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IMMUNOLOGICAL COMPARISON BETWEEN THE PARAMYOSINS FROM ADDUCTOR MUSCLE OF BIVALVES AND FROM FOOT MUSCLE OF A GASTROPOD

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Abstract—1. α-Paramyosin was isolated and purified from three molluscan species; clam *Meretrix meretrix*, scallop *Patinopecten yessoensis* and abalone *Notohaliotis discus*. Rabbit anti-α-paramyosin polyclonal antisera were raised separately against the three paramyosins.

2. Immunological cross-reactivity, as elicited by immunodiffusion and enzyme-linked immunosorbent assay tests showed distinct variations in the antigenic structure of the paramyosins of bivalve catch muscles from that of the paramyosin of non-catch foot muscle of abalone.

INTRODUCTION

Whereas the adductor muscle of bivalves is identified as a catch muscle, the foot muscle of gastropods is considered as one which may not exhibit catch contraction. The phosphorylation rate of α-paramyosin present in the adductor muscle of bivalves seemed to have some functional significance in the exhibited catch contraction. The preceding paper (Watabe *et al.*, 1990) showed evidence that the phosphorylation rate of paramyosins isolated from adductor muscles of bivalves *Meretrix* and *Patinopecten* was higher than the phosphorylation rate of the paramyosin isolated from the foot muscle of abalone *Notohaliotis*.

Anitigenic cross-reactivity is a convenient probe to identify the structural difference between the paramyosins of catch muscle and non-catch muscle. However, with the exception of the reports of Waterston *et al.* (1974) and Goldfine (1985), data on the anti-paramyosin antisera raised from any molluscan species remain scanty. Therefore, an attempt was made in this study to investigate comparatively, the immunological interactions of paramyosins isolated from adductor muscles of bivalves *Meretrix meretrix* and *Patinopecten yessoensis* with the paramyosin isolated from the foot muscle of gastropod *Notohaliotis discus*.

MATERIALS AND METHODS

Animals

Live specimens of clam *Meretrix meretrix*, scallop *Patinopecten yessoensis* and abolone *Notohaliotis discus* were purchased at the Tokyo Central Wholesale Fish Market and brought to the laboratory.

Isolation of α-paramyosin

α-Paramyosin was isolated from the adductor muscles of *Meretrix* and *Patinopecten* as well as from the foot muscle of *Notohaliotis*, according to the method of Merrick and Johnson (1977). All the procedures were carried out at 0–4°C and all solutions and buffers contained 0.5 mM dithiothreitol (DTT) to minimize proteolysis (Levine *et al.*, 1982).

Immunological methods

Rabbit anti α-paramyosin polyclonal antisera were raised by subcutaneous injections of 2 mg of *Meretrix*, *Patinopecten* and *Notohaliotis* α-paramyosin antigens in different rabbits (Hurn and Chantler, 1980). Ouchterlony immunodiffusion test (Ouchterlony and Nilsson, 1978) and enzyme-linked immunosorbent assay (Engvall, 1980) were carried out as described in the preceding paper (Watabe *et al.*, 1990) for the three α-paramyosin antigens against the three anti α-paramyosin antisera.

Protein concentration

Using bovine serum albumin (BSA) as the standard, protein concentration of the paramyosin preparation was measured by the Folin phenol colorimetric method (Lowry *et al.*, 1951).

RESULTS AND DISCUSSION

Immunodiffusion

The comparison of Ouchterlony immunodiffusion analysis of anti-*Meretrix*, anti-*Patinopecten* and anti-*Notohaliotis* paramyosin antisera to respective antigens are shown in Fig. 1. The precipitin arcs developed in Fig. 1A and Fig. 1C (anti-*Meretrix* α-paramyosin antiserum and anti-*Notohaliotis* α-paramyosin antiserum respectively) showed the existing immunological variation among the three paramyosin antigens. The precipitin arcs (spurs) are visible in Fig. 1A and Fig. 1C, however, the precipitin arcs developed with anti-*Notohaliotis* α-paramyosin antiserum showed the *Notohaliotis* paramyosin's distinct partial identity to the paramyosin antigens of *Meretrix* and *Patinopecten*. The immunological

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Abbreviations used—BSA, bovine serum albumin; DTT, dithiothreitol; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin.

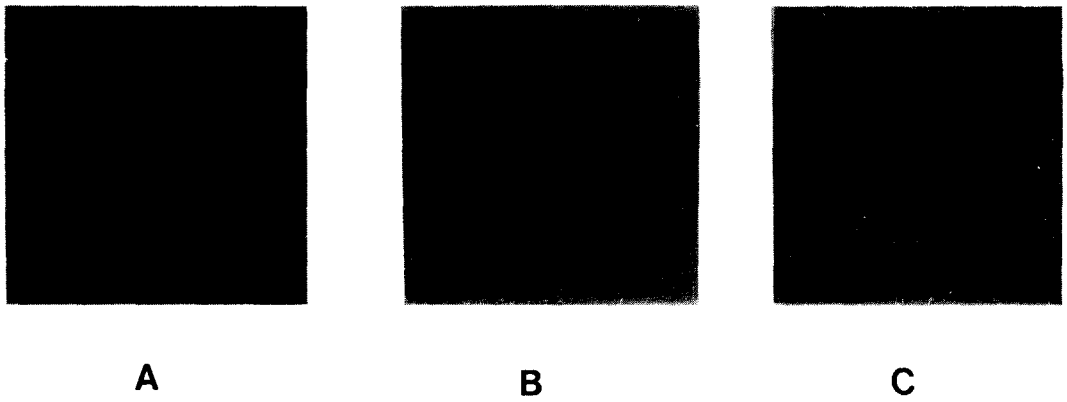

Fig. 1. Comparison of Ouchterlony immunodiffusion analyses of anti-*Meretrix*, anti-*Patinopecten* and anti-*Notohaliotis* α-paramyosin antisera to respective homologous and heterologous antigens. Center wells; A, anti-*Meretrix* α-paramyosin antiserum (AbM); B, anti-*Patinopecten* α-paramyosin antiserum (AbP); C, anti-*Notohaliotis* α-paramyosin antiserum (AbN). Antisera were used as *per se*, without dilution. Paramyosin antigens in the peripheral wells; *Meretrix* α (m); *Patinopecten* α (p) and *Notohaliotis* α (n). Protein concentration in each peripheral well was 5 μg. The well on top left corner was used as a negative blank and left unfilled. Arrowheads indicate the spurs developed between different paramyosin antigens.

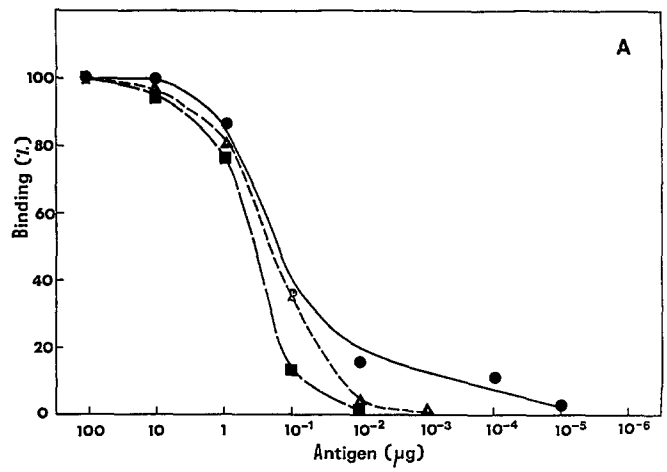

Fig. 2(a).

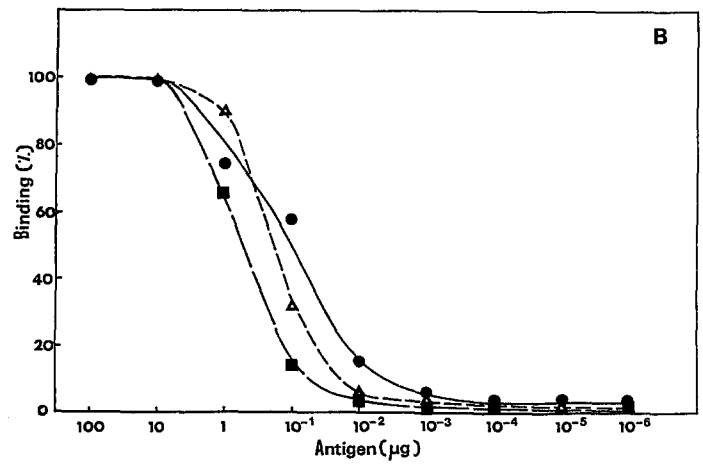

Fig. 2(b).

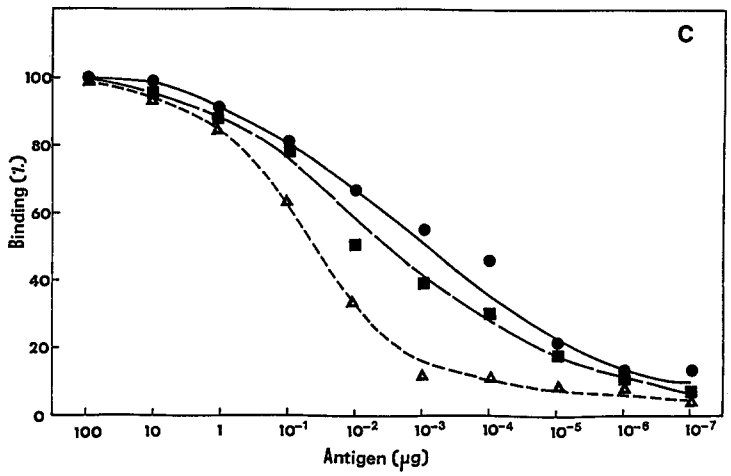

Fig. 2(c).

Fig. 2. Comparison of ELISA immunological cross-reactivity among anti-*Meretrix*, anti-*Patinopecten* and anti-*Notohaliotis* α-paramyosin antisera to respective antigens. A, anti-*Meretrix* α-paramyosin antiserum; B, anti-*Patinopecten* α-paramyosin antiserum; C, anti-*Notohaliotis* α-paramyosin antiserum. Paramyosin antigens used are; *Meretrix* α (●); *Patinopecten* α (△); *Notohaliotis* α (■). Antisera used at, 1:1000 dilution; anti-rabbit IgG alkaline phosphatase conjugate, 1:2000 dilution.

cross-reaction pattern seen in Fig. 2B revealed that the *Patinopecten* paramyosin does not share any immunological identity with either *Meretrix* or *Notohaliotis* paramyosin antigens.

Enzyme-linked immunosorbent assay (ELISA)

Figure 2 provides a comparison of ELISA immunological cross-reactivity among anti-*Meretrix*, anti-*Patinopecten* and anti-*Notohaliotis* α-paramyosins antisera to respective homologous and heterologous antigens. The phylogenetic resemblance between *Meretrix* and *Patinopecten* (both bivalves) against the gastropod *Notohaliotis* is evident from the half-maximal binding responses (Figs 2A, 2B) of the three paramyosin antigens to the anti-*Meretrix* and anti-*Patinopecten* antisera. However, at the half-maximal binding level, the *Meretrix* paramyosin antigen showed a higher immunobinding response to the anti-*Notohaliotis* α-paramyosin antiserum than its homologous antigen, *Notohaliotis* paramyosin (Fig. 2C). This response is difficult to explain.

Many of the immunological cross-reactivity studies of paramyosins reported so far have been based on anti-paramyosin polyclonal antisera raised from *Limulus* (Levine *et al.*, 1972, 1974; Elfvin *et al.*, 1976, 1979) and *Lethocerus* (Bullard *et al.*, 1977) of Arthropoda. Recently, Ardizzi and Epstein (1987) have reported studies on the monoclonal antibodies raised for paramyosin of *Caenorhabditis elegans* of Nematoda. These reports identified that paramyosin is localized exclusively in the A bands of several invertebrate striated muscles.

Waterston *et al.* (1974) had reported raising an anti-scallop paramyosin antiserum which cross-reacted with the paramyosin of nematode *Caenorhabditis elegans* in the immunodiffusion test. The scientific name of the scallop used in this study was not mentioned. So the immunobinding responses obtained with scallop *Patinopecten yessoensis* in the present study cannot be compared with the results of Waterston *et al.* (1974). The unpublished study of Goldfine (1985) had dealt with the immunological cross-reactivity of anti-*Mercenaria* α-paramyosin antiserum (*Mercenaria* is a bivalve similar to *Meretrix*). According to the results reported by Goldfine (1985), the anti-*Mercenaria* α-paramyosin antiserum showed positive cross-reactions with its homologous antigen, as well as to paramyosin antigens of other bivalves *Mytilus* and *Aquepecten*. Though no immunological cross-reactivity was noticed with the paramyosin antigens of six species of Arthropoda and sea urchin (an echinoderm), the anti-*Mercenaria* α-paramyosin antiserum showed positive cross-reactions with *Golfingia* (a sipunculid worm) and *Lumbricus* (an annelid worm) as well.

The reports of Waterston *et al.* (1974) and Goldfine (1985) as well as the data present in this study show that additional investigation on the immunological cross-reactivity among various paramyosin antigens to the anti-molluscan paramyosins antisera are needed to clarify the immunological relationships of molluscan paramyosins.

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