml of TBST2 (10mM Tris-HCl buffer, pH 7.5, 0.5M NaCl, 0.02% Tween 20) containing 3% gelatine and shaken for 1 hr at room temperature to block nonspecific protein-binding sites. The filter was then incubated in 50 ml of TBST5 (10mM Tris-HCl buffer, pH 7.5, 0.5M NaCl, 0.05% Tween-20) containing 1% gelatine and PL 15.2 monoclonal antibody for 2 days. After washing the filter four times at 5-min intervals with 100 ml of TBST2, the filter was incubated in 50 ml of TBST5 containing 1% gelatine and rabbit anti-mouse IgG coupled with horseradish peroxidase for 2 days. Finally, the filter was washed four times at 5-min interval with 100 ml of TBST2 and with 150 ml of

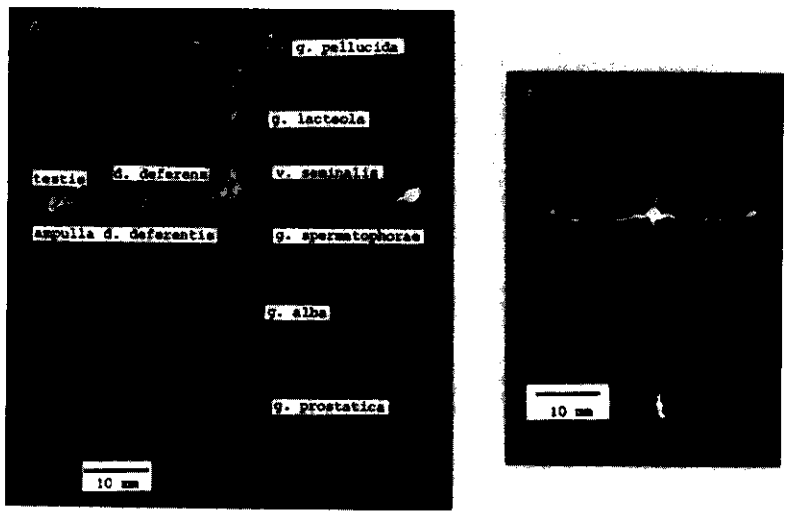

Fig. 1. Male adult reproductive system of Bombyx mori (A) and Bombyx mandarina (B).

Immunocross-reactivity

There were some strong cross-reactions between the proteins of reproductive organs of silkworms and PL 15.2 monoclonal antibody which recognizes an antigen in the tubular accessory gland of T. molitor, when homogenates of male reproductive organs of silkmoths were subjected to electrophoresis on an SDS slab gel and then to Western blotting (Figs. 3 and 4). As a matter of convenience, approximate molecular weights on durapore filter were estimated by comparing them with molecular weight standards on an SDS gel.

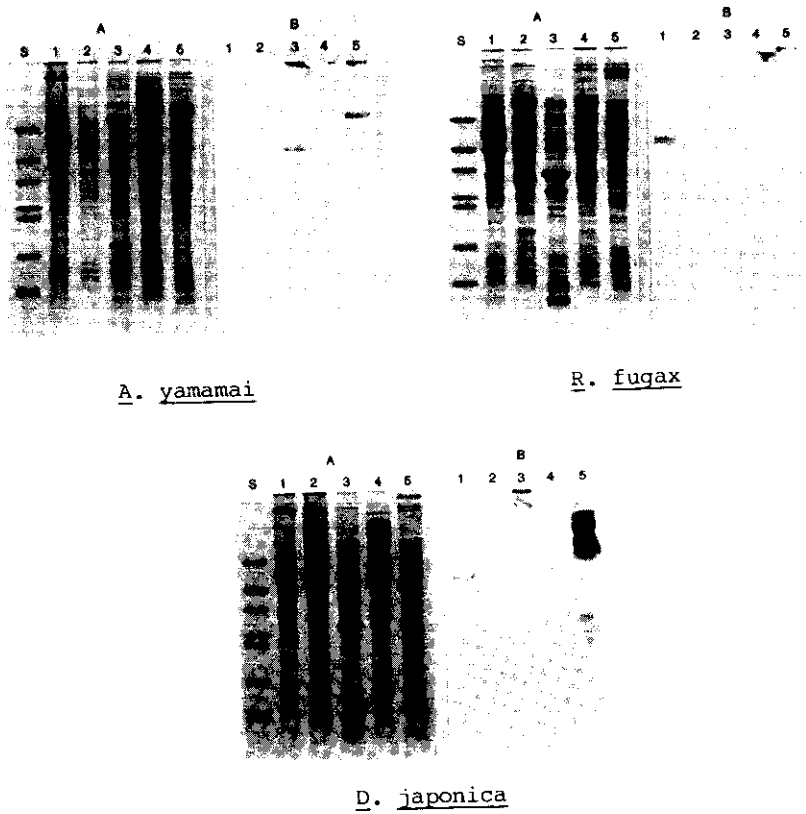

Fig. 4. SDS-polyacrylamide slab gel electrophoresis (A) and corresponding immunoblot (B) of male reproductive organ homogenates of A. yamamai, R. fugax and D. japonica. Footnotes same as Fig. 3.

In B. mori, PL 15.2 antibody recognized bands from accessory gland and ejaculatory duct; especially stronglyreactive bands were seen at about 23 kDa in accessory gland. No reactive proteins were detected in homogenates of other reproductive organs.

In B. mandarina, two reactive bands were seen at about 60 kDa and higher mass in ejaculatory duct, whereas no reactions were observed in homogenates of other organs.

In A. pernyi, PL 15.2 antibody recognized a single discrete band at about 30 kDa from accessory gland, and weakly reacted with bands at higher masses from ejaculatory duct.

In S. cynthia pryeri, weak reactions at relative high masses were observed only in ejaculatory duct.

In A. yamamai, PL 15.2 antibody recognized bands from accessory gland and ejaculatory duct. In accessory gland, two bands were seen at about 50 kDa and the origin of the gel, and two bands were seen at about 80 kDa and the origin of the gel in ejaculatory duct.

In R. fugax, PL 15.2 antibody recognized bands at about 50 kDa from testis, and also reacted with a band at the origin of the gel from ejaculatory duct.

In D. japonica, PL 15.2 antibody recognized bands from testis, accessory gland and ejaculatory duct. In ejaculatory duct, very strong reactive bands were seen at relative high masses, and weaker reactions between about 23 and 30 kDa were also observed. In testis, bands were seen at about 50 kDa, and bands were detected at high masses in accessory gland.

DISCUSSION

The monoclonal antibody, PL 15.2, made against antigens of the male accessory reproductive glands of a mealworm beetle, T. molitor, is strongly and specifically reactive against discrete organ-specific antigens in the male reproductive organs of saturniid and bombicid moths. In B. mori, the reactive moth antigens is absent in the young pupa and therefore appears to be an adult-specific product which can serve as a marker of the differentiated state (Shinbo and Happ, 1989).

In the present paper, the molecular weights and organ specificities of the antigens differ markedly among the different moth species. It seems unlikely that the epitopes recognized were found in highly-conserved proteins, but that possibility can only be explored after the antigens are isolated and characterized. Perhaps more encouraging is the possibility that for these many species of moths, as for B. mori, the antibody can detect a differentiation-specific antigen and thus can allow experimental scoring of the differentiated state.

The transfer of sperm is required for growth of feral populations and for maintenance of laboratory or agricultural cultures. An understanding of the physiology of sperm transfer, of the assembly of the spermatophore, and of the hormones that control the maturation and function of the male secretory systems may allow better evaluation of alternative culture procedures and, by comparisons among different strains, the proper choice of laboratory strain. We belive that the biochemistry, physiology, and development of the male system is a fertile area for future study in wild and domestic silkmoths.

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Relationship between the activities of inducible antibacterial substances and resistance to APNPV infection of the Chinese oak silkworm, *Antheraea pernyi*

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ABSTRACT Diapausing pupa of Antheraea pernyi were found to produce a series of inducible antibacterial substances in their hemolymph after injection of certain dead or viable bacteria. The variation of inducible antibacterial activity of diapausing pupae from 29 races, which include bivoltine and univoltine races, was compared, and significant differences were observed. The investigations of the relationship between the inducible antibacterial activity and the resistance to ApNPV infection were made by a half-batch determination method. Preliminary results showed that the inducible antibacterial activity was higher in the pupal stage, while the resistance to ApNPV was stronger in the young larval stage. This seems to indicate that the humoral immunity of A. pernyi is related to its resistance to ApNPV infection.

It is well known that insects have special defense systems to protect themselves from the invasion of various pathogens. Compared to the cellular defense system, humoral immunity is more interesting for it can be induced by injection of some dead or live bacteria to produce a series of antibacterial substances which can kill and lyse a variety of Gram-positive and Gram-negative bacteria (1-4).

Previously, we reported that the humoral immunity of the Chinese oak silkworm can be induced with some pathogenic bacteria and with inactivated nuclear polyhedrosis virus of A. pernyi (ApNPV). It is more interesting that when the pupae of A. pernyi were injected with viable ApNPV virus, antibacterial activity was also found in the hemolymph 3-4 days post-injection. This phenomenon suggested the possibility that when a pupa was infected with ApNPV virus, its humoral immunity defense systems were activated, producing a series of active substances to resist the invasion of the pathogen. However, we