Genetic variant and inheritance of the 35K protease in digestive juice of silkworm, *Bombyx mori*

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The digestive juice of the larvae of the silkworm, *Bombyx mori* include many proteases. Some were isolated and characterized (EGUCHI and IWAMOTO, 1982; Sasaki and Suzuki, 1982; Kido *et al*., 1986; Kotani *et al*., 1999), but their heterogeneity has not been investigated, for except one case (Eguchi and Yositake, 1967).

We purified an enzyme named as 35K protease from silkworm digestive juice (Jiang *et al*., 2000). This enzyme is a chymotrypsin-like protease and was inhibited by CI-8 (Shirai *et al*., 1997) in the larval hemolymph. During our investigation, we discovered polymorphism of the 35K protease from digestive juice of silkworm. In this paper, we focus on polymorphism and the mode of inheritance of 35K protease.

**Materials and Methods**

Experimental animal and preparation of digestive juice: The silkworm strains stocked by the Institute of Genetic Resources, Kyushu University were reared on mulberry leaves at 25°C. Digestive juice was collected individually from five to ten larvae per each strain on the 3rd day of the 5th instar, after starvation for approximately four hours, by applying an electric shock, and stored at −20°C until use.

Polyacrylamide gel electrophoresis: Digestive juice was analyzed for protease by native polyacrylamide gel electrophoresis (native-PAGE, under undenaturing condition). Native-PAGE were prepared with 7.5% polyacrylamide as described previously (Jiang *et al*., 2000). The volume of sample applied was 6μl per lane. After electrophoresis, the gels were washed using distilled water and dried for 5 minutes at room temperature. The staining solution containing 1mg/ml of N-acetyl-D, L-phenylalanine-β-naphthylester dissolved in N, N'-dimethylformamide and 1mg/ml of tetrazotized orthodianisidine dissolved in 0.04 M sodium phosphate buffer, pH 7.4, was poured onto the gels, which were then incubated at room temperature for 10 minutes. The 35K protease activity was observed as a deep red band on a light red background.

**Results and Discussion**

Two electrophoretic variations in 35K protease were observed by polyacrylamide gel electrophoresis of digestive juice samples from the larvae of 60 strains. The two types of the 35K protease dealt with in this study were designated as Type A and Type B (Fig. 1), for the former migrated faster toward an anode and the
latter slower. Minor difference in the Type B is also shown on the gel. The bands of \( w_{42}, b_{31} \) and \( i_{90} \) strains (lanes 2, 6 and 7 in Fig. 1) migrate slightly faster toward the anode than those of \( w_{43} \) and \( p_{531} \) strains (lane 3 and 5 in Fig. 1). The \( F_1 \) individuals from the cross between minor different types gave single band, and in the \( F_2 \) progenies heterogeneities were not recognized. From these results, the minor differences observed in Type B could not be distinguished genetically.

To analyze the inheritance of 35K protease in the digestive juice, the phenotypes of individuals obtained from the following crosses were investigated. Reciprocal crosses between Type A (strain \( c_{60} \)) and Type B (strain \( p_{531} \)) always gave \( F_1 \) progenies with both Type A and Type B, named two band (AB) type. The \( F_1 \) female with AB type was backcrossed to the male with Type A (strain \( c_{60} \)) or Type B (strain \( p_{531} \)), respectively. The phenotypes of the \( BF_1 \) progenies are shown in Fig. 2. In the \( BF_1 \) progenies from the cross of Type AB × Type A, the segregation of Type A and two band type was 12:8. In the cross of Type AB × Type B, individuals with Type B and Type AB segregated 26:24. On the other hand, in \( F_2 \) progenies of the cross between Type A and Type B individuals with Type A, Type B and Type AB segregated 4:3:13. From these facts, we presume that the 35K protease of the digestive juice was controlled by codominant alleles. The gene was designated “35K protease” with the gene symbol Pro-35K. Type A and Type B of 35K protease were represented as Pro-35K\(^A\) and Pro-35K\(^B\).

**Fig. 1.** Zymogram of polymorphic variation of 35K protease shown by native polyacrylamide gel electrophoresis. 1, \( w_{34} \); 2, \( w_{42} \); 3, \( w_{43} \); 4, \( c_{60} \); 5, \( p_{531} \); 6, \( b_{31} \); 7, \( i_{90} \). A and B indicate type A and type B, respectively.

**Fig. 2.** Polyacrylamide gel electrophoresis patterns of 35K protease. a, (AB)×A; b, (AB)×B. 1, \( c_{60} \); 2, \( p_{531} \); 3-12, the individuals of \( BF_1 \) progenies.

**References**


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