Involvement of lectin-like factors in larval settlement and metamorphosis in the abalone, Haliotis discus hannai

Tohoku Journal of Agricultural Research
Vol. 51 No. 3-4, March 2001
Printed in Japan

Involvement of lectin-like factors in larval settlement and metamorphosis in the abalone, *Haliotis discus hannai*

Takeshige MATSUTANI, Kaoru MORISHITA,
Tetsuo SEKI*, and Katsuyoshi MORI

Laboratory of Aquacultural Biology
Graduate shchool of Agricultural Science, Tohoku University,
Sendai 981-8555, Japan
**National Research Institute of Aquaculture*
Nansei Mie, 561-0108, Japan

(Received, Mar. 2, 2001)

Summary

The planktonic larvae of the gastropod mollusk *Haliotis discus hannai* are effectively induced to settle and metamorphose by contact with the seed collecting plate which was treated with the abalone mucous trail and the algae, such as the diatom, Cocconeis spp.. This process appears to be medated by lectins, because monosaccharide, glucose or mannose blocked the metamorphosis and larvae did not metamorphose on the substrate treated with the lectin, concanavalin A. It was suggested that there was the Con A binding site on the surface of the substrates, and the sugers on the substrates might play an important role in the metamorphosis of the larvae.

Many sessil invertebrates have larvae that are free-swimming until they are ready or competent to metamorphose. The larvae of *Haliotis* have been investigated very well. The larvae of *Haliotis rufescencs* living in the coast of California U.S.A. were induced to settle and metamorphose by γ-aminobutyric acid (GABA) (1). And it was reropted that there were GABA-mimetic inducers at the surfaces of crustose red alge (2). On the other hand GABA did not induce to settle and metamorphose in the larvae of *Haliotis discus hannai*, and only made to stop the velum of the larvae (3). *Haliotis diversicolor* was not induced to settle by GABA (4).

The settlement and metamorphosis of larvae of *Haliotis discus hannai* were reported to be induced by mucous trail secreted by crawling adult abalone (5) or sessil microalgae (6). In order to utilize this effect, the seed collecting plate (SCP) are produced, and the planktonic larvae are effectively induced to settle and metamorphose by contact with the SCP. In this study, we suggest that the

mechanism of induction of settlement and metamorphosis by the SCP, mucous trail and sessil microalge involves lectin-like factors.

Materials and Methods

Larvae, substrates, microalgae, and assay

Larvae of the abalone *Haliotis discus hannai* were obtained from Oyster Research Institute Moune Research Center, Miyagi Prefecture. The larvae used had been maintained until the total effective temperature (the index of larval development,) reached 1,500~1,600°C•h. The SCP (substrate), mucous trail and sessil microalgae such as *Cocconeis sculttum*, Carteria sp. and *C. scutllum* were used. These algae were obtained from Tohoku National Fisheries Research Institute and Oyster Research Institute.

Assays were conducted in 6 wells tissue culture plate (Corning). The microalgae were sessiled on the bottom of the well. The mucous trail were obtained by putting three young abalone into the each well overnight. Thirty to fifty veligers were placed into each well containing 5 ml of ultraviolet rays-irradiated sea water (UVSW), and the number of the individuals settling and metamorphosing were recorded after certain time.

Effects of heat and proteolytic enzymes

The heat treatment was conducted at 25–100°C. The substrates were exposed to UVSW for 30 min. The proteolytic enzymes used were trypsin, papain, and pepsin. The substrates were exposed to each enzyme dissolved in UVSW (1.0 mg/ml, pH 7.4). The substrate was rinced with UVSW three times after the enzyme-treatment.

Effects of sugars and lectins

It was suggested that the inducer of settlement and metamorphosis of larvae might be sugar, so the settlement and metamorphosis of the larvae on sugar-treated substrates was investgated. Processes mediated by lectins are blocked by some sugars. Various sugars were added to UVSW containing the larvae in order to test whether settlement and metamorphosis could be inhibited or not.

Effects of Concanavalin A (Con A)

The substrates used were mucous trail and sessile maicroalgae. The substrates were sessiled on the non-flourescent glass slides. Each sbstrate was exposed for 30 min. at 18°C to 50 ml of FITC-Con A (50 μg/ml) in Tris-buffered artificial seawater (pH 8.0, 20 mM Ca) and was observed by fluorescent microscope after rinse with the Tris-buffered artificial seawater.

Results

heat and proteolytic enzymes

The mucous trail and sessil maicroalgae induced the settlement and metamorphosis of the larvae. The rate of settlement and metamorphosis on each substrate after 48h were 98.4±3.2%, 74.1±17.4% on the mucous trail, 76.4±15.4%, 59.7±18.9% on *Cocconeis scutllum*, and 86.4±3.0%, 15.4±5.8% on *Carteria* sp.. On the control without those substrates, the rate was less than 5%.

The substrates which had been exposed to high temperatue (90°C) for 30 min. did not lose their effect (Fig. 1). Heat stability of each substrates were high. Moreover the proteolytic enzymes did not affect of efficancy of the substrates (Fig. 2). These results may suggest that inducer is not a high molecular protein.

Next, the possibility that sugars are implicated in the mechanism was tested, because the mucous trail and cell wall of sessile microalgae contain polysaccaride.

sugars and lectins

Glucose or mannose inhibited larval metamorphosis on the mucous trail. Metamorphosis was normal in the presence of D(+)-galactose, D(+)-fucose, D(−)-arabinose, D(+)-ribose, D(+)-xylose, D(+)-glucuronic acid, D(+)-glactonic acid and N-acetyl-D-glucosamine at a concentration of 25 mM (Fig. 3). Only mannose significantly inhibited larval metamorphosis on SCP and the sessil microalgae. Inhibition of metamorphosis increased with higher glucose concentrations (Fig. 4).

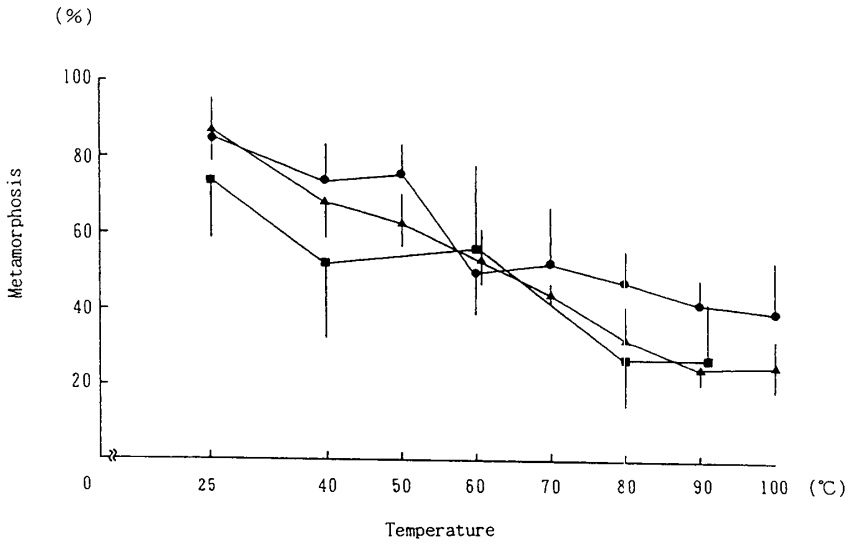

FIG. 1 Heat stability of metamorphosis-inducing factors in each substrates.
●, mucous trail (after 48 h); ▲, S.C.P. (after 48 h); ■, *Cocconeis scutllum* (after 96 h).
Each value represents the mean±S.E. of six experiments.

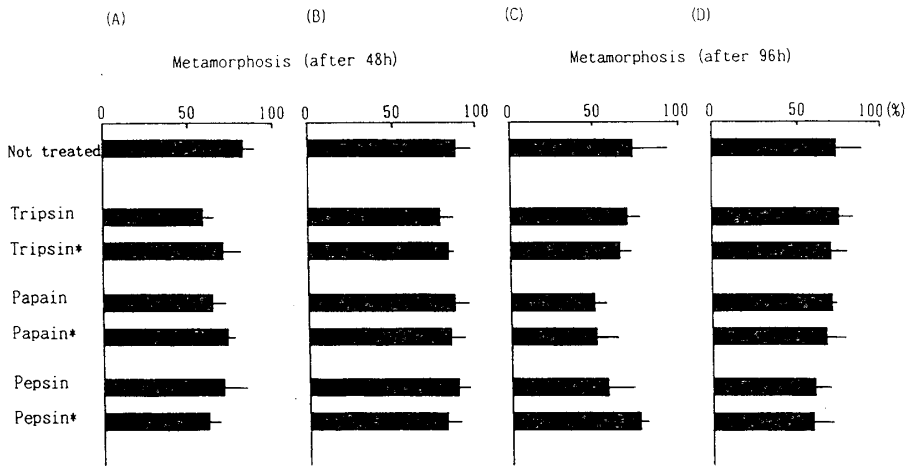

FIG. 2 Effects of proteolytic enzymes on metamorphosis induced by mucous trail (A), S.C.P. (B), *Carteria* sp. (C) and *Cocconeis scutllum* (D). Each value represents the mean±S.E. of six experiments. * ; Treated with 100°C, 30 min.

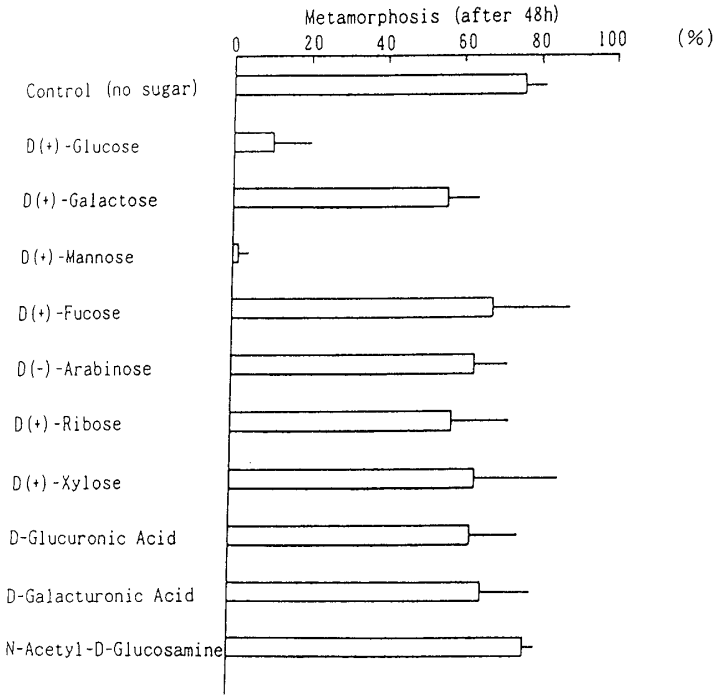

FIG. 3 Inhibition of mucous trail-induced metamorphosis by simple sugars. Each value represents the mean±S.E. of six experiments.

Concanavalin A (Con A)

Con A inhibited larval metamorphosis on the mucous trail and the sessil microalgae, but not on the SCP (Fig. 5). So, inhibition of metamorphosis by glucose was tested in detail. In the larvae metamorphosis, peristomal shell formation, was inhibited, but settlement and velum abscission were normal (Fig.

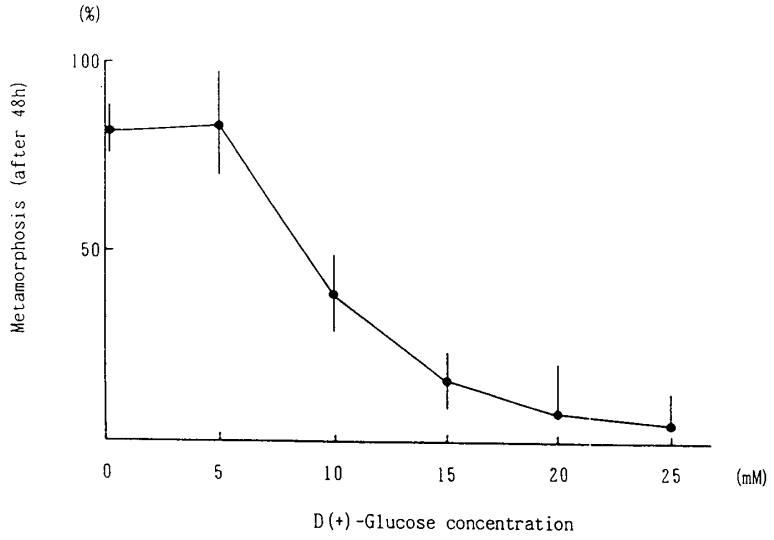

FIG. 4 Effect of D(+)-glucose on metamorphosis induced by mucous trail. Each value represents the mean±S.E. of six experiments.

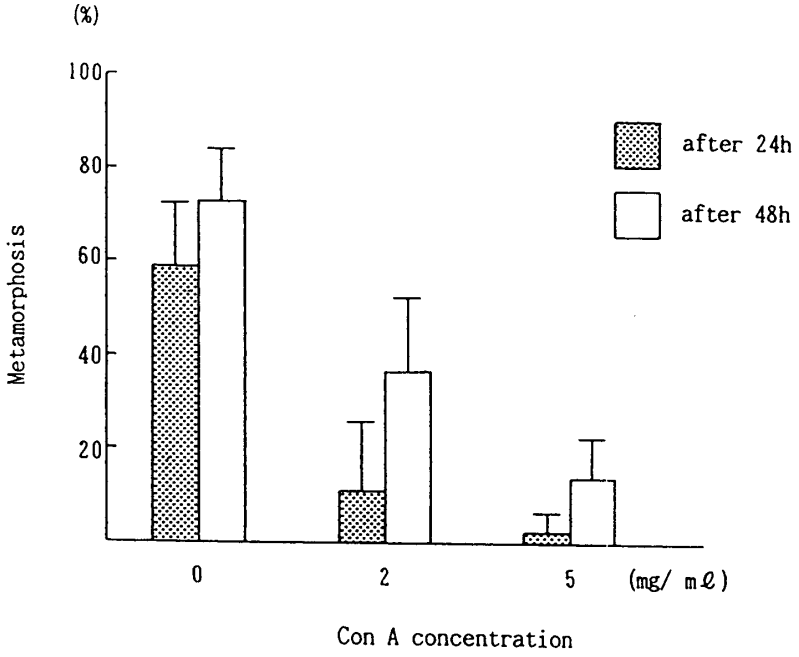

FIG. 5 Effect of Con A concentration on metamorphosis induced by mucous trail. Each value represents the mean±S.E. of six experiments.

6). In investigation using FITC-Con A, Con A-biding site was not observed in the mucous trail, but there were Con A-biding sites in the sessil microalgae, *Cocconeis scutllum* and *Carteria* sp.. It was, therefore, suggested that there were sugars with affinity for Con A on the sessil microalgae.

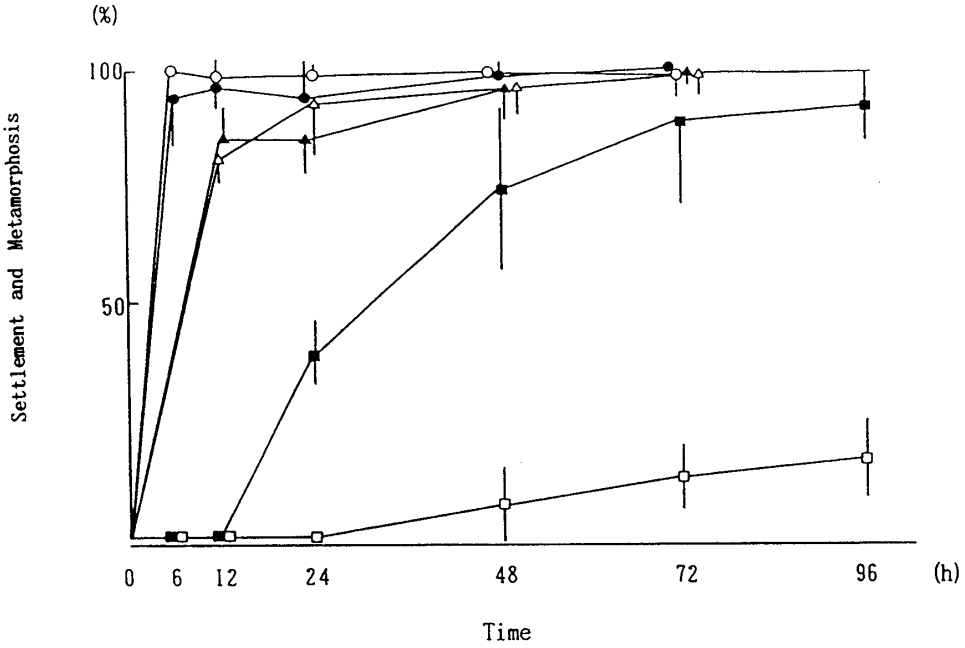

FIG. 6 Effect of D(+)-glucose (Glc) on settlement and metamorphosis induced by mucous trail. Settlement; ●, no Glc and ○, 25 mM Glc. Velum abscission; ▲, no Glc and △, 25 mM Glc. Peristomal shell formation; ■, no Glc and □, 25 mM Glc.
Each value represents the mean±S.E. of six experiments.

Discussion

It was suggested that mechanism of induction of settlement and metamorphosis in larvae of *Haliotis discus hannai* might involves Concanavalin A-binding sites, because the inducers contained in mucous trail and sessil microalgae were stable for heat and proteolytic enzymes. Larvae of spirobid polychate *Janua brasiliensis* settles and metamorphosis on surfaces coated with bacteril films. It was suggested that settlement and metamorphosis of the *Juana* was induced by extracellular polysaccharide (7).

In *Haliotis discus hannai* D(+)-glucose and D(+)-mannose inhibited the larval metamorphosis but velum abscission was not inhibited. Larvae may produce species-specific lectins that bind to polysaccharides or blycoprotrains in substratum on which the larvae settle and metamorphose. The metamorphosis involving peristomal shell formation in larvae of *Haliotis discus hannai* was inhibited by some simple sugars but the settlement and velum abscission process was normal. Inducer may be different from that in *Junua brasiliensis*. In the control, D(+)-glucose and D(+)-mannose did not induce the larval settlement and metamorphosis.

The larval metamorphosis was inhibited by the treatment with Con A, and glucose and mannose were present on surface of substrates used. Therefore these

sugars on the substrates may play an important role in metamorphosis in larvae of *Haliotis discus hannai*.

References

1) Morse, D.E., Hokker, N., Duncan, H. and Jensen, L., γ-Aminobutyric acid, a neurotransmitter, inducers planktonic abalone larvae to settle and begin metamorphosis. *Science*, **204**, 407-410 (1979)
2) Morse, A.N.C. and Morse, D.E., Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surfaces of crastose red algae. *J. Exp. Mar. Biol. Ecol.*, **75**, 191-215 (1984)
3) Akashige, S., Sseki, T., Kanno, H. and Nomura, T., Effects of γ-Aminobutyric acid and some neurotransmitters on induction settlement and metamorphosis in larvae of *Haliotis discus hannai*. *Bull. Tohoku Reg. Fish. Res. Lab.*, **43**, 37-45 (1981) (in Japanese)
4) Liu, L.L., Chu, L.S., Chang, K.H. and Chia, F.S., Negative effect of γ-Aminobutyric acid (GABA) on the settlement of larvae of the small abalone, *Haliotis diversicolor* Sepertexta Lischke. *Bull. Inst. Zool., Academica Sinica*, **25**, 1-5 (1986)
5) Seki, T. and Kan-no, H., Induced settlement of the Japanese abalone, *Haliotis discus hannai*, veliger by the mucous trails of the juvenile and abult abalones. Bull. Tohoku Reg. Fish. Res. Lab., **43**, 29-36 (1981) (in Japanese)
6) Kawamura, T, Asano, M., Kikuchi, S and Inoguchi, N., Sessilmicroalgae as substrate for settlement in the larvae of *Haliotis discus hannai*. *Bull. Tohoku Reg. Fish. Res. Lab.*, **53**, 194 (1991)
7) Kirchman, D., Graham, S., Reich, D. and Mitchell, R., Lectins may mediate in the settlement and retamorphosis of *Janua* (Dexiospira) *brasiliensis*. *Mar. Biol. Lett.*, **3**, 131-142 (1982)