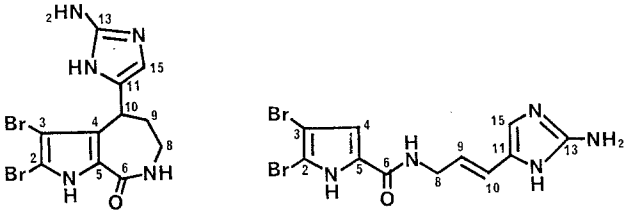

1 2

Structures of hymenin **1** and oroidin **2**.

and dibromophakelin[16] from the marine sponge *Phakellia flabellata,* and hymenialdisine[11] from the marine sponge *Hymeniacidon aldis*. Hymenin **1** exhibits a potent α-adrenoceptor blocking activity. In the isolated rabbit aorta, the contractile response to norepinephrine (10^{-7}M) was abolished by **1** (10^{-6}M), whereas the responses to potassium chloride (4×10^{-2}M) and serotonin (10^{-6}M) were not affected by **1** (10^{-6}M). The only α-adrenoceptor blocking agent of marine origin which has previously been found is aaptamine from the marine sponge *Aaptos aaptos*[17]; the molecular skeleton of this substance is benzo-naphthyridine, and is therefore quite different from that of **1.** In addition, hymenin is an antibacterial agent, giving 12 and 13 mm zones of inhibition at a concentration of 10 μg/disc against *Bacillus subtilis* and *Escherichia coli,* respectively. Further clarification of the stereochemistry and the pharmacological properties of **1** is in progress.

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10 Oroidin **2** was previously isolated with keramadine from the Okinawan sponge *Agelas nemoechinata* and the ^{1}H and ^{13}C NMR spectra were as follows; ^{1}H NMR (DSMO-d_6, 70 °C) δ 3.96 (2 H, dd, J = 3.9, 5.6 Hz, H–8), 6.18 (1 H, dt, ABX_2, J = 3.9, 16 Hz, H–9), 6.19 (1 H, d, AB, J = 16 Hz, H–10), 6.82 (1 H, s, H–15), 6.93 (1 H, s, H–4), 7.27 (2 H, brs, H–13), 8.43 (1 H, t, J = 5.6 Hz, H–7) and 12.4 (3 H, brs, H–1, H–12, H–14); ^{13}C NMR (DSMO-d_6) δ 39.8 (d, C–8), 97.8 (s, C–3), 104.4 (s, C–2), 110.8 (d, C–15), 113.0 (d, C–9), 116.2 (d, C–4), 124.7 (s, C–11), 126.8 (d, C–10), 128.0 (s, C–5), 147.5 (s, C–13) and 158.6 (s, C–6).
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Aplysianin-A, an antibacterial and antineoplastic glycoprotein in the albumen gland of a sea hare, *Aplysia kurodai*

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Summary. Aplysianin-A, an antibacterial and antineoplastic factor in the albumen gland of the sea hare *Aplysia kurodai,* was isolated. It had a molecular weight of approximately 320 kD and consisted of subunits with a molecular weight of 85 kD. It contained 9.8% neutral sugar. Aplysianin A showed 50% inhibition of *Bacillus subtilis* growth at a concentration of 4 μg protein/ml and 50% lysis of murine MM46 tumor cells at 14 ng protein/ml. A partial identity of antigenic specificity of the purified specimen with an antineoplastic factor from *Aplysia* eggs was observed in immunodiffusion tests.
Key words. Antibacterial factor; antineoplastic factor; *Aplysia;* opisthobranch; albumen gland.

Sea hares, which are opisthobranch molluscs, have attracted the interest of many workers investigating chemical defense substances[2,3]. Most of these substances are low molecular weight compounds derived from algal diets. We found previously the presence of glycoproteins with antibacterial and antineoplastic activity in eggs and albumen glands of *Aplysia kurodai,* but not in mucous gland and other tissues[4,5]. The main active substance in eggs of *Aplysia* species was isolated as a glycoprotein[6]. Recently, we have found that the physiocochemical properties of the active factor in the albumen gland are different from those of the factor in the eggs laid. The latter is termed hereafter aplysianin-E, from the name of the suborder *Aplysiacea.* In this report, we describe the purification and characterization of the other active component, aplysianin-A, in the albumen gland of *A. kurodai.*

A. kurodai were collected from Lake Hamana, Shizuoka, in their spawning season (May and June), and from Okkirai Bay, Iwate, Japan in December 1984. Anterior genital mass, which is composed of the globular albumen gland and surrounding mucous gland, was removed from freshly dissected animals and was used for the extraction of active substances. Antibacterial activity was determined turbidometrically using *Bacillus subtilis,* as reported previously[4]. Antineoplastic activity of the purified aplysianin-A was measured in vitro using ^{51}Cr labeled murine MM46 cells[5].

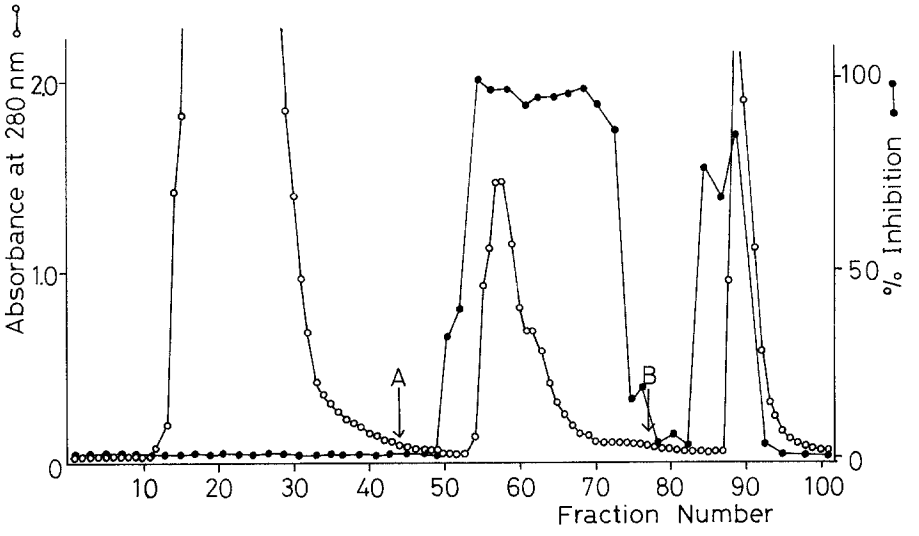

Figure 1. Chromatography of *Aplysia* antibacterial factors on Butyl-Toyopearl 650S. The crude sample in 40%-saturated ammonium sulfate solution was applied onto a column of Butyl-Toyopearl 650S (2.2 × 26 cm). After washing the column with 40%-saturated ammonium sulfate, it was eluted with 20%-saturated ammonium sulfate (arrow A) and with 50 mM phosphate buffer, pH 7.0 (arrow B). Fractions (8 ml) were analyzed for absorbance at 280 nm (○) and antibacterial acticity against *Bacillus subtilis* (●). Active fractions (horizontal bar) were pooled.

Anterior genital mass was largest prior to egg laying. After the spawning season it shrank markedly, and the specimens obtained in December did not show any antibacterial activity. Aplysianin-A was purified according to our previous method with a slight modification adopted for aplysianin-E[6]. Pooled anterior genital mass was homogenized with 50 mM phosphate buffered saline, pH 7.0. Solid ammonium sulfate was added to the extract to give a final concentration of 40% saturation. After centrifugation the supernatant was applied to a column of Butyl-Toyopearl 650S, a hydrophobic adsorbent (Toyo Soda Manufacturing Co., Tokyo). As shown in figure 1, antibacterial substances were adsorbed on the gels, while a large amount of inactive and highly viscous substances in the homogenate of the raw material was not adsorbed. The active principle was eluted mainly with 20%-saturated ammonium sulfate solution from the column. Antibacterial activity was also recognized in the fractions eluted with phosphate buffer.

The main active fractions (horizontal bar in figure 1) were pooled, ultrafiltered using UK-10 membrane (molecular cut 10 kD), and purified by column chromatography on Sephacryl S-300 (3.2 × 80 cm, Pharmacia, NJ) with the phosphate buffered saline. Fractions with antibacterial activity were loaded onto a column of DEAE-Toyopearl 650S (1.2 × 10 cm) previously equilibrated with starting buffer (10 mM phosphate buffer, pH 7.4). The column was eluted with starting buffer and subsequently with a linear NaCl gradient (0–200 mM). The active factor was further purified by chromatofocusing on a column of PBE 94 (2 × 40 cm, Pharmacia, NJ) using 25 mM histidine-HCl buffer, pH 6.2 as starting buffer, and polybuffer 74-HCl, pH 4.0 as eluting buffer. The purified sample showed one major protein band in polyacrylamide disc gel electrophoresis as shown in figure 2A, and the antibacterial activity was recovered from the

Amino acid composition of aplysianin-A

Amino acid	Mol %	Amino acid	Mol %
Asx	9.9	Ile	2.8
Thr	4.7	Leu	8.2
Ser	7.0	Tyr	5.8
Glx	10.3	Phe	4.2
Gly	8.1	Trp	ND*
Ala	6.7	His	3.9
Cys(half)	0	Lys	8.9
Val	6.8	Arg	7.3
Met	1.5	Pro	4.1

*Not determined.

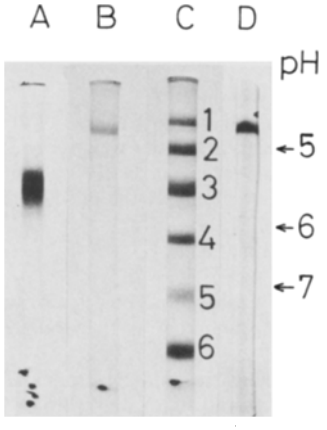

Figure 2. Polyacrylamide disc gel electrophoresis of the purified aplysianin-A. *A* Electrophoresis in 5% gel at pH 9.5; *B* SDS-polyacrylamide gel electrophoresis with 2-mercaptoethanol in 10% gel; *C* Markers (1, phosphorylase B 94 kD; 2 bovine serum albumin 67 kD; 3, ovalbumin 43 kD; 4, carbonic anhydrase 30 kD; 5, soybean trypsin inhibitor 20 kD; 6, α-lactoalbumin 14 kD); and *D* Isoelectric focusing in 5% gel. Gels were stained by Coomassie brilliant blue G-250.

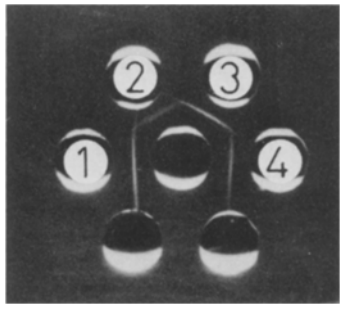

Figure 3. Ouchterlony double-diffusion test in 1.2% agarose. Center well, rabbit antiserum against aplysianin-E, an antineoplastic factor in eggs. Purified aplysianin-E, 1 and 3 (95 µg/ml, 10 µl); Purified aplysianin-A, 2 and 4 (58 µg/ml, 10 µl).

band. In a typical run, 100 g of anterior genital mass yielded 11 mg of aplysianin-A.

The aplysianin-A obtained showed similar physiological activity to that of aplysianin-E, an active factor in eggs. It showed a 50% inhibition of *B. subtilis* growth at a concentration of 4 µg protein/ml. The concentration required for 50% lysis of murine MM46 tumor cells was as low as 14 ng protein/ml. Physicochemical properties were, on the other hand, quite different from those of aplysianin-E.

Aplysianin-E is a glycoprotein (8% neutral sugar content) of 250 kD and is composed of three distinct subunits which have molecular weights of 76, 88, and 102 kD, respectively[6]. In contrast, the molecular weight of aplysianin-A was estimated to be approximately 320 kD by high speed gel-filtration on Toyo Soda TSK G-3000SW. In SDS-polyacrylamide disc gel electrophoresis it showed a major band corresponding to 85 kDa with or without 2-mercaptoethanol (fig. 2B). The ratio of the molecular weight of the intact aplysianin-A to dissociated proteins was very close to 4. In isoelectric focusing, it gave a single band at pI 4.7 (fig. 2D). The neutral sugar content was determined to be 9.8% by phenol-sulfuric acid method using glucose as a standard. The amino acid composition of aplysianin-A is listed in the table. The absence of half cystine suggested that the subunits are linked by non-covalent bonds. In spite of these physicochemical differences, a similarity of the molecule to aplysianin-E was found by immunological tests using rabbit antiserum against aplysianin-E. As seen in figure 3, Ouchterlony double-diffusion tests showed the spur of a cross reaction suggesting that aplysianin-A was partly identical in antigenic specificity with aplysianin-E.

The antibacterial activity of the aplysianins was found to be resistant to treatment with proteinases, such as trypsin, papain, and pronase. The aplysianins did not show significant inhibitory activity against these proteinases. It is of interest from the comparative physiological point of view that certain prosobranch snails are known to possess polyvalent inhibitors of proteinases, as well as agglutinins in albumen gland[7,8], whereas the sea hare, an opisthobranch mollusc, contains potent antibacterial glycoproteins in the albumen gland. The physiological function of aplysianins remains unknown.

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Announcements

Switzerland

23rd EUCHEM conference on stereochemistry

Bürgenstock, near Lucerne, May 3-9, 1987

The conference covers a wide range of topics in chemistry with emphasis on its interdisciplinary character in natural sciences. Inquiries and applications (no special forms are required) should be addressed, before January 10, 1987, to the President, Prof. D. Seebach, Laboratorium für Organische Chemie, ETH-Zentrum, CH-8092 Zürich/Switzerland.

Hungary

International Society for Heart Research

Budapest, September 13–16, 1987

The main topics of this 8th European Section Congress will be: Endothelial control of myocardial circulation; Physiology and pathophysiology of cardiac membranes; Theoretical and clinical aspects of arrhythmias; Diabetic heart; Biochemistry and electrophysiology in reperfusion.

Detailed information by G. Pogatsa, Research Department, National Institute of Cardiology, P.O. Box 9-00, H-1450 Budapest/Hungary.

Romania

4th international conference on water and ions in biological systems

Bucharest, 24–28 May 1987

For information, please contact Prof. V. Vasilescu, Romanian Biophysical Society, c/o Union of the Societies for Medical Sciences, str. Progresului 10, R-70754 Bucharest/Romania.

4th Basel Psi Days 86

Basel, October 30–November 2, 1986

Ethnoparapsychology – a new scientific discipline? 'Exotic Psi – the paranormal in alien cultures' is the title of this year's International Congress on Interdisciplinary Discussion of Border Area Problems of Science.

The detailed program of the Basel Psi Days 86 is being compiled under the guidance of Prof. Dr. Manfred Schuster, Head of the Ethnological Institute of the University of Basel. It will contain a first, theoretical part (30/31.10.) whilst the second, practical part (1./2.11.) will comprise workshops for the demonstration and discussion of paranormal phenomena in alien cultures. Sensitive subjects from all the continents of the world are expected to join in these demonstrations. The program is available from the Secretariat Basel Psi Days 86, c/o Congress Service, Swiss Industries Fair, P.O. Box, 4021 Basel, Switzerland, telephone 061/26 20 20, telex 962 685 smm-ch, telefax 061/32 06 17.

Courses

Mexico

International training course on biological membranes: Principles, techniques and application to parasitic diseases

Mexico City, October 13–25, 1986

Information by Dr Armando Gomez-Puyou, Universidad Nacional Autonoma de México, Instituto de Fisiologia Celular, Apartado Postal 70–600, 04510 México, D.F., México.