Are Echinoderms of Interest to Biotechnology?

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Abstract. The huge potential of echinoderms as a so far fairly untapped source of bioactive molecules is described. Examples are presented that show the usefulness of echinoderm-derived molecules for therapeutic application in selected fields of cancer research, in the control of bacterial growth as substances with new antibiotic properties, and finally in the context of technical applications such as antifouling substances. The molecules described here are but the mere beginning of a commercial exploitation of echinoderms and may incite a deeper involvement of biotechnology-oriented research in this material.

Echinoderms have been used as embryos since the dawn of cell biology as model systems to study basic phenomena such as mitosis, cell division, differentiation, and organ formation, and are linked to the great cell biologists of the 19th and 20th centuries; among many others, Boveri, Heilbrunn, Mazia, Monroy, Wilson, Hertwig, and Brachet may be cited. Today, echinoderm embryos continue to be the model of choice for many cell and molecular biologists, offering exciting overtures on the way from molecular to cell biology (e.g. Arnone et al. 1997). At the same time they serve as a sensitive test system for toxicological and environmental studies (Matranga et al. 2000).

This chapter is not intended to cover the many applications of the peculiarities of the echinoderm embryonic system in cell and molecular biology; rather it will give examples of echinoderm-derived substances that may have biotechnological value.

Without any doubt the marine environment has huge potential as a source of new compounds to be used in so far unknown strategies in the combat of many pathological situations. In the course of millions of years of evolution, many trials and errors have been made to protect the individual, either the cell

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or the entire organism, and to gain advantage in the face of possible competitors. Substances have evolved that interfere with signalling or are simply poisonous to other cells. Such products, to be discussed later, could be used in the human environment to attack key problems such as cancer, bacterial infections, or, more practically, biofouling. Surprisingly, echinoderms appear to be a rather untapped source in the pursuit of the identification of new and useful products.

Successful identification depends to a large extent on the test system used and on subsequent purification techniques. Probably one of the most direct approaches is the use of whole animals or pieces of an animal to test whether there is any antibacterial or cytotoxic activity.

Sasaki et al. (1985) identified in the macromolecular fractions of aqueous extracts from the sea urchin *Strongylocentrotus nudus* antitumor activities that inhibited transplanted sarcoma 180 solid form in ICR mice. Unfortunately, this interesting work was apparently not followed up. Earlier on, it was shown in an elegant study that triterpene glycoside isolated from 19 holothurian species of the Pacific tropical zone exhibited cytotoxic activity against yeast and tumour cells, whereas bacteria were not affected. Out of these glycosides the stichoposides, theoturins, and oligosides of Holothuria of the genus *Bohadschia* were found to be the most active versus fungal and yeast microflora and tumour cells (Kuznetsova et al. 1982).

Haug et al. (2002) isolated from the sea urchin, *Strongylocentrotus droebachiensis*, the sea cucumber *Cucumaria frondosa*, and the starfish *Asterias rubens* antibacterial activities that were detected in extracts from several tissues in all species tested, but mainly in the coelomocyte and body wall extracts. High antibacterial activity was also found in gastrointestinal organs and eggs from *A. rubens* and in eggs from *C. frondosa*. If differences in hydrophobicity and sensitivity to heat and proteinase K treatment were compared between active extracts, it was observed that several different compounds were responsible for the antibacterial activities detected. Lysozyme-like activity was identified in several tissues from *A. rubens*. Haemolytic activity could be detected in all species tested, especially in the body wall extracts. These results indicate that echinoderms may serve as a useful source when searching for novel antibiotics.

Carballo et al. (2002) used two brine shrimp assays to identify potential cytotoxic substances useful in cancer therapy. They incubated whole body extracts from three echinoderms (*Holothuria impatiens*, *Pseudoconus californica*, and *Pharia pyramidata*) that showed a strong cytostatic (growth inhibition) and cytotoxic effect against two human cell lines, lung carcinoma A-549 and colon carcinoma HT-29. Palagiano et al. (1996) isolated up to 20 steroid glycosides from the starfish *Henricia downeyae* that caused growth inhibition in bacteria and fungi. In this work, it is remarkable that the biological activity originally identified in ethanolic extracts was related to single compounds whose molecular structures were even identified. Aminin et al. (1995) first identified in the Pacific brittle star *Ophiopholis aculeata* disulfated polyhydroxysteroids that turned out to be potent Ca^{2+} agonists in mammalian cell systems (Aminin et al. 1995; Agafonova et al. 2002).

Even in the main constituents of the immune systems of echinoderms, cytotoxic substances are found. Coelomocytes are intriguing entities expressing variable effector mechanisms that are elicited specifically and are repeatable after a variety of non-self challenges (Glinski and Jarosz 2000; Lin et al. 2001). Stabili et al. (1996) were able to isolate from coelomocytes of the sea urchin *Paracentrotus lividus* a bactericidal protein and purified it to a single polypeptide chain with a molecular weight of 60 kDa.

Epibiosis, the colonization of biogenic surfaces by epibiotic organisms such as bacteria, filamentous algae, and sessile invertebrates, poses a major threat to the fitness and survival of macroorganisms which could potentially be fouled. Fouling of artificially submerged structures (e.g. ship hulls) can also cause severe economic problems, establishing the need for refined bioassays to determine the efficacy of potential antifouling compounds. Palagiano et al. (1996) identified steroid glycosides from the starfish *H. downeyae* with profound antifouling activities, compounds that were also found in several species of the family Echinasteridae. Iken et al. (2003) monitored the brown algal spore swimming behaviour in the presence of echinoderm extracts in order to identify possible antifouling activities. They tested different concentrations of aqueous and organic extracts from body walls of sympatric echinoderms (starfish *Luidia* and *Astropecten* and the brittle star *Astrocyclus*). They found significant effects of those extracts on spore swimming behaviour at concentrations three orders of magnitude lower than that present naturally in the echinoderm body walls.

Sulfated fucans are among the most prominent of all the sulfated polysaccharides of non-mammalian origin that exhibit biological activities in mammalian systems. Pereira et al. (1999) investigated the anticoagulant activity of echinoderm fucans in comparison with that of several species of brown algae and found that the linear sulfated fucans from echinoderms had an anticoagulant action resembling that of mammalian dermatan sulfate, whereas the branched fucans from brown algae were direct inhibitors of thrombin. Such differences have also been described for the linear sulfated fucans derived from sea cucumbers compared to algal fucans (Mulloy et al. 2000).

Glycosphingolipids and glycopeptides are normally occurring constituents of various cell membranes in a wide variety of organisms. Surprisingly, these compounds derived from echinoderms exhibit biological function that might render them useful in medical applications. Glycosphingolipids have been isolated from sea cucumbers that had neuritogenic activity in the rat pheochromocytoma cell line PC-12, i.e. they were able to induce neurite differentiation in the same way as can be achieved by the addition of nerve growth factor (Yamada 2002). It remains to be seen whether these compounds find medical applications, but even today they are of economic interest in view of the high price of commercially available nerve growth factor.

Several unique lectins are found in echinoderms. In marine invertebrates lectins may be considered as humoral factors in the defence mechanism, as are immunoglobulins in vertebrates, resulting in activation of phagocytes. On the other hand, direct haemolytic activity has recently been found in a galactose-specific lectin from the sea cucumber *Cucumaria echinata* (Hatakeyama et al. 1999). After binding to the specific carbohydrate chains on the erythrocyte surface, these lectins damage the cell membrane, leading to cell lysis. A lectin with biological activities such as mitogenic and chemotactic characteristics was also described in the venom of the pedicellariae of the sea urchin *Toxopneustes pileolus* (Nakagawa and Kimura 1982) as had been described with other bioactive substances many years earlier by Alender (1967) occurring in the spines of *Diadema* sea urchins.

A rich source of useful venoms has been found in the crown-of-thorns starfish *Acanthaster planci*. One of its deadly venoms has been identified as a myotoxic phospholipase A (Mebs 1991), and several other candidates for such effects have been identified (Shiomi et al. 1985, 1988; Mebs 1989).

A not yet commercially exploited area is the phenomenon of bioluminescence in the brittle star *Amphipolis squamata*. The photocytes of this animal produce light dependent on cyclic nucleotide and IP3, and the system may become as useful and widespread as the luciferin–luciferase system is today (De Bremaeker et al. 2000; Deheyn et al. 2000a–e).

Finally, an example may be cited of how echinoderms may become useful even in bionics. It has been recently discovered by Aizenberg et al. (2001) that in the brittle star *Ophiocoma wendtii* single calcite crystals are arranged in such a way that they function as lenses. These lenses focus light onto nerve bundles that run behind them, which presumably receive the signal to be further processed. Thus, these thousands of lenses form a compound eye that covers the upper surface of the animal, resulting in a function similar to a digital camera that builds up the picture pixel by pixel. At present, engineers in the photonic industry try to imitate the perfect calcite lenses and their use in signal reception.

This short overview was intended to stimulate the search for bioactive compounds in echinoderms. It is surprising how little work has been done to identify promising candidates for applied research, especially in view of the widespread availability of the animals. It is to be hoped that the urgent need for new strategies in cancer research or for new candidates for antibiotics in view of the fast development in resistance of harmful bacteria will lead to a renaissance in the field. After all, the potential rewards are of considerable magnitude.

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