Bull. Environ. Contam. Toxicol. (1991) 47:751–757

Bulletin of Environmental Contamination and Toxicology

Inhibition of Release of Phospholipase A_2 from Sponge Cells (*Geodia cydonium*) by Detergent-Polluted Sea Water. A Sensitive Method to Monitor Marine Pollution

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The siliceous sponge *Geodia cydonium*, which lives at relatively unpolluted sites at the bottom of the Northern Adriatic, has been established as a suitable model for studying (i) the biological effects of sea water pollution (Zahn et al. 1977; Ugarkovic et al. 1990) and (ii) the biochemical processes occurring during cell aggregation (for a review, see Müller et al. 1990). Whole sponges can be readily dissociated into single cells, which reaggregate in Ca^{2+}-containing artificial sea water in the presence of the soluble aggregation factor (AF) to form large aggregates, in which the cells rearrange within 1 to 3 d under regeneration of a functional sponge. The specific cell binding fragment of the AF particle has been identified and isolated (Gramzow et al. 1986). This molecule interacts with the aggregation receptor (AR) of *G. cydonium*, which is present on the cell surface (Müller et al. 1990). The AF particle acts as a growth factor (Müller et al. 1987). After binding of AF to AR, the *Geodia* cells start to produce a voluminous extracellular matrix (Müller et al. 1990). One component of the matrix is the *Geodia* lectin that is specific for D-galactose (Conrad et al. 1984). The matrix lectin is the major growth-regulatory molecule at a later stage (after 15 hr) of formation of sponge cell aggregates (Gramzow et al. 1989). The *Geodia* lectin binds to the membrane-bound lectin receptor (Müller et al. 1990). We could demonstrate that, within sponge cells, transduction of mitotic signals of the AF and the lectin occurs through two mechanisms: (*i*) AF-caused and protein kinase C-mediated signal pathway and (*ii*) subsequent lectin-caused and phospholipase A_2 (PLA_2)/arachidonic acid-mediated signalling (Gramzow et al. 1989). The

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latter process, which depends on the preceding activation by the AF (Müller et al. 1987), is initiated by the interaction between the lectin and the lectin receptor and results in the release of PLA_2 into the extracellular space (Gramzow et al. 1989). Extracellularly, PLA_2 cleaves phosphatidylethanolamine and phosphatidylcholine, which are present in the extracellular space. The arachidonic acid, formed during this reaction, is then taken up by the cells and intracellularly metabolized to prostaglandin E_2. This results in an increase in ornithine decarboxylase level and, ultimately, in DNA synthesis (Gramzow et al. 1989).

Detergents are important components of the total pollution load of sea water, particularly in the coastal regions in many parts of the world. Many studies have been published on the aquatic effects of detergents, but little is known on their effects on cell-cell interaction and gene induction in the low-concentration range. Regenerating sponge cubes of *G. cydonium* have previously been shown to be a sensitive indicator model in the investigation of pollution of sea water by detergents and by genotoxic xenobiotics (Zahn et al. 1977). Previously we demonstrated that transplantation of regenerating *Geodia* cubes to differently polluted sites in the sea resulted in pronounced changes in the intracellular signal transduction chain, compared to controls exposed to unpolluted environments (Ugarkovic et al. 1990). Our results indicated that the intracellular signalling system within sponge cells is activated in response to moderate pollution, but it is depressed in heavily polluted environment. In this paper, the effect of detergents, representing one major component pollutant in the coastal waters of the sea, on the second signal transduction pathway present in *Geodia* cells, the sponge's lectin-induced PLA_2/arachidonic acid pathway, was studied. The anionic detergent sodium dodecyl sulfate (SDS) and the cationic detergent cetyltrimethylammonium bromide (CTAB) were used in the concentration range from 1×10^{-10} g/mL (0.1 ppb) to 1×10^{-5} g/mL (10 ppm).

MATERIALS AND METHODS

1-Acyl-2-[1-^{14}C]arachidonyl glycerophosphoethanolamine (specific activity 55 mCi/mmol) was purchased from Amersham Buchler International (Buckinghamshire, England); PLA_2 (from porcine pancreas) and sodium dodecyl sulfate (SDS) were from Sigma (St. Louis, Missouri, USA); and cetyltrimethylammonium bromide (CTAB) was obtained from E. Merck (Darmstadt, Germany). Primary stock solutions of 10^{-3} g/mL of SDS or CTAB were prepared; from them secondary stock solutions of 10^{-5} g/mL and 10^{-7} g/mL were diluted immediately prior to use.

Live specimens of *Geodia cydonium* Jameson (Demospongiae: Tetractinomorpha: Geodiidae) were collected by diving from a depth of 20–30 m at an unpolluted site of the sea near Rovinj (Yugoslavia).

The isolation and purification of the AF particle of *G. cydonium* have been described previously (Conrad et al. 1984). The specific cell binding fragment was isolated from the AF as described (Gramzow et al. 1986).

The homologous lectin of *G. cydonium* was isolated and purified by affinity chromatography (Conrad et al. 1984). Where indicated, the lectin solution (30 µg/mL) was adsorbed with anti-lectin antibodies (5 mg/mL) for 24 hr at 2°C. Then the sample was centrifuged (100,000 x g, 5°C, 30 min), and the supernatant was used for the experiments. Antibodies to *G. cydonium* lectin were prepared as described (Conrad et al. 1984).

Tris-buffered Ca^{2+}- and Mg^{2+}-free seawater was made as described (Müller et al. 1987). The distilled water used for preparing the solutions had been passed over a column of Chelex 100 (Bio-Rad).

The preparation of viable sponge single cells has been described earlier in detail (Müller and Zahn 1973); mucoid cells, archaeocytes, and choanocyte clusters were obtained from the total cell populations (Gramzow et al. 1989). The cells were used for the experiments 6 hr after dissociation.

In the standard incubation assay (3 mL volume) (Müller and Zahn 1973), 2 µg/mL of cell binding fragment or 30 µg/mL of AF particle was added to a suspension of 25 ± 5 x 10^6 cells per mL of Tris-buffered Ca^{2+}- and Mg^{2+}-free seawater. The cell suspensions were placed into glass tubes and rolled at 35 rpm for 60 min at 20°C. Where indicated, 10^{-10} to 10^{-5} g detergent/mL was added to the suspensions and incubated along with the controls. After a subsequent incubation for 10 hr without moving, 1 µg/mL of untreated lectin or adsorbed lectin was added to the cell suspensions and incubation was continued for a period of up to 1 h.

The standard reaction mixture for determination of PLA_2 activity containing in a final volume of 300 µl, 50 m*M* Tris-HCl pH 8.5, 5 m*M* $CaCl_2$, and 10 µM 1-acyl-2-[1-^{14}C]arachidonyl glycerophosphoethanolamine. The incubation was performed at 30°C for 0–15 min. The reaction was terminated by addition of Dole's reagent, and the released fatty acid was extracted and quantified as described (Gramzow et al. 1989). One enzyme unit corresponds to 1 nmol of fatty acid released per min.

Protein and DNA were determined as described previously (Gramzow et al. 1989). Vital staining was performed as described (Müller et al. 1978).

Student's *t* test was used to determine significance (Sachs 1984).

RESULTS AND DISCUSSION

Two detergent types were selected: SDS, an anionic detergent which is present in many laundry and household effluents, and CTAB, a monovalent cation, which has previously been shown to inhibit the calcium-dependent aggregation of sponge cells (Müller et al. 1978). The detergent concentrations tested (1×10^{-10} to 1×10^{-5} g/mL) were below and above those found in the sea water in various coastal regions of the world (Kozarac et al. 1975; Zvonaric et al. 1973). The total concentrations of compounds with anionic detergent character in the Northern Adriatic have been found in the range of 10^{-8} to 6×10^{-7} g/mL SDS equivalents (Kozarac et al. 1975). However, in many places the concentrations of detergents introduced into the sea may often be considerably higher (Zimmermann 1965). At the concentrations used, SDS and CTAB were not toxic to the cells as checked by vital staining.

As shown in Fig. 1, dissociated *Geodia* cells that had been preincubated with the cell binding fragment of AF released PLA_2 into the extracellular medium after addition of homologous lectin. The release of PLA_2 was a very rapid process and was found to be maximal already after an incubation period of 1 min; at this time it amounted to 1.1 ± 0.2 U/mL. The preincubation of the cells with the cell binding fragment was performed for 10 h; shorter preincubation periods resulted in the release of a smaller amount of PLA_2 (not shown; see also Gramzow et al. 1989). Preincubation of the cells in the presence of low concentrations of detergent caused a strong decrease in the release of PLA_2; at 1×10^{-8} g/mL of SDS and 1×10^{-7} g/mL of CTAB, the extent of enzyme release in the extracellular medium was decreased by about 65% and 55%, respectively (1 min after addition of the lectin stimulus; Fig. 1). No stimulation of enzyme release was observed after incubation of the cells with an antibody-adsorbed lectin preparation (Fig. 1). The dependence of inhibition of PLA_2 release on detergent concentration is shown in Table 1. The inhibition became significant already at a concentration of 1×10^{-9} g/mL of SDS and 1×10^{-8} g/mL of CTAB. Control assays with commercially available PLA_2 or incubation supernatants from detergent-free assays revealed that at the concentrations used the detergents have no effect on enzyme activity (not shown).

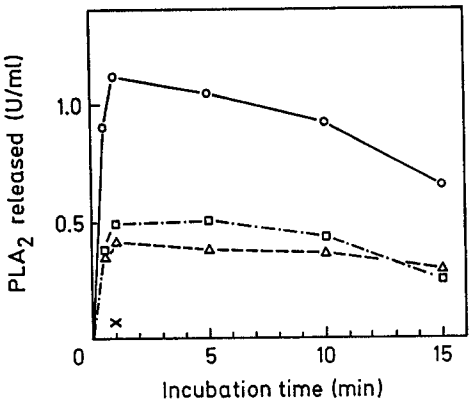

Fig. 1. Effect of SDS and CTAB on release of PLA_2 from intact *G. cydonium* cells after addition of homologous lectin. The cells were preincubated with the cell binding fragment of AF for 10 hr in the absence (o) or presence of 1 x 10^{-8} g/mL of SDS (Δ) or 1 x 10^{-7} g/mL of CTAB (□). Then the cells (8.1 x 10^6 cells/mL) were stimulated in the standard incubation assay with 1 µg/mL of lectin. After an incubation period of 0.5-15 min the cell suspension was centrifuged (2000 x g, 22°C, 10 min), and the PLA_2 activity was determined in the supernatant. In one control assay, a lectin sample (1 µg/mL) that had been preadsorbed with anti-lectin antibodies was added to the cells after the preincubation step (x).

It should be noted that the reported effects of detergents on PLA_2 release occur at low and pollution-relevant concentration levels, never tested in other systems. Studies reported in the literature usually used considerably higher concentration levels (for a survey, see Zahn et al. 1977).

Previously we determined that exposition of regenerating sponge tissue to detergent also caused pronounced alterations in the chain of programmed synthesis (Zahn et al. 1977). It was found that SDS at a concentration of 10^{-7} g/mL and higher decreases the uptake of the precursors thymidine, uridine and phenylalanine into the acid-soluble sponge fraction. The incorporation into the acid-insoluble fraction was already altered at a concentration of 10^{-8} g/mL. However, the effect of detergents on PLA_2 release occurs at a 10-fold lower concentration than that on macromolecular synthesis. Thus, measurement of the release of PLA_2 from dissociated sponge cells provides a highly sensitive assay for testing environmental effects caused by detergents, in

Table 1. Inhibition of release of PLA_2 from *G. cydonium* cells induced by homologous lectin in dependence on detergent concentration. Sponge cells were preincubated with the cell binding fragment of AF for 10 hr in the absence or presence of the indicated concentrations of SDS or CTAB. The cells were then stimulated in the standard incubation assay with 1 µg/mL of lectin, and the PLA_2 activity released into the supernatant (2000 x g , 22°C, 10 min) after an incubation time of 1 min was determined. n = 6.

Additional compound (g/mL)		PLA_2 released	Differences* (U/mL)
None		1.1 ± 0.2	
SDS,	10^{-10}	1.0 ± 0.2	0.1 ± 0.4[a]
	10^{-9}	0.7 ± 0.2	0.4 ± 0.3[b]
	10^{-8}	0.4 ± 0.1	0.7 ± 0.3[c]
	10^{-7}	0.3 ± 0.1	0.8 ± 0.3[c]
	10^{-6}	0.3 ± 0.1	0.8 ± 0.3[c]
	10^{-5}	0.2 ± 0.1	0.9 ± 0.3[c]
CTAB,	10^{-10}	1.2 ± 0.2	
	10^{-9}	1.1 ± 0.2	0.1 ± 0.4[a]
	10^{-8}	0.8 ± 0.1	0.4 ± 0.3[b]
	10^{-7}	0.5 ± 0.1	0.7 ± 0.3[c]
	10^{-6}	0.3 ± 0.1	0.9 ± 0.3[c]
	10^{-5}	0.3 ± 0.1	0.9 ± 0.3[c]

*Shown are the differences between values for exposed cells compared to controls (assays without detergent). Significance: [a] not significant, [b] $p<0.01$, [c] $p<0.005$

a concentration range apparently not uncommon in the sea (Zvonaric et al. 1973; Kozarac et al. 1975). This bioassay method is also practical and the procedures are not highly complicated, so that a number of other laboratories could try to use it. The sponge specimens can also be obtained by dredging from a depth of 15-20 m. Moreover, *G. cydonium* can be cultured routinely in the laboratory to be used for testing. Preliminary results showed that under these conditions effects of detergents on PLA_2 release already occurred within incubation periods of less than 24 hr.

Acknowledgments. This work was supported by a grant from the Bundesministerium für Forschung und Technologie (Yugoslav - German cooperation program, coordinated by GKSS, Internationales Büro, Geesthacht).

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Received November 12, 1990; accepted March 21, 1991.