Starfish Gonad: Action and Chemical Identification of Spawning Inhibitor

Abstract. *The gamete-shedding substance obtained from the radial nerves induces spawning when it is applied to the gonads of mature starfish in vivo and in vitro. A substance that inhibits the action of this spawning factor is present in both ovary and testis; it has been isolated from testis of* Asterina pectinifera *and chemically identified as* L-*glutamic acid.*

That spawning of starfish is induced by the action of a gamete-shedding substance present in the radial nerves has been well established (*1*). The active substance, a polypeptide having a relatively low molecular weight (*2*), seems to be liberated into the body cavity in which the gonads are suspended (*3*) and then to act on the ovary to free the ovarian eggs from adherence to each other, or to the gonadal wall, by dissolution of the intercellular cementing substance among the eggs (*4*). The discharge of eggs from the gonopore takes place by contraction of the gonadal wall, which cannot occur before this cement is dissolved. On the other hand, it is known that the gonad fails to react to the shedding substance earlier in the breeding season; it becomes more reactive toward the end of the season. This fact suggests that a mechanism that controls spawning operates also within the gonad. We now describe experiments designed to elucidate this mechanism; they revealed the presence of a spawning inhibitor in the gonad and identified it chemically.

Isolated ovaries and testes of *Asterias amurensis* early in the breeding season were separately lyophilized and then homogenized in sea water; they were centrifuged for 50 minutes at 30,000*g*. The supernatants were serially diluted, at concentrations of (dry tissue) from 40 to 2.5 mg/ml, with sea water containing the gamete-shedding substance (*5*).

For the assay, ovarian fragments, isolated from starfish at the height of the breeding season, were placed in small petri dishes containing these solutions, and the degree of their spawning was observed. The result showed that both ovary and testis contain some substance that inhibits spawning, thus opposing the action of the gamete-shedding substance (Fig. 1): dry ovary or testis at 10 mg/ml effectively inhibits the action of the gamete-shedding substance at the concentration used. This finding was confirmed in another species, *Asterina pectinifera*: dry ovary at 1.3 to 5 mg/ml or dry testis at 2.5 to 10 mg/ml was effective.

This spawning inhibitor was next purified by use of the testis of *A. pectinifera* early in the breeding season, since extract of testis contains fewer contaminating substances than does that of ovary. Acetone powder (167 g), obtained from lyophilized testes of 269 starfish, was extracted with cold 0.5*M* sodium chloride and centrifuged for 1 hour at 25,000*g* and 2°C. After heating to 80° to 90°C for 8 minutes, the supernatant was again centrifuged (1 hour at 77,000*g* and 2°C). To the supernatant, concentrated to 200 ml with a rotary evaporator kept below 45°C, 500 ml of cold methanol was added before centrifugation for 1 hour at 25,000*g* and 2°C. The supernatant was concentrated and then fractionated by gel-filtration on a Sephadex G-25 column (3.9 × 110 cm) in 0.1*M* sodium chloride (fraction size, 15 ml; 3°C).

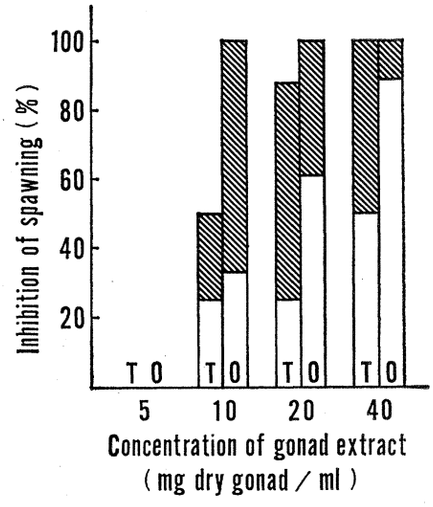

Fig. 1. Inhibition of spawning by extracts of ovary (*O*) and testis (*T*) in *Asterias amurensis*. Ovarian fragments were placed in sea water containing various concentrations of gonad extract together with nerve extract (10 μg of lyophilized nerve per milliliter). Shaded columns show incomplete inhibition. Percentage inhibition was calculated from the results of 18 tests for ovary extract and eight tests for testis extract.

Portions (0.2 ml) taken from each fraction were diluted with sea water (3.8 ml) containing the shedding substance, and their activity in inhibition of spawning was assayed against ovarian fragments taken from starfish cultured in cold circulating sea water in the laboratory.

The active fractions, collected and concentrated, were fractionated on a Sephadex G-15 column (2.5 × 89 cm) in 0.01*M* sodium chloride (fraction size, 15 ml; 3°C). The concentrated active fraction was applied again to the same Sephadex G-15 column in order to lower the salt concentration (fraction size, 5 ml; 3°C). The resultant active fraction, totaling 20 ml, was dried and dissolved in 10 ml of 0.01*M* phosphate buffer (*p*H 8.0) containing 2 percent *n*-butanol, and fractionated on a DEAE-Sephadex A-25 column (2.5 × 96 cm; 23°C) equilibrated with the same buffer by stepwise elution with sodium chloride (six steps from 0.1 to 1.0*M*). The active fractions were collected and centrifuged. The supernatant was again gel-filtered on a Sephadex G-10 column (2.5 × 95 cm) in 0.06*M* ammonium acetate buffer (*p*H 5.6) containing 1 percent *n*-butanol (fraction size, 4 ml; 23°C). The active fractions, containing little inorganic phosphate and sodium chloride, were collected and concentrated. After sublimation of the ammonium acetate under partial vacuum (10^{-2} mm-Hg) at 40°C, addition to the concentrate of 95 percent ethanol resulted in formation of colorless, fine, needle-like crystals (105 mg). These were further twice recrystallized with the ethanol-water system, and 64 mg of the purified material (platy crystal) was obtained. Determination of the spawning-inhibitive activity of this sample, by serial dilution of the solution, showed that the minimum effective dose lay between 4.4 and 44 μg/ml, against 20 μg/ml for lyophilized nerve.

The purity of this sample was next examined in various ways. Thin-layer chromatography [Kieselgel G (*Merck*); solvents: 63 percent *n*-propanol, and *n*-butanol–acetic acid–water, 3 : 1 : 1 by volume], paper chromatography (*n*-butanol–acetic acid–water, 12 : 3 : 5 by volume), and paper electrophoresis (*p*H 3.5, 0.1*M* pyridine-acetate buffer) each gave a single spot with ninhydrin spray. Further analysis, with an amino acid autoanalyzer (*6*) without previous

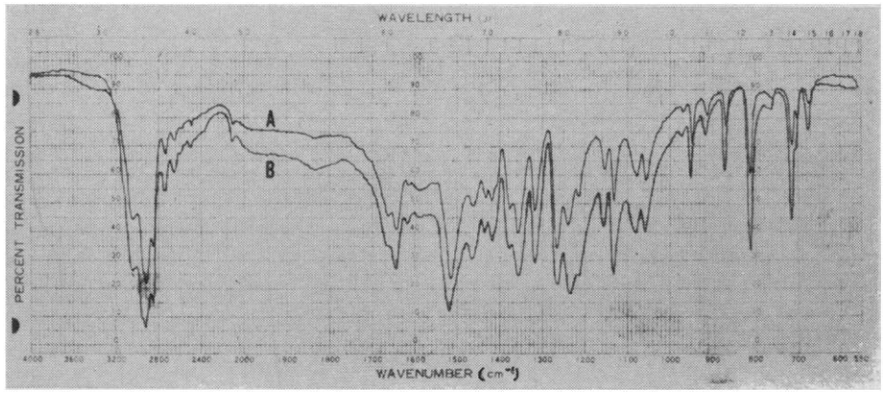

Fig. 2. Infrared spectra of spawning inhibitor obtained from *Asterina* testis (A) and of L-glutamic acid (B) in Nujol.

hydrolysis, revealed that the sample was pure and contained nothing but glutamic acid.

This identification was next checked by comparison of the sample with authentic L-glutamic acid. Amino acid–autoanalyzer diagrams of the sample and of the sample mixed with standard L-glutamic acid both showed single peaks at the same position. The infrared-absorption spectrum (Fig. 2) and the nuclear magnetic resonance spectrum of the sample were in good agreement with those of the authentic L-glutamic acid. Both sample and standard showed the same optical rotation: $[\alpha]_D$, +29° (6*N* HCl, 25°C). All these results clearly showed that the purified sample was L-glutamic acid.

Furthermore biological assay of the authentic L-glutamic acid revealed that 5 to 50 μg/ml effectively inhibited the activity of 20 μg of lyophilized nerve per milliliter in inducing spawning. This activity corresponded well with that of the purified sample. The effectiveness of amino acids other than glutamic acid was also examined by use of the following 18 amino acids: glycine, alanine, serine, cystein, threonine, valine, methionine, leucine, isoleucine, phenylalanine, tyrosine, proline, hydroxyproline, trytophane, aspartic acid, arginine, lysine, and histidine. Ovarian fragments of *A. pectinifera* were placed in sea water containing each of these amino acids at a concentration of 10^{-2} *M* together with nerve extract (20 μg/ml), and the degree of spawning was observed. None of the amino acids except aspartic acid had inhibitive effect (the inhibitive activity of aspartic acid was less than 10 percent of that of glutamic acid).

Finally determination with the amino acid autoanalyzer of the contents of free amino acids in the acetone powder of testis material of *A. pectinifera*, by use of a trichloroacetic acid extract (*7*), showed that 4.9 μg of glutamic acid and 0.2 μg of aspartic acid were present in 1 mg of the acetone powder. Inhibition of spawning by testis extract (2.5 to 10 mg of dry tissue per milliliter) can therefore be explained by the inhibitive action of glutamic acid contained in the testis. Although free aspartic acid also is present in starfish gonad as we have explained, its low content and its low activity in inhibiting spawning suggest that the representative inhibitor of spawning in the testis of *A. pectinifera* is L-glutamic acid. Considering the normal proportion by weight of testis to radial nerve (about 90 : 1) in a starfish, only a part of the glutamic acid present in the gonad seems to act as an inhibitor of spawning under natural conditions. The mechanism of this inhibition remains unknown.

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5. Sea water containing gamete-shedding substance was prepared as follows: several milligrams of lyophilized radial nerves were homogenized in a few milliliters of deionized water and centrifuged for 1 hour at 30,000*g*. The supernatant was diluted with sea water to a concentration of 10 μg of lyophilized nerve per milliliter.
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8. Detailed results of this study will be reported elsewhere.
9. We thank H. Shirai for technical assistance, J. C. Dan and H. A. Bern for encouragement and advice, and the director and staff of Asamushi Marine Biological Laboratory where part of this work was carried out.

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Staurolite: Sectoral Compositional Variations

Abstract. *Electron-microprobe analyses across six successive sections of a single staurolite crystal show that the staurolite has three crystallographically controlled sectors, each with a distinctive content of Al, Si, Ti, Fe, Mg, and Mn. The sector distribution of elements raises the question of whether the staurolite acts as one or several phases during growth. Total analyses of the separate sectors suggest that: (i) the staurolite has tetrahedral Al substitution for Si, and (ii) the hydroxyl content varies beween sectors.*

Electron-microprobe analyses of staurolite from the Kwoiek area, British Columbia, indicate that the individual staurolite crystals have markedly different compositions between sectors, which result from growth in different crystallographic directions; the compositions within any one sector are relatively homogeneous. This finding contrasts with compositional zoning patterns of garnet from the same rocks, which can be interpreted in terms of a depletion model (*1*). Both minerals are from the assemblage chlorite-garnet-biotite-staurolite-ilmenite-quartz-plagioclase-graphite.

The volumes of the several sectors are defined by chiastolite-type patterns of inclusions, often described in staurolite (*2*). Figure 1, modified from Harker (*2*), illustrates in three dimensions the sectors in staurolite that have the crystallographic forms (010), (110), and (001). In the following discussion,