J. Mar. Biol. Ass. India, **53** (1) : 149 - 152, January - June 2011

Short Communication

Comparative studies on the lysosomal lability index of three animals belonging to different phyla

*L. P. Rema and [1]Babu Philip

Department of Zoology, Maharaja's College, Ernakulam, Kerala, India.
**E-mail: remakannampilly@rediffmail.com*
[1]Department of Marine Biology, Microbiology & Biochemistry, School of Marine Sciences, Cochin University of Science & Technology, Cochin, Kerala, India.

Abstract

Lysosomes are sensitive to environmental stresses. Lysosomal membrane stability measured using lysosomal enzyme release assay (LERA) and expressed in terms of lysosomal lability index is employed as an early warning system for detection of disturbances in the surrounding environment. In the present study, lysosomal membrane stability of animals belonging to different phyla namely, *Rattus norwegicus* (mammal), *Oreochromis mossambicus* (fish), and *Sunetta scripta* (mollusc) was determined using LERA technique. Results show that lysosomal lability index is high for *Sunetta scripta* and low for *Rattus norwegicus*. The data obtained confirm that molluscs are sentinel organisms. In higher animals, higher stability of lysosomal membrane is noticed.

Keywords: Lysosomal enzyme release assay, sentinel organism, lysosomal lability index

Introduction

Lysosomal stability index, as a measure of cellular condition and catabolic potential, is provided by positive correlation between this index and physiological scope for growth (Bayne *et al*., 1976, 1979). The level of destabilisation bears a quantitative relationship to the degree of stress (Bayne *et al*., 1976; Moore and Stebbing, 1976; Moore *et al*., 1978; Cajaraville *et al*., 2000; Camus *et al*., 2000; Marchi *et al*., 2004). Investigations at the sub-cellular level reveal alterations at an early stage of response, before integrated cellular damage shifts to the level of organ or whole organism. In many instances, the earliest detectable alterations are associated with the lysosomes (Moore, 1985). The sensitivity of lysosomes to environmental pollutants ranks lysosomal responses as early warning systems for the detection of disturbances in the surroundings. The lysosomal stability measured in terms of lysosomal enzyme release assay (LERA), can clearly reflect any breakdown in the adaptive capacity of the organism to toxic injury. The utility of lysosomal membrane lability assay as a health monitoring tool has been suggested by Chvapil *et al*. (1972).

The lysosomal enzyme release assay has been substantiated as a sensitive indicator of numerous environmental stresses in molluscs (Bayne *et al*., 1976; Widdows *et al.,* 1982; Rigerta *et al*., 2010). However, only a few studies have been conducted in aquatic vertebrates (James, 1986; Kohler *et al.,* 1986; Kohler, 1989, 1990). The measurement of lysosomal perturbations in fish liver as an integrative biological warning system for biological effect monitoring was suggested by Kohler (1990, 1991).

In this paper the lysosomal membrane stability of animals belonging to different phyla, *Rattus norwegicus* (mammal), *Oreochromis mossambicus* (fish) and *Sunetta scripta* (mollusc) are compared. The study is aimed at critically evaluating the validity of mussels as early indicators of stress and also to study the evolutionary status and adaptive strategy of the selected animals.

Material and Methods

Sunetta scripta was collected from fishermen of Murikkumpadam, Cochin, *Oreochromis mossambicus* from Rice Research Institute (ICAR), Cochin and *Rattus norwegicus* collected from College of Animal and Veterinary Science, Kerala Agricultural University, Mannuthy, Thrissur. The experimental animals were acclimatised in the laboratory. Hepatopancreas of *Sunetta scripta* and liver of *Rattus norwegicus* and *Oreochromis mossambicus* were dissected out. The tissues were blotted off the adhering water. A fixed weight of the sample tissues obtained by pooling sample from six different animals was homogenised in ice cold isotonic sucrose solutions containing 2 mM mercaptoethanol. Isotonic sucrose solutions were 0.45 mM for mollusc, 0.35 mM for fish and 0.25 mM for mammal.

Lysosomal lability index (LLI) is the activity of acid phosphatase released and is expressed as the percentage of the total activity of lysosomal acid phosphatase. Lysosomal lability index was determined following the procedure of Philip and Kurup (1977, 1978) and Rao and Sisodia (1986) with minor modification. The quantity of acid phosphatase, the marker enzyme of the lysosome, leaking out was measured following Anon (1963). Specific activity of acid phosphatase is expressed as millimoles of para-nitrophenol formed per hour per gram protein. Bovine serum albumin was used as the standard for protein estimation**.**

Results and Discussion

The stability of lysosomes against time of incubation is shown in Table 1. At 0 time of incubation LLI is 11.01 for *Rattus norwegicus,* 18.72 for *Oreochromis mossambicus* and 35.76 for *Sunetta scripta*. As time of incubation increases, LLI increases in all the three cases. At 60 minutes of incubation, LLI is 24.51 for *Rattus norwegicus,* 23.87 for *Oreochromis mossambicus* and 39.44 for *Sunetta scripta.* The data obtained were analysed using ANOVA. The analyses showed a significant difference at 5% level between the lysosomal stability of *Rattus norwegicus*, *Oreochromis mossambicus* and *Sunetta scripta* ($p< 0.001$). A significant difference was noticed between the lysosomal lability index due to different time of incubation also ($p<0.01$). The least significant difference between the species is 1.006. The lysosomal stability is high for *Rattus norwegicus*, whereas, it is low for *Sunetta scripta.* The least significant difference for time of incubation is 0.616.

Lysosomes play a crucial role in the isolation and sequestration of xenobiotics. Thus they relieve biological machinery from the toxic effects. But if the storage capacity is exceeded, there ensues leakage of hydrolytic enzymes into the cytoplasm and nucleoplasm bringing about derangement of cell functions.

An index of lability or latency – the lysosomal property requiring membrane alterations for enzyme expression – permits the comparison of the relative amount of enzyme leaking out of the lysosomal preparation. This lysosomal enzyme release assay (LERA) is a sensitive signal of the functional state of the lysosomes. Lysosomal lability index of *Oreochromis mossambicus* is less than that of *Sunetta*

Table 1. Stability of lysosomal membrane of three animals as a function of time at 37^0 C. Stability of lysosomes is assessed by following the activity of acid phosphatase (millimoles of para nitrophenol formed/ hour/gm protein) released; values are mean of six different experiments ± SD

Time	*R. norwegicus*		*O. mossambicus*		*S. scripta*	
(minutes)	Activity	LLI	Activity	LLI	Activity	LLI
00	01.76 ± 0.14	11.01	01.67 ± 0.05	18.72	21.14 ± 0.32	35.76
10	01.92 ± 0.27	12.00	01.85 ± 0.16	20.72	21.84 ± 0.19	36.95
20	02.20 ± 0.04	13.74	02.03 ± 0.15	22.70	22.97 ± 0.43	38.86
30	03.01 ± 0.08	18.80	02.13 ± 0.18	23.81	23.01 ± 0.92	38.93
40	03.61 ± 0.19	22.56	02.09 ± 0.11	23.42	23.33 ± 1.39	39.47
60	03.92 ± 0.38	24.51	02.13 ± 0.18	23.87	23.31 ± 0.42	39.44

scripta, but greater than that of *Rattus norwegicus*. This indicates that as evolution proceeds, the stability of lysosomal membrane to the self contained enzymes increases. The higher lysosomal lability index of molluscan lysosomes may be because of the fact that molluscs have comparatively more number of lysosomes that are very sensitive to environmental alterations. The high lysosomal lability index of *Sunetta scripta* probably indicates that molluscs have a limited capacity to metabolise the xenobiotics and to encounter stress. Hence, a minor change in the surrounding environment may be reflected in its physiology. Fishes have relatively low number of lysosomes which are physiologically more tolerant, whereas mammalian lysosomes are very few and are the most resistant of the three. The results of the present study thus confirm the mussels as sentinel organisms.

References

Anon., 1963. The colorimetric determination of phosphatase. Sigma Chemicals Company, St.Louis, U.S.A., *Sigma Tech. Bull.,* No.104.

Bayne, B. L., D. R. Livingstone, M. N. Moore and J. Widdows. 1976. A cytochemical and biochemical index of stress in *Mytilus edulis* (L). *Mar. Pollut. Bull*., 7: 221-224.

Bayne, B. L., M. N. Moore, J. Widdows, D. R. Livingstone and P. Salkeld. 1979. Measurement of the responses of individual to environmental stress and pollution: Studies with bivalve mollusks. *Philos. Trans. R. Soc. London*. B., 286: 563-581.

Cajaraville, M. P., M. J. Bebianno, J. Blasco, C. Porte, C. Sarasquete and A. Viarengo. 2000. The use of biomarkers to assess the impact of pollutions in coastal environments of the Iberian peninsula: a practical approach. *Sci. Total Environ. Mar*. 247: 295-311.

Camus, L., B. E. Grovik, J. F. Borseth, M. B. Jones and M. H. Depledge. 2000. Stability of lysosomal and cell membranes in hemocytes of common mussel (*Mytilus edulis*): effect of low temperatures. *Mar. Environ. Res*. 50: 325-329.

Chvapil, M., J. Ryan and C. F. Zukosi. 1972. The effect of zinc and other metals on the stability of lysosomes. *Proc. Soc. Exp. Biol. Med*., 40: 642-646.

James, V. D. 1986. Lysosomal membrane stability, histopathology and serum enzyme activities as sub lethal bioindicators of xenobiotic exposure in the blue gill sunfish (*Lepomis machrochirus* Rafinesque). *Chemistry, Biochemistry Dissertation Abstracts International*, 46(5): p.1552.

Kohler, A. 1989. Experimental studies on the regeneration of contaminant induced liver lesions in flounder from the Elbe estuary – steps towards the identification of cause effect relationships. *Aquat. Toxicol*., 14: 203-232.

Kohler, A. 1990. Identification of contaminant induced cellular and sub cellular lesions in the liver of flounder (*Platichthys flesus* L.) caught at differently polluted estuaries. *Aquat. Toxicol.,* 16: 271-294.

Kohler, A., 1991. Lysosomal perturbations in fish liver as indicators for toxic effects of environmental pollution. *Comp. Biochem. Physiol. C*, 100: 123-127.

Kohler, A., U. Harms and B. Luckas. 1986. Accumulations of organochlorines and mercury in flounder – an approach to pollution assessments. *Helgolander Meeresun*, 40: 431-440.

Marchi, B., B. Burlando, M. N. Moore and A. Viarengo. 2004. Mercury and copper induced lysosomal membrane destabilization depends on $[Ca^{2+}]_i$ dependent phosphlipase A_2 activation. *Aquat. Toxicol.,* 66: 197-204.

Moore, M. N. 1985. Cellular responses to pollutants. *Mar. Pollut. Bull*., 16: 134-139.

Moore, M. N. and A. R. D. Stebbing. 1976. The quantitative cytochemical effects of three metal ions on a lysosomal hydrolase of a hydroid. *J. Mar. Biol. Asso. U. K.*, 56: 995-1005.

Moore, M. N., D. M. Lowe and P. E. M. Feith. 1978. Lysosomal responses to experimentally injected anthracene in the digestive cells of *Mytilus edulis*. *Mar. Biol*., 48: 297-302.

Philip, B. and P. A. Kurup. 1977. Cortisol and lysosomal stability in normal and atheromatous rats. *Atherosclerosis*, 27: 129-139.

Philip, B. and P. A. Kurup. 1978. Adrenocortical suppression and lysosomal stability. *Indian J. Biochem. Biophys.*, 15: 193-195.

Rao, A. B. and P. Sisodia. 1986. Lysosomal membrane stabilization by enfenamic acid. *Indian J. Exp. Biol.*, 24: 771-772.

Rigerta, S., E. Panariti and D. Arapi. 2010. Monitoring of toxic residues in bivalve mollusks along the Adriatic coastal line of Albania. *Natura Montenigrina Podgorica*, 9(3): 321-329.

Widdows, J., T. Bakke, B. L. Bayne, P. Donkin, D. R. Livingstone, D. M. Lowe, M. N. Moore, S. V. Evans and S. L. Moore. 1982. Responses of *Mytilus edulis* L. on exposure to the water accommodated fraction of North Sea oil. *Mar. Biol*., 67: 15-31.

Received : 03/01/11
Accepted : 28/02/11
Published : 15/06/11