Chromatogram of Humoral Lectin of *Bombyx mori* by FPLC.

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INTRODUCTION

Biological phenomena based on the specific recognition of sugar chains have attracted much less attention in comparison with those of nucleic acids and proteins. Accordingly, the biological significance of glycoconjugates is not yet fully understood, and too many questions remain unanswered (KASAI and HIRABAYASHI, 1996).

A lectin-protein has the specific property reacting reversibly with a specific sugar residue. For this specificity, various animal lectins seem to play fundamental roles in the living body ; for instance, the function as specific endocytosis, the fluid defence factor, the opsonin-like action and so on (KAWASAKI, 1993).

On the other hand, invertebrate lectins play an important role in a non-specific self defence mechanism, as invertebrates do not synthesize specific antibodies (NATORI, 1991 ; KURATA, 1992 ; KOTANI *et. al*., 1995 a, 1995 b).

We recently reported that the humoral lectin (130 K-glycoprotein) played physiological role through metamorphosis of the silkworm, *Bombyx mori* (KATO *et al*., 1991, 1994). We also reported on the appearance and the disappearance of the lectin activity (NAKAMURA and KATO, 1994, 1995). However, it still remains obscure where the lectin-protein is produced and activated in the living body.

In the previous paper (NAKAMURA and KATO, 1996), we tried to confirm the tissue distribution of sialic acid relating to the humoral lectin activity in various tissues of *Bombyx mori*. The purpose of the present work is to make a comparative study of the lectin-protein obtained from haemolymph and the one obtained from fat body, by means of FPLC system. This investigation will provide useful information for understanding the production and activation mechanisms 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, haemolymph and fat body were collected daily. The haemolymph was collected by cutting pleopods and abdominal legs of larvae. The fat body was collected from the dissected larvae, 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 resultant supernatant was lyophilized.

2. Con A-Sepharose affinity chromatography

Freeze-dried sample was dissolved in 0.02 M Tris-HCl buffer solution (pH 7.4, 0.5 M NaCl) and applied to a column of Con A-Sepharose 4 B. After washing thoroughly with the same buffer solution, 0.3 M α-methyl-D-glucoside was added.

3. FPLC Mono Q ion exchange chromatography

The concentrated sample was applied on to a column of Mono Q HR 5/5 (Pharmacia, Sweden) previously equilibrated with 0.05 M Tris-HCl buffer solution (pH 8.0). Elution was performed with a linear gradient of 0 to 0.5 M or 1.0 M NaCl in 0.05 M Tris HCl buffer solution (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.

4. Superdex 200 gel filtration

Gel filtration on Superdex 200 pg (Pharmacia, Sweden) was performed using 0.05 M Tris-HCl buffer solution (pH 8.0) containing 0.5 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 2.5 ml per minute. Column eluates were collected in 4 ml fractions and measured at 280 nm.

5. SDS (sodium dodecyl sulfate) polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis (SDS-PAGE) of proteins on Phast Gel Gradient 10–15 was performed by means of Pharmacia Phast System. The proteins were located with Phast gel blue R.

RESULTS and DISCUSSION

Figures 1 and 2 show the results of Con A-Sepharose affinity chromatography. Figure 1-A illustrates the chromatogram of mature larval haemolymph, while an elution profile of the chromatogram is shown in Fig. 1-B for mature larval fat body. The humoral lectin-protein (130 K-glycoprotein) was thus obtained as the adsorbed fraction to Con A-Sepharose, and the product lyophilized after desalting with a Pharmacia Biotech PD-10 column containing Sephadex G-25 M. Moreover, Fig. 2-A shows the chromatogram of pupal haemolymph, while Fig. 2-B shows the chromatogram of pupal fat body.

Table 1 compares the amounts of each fraction. Fifty five mg of Con A-adsorbed fraction was obtained from 500 mg of the freeze-dried mature larval haemolymph, while 37 mg of the fraction was obtained from the mature larval fat body. Thus the mature larval haemolymph contained more Con A-adsorbed fraction than the mature larval fat body did. On the other hand, both of the fraction from the pupal haemolymph and one from the pupal fat body seemed similar amounts as shown in Table 1.

Figures 3 and 4 show the results of Mono Q ion exchange chromatography on the

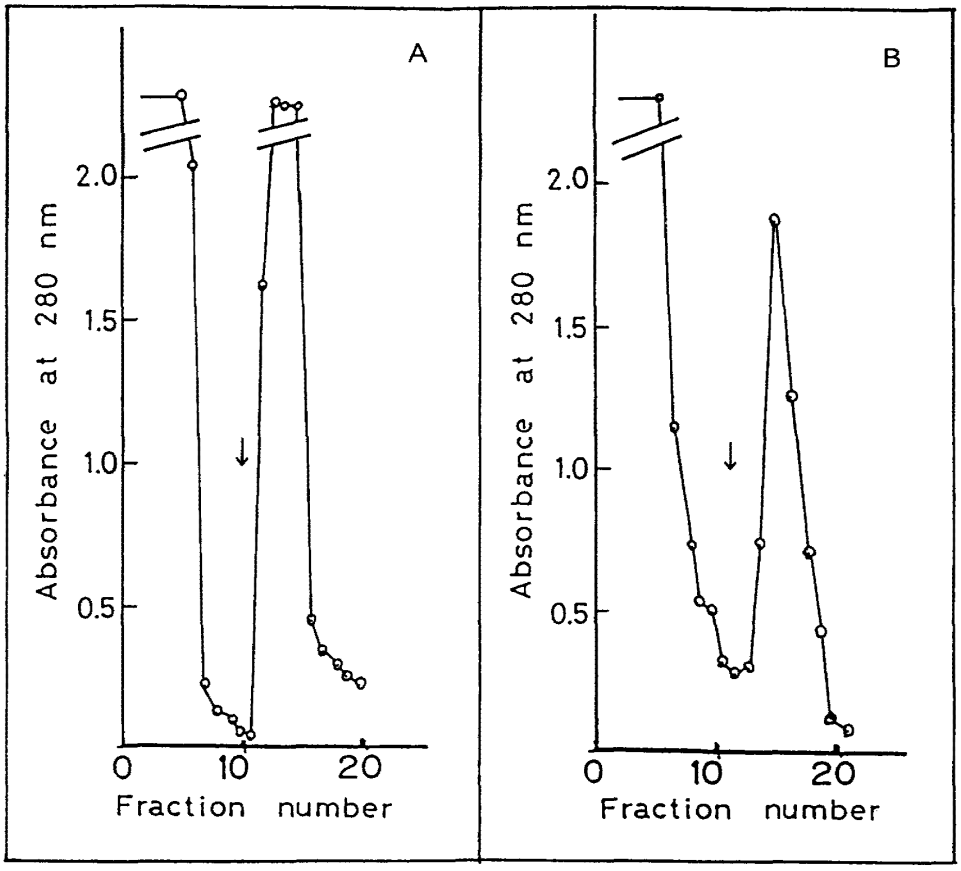

Fig. 1 Con A-Sepharose affinity chromatography of mature larvae
A : Haemolymph, B : Fat body

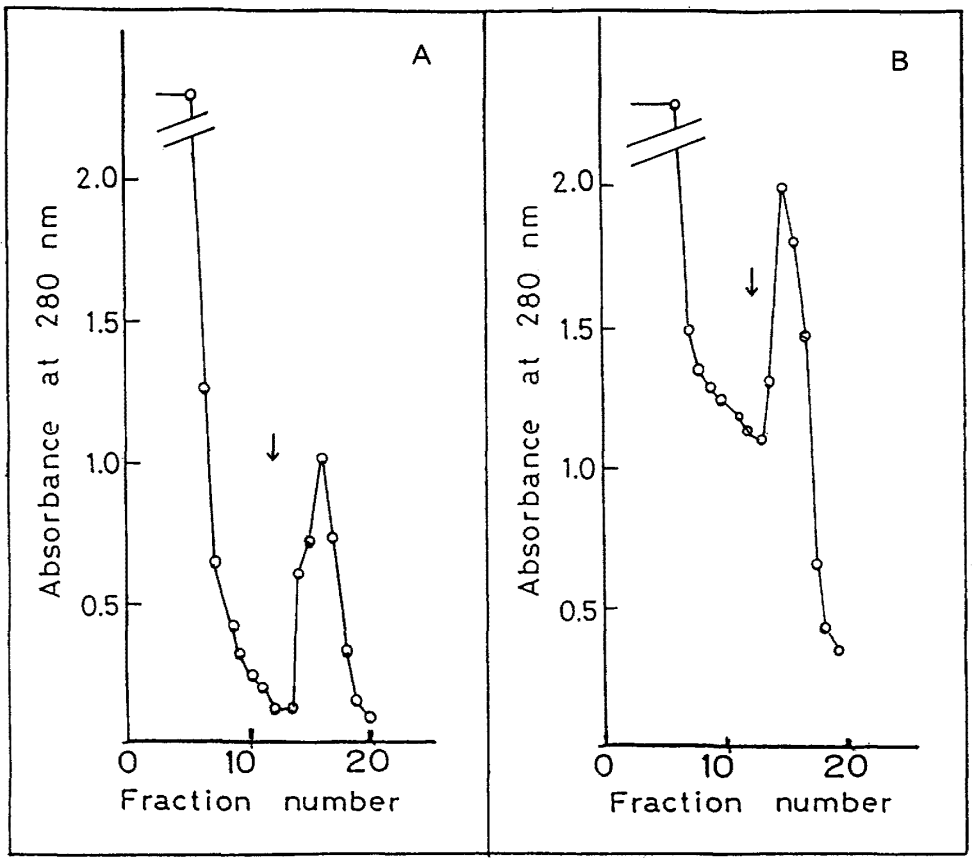

Fig. 2 Con A-Sepharose affinity chromatography of pupae
A : Haemolymph, B : Fat body

Table 1 Yields of the fraction obtained by Con A-Sepharose affinity chromatography

	Con A-adsorbed fraction	
	Haemolymph	Fat body
Mature larvae	55 mg	37 mg
Pupae	42 mg	42 mg

Con A-adsorbed fractions shown in Table 1, by means of FPLC system. After dissolved in 0.05 M Tris-HCl buffer solution (pH 8.0), each fraction was applied on to a column of Mono Q HR 5/5 previously equilibrated with the same buffer solution. Elution was performed with a linear gradient of 0 to 0.5 M or 1.0 M NaCl in 0.05 M Tris HCl buffer solution (pH 8.0). An elution profile of the chromatogram is shown in Fig. 3-A for the mature larval haemolymph. The chromatogram revealed a single peak at the salt concentration of 0.3 M. Figure 3-B shows the chromatogram of the mature larval fat body. The chromatogram also revealed a single peak at the salt concentration of 0.3 M. Namely, it seems that similar elution patterns were obtained. Furthermore, Fig. 4-A shows the chromatogram of the pupal haemolymph. The chromatogram revealed a single peak at

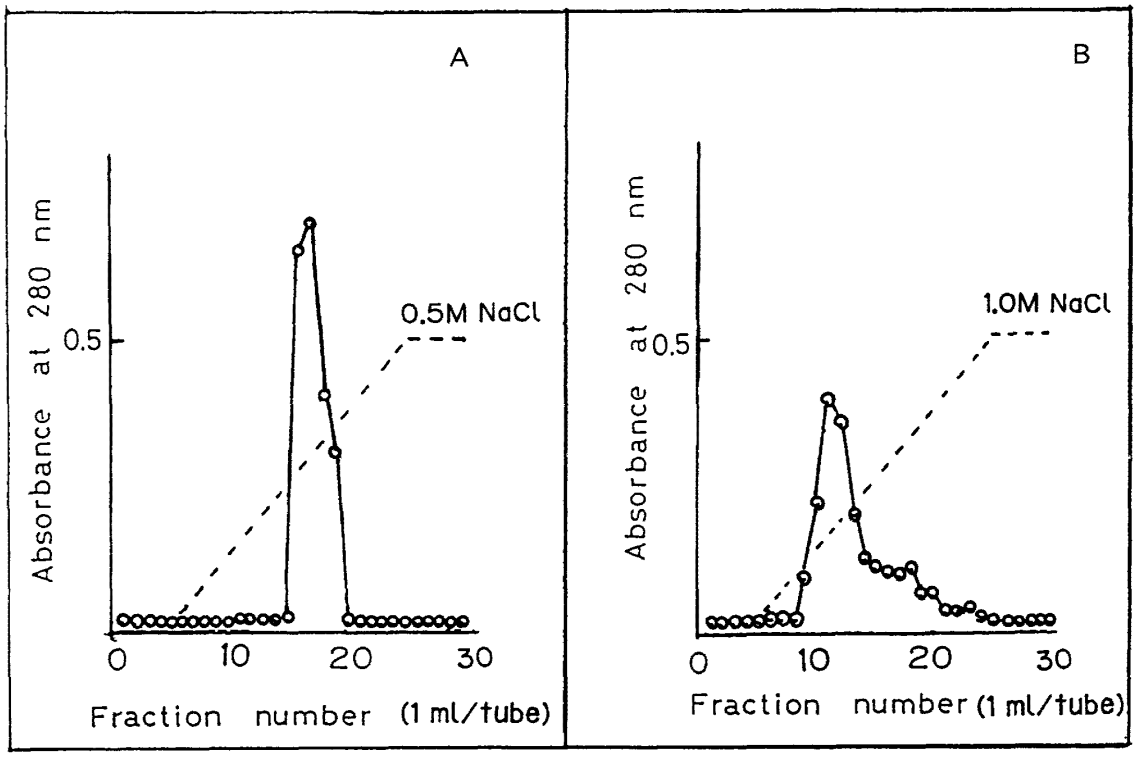

Fig. 3 FPLC Mono Q ion exchange chromatography of the Con A-adsorbed fractions of mature larvae
A : Haemolymph, B : Fat body

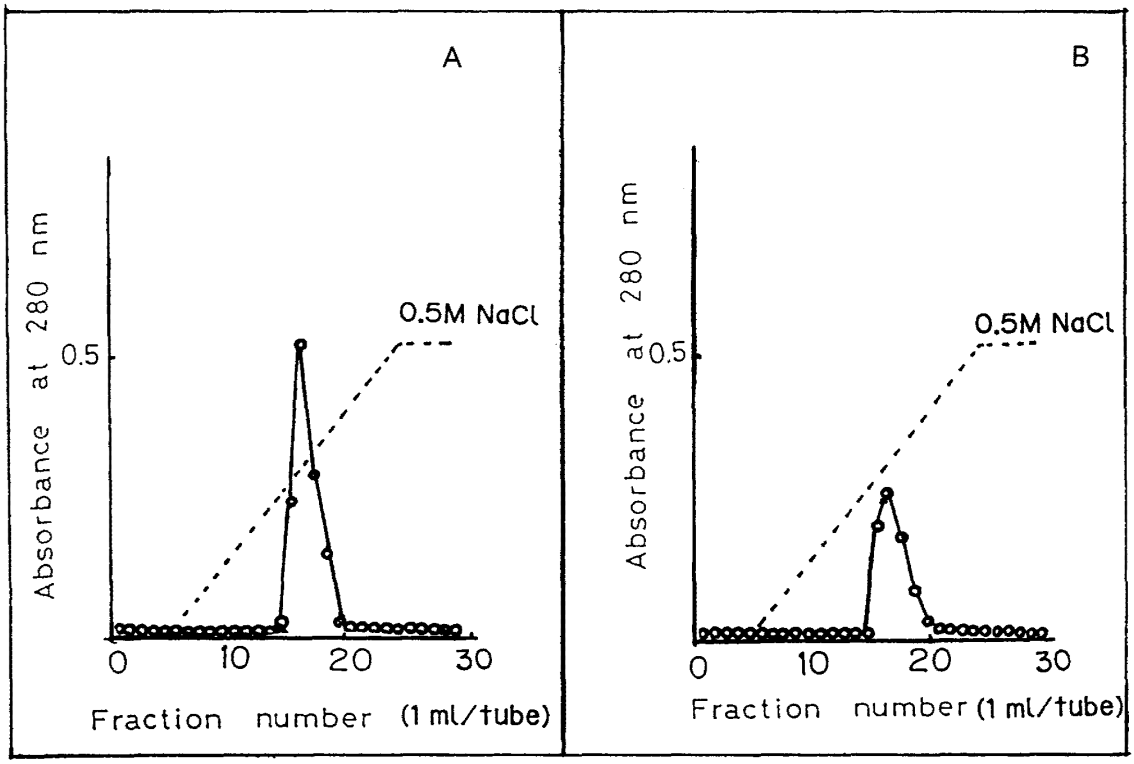

Fig. 4 FPLC Mono Q ion exchange chromatography of the Con A-adsorbed fractions of pupae
A : Haemolymph, B : Fat body

the salt concentration of 0.3 M. Figure 4-B shows the chromatogram of the pupal fat body. The chromatogram also revealed a single peak at the salt concentration of 0.3 M. Accordingly, it was evident that similar elution patterns were obtained on these occasions.

On the other hand, Superdex 200 pg gel filtration was tried on each Con A-adsorbed fraction shown in Table 1. A column of Superdex 200 pg was equilibrated with 0.05 M Tris-HCl buffer solution containing 0.5 M NaCl (pH 8.0) and eluted with the same buffer solution. As shown in Fig. 5, three fractions, Fraction I (Fr-I), Fraction II (Fr-II) and Fraction III (Fr-III), were obtained by gel filtration of the Con A-adsorbed fraction from the mature larval haemolymph. Gel filtration of Blue Dextrane 200 and four standard proteins (Ferritin ; MW 440,000, Aldolase from rabbit muscle ; MW 158,000, Ovalbumin ; MW 43,000, Ribonuclease A ; MW 13,700) was performed at the same condition. The result showed that Fr-III in Fig. 5 corresponded to the fraction containing the humoral lectin-protein (130 K-glycoprotein) of *Bombyx mori*. Accordingly, Mono Q ion exchange chromatography was performed on Fr-III, by means of FPLC system. The result shows in Fig. 6.

The chromatogram of Fr-III revealed a single peak at the salt concentration of 0.3 M as shown in Fig. 6. It was evident that the elution pattern was much almost same as one of Mono Q ion exchange chromatography on the Con A-adsorbed fraction from the

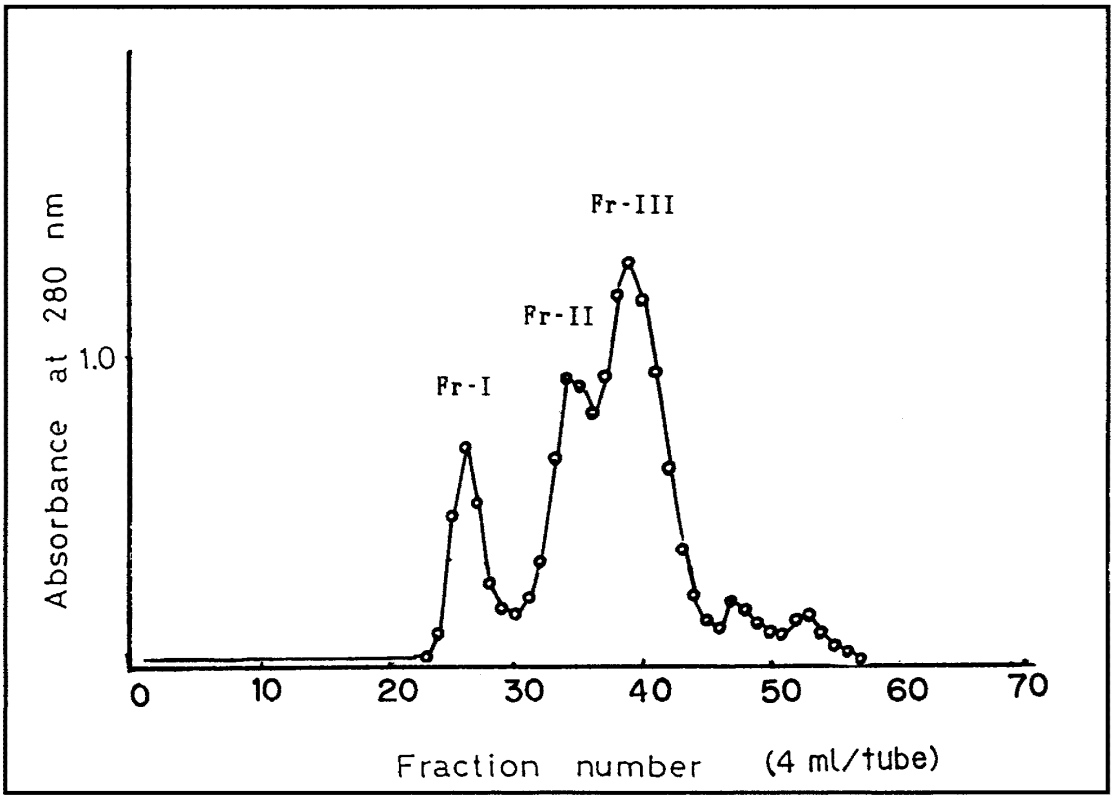

Fig. 5 Gel filtration on Superdex 200 pg column

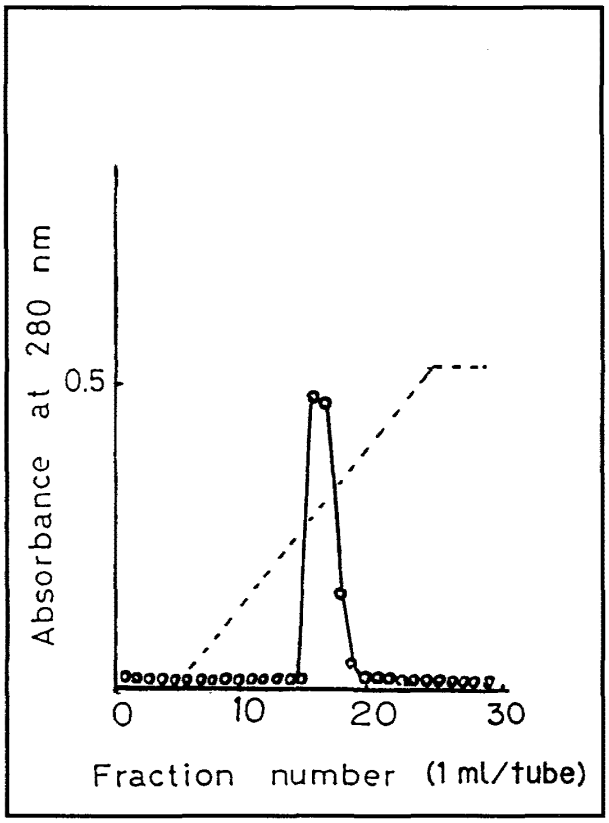

Fig. 6 FPLC Mono Q ion exchange chromatography of Fr-III

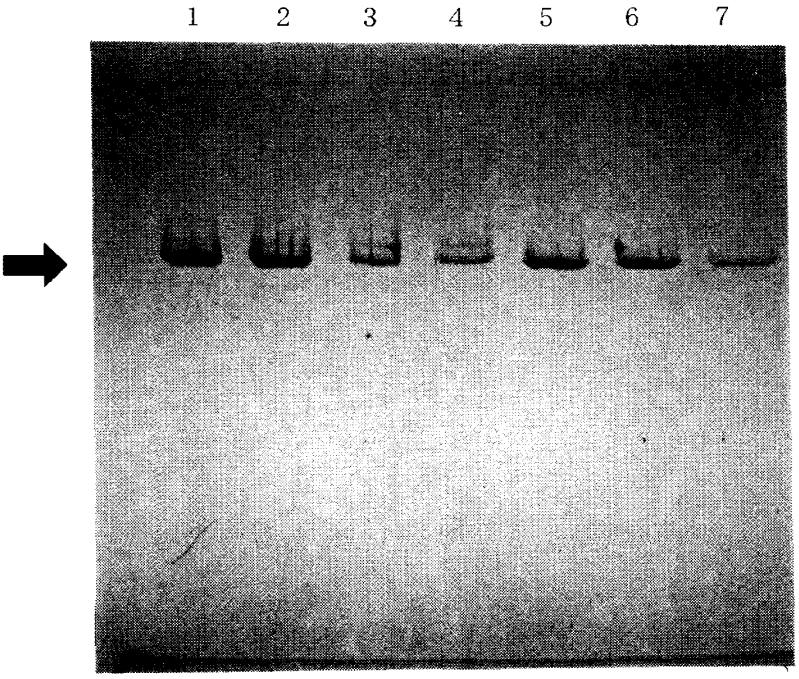

Fig. 7 Analysis of SDS-PAGE

haemolymph or the fat body in Figs. 3 and 4.

Figure 7 depicts the result of SDS-PAGE analysis on the fraction obtained by Mono Q ion exchange chromatography. Lanes 1~4 in Fig. 7 show the fractions obtained by

Mono Q ion exchange chromatography on the Con A-adsorbed fraction, while lanes 5～7 of the figure show the fractions obtained by Mono Q ion exchange chromatography on Fr -III. The result shows that each lane gave a single band at the position of the active 130 K-glycoprotein isolated from the control mature larval haemolymph. The fact was observed on the all Con A-adsorbed fractions shown in Table 1.

In conclusion, all chromatograms by means of FPLC system revealed similar patterns on the fractions obtained from the haemolymph and fat body of each periods shown in Table 1, and the result of SDS-PAGE analysis showed similar electrophoretic patterns on the fractions. Accordingly, we emphasize the possibility that the humoral lectin-protein (130 K-glycoprotein) is produced and activated in the fat body of *Bombyx mori*, and that it is moved into the haemolymph from the fat body. In future, we try to research some factors which activate the lectin-protein in the fat body, to provide a clue to its activation mechanism *in vivo*.

SUMMARY

Mono Q ion exchange chromatography was performed on Con A-adsorbed fractions from haemolymph and fat body of *Bombyx mori*, by means of FPLC system. The result showed that both of the chromatograms revealed similar patterns. On the other hand, Superdex 200 pg gel filtration was tried on the each Con A-adsorbed fraction and Mono Q ion exchange chromatography was performed on Fr-III obtained by means of gel filtration. The result showed that the elution pattern was much almost same as one of Mono Q ion exchange chromatography on the Con A-adsorbed fraction from the haemolymph or the fat body. Then, the result of SDS-PAGE analysis on the fractions showed that each lane gave a single band. These results strongly suggest that the humoral lectin-protein is produced and activated in fat body, and that it is moved into haemolymph.

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