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Satiety Substance Supposed in the Hemolymph of the Prawn *Penaeus japonicus*

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Abstract

To investigate a satiety substance in the hemolymph of the prawn *P. japonicus*, chemical analysis of saccharide group under different molting stages and feeding conditions was done using paper and thin-layer chromatographies. Hemolymph patterns of saccharides, regardless of the individual prawn's condition, showed a prominent glucose, and an especially weak reaction which corresponded to maltotriose.

The oligosaccharide was identified as maltotriose by the thin-layer chromatography of hemolymphs collected from four prawns. These prawns were injected with glucose solution through the oesophagus into the stomach cavity and kept alive for 4.5 h.

In contrast to glucose, maltotriose showed an inhibitory effect on feeding during the postmolt period after its abdominal injection.

Further, maltotriose indicated an accelerative effect on the nerve cells in the ventro-anterior portion of the suboesophageal ganglion, because of the increase in their nucleo-cytoplasm ratio after its abdominal injection.

As a result, the oligosaccharide maltotriose seemed to perform as a satiety substance which originated in the midgut gland of the prawn.

Feeding pattern of the prawn *P. japonicus* has indicated some existence of a humoral regulator for the feeding activity performed by the nervous mechanism[1]. This function is related to the concentration of some specific nutriment in the hemolymph. Such a humoral regulator named commonly as satiety substance has been studied mainly in mammals, and corresponds to glucose, 2-deoxytetronic acid or TRH-like tripeptide, pGlu-His-Gly-OH[2,3]. For the invertebrates, there have been few reports about this subject.

In this report for the prawn *P. japonicus*, the detection of the satiety substance was treated for hemolymph saccharides, and its effects to feeding behaviour or nerve cells related to the feeding mechanism were investigated by abdominal injection of the saccharide.

Materials and Methods

The prawns *Penaeus japonicus* used in the present experiments were about 10 g.

To detect the occurrence of a satiety substance, chemical analyses by paper and thin-layer

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chromatographies were undertaken for the hemolymph saccharides of prawns reared under different feeding conditions, fed and starved, or prawns of different molting stages, inter-, pre- and postmolt. The solvents of chromatographies were BuOH : acetic acid : H_2O (3 : 1 : 1). After repeated treatments of development at room temperature, they were subjected to the color development with anilin-phthalic acid at 105℃. For the thin-layer chromatography, avicel plates were used. As for rearing, prawn pellets were given quantitatively for individuals during feeding experiment. Some prawns were also reared under starvation of 10 days. Temperature during these rearings was 20. 0-23. 5℃. Hemolymphs were sampled by cutting the tail of each prawn. Before chromatographic analysis, 4 % trichloroacetic acid was added to the hemolymph of the individuals, centrifuged to exclude proteins, and then, passed through an ion-exchange resin to obtain the desalted solution.

To identify the oligosaccharide, maltotriose, as the satiety substance, hemolymphs were collected from four prawns treated with an oral injection of glucose solution. 1 M solution of glucose was injected into the stomach cavity upto its capacity, via the oesophagus. The injection was inserted into the mouth using a guiding vinyl-tube. The prawns were wrapped in a moist cloth and left as was in a somewhat stationary condition for 4. 5h. Then their hemolymphs were subjected to the thin-layer chromatography as previously stated. For reference, the culture medium of the extirpated stomach together with the midgut gland and intestine were similarly treated. The end of the intestine was tied to prevent the outflow of injected solution. For the control, the hemolymphs of normal prawns were applied.

Secondary rearings to observe the feeding pattern were undertaken quantitatively for molted prawns which were injected with maltotriose solution into their abdominal muscle. This seemed to occur in the hemolymph and related to the nutritional condition of individual prawns. The concentrations were 0. 7 mM, 7 mM and 70 mM. For comparison of the effect, injections of 200 mM glucose and 200 mM glycine were undertaken. Injection volume of each solution was constantly 2 % of the body weight. Feeding was set just after the daily injection at around 23 : 00-24 : 00. After 3 days of the daily injection, rearing was continued without the treatment. Feeding consumptions during injected and non-injected periods were compared to detect the inhibitory effect of the injection on the feeding. Temperature during the experiment was 22. 5-24. 0℃.

Further, injections of maltotriose into the abdominal muscle of prawns were treated to determine its influence on the nerve cells in the suboesophageal ganglion. The concentration of the solution was 70 mM ; and 200 mM glucose was prepared for reference. Injected volume of each solution was the same as previous treatment. After injections of each solution, samplings were done at 0, 5-6, 8 and 9-10 h. Non-injected individuals sampled at 0 and 8 h were treated as the control specimen. Temperature during the experiment was the same as the previous injection. For the histological observation, the suboesophageal ganglions of the prawns were extirpated at each sampling time, and immersed in Bouin's fixative. They were dehydrated with BuOH series, then, embedded in paraffin for sectioning. After staining with PAS-hematoxylin, the preparations were observed for the estimation of cellular activity using a micrometer. Since it was considered that the injection would affect the nerve cell's function related to the feeding mechanism supposed in the

suboesophageal ganglion, and if the saccharide had a satiety character for the prawn, its influence would be expected to result in the morphological change of the cell as the variance of the nucleo-cytoplasm ratio dealt in previous report[4]. The cells chosen were large-typed ones of the ventro-anterior portion of the ganglion, due to their distribution near mouth-parts appendages and measuring convenience.

Results and Discussion

Paper chromatographic pattern of hemolymph saccharides was shown in **Fig. 1.** Glucose

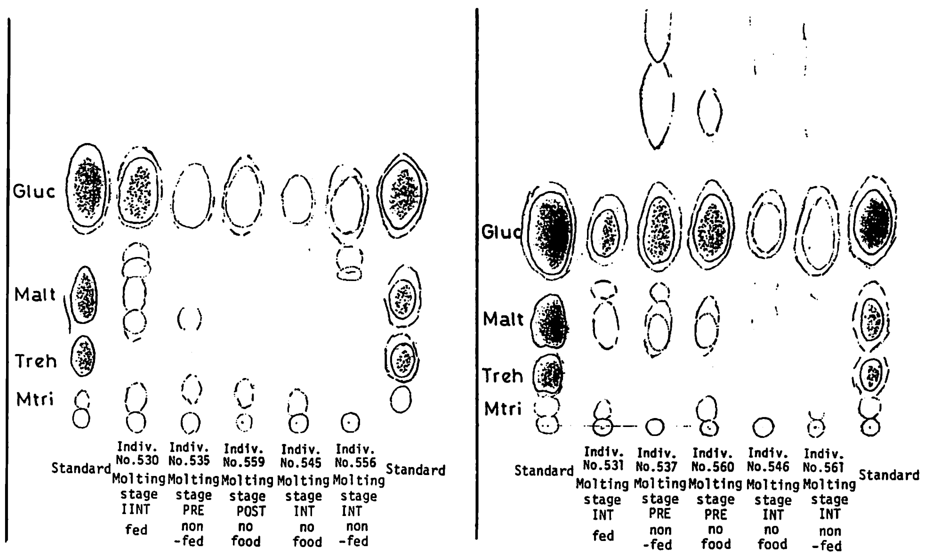

Fig. 1. Paper chromatographic patterns of hemolymph saccharides of individual prawns under different molting stages and feeding conditions. Gluc, glucose ; Malt, maltose ; Mtri, maltotriose ; Treh, trehalose.

was a prominent constituent of hemolymph saccharide of the prawn, regardless of the molting stage and feeding condition. Trehalose was undetected, differing from its dominant appearance in insects[5]. And trehalose in insects was in some cases thought to function as satiety substance like the blood glucose in mammals. In spite of its extremely weak reaction, oligosaccharide occurred near the individual origin. Its occurrence did not show any relation to the molting stage nor feeding condition, at least, in this chromatogram. Based on its Rf value, it seemed to be maltotriose. Indentification of this substance was achieved from the thin-layer chromatography as shown in **Fig. 2.** Hemolymph of the prawns injected with glucose yielded a substance corresponding to maltotriose Rf (Hem in **Fig. 2**). However, medium after cultivating the extirpated organs or hemolymph of the control did

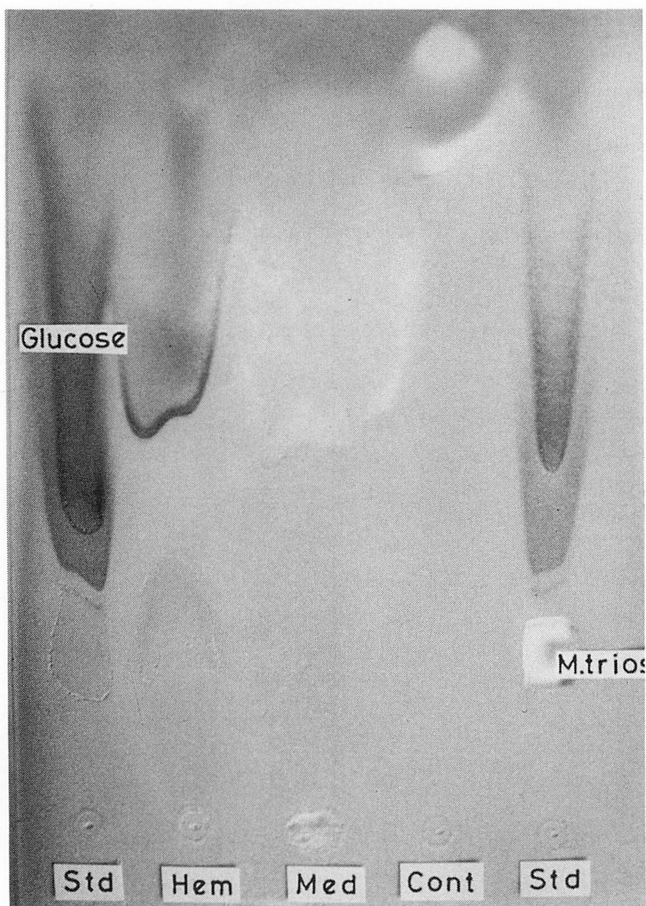

Fig. 2. Thin-layer chromatographic patterns for saccharides of hemolymph of the normal prawn (Cont), or glucose-injected prawn (Hem), and of the medium after cultivation of the extirpated stomach filled with glucose solution and provided with the midgut gland (Med). Std, standard.

not show such a substance (Med or Cont in **Fig. 2**, respectively). It might be deduced that maltotriose-like substance resulted from absorbed glucose in the midgut gland, and absence in the specimen Med indicated the unsuitable condition for conversion of glucose to oligosaccharide by the midgut gland.

Comparison of the feeding patterns of prawns injected with different concentrations of maltotriose, glucose and glycine was shown in **Fig. 3.** Inhibitory effect on feeding was recognized only in prawns injected with 70 mM of maltotriose solution. For glucose injection, that effect was not detected. Therefore, glucose was not likely to be the satiety substance in the prawn.

The cells for the measurement of the nucleo-cytoplasm ratio were 40-70 μm. The injection effect of maltotriose and glucose on the value of the measurements was represented in **Fig. 4.** The value increased gradually after the maltotriose injection, in contrast to a decrease or no change for glucose injection or the control, respectively. It suggested that the cellular function had been elevated by injected oligosaccharide. That is, if the cell acted as inhibitor of the feeding mechanism in the suboesophageal ganglion, its acceleration of function would bring about the suppressed feeding, likewise observed in the previous result. Hereupon, it was supposed that the function of the large-typed cells in the ventro-anterior portion of the suboesophageal ganglion, which belonged to the inhibitory component of the feeding mechanism of the prawn, would be regulated by maltotriose-like substance which originated in the midgut gland and performed like a satiety substance.

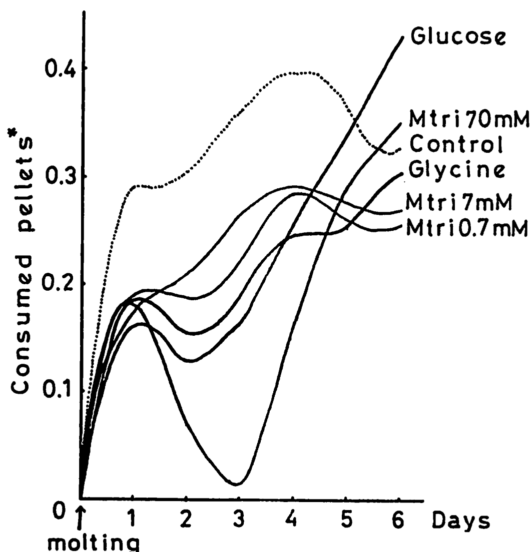

Fig. 3. Feeding patterns of the prawns injected individually with the solution of 70 mM, 7 mM or 0.7 mM maltotriose (Mtri 70 mM, Mtri 7 mM or Mtri 0.7 mM, respectively), as well as 200 mM glucose or 200 mM glycine. Non-injected control is represented as dotted line. Each injection has been treated during 3 days after the molting. *diurnally consumed pellets (unit: g)

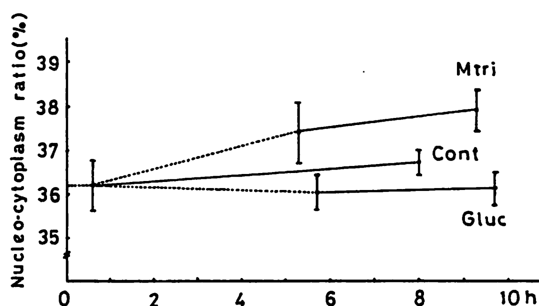

Fig. 4. Injection effect on the nucleo-cytoplasm ratio* of the large cell of the ventro-anterior of the suboesophageal ganglion.
*Nuclear diameter x100/its cytoplasmic diameter. Cont, non-injected; Gluc, 200 mM glucose injected; Mtri, 70 mM maltotriose injected.

References

1) NAKAMURA, K. (1986): Feeding and its mechanism related to midgut gland of the prawn *Penaeus japonicus*. *Bull. Japan. Soc. Sci. Fish.* (submitted).

2) MAYER, J. and N. MARSHALL (1956): Specificity of gold thioglucose for ventromedial hypothalamic lesions and hyperphagia. *Nature*, **178**, 1399.

3) OOMURA, Y. and K. YAMABE (1982): Feeding and appetite. in "Mechanism of Feeding Behaviour" (ed. by H. MORITA and K. KUBOTA), Sangyo-tosho, Tokyo, pp. 1-25 (in Japanese).

4) NAKAMURA, K. (1986): Biological clock supposed in the optic ganglion of the prawn . *Bull. Japan. Soc. Sci. Fish.* (accepted).

5) JEUNIAUX, C. (1971): Hemolymph-Arthropoda. in "Chemical Zoology" (ed. by M. FLORKIN and B. T. SCHEER), Vol. 6, Academic Press, New York and London, pp. 63-118.