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Effect of Triton X 100 on Isolation of a *Bombyx* Humoral Lectin Activating Enzyme.

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INTRODUCTION

A lectin is a functional protein which has the specific property reacting reversibly with a specific sugar residue on the cellular membrane. For this specificity, various animal lectins seem to play fundamental roles in the living body (KAWASAKI, 1998). For instance, invertebrate lectins play a significant role in a non-specific self defense mechanism (HATAKEYAMA, 1997) and in an apoptosis mechanism (HIRABAYASHI and KASAI, 1998). Especially, humoral lectins of insects play a significant role through metamorphosis of insects and will be useful defense substances for human being in future (KOTANI *et al*., 1995 ; NATORI, 1998 ; FUJITA *et al*., 1998).

In a previous study, we reported that a humoral lectin-protein (130K-glycoprotein) played a physiological role through metamorphosis of the silkworm, *Bombyx mori*, as it always seemed to possess the highest lectin activity on spinning day (KATO *et al*., 1994). We also reported on the appearance and the disappearance of the lectin activity. We emphasized the possibility that the humoral lectin-protein was produced and activated in fat body of *Bombyx mori*, and that it was secreted into haemolymph (KATO and NAKAMURA, 1997 ; KATO *et al*., 1998). Moreover, we researched a *Bombyx* humoral lectin activating factor in the fat body by means of FPLC system, and obtained neuraminidase-like enzyme from the fat body in the previous papers (KATO and NAKAMURA, 1998, 1999). On that occasion, it seemed needful to obtain more enzyme fraction for researching various properties of the fraction isolated by gel filtration.

In this paper, we report an effective method to obtain more enzyme fraction by means of Triton X 100 which is a kind of surface activator. Moreover, we report a comparative study between the haemolymph and the fat body on change of neuraminidase activity due to the difference of time. This investigation will provide useful information for understanding the activation mechanism of the lectin-protein *in vivo*.

MATERIALS and METHODS

1. Preparation of samples

A hybrid race, Shunrei×Shougetu, of the silkworm, *Bombyx mori*, was used in this experiment. The larvae were reared on mulberry leaves.

In preparing the samples for this research, fat body was collected daily. The fat body was collected from the dissected larvae and pupae, washed with cold 0.7% NaCl solution and homogenized in a glass homogenizer with a Teflon pestle. After centrifuging them at 3,500 rpm for 15 min at 4℃, each of resultant supernatant and precipitation was lyophilized.

2. Superdex 200 gel filtration

Gel filtration on Superdex 200 (Pharmacia) was performed using 0.1 M Tris-HCl buffer (pH 8.0) containing 0.1 M NaCl as the eluting buffer. The column (2.6×60 cm) was eluted with the buffer solution and a flow rate was kept at 150 ml per hour. The effluent was collected in 5 ml fractions and measured at 280 nm with a Shimadzu spectrophotometer type UV 1200.

3. FPLC Mono Q ion exchange chromatography

Protein was applied on to a column of Mono Q HR 5/5 (Pharmacia) previously equilibrated with 0.05 M Tris-HCl buffer (pH 8.0). Elution was performed with a linear gradient of 0 to 0.5 M NaCl in 0.05 M Tris HCl buffer (pH 8.0) and the flow rate was 1.0 ml per minute. Column eluates were collected in 1 ml fractions and measured at 280 nm.

RESULTS and DISCUSSION

Figure 1 shows comparative results on changes of optical density of fractions from the fat body of *Bombyx mori* due to the difference of the density of Triton X 100 in 0.1 M Tris-HCl buffer containing 0.1 M NaCl. It was recognized that neuraminidase existed in the enzyme fraction obtained by gel filtration in the previous paper (KATO and NAKAMURA, 1999). However, it seemed needful to obtain more enzyme fraction for researching various properties of the enzyme and existence of other enzymes in the fraction on that occasion. Accordingly, we tried to obtain more enzyme fraction by means of Triton X 100 which was a kind of surface activator. Figure 1-A shows the result on change of optical density of the supernatant fraction from the fat body of due to the difference of the den-

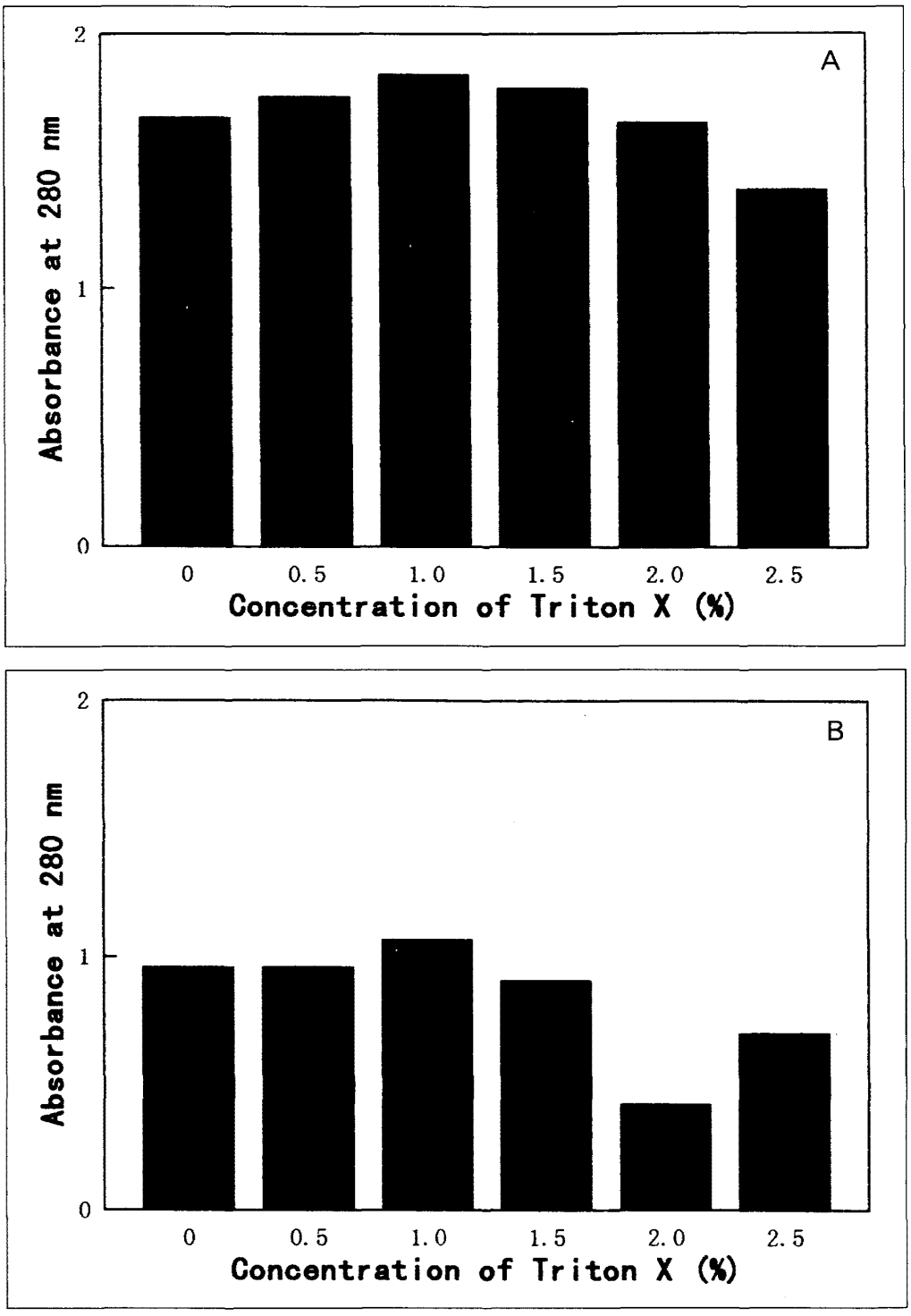

Fig. 1 The best concentration of Triton X 100
A : Supernatant fraction of fat body in 0.1 M Tris-HCl buffer containg 0.1 M NaCl,
B : Precipitation fraction of fat body in the buffer.

sity of Triton X 100 in 0.1 M Tris-HCl buffer containing 0.1 M NaCl. The result showed the highest absorbance when the density of Triton X 100 was one percent in the buffer as shown in Fig. 1-A. On the other hand, Fig. 1-B shows the result on change of optical density of the precipitation fraction from the fat body due to the difference of the density of

Triton X 100 in the buffer, for researching to obtain the enzyme fraction from the precipitation fraction of the fat body. The result showed the highest absorbance when the density of Triton X 100 was one percent in the buffer as shown in Fig. 1-B, although the precipitation fraction showed lower absorbance than the supernatant fraction. Namely, both the supernatant fraction and the precipitation one from the fat body showed the highest absorbance when the density of Triton X 100 was one percent in the buffer.

Figure 2 shows comparative elution patterns of gel filtration on the precipitation fraction obtained from the pupal fat body of *Bombyx mori*. A column of Superdex 200 pg was equilibrated with 0.1 M Tris-HCl buffer containing 0.1 M NaCl (pH 8.0) and eluted with the same buffer. Figure 2-A shows an elution pattern of the precipitation fraction eluted with the buffer on a column of Superdex 200. No enzyme fraction was obtained by gel filtration as shown in Fig. 2-A. On the other hand, Fig. 2-B shows a pattern of the fraction eluted with the buffer containing 1% Triton X 100 on a column of Superdex 200. The result showed that the chromatogram revealed a peak containing neuraminidase showed as an arrow in the figure, as the peak corresponded to the fraction containing neuraminidase in the previous paper (KATO and NAKAMURA, 1998). Namely, the enzyme fraction was obtained from the precipitation fraction of the fat body by means of Triton X 100.

Figure 3 and 4 show the results of Mono Q ion exchange chromatography on some fractions of the pupal fat body obtained by gel filtration. After dialyzed against 0.05 M Tris-HCl buffer (pH 8.0), each fraction was applied on to a column of Mono Q HR 5/5 previously equilibrated with the same buffer. Elution was performed with a linear gradient of 0 to 0.5 M NaCl in 0.05 M Tris-HCl buffer (pH 8.0). Figure 3 shows the chromatograms of five gel filtration fractions of the supernatant as shown in the previous paper (KATO and NAKAMURA, 1999). Each chromatogram revealed similar peaks at No. 12 except Fig. 3-A. On the other hand, Fig. 4 shows the chromatograms of four fractions of the precipitation eluted with the buffer containing 1% Triton X 100 as shown in Fig. 2-B of this paper. All chromatograms revealed low peaks at No. 12. Accordingly, it seemed needful to research again on the suitable condition for isolating the enzyme fraction from the precipitation fraction.

Next, we tried comparative studies between the haemolymph and the fat body on change of neuraminidase activity due to the difference of time. Figure 5 shows comparative elution patterns of gel filtration on the fracions from larval fat body on day 7 in the fifth instar, when neuraminidase showed low activity in the haemolymph as described in the previous paper (NAKAMURA and KATO, 1994). Figure 5-A shows the elution pattern of gel filtration on the supernatant fraction from the larval fat body eluted with the buffer

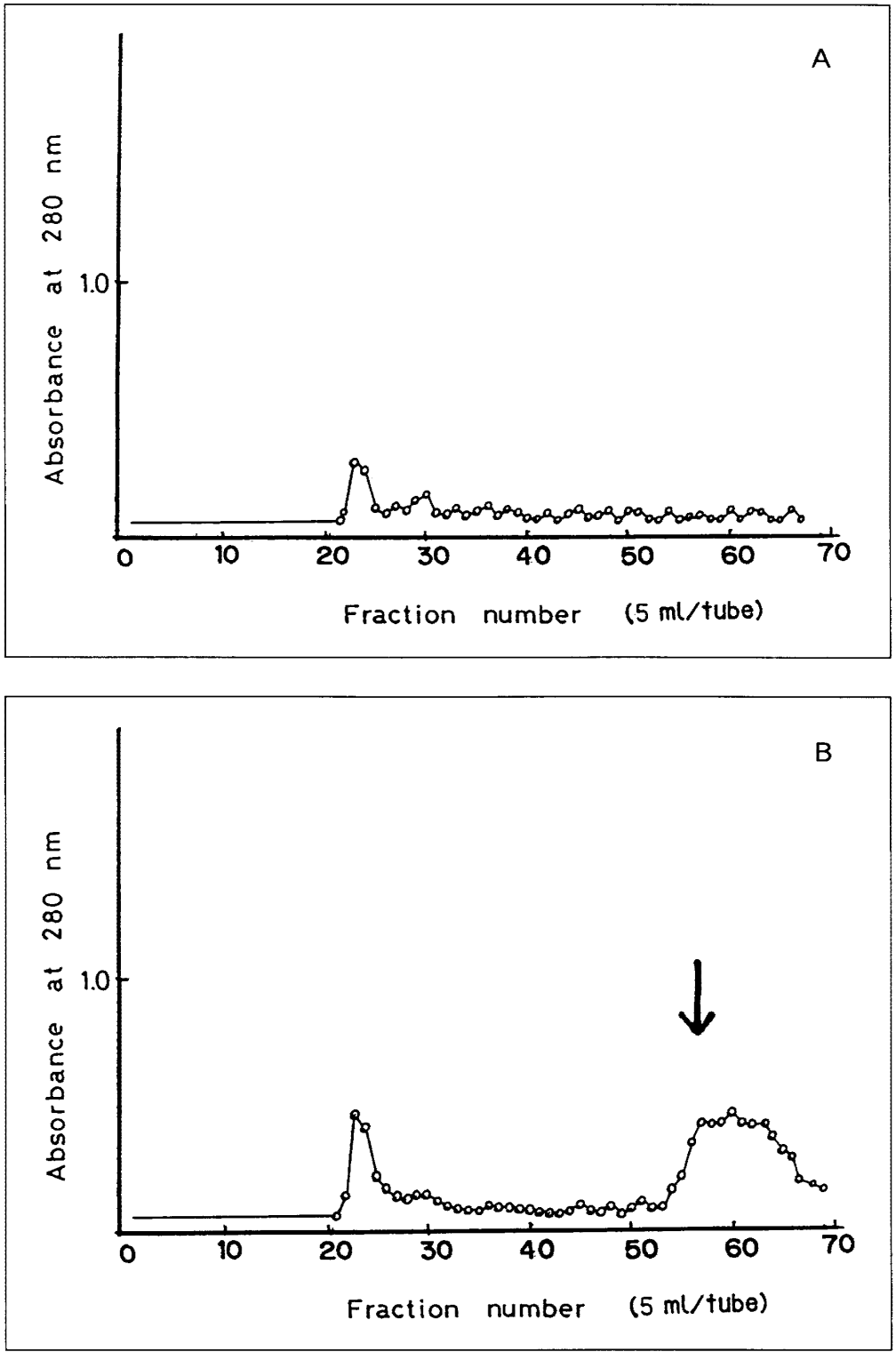

Fig. 2 Elution patterns of gel filtration on a Superdex 200 column
A: Precipitation of pupal fat body in the buffer,
B: Precipitation of pupal fat body in the buffer containing 1% Triton X 100.

containing 1% Triton X 100 on a column of Superdex 200. The result showed that the chromatogram revealed a peak containing the enzyme showed as an arrow in the figure. On the other hand, Fig. 5-B shows the pattern of the precipitation fraction from the lar-

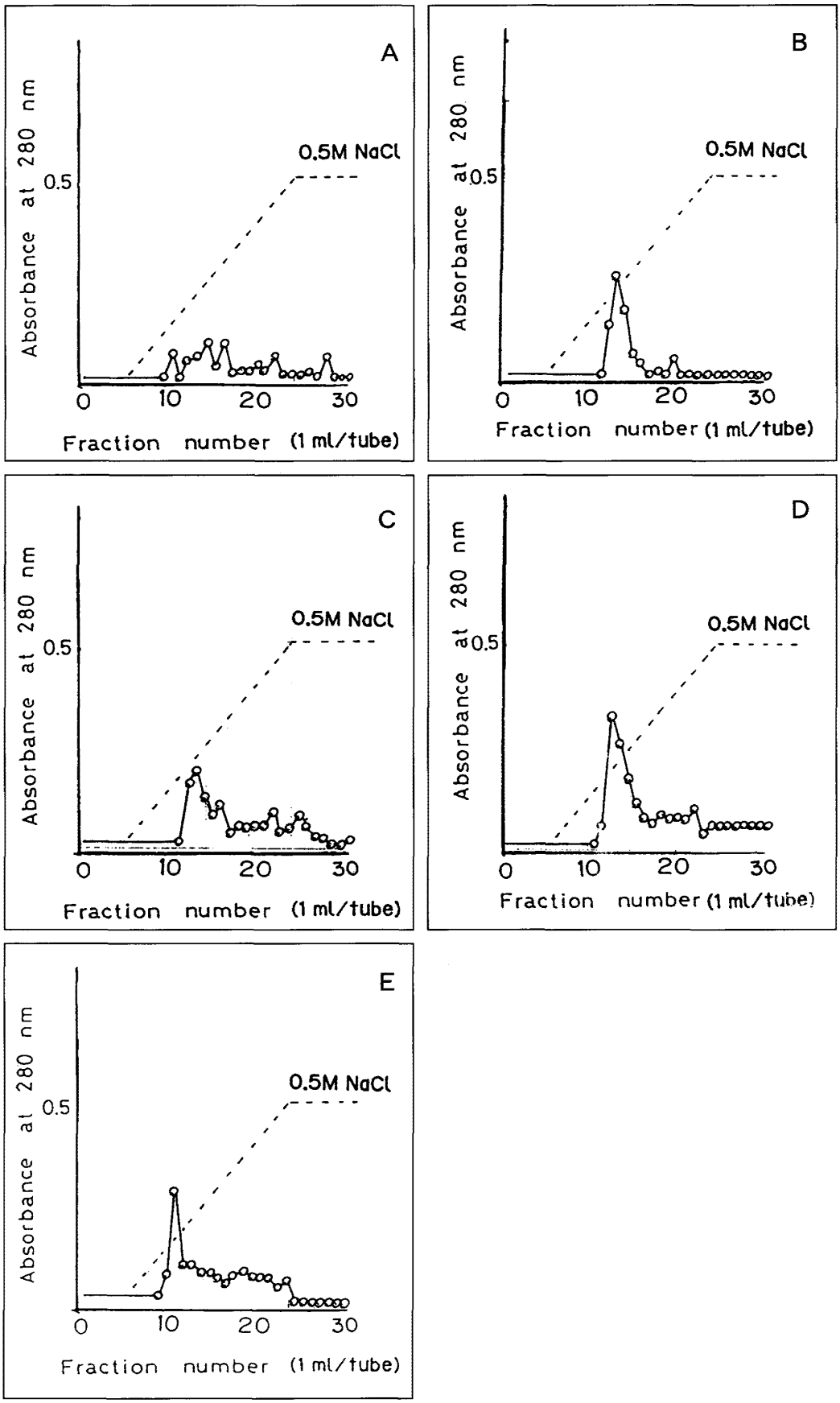

Fig. 3 Elution patterns of FPLC Mono Q chromatography
-Gel filtration fractions of supernatant of pupal fat body in the buffer-
A : No. 23~25, B : No. 28~32, C : No. 35~38, D : No. 50~53, E : No. 62~64.

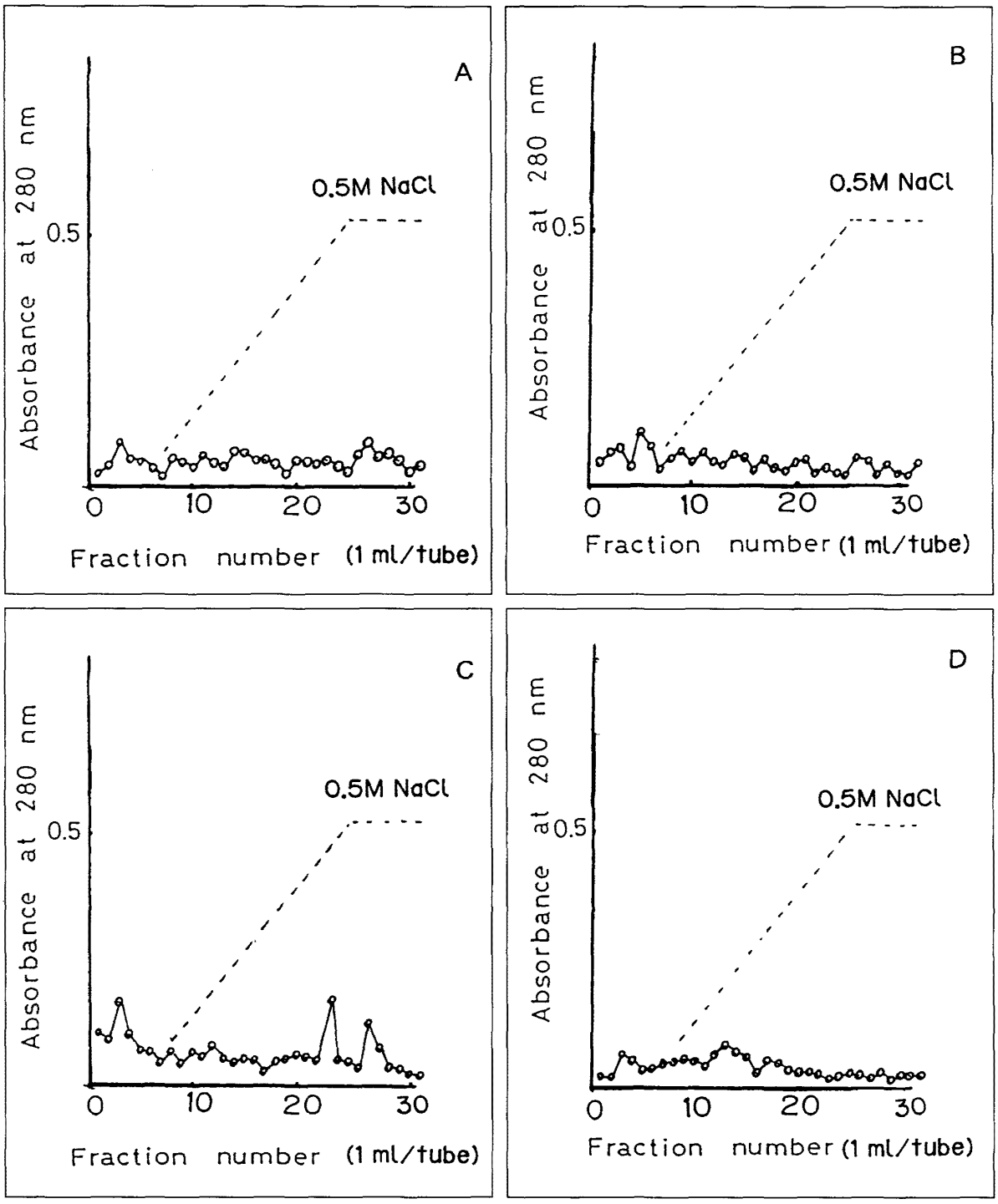

Fig. 4 Elution patterns of FPLC Mono Q chromatography
–Gel filtration fractions of precipitation of pupal fat body in the buffer containing 1% Triton X 100–
A: No. 23~25, B: No. 29~30, C: No. 56~60, D: No. 61~66.

val fat body eluted with the buffer containing 1% Triton X 100. The result showed that the chromatogram revealed a peak containing the enzyme showed as an arrow in the figure, as same as the supernatant fraction. Figure 6 shows the result of Mono Q ion exchange chromatography on the enzyme fraction of the larval fat body, on day 7 in the fifth instar, obtained by gel filtration. Figure 6-A shows the chromatogram of the fraction

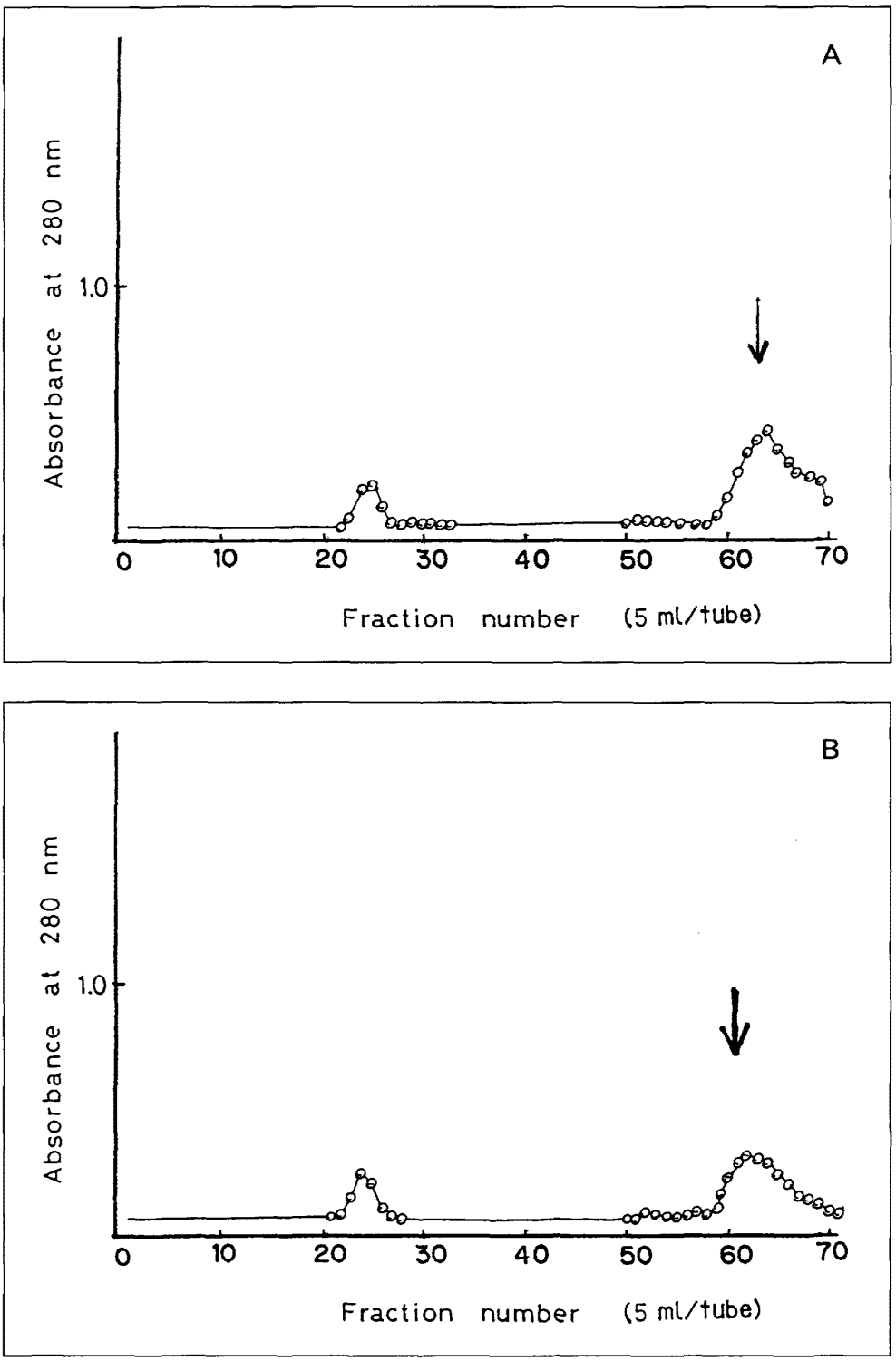

Fig. 5 Elution patterns of gel filtration on a Superdex 200 column
A : Supernatant of larval fat body on day 7 in the buffer containing 1% Triton X 100,
B : Precipitation of larval fat body on day 7 in the buffer containing 1% Triton X 100.

of the supernatant. The chromatogram revealed a peak containing the enzyme showed as an arrow in the figure. On the other hand, Fig. 6-B shows the chromatogram of the fraction of the precipitation. The chromatogram revealed no peak containing the enzyme. Namely, the supernatant fraction from the larval fat body, on day 7 in the fifth instar,

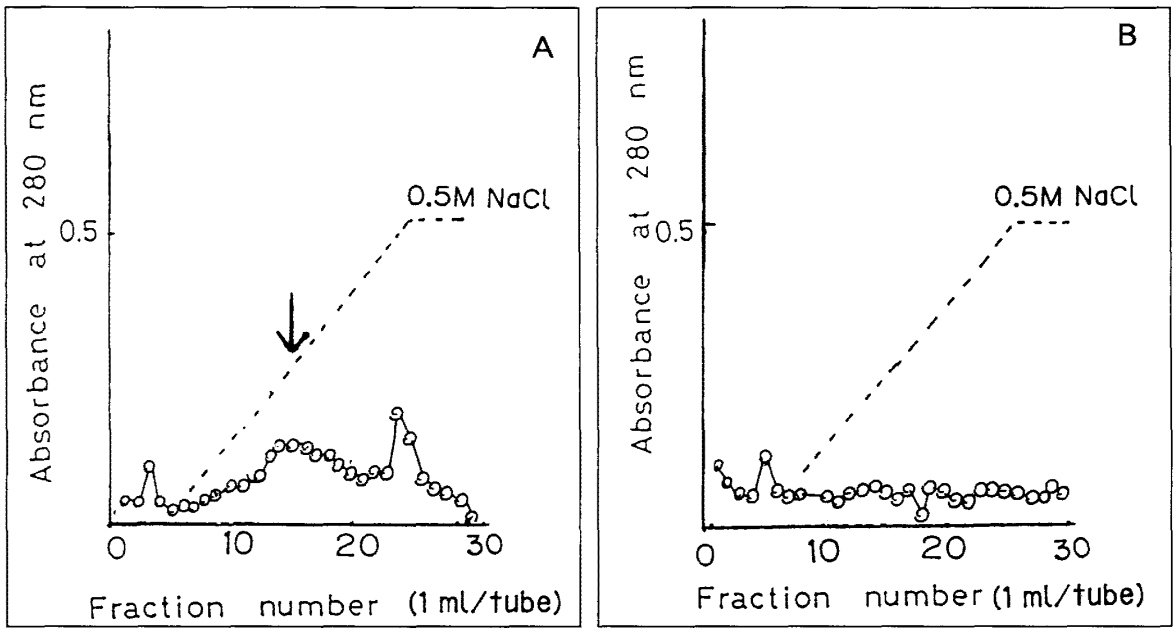

Fig. 6 Elution patterns of FPLC Mono Q chromatography
A: Gel filtration fraction (No. 62~66) of supernatant of larval fat body on day 7.
B: Gel filtration fraction (No. 60~65) of precipitation of larval fat body on day 7.

revealed a remarkable peak of the enzyme fraction. The fact showed the possibility that the change of neuraminidase activity due to the difference of time differed between larval haemolymph and fat body. This provides useful information for understanding the activation mechanism of the lectin-protein *in vivo*. On the other hand, it seemed needful to research again on the suitable condition for isolating the enzyme fraction from the precipitation fraction, because the precipitation fraction revealed no clear peak as shown in Fig. 6-B.

In future, we will try comparative studies between neuraminidase and galactosidase in the haemolymph and the fat body of *Bombyx mori*, because galactosidase seems needful to convert the inactive lectin form from the active lectin form as described in the previous paper (KATO and NAKAMURA, 1987).

SUMMARY

Both the supernatant fraction and the precipitation one from the fat body of *Bombyx mori* showed the highest optical density when the density of Triton X 100 was one percent in 0.1 M Tris-HCl buffer containing 0.1 M NaCl. Accordingly, it was studied on gel filtration eluted with the buffer containing 1% Triton X 100 on a column of Superdex 200. The result showed that this method was more effective than the former method for obtaining the enzyme fraction from the fat body by gel filtration. Moreover, more enzyme

fraction was obtained from larval fat body than from larval haemolymph on day 7 in the fifth instar by gel filtration. The fact showed the possibility that the change of neuraminidase activity due to the difference of time differed between the larval haemolymph and the fat body.

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