THE BIOACTIVATION OF PREMUTAGENS IN THE SEA-URCHIN ECHINUS MELO MEASURED BY THE SALMONELLA-MICROSOMAL ASSAY

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Tissues of lower marine animals generally fail to show the presence of microsomal mixed function oxygenases(MFO). The meaning of this absence has been largely ignored although the consequence of the lack of MFO-system is crucial for the understanding of both the environmental fate and the biological effects of xenobiotics in the marine ecosystem. However, judging on the increase of the frequency of chromosomal aberrations and the increased frequency of neoplasia in avertebrates from polluted areas, the presence of the capacity for the biotransformation of premutagens into their ultimate forms is obvious. The activity of MFO is usually measured by the production of fluorescent phenolic products from the polycyclic aromatic hydrocarbon benzo(a)pyrene(BaP). Recently we have developed a new technique using the avertebrate microsomes instead of microsomes from the rat liver as the bioactivating system in the S-9 fraction of the Ames-Salmonella test, which enables the biological measurements of formation of mutagenic products. We have applied this method to the measurement of biotransforming activity in the intestine of sea-urchins, since their wide distribution, availability and the ease in laboratory maintenance make them ideal in the pollutional oriented research.

The specimens of sea-urchins (Echinus melo) were collected at an unpolluted site ("naive" specimens) in the vicinity of Rovinj, the Northern Adriatic. Animals were analyzed either immediatelly or after 3 days of exposure to seawater saturated with Diesel 2 oil ("exposed" specimens) in a flow-system design. The activity of benzo(a)pyrene monooxygenase(BaPMO) was determined in the postmitochondrial fraction of the intestine as described previously (Kurelec et al,1977). The activity was expressed in pmol of BaPOH/mg protein/min. A portion of postmitochondrial fraction was filtered subsequently through Whatman Glass Fibre Paper GF/C, Millipore HA 0.45 µm, GS 0.22 µm and finally Millex-GS 0.22 µm Filter Unit. Filtrate was used for the preparation of S-9 Mix (Maron and Ames, 1983). BaP was used as a standard precancerogen and S. typhymurium TA 100 as a tester strain. Filtrate from the liver of mullets (Mugil aureatus) induced in the polluted area of Rovinj served as positive, and heated filtrate of the sea-urchin intestine as negative control. The bioactivating potential was expressed in the number of his^+ rebertants/mg of protein/plate.

The results of the BaPMO determinations are presented in Table 1. Note that 10^{-5}M benzoflavone, an in vitro inhibitor of cytochrome P-448 dependent BaPMO strongly inhibits the activity of mullet enzyme. Sea-urchin intestine fails to show activity in both conditions.

TABLE 1. BaPMO activity in sea-urcin intestine and mullet liver

Organism	BaPMO	BaPMO+benzoflavone
Enzyme blank	1.59+-0.34	1.64+-0.27
Naive sea-urchin	1.62+-0.12	1.80+-0.21
Exposed sea-urchin	1.64+-0.13	1.75+-0.31
Mullet	26.30+-2.04	2.75+-0.36

However, the same postmitochondrial fraction do produce ultimate mutagens from the BaP, as is shown in Table 2. Exposure to Diesel 2 oil inhibits this activity.

TABLE 2. Potential of sea-urchin intestine and mullet liver to bioactivate benzo(a)pyrene

Metabolic system	mg protein/plate	his^+revertants/6.8 µg BaP
Naive sea-urchin	0.00	152; 148; 155
	0.35	164; 153; 149
	0.70	205; 215; 196
	2.10	330; 350; 345
Heated naive sea-urchin	2.10	153; 167; 149
Exposed sea-urchin	0.00	167; 152: 148
	0.35	174; 163; 181
	0.70	250: 220; 210
	2.10	243; 225; 228
Mullet	0.00	163; 157; 155
	0.80	405; 420; 425
	1.60	620; 676; 648

The results presented indicate that the potential to bioactivate premutagens from the marine environment into their ultimate mutagenic form is present also in at least one representative of the marine avertebrate species.

LITERATURE

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