Contribution of Glycosidases to Lectin Activity in Haemolymph of *Bombyx mori*  

Teruko NAKAMURA · Yasuo KATO

**INTRODUCTION**

Recently, various animal lectins have been of interest because they seem to play fundamental roles in the living body; for instance, the function as specific endocytosis in the liver or the macrophage, the fluid defence factor in the living body, the action of the activation for the complement, the opsonin-like action and so on (KAWASAKI, 1992). On the other hand, soluble lectins in the haemolymph of some invertebrates have been of interest for the defence and differentiation mechanisms of the living body because they seem to serve for Ig G (NATORI, 1991). In addition, KURATA (1992) emphasized the importance of insect lectins for the metamorphosis and self-non self recognition.

We reported previously (KATO *et al.*, 1991, 1994) that the lectin activity in the haemolymph of *Bombyx mori* may be influenced by the administration of terpenoid imidazole (KADONO-OKUDA *et al.*, 1987) and juvenile hormone analogue (KAJURA *et al.*, 1987). Moreover, it has been shown that the lectin played physiological role through metamorphosis of *Bombyx mori* because it seems to possess the highest activity on spinning day at all times. However, the activation mechanism of the lectin still remains obscure (NAKAMURA and KATO, 1994).

In the present investigation, we try to study the contribution of glycosidases to the lectin activity of *Bombyx mori*, in order to obtain further information on the appearance mechanism of the lectin activity *in vivo*.

**MATERIALS AND METHODS**

1. Preparation of samples

A hybrid race, Shunrei × Shogetu, of the silkworm, *Bombyx mori*, was used in this experiment. Thirty μg/larva of methoprene (JHA: Ohtsuka Pharmaceutical Co.) was applied topically to larval skin on the second day of the fifth instar. After the application
of JHA, the larvae were reared on mulberry leaves, while the control larvae were reared on mulberry leaves without application of JHA.

In preparing the samples for this research, larval haemolymph was collected daily. After centrifuging the haemolymph at 3,500 rpm for 15 min at 4°C, the supernatant that resulted was lyophilized.

2. High performance liquid chromatography (HPLC)

The sialic acid analysis was performed with a Shimadzu HPLC system (Murakita, 1987). A column of Shim-pack SCR 101H or ISA-07/S2504 was used. Elution was performed with aqueous solution of phosphoric acid (pH 1.5) and the flow rate was 1.0 ml per minute. Column eluates were monitored at 205 nm with an ultra violet spectrophotometric detector SPD-6A.

The neutral sugar analysis was also performed with a Shimadzu HPLC system which combined the boric acid complex anion exchange method and the post column fluorometric detection method using a mixture of arginine and boric acid as detection agent (Mikami and Ishida, 1983). A column of ISA-07/S2504 was used. Elution was performed with 0.3 M boric acid (pH 8.0) and the flow rate was 0.6 ml per minute. A mixture of 1% arginine and 3% boric acid as the reaction solvent was moved at a flow rate of 0.5 ml per minute and the reaction temperature was 150°C. Carbohydrates eluted from a column were treated with the reaction reagent, a mixture of arginine and boric acid, to produce highly fluorescence derivatives, and monitored with a fluorescence detector RF-530 (Ex. 320 nm, Em. 430 nm).

RESULTS AND DISCUSSION

Figure 1 illustrates the growth profile of larvae of Bombyx mori. The result indicated that JHA, methoprene, exerted effects on the growth of the fifth instar larvae, and that the larvae of Bombyx mori which were treated with methoprene (JHA, 30 µg/larva) on day 2 of the fifth instar characterized a prolonged feeding period. Figure 1 also shows the haemagglutination activity (the lectin activity), the content of sialic acid and the neuraminidase activity (the sialidase activity) in the haemolymph of the larvae treated with methoprene, relatively. They changed through the larval maturation as shown in Fig. 1. They kept low level on day 4 and on day 7, but they increased at the larval maturation. In fact, the neuraminidase activity increased at the larval maturation, but it decreased on spinning day. Especially, it was evident that the activity decreased remarkably just after maturation. The facts suggest that the lectin activity in the haemolymph became high.
Fig. 1. Relative growth profile of JHA-treated larvae (○) and control larvae (●).
- Sialic acid content
- Neuraminidase activity
- Haemagglutination activity

Days after 4th ecdysis

Fig. 2. Chromatogram of standard N-acetyl neuraminic acid (NANA) by using the ISA-07/S2504 column.

Fig. 3. Chromatogram of sialic acid in the larval haemolymph by using the ISA-07/S2504 column.
after the terminal sialic acid in the sugar chains of the lectin-protein was removed with the neuraminidase activated with the ecdyson *in vivo*.

N-acetyl neuramin lactose from bovine colostrum was used as substrate for assay of the neuraminidase activity in the haemolymph, and the activity was estimated according to determine free sialic acid separated from N-acetyl neuramin lactose treated with the haemolymph of *Bombyx mori*. Then, analysis of sialic acid was performed by means of high performance liquid chromatography (HPLC). The results indicated that the sialic acid existed in the larval haemolymph. Therefore, we tried to estimate the sialic acid in detail. Namely, the analysis of sialic acid was performed by using the ISA-07/S2504 column this time, while the analysis of sialic acid was performed by using the SCR 101H column until now. Figure 2 shows a chromatogram of N-acetyl neuraminic acid (NANA) obtained by using the ISA-07/S2504 column. NANA was used as standard sialic acid. The chromatogram revealed a peak caused by NANA, which had a retention time of 25.98 minutes. Figure 3 shows a chromatogram of sialic acid in the larval haemolymph by using the ISA-07/S2504 column. The chromatogram revealed a small peak caused by NANA, which had a retention time of 25.88 minutes. Figure 4 shows a chromatogram of sialic acid in the larval haemolymph by using the ISA07/S2504 column, when NANA was added to the haemolymph sample. The chromatogram revealed a larger peak caused by NANA, which had a retention time of 26.14 minutes. The results indicated that the sialic acid existed in the larval haemolymph, exactly. Accordingly, it was confirmed that the neuraminidase contributed to the activation of the lectin-protein in the haemolymph of *Bombyx mori*.

Figure 5 (A) and Fig. 5 (B) show chromatograms of neutral sugars of the active lectin and the inactive one obtained by means of HPLC, respectively. Both of them showed some peaks corresponding to maltose, L-rhamnose, D-mannose, D-arabinose, D-galactose, D-xylose and D-glucose, respectively. The content of galactose seems to decrease, judging from the two chromatograms. However, this analysis was performed on the
equivalent amount of glycoproteins. Therefore, the active lectin contained total neutral sugars more than the inactive one. Accordingly, it is considered that the content of galactose decreased as shown in Fig. 5. Moreover, the active lectin seemed to contain total proteins less than the inactive one by means of Lowry method (Lowry et al., 1951), while the active lectin seemed to contain total hexoses more than the inactive one by means of Dubois method (Dubois et al., 1956).

Next, a chromatogram of the active lectin in the insect saline revealed no peak caused by neutral sugars. A chromatogram of galactosidase in the insect saline revealed no peak caused by neutral sugars, too. Then, the active lectin was pretreated with the galactosidase solution for 1 hr at 37°C and analysis of neutral sugars was performed by means of HPLC. The result showed that the chromatogram revealed a peak caused by \( \alpha \)-galactose. It seems to coincide with the result described in the previous paper (Nakamura and Kato, 1989). These facts suggest the possibility that the terminal sugar in the sugar-chains of the active lectin was \( \alpha \)-galactose. Moreover, we studied on the relationship between \( \alpha \)-galactose and the lectin activity. The result showed that the haemagglutination activity of the galactosidase-treated section was weaker than one of the control section. It seems to coincide with the result described in the previous paper (Nakamura and Kato, 1990). These facts indicate that \( \alpha \)-galactose influence on the haemagglutination activity of the lectin-protein, and that the reason why the active lectin
changed into the inactive one is because the terminal  β-galactose in the sugar chains was removed with galactosidase.

In the present investigation, we have shown the possibility that the appearance and the disappearance of the lectin activity were controlled with neuraminidase and galactosidase, because these enzymes seemed to be activated with the ecdyson in the mature larval haemolymph of *Bombyx mori*.

**SUMMARY**

The contribution of glycosidases, neuraminidase and galactosidase, to the lectin activity in the haemolymph of *Bombyx mori* was investigated. The neuraminidase activity was estimated according to determine sialic acid separated from N-acetyl neuramin lactose treated with the haemolymph of *Bombyx mori*, by means of high performance liquid chromatography (HPLC). Namely, the analysis of sialic acid was performed by using the ISA-07/S2504 column this time, while the analysis of sialic acid was performed by using the SCR 101H column until now. On the other hand, the galactosidase activity was estimated according to determine the content of β-galactose in the active lectin and in the inactive one by HPLC, comparatively. The results suggested that neuraminidase and galactosidase were activated with the ecdyson in the mature larval haemolymph of *Bombyx mori*, therefore that the appearance and the disappearance of the lectin activity were controled with these enzymes.

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**REFERENCES**


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