Bull. Environ. Contam. Toxicol. (1990) 44:205–209

Bulletin of Environmental Contamination and Toxicology

Use of Carbonyl Iron to Induce Iron Loading in the Mussel *Mytilus edulis*

N. Bootsma,[1] D. J. Macey,[1] J. Webb,[2] and V. Talbot[3]

[1]School of Biological and Environmental Sciences and [2]School of Mathematical and Physical Sciences, Murdoch University, Murdoch, Western Australia 6150 and [3]60 Vincent Street, Nedlands, Western Australia 6009, Australia

It is now recognized that in organisms such as marine mussels, the prior presence of one metal can be important in determining the ultimate toxicological response to a second challenge by a different metal species (Cunningham 1979; Elliott et al. 1986). Thus, for example, the presence of iron in the mussel Mytilus edulis profoundly affects the subsequent accumulation of zinc while iron in the oyster Saccostrea cucullata affects the mechanism of zinc storage (Lobel 1981; Webb et al. 1985). To determine these synergistic (or indeed antagonistic) effects in an organism such as the mussel, it is important to be able to both load the animal rapidly, and ensure that the metal ends up in a form which is ultimately the same as that found in the animal in the natural environment. Unfortunately, considerable problems have arisen with the form in which iron has been loaded into mussels. For example, while soluble iron(III) chloride has been used to induce iron loading in mussels (e.g. Hobden 1969, Pentreath 1973), this iron compound in seawater is rapidly converted to iron(III) hydroxide which precipitates, leading to uncertainties in the actual dosage received by the animals. Other studies on mussels have used iron(III) hydroxide as the medium of iron delivery, even though the bioavailability of iron in this form is limited (George et al. 1976). Others have used soluble iron complexes (George and Coombs 1977). Recently, Bacon et al. (1983) have successfully used carbonyl iron to induce iron loading in rats. This form of iron is prepared by reacting elemental iron at high temperatures with carbon monoxide to form iron pentacarbonyl which, on further heating, deposits relatively pure metallic iron as microscopic spheres less than 5 μm in diameter (Figure 1). Carbonyl iron was readily accepted by rats when mixed with food and produced iron loading in a relatively short period of time when compared with other methods.

This study was thus undertaken to determine whether carbonyl iron could be used for the rapid non-toxic iron loading of the mussel Mytilus edulis. Such loading could subsequently be used for the investigation of synergistic metal accumulation in mussels, a topic of considerable interest due to their use as marine pollution

send reprint requests to D.J. Macey at the above address

indicator organisms. Biochemical aspects of this tissue iron loading, including the isolation and characterization of the major metal-binding protein ferritin, have been reported previously (Bootsma et al. 1988).

MATERIALS AND METHODS

Specimens of Mytilus edulis, between 50 and 60 mm total shell length, were collected from near the Perth metropolitan region (Lat 32^{o}S, Long 116^{o}E). Following cleaning of the shell, animals were placed in well-aerated (approx. 10mg O_2/l) seawater at a density of not more than four per litre and at temperature and light regimes paralleling those found in the field. Carbonyl iron (Sigma Chem. Co.), mixed in a 1:4 ratio with finely ground commercial fish food, was added to the water daily at a dosage equivalent to 10 mg/kg. In this form the carbonyl iron did not settle out to any great extent, allowing ambient levels to remain fairly constant. The water was routinely filtered overnight to remove any carbonyl iron that remained, together with any faeces and other particulate matter. Iron loading was carried out over a 16 day period with animals being removed at 0, 2, 4, 8, 12 and 16 days for analysis. At least 9 animals were used at each sampling point. All animals were depurated for 48 hours prior to analysis to prevent unabsorbed iron affecting the results of subsequent analysis. Tissues were freeze-dried, ground and an aliquot digested using nitric and perchloric acid. Samples were then diluted with distilled water before being assayed for iron using atomic absorption spectrophotometry by direct aspiration into an acetylene-air flame. Reagent blanks made up 5% of all samples assayed and each digestion batch included standard reference material (National Institute for Environmental Studies, Japan certified reference material No. 6-Mussel). In all assays the iron content of the reference material was within the certified value.

RESULTS AND DISCUSSION

Results from preliminary experiments showed that the kidney, hepatopancreas and, to a lesser extent the gill, accumulated large amounts of iron in comparison to other organs such as the foot, gonad, mantle margin and adductor and pedal muscles. However, by far the greatest amount of iron on a total weight basis was found in the hepatopancreas. As such, subsequent work concentrated on this organ. This distribution of iron following loading agrees with data obtained by other workers using different forms of iron, which also showed that the greatest accumulation of iron occurred in the kidney and hepatopancreas (Pentreath 1973, George et al. 1976, George and Coombs 1977).

Median iron concentrations in the hepatopancreas after only two days of iron loading and following the 48-hour depuration period were dramatically higher than those found in the control animals (1.149 and 0.263 mg g^{-1} dry weight respectively), indicating a rapid accumulation of carbonyl iron (Figure 2). While some individual animals had iron concentrations almost as high as the

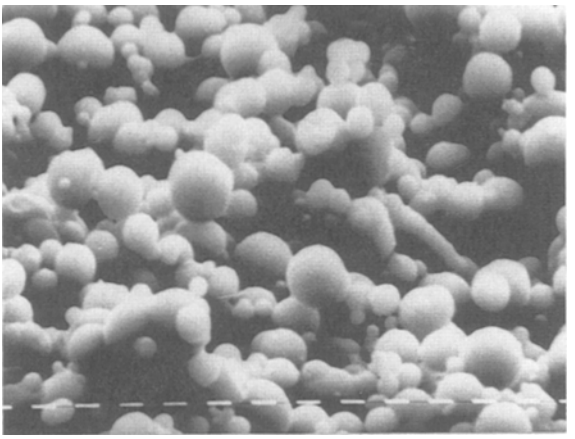

Figure 1. Scanning electron micrograph of carbonyl iron. Scale shows divisions of 1 μm.

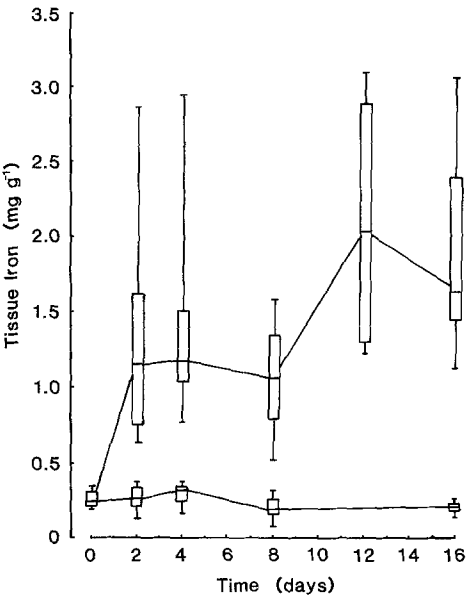

Figure 2. Iron concentration in the hepatopancreas of iron-loaded (upper line) and control Mytilus edulis. The plot line joins the median values at each time, while the boxes indicate 25% either side of the median and the whiskers the maximum and minimum values obtained. A minimum of nine animals was sampled at each point.

maximum achieved as early as day 2, the median iron concentration peaked at day 12 before falling back slightly by day 16 (Figure 2). These fluctuations may well represent different responses by the mussel to the iron load over the time course of the experiment. However, without further data it is impossible to speculate upon them. By the end of the sixteen-day loading period, hepatopancreas iron concentrations as high as 3.058 mg g^{-1} were achieved in some

animals, approximately fifteen times the median value found in the control animals at this time (Figure 2).

The dramatic increase in iron in the tissues of M. edulis parallels the situation described for rats in which carbonyl iron was able to produce rapid hepatic iron loading leading to high final concentrations in the rat liver (Bacon et al. 1983). The form and size of carbonyl iron (less than five microns in diameter) falls well within that accepted as food material by M. edulis (Lucas et al. 1987), and so should be readily trapped by the gills. It should be noted that in a separate series of experiments, iron loaded animals analysed without a depuration period were found to have approximately four times the gill-iron concentration of animals that had undergone depuration, confirming that carbonyl iron was trapped by the gill's filtering apperatus. The subsequent distribution of the iron suggests that it is then transported to the hepatopancreas prior to ingestion. Indeed, other workers have shown that while some particulate iron is taken up by the gills directly, the major entry route for such iron following capture is via endocytosis in the hepatopancreas, prior to storage in lysosomes (George et al. 1976; Janssen and Ertelt-Janssen 1983). Histological analyses of the hepatopancreas conducted at both the light and electron microscope level, failed to reveal the presence of any spheres of carbonyl iron in loaded mussels even though considerable amounts of iron were present in the tissue. Subsequent biochemical analysis of this tissue identified ferritin as the major iron-binding protein present in both control and iron-loaded animals, albeit more abundant in the latter group (Bootsma et al. 1988). This protein is a well known naturally occurring iron storage protein and has been isolated from other molluscs (Webb et al. 1985; Kim et al. 1986). Its presence implies that the iron had been converted by the hepatopancreas into a biologically acceptable form and that the processing of the iron was fairly rapid.

The method of iron loading described herein has considerable advantages over those used previously in that a relatively high concentration of iron in a bioavailable form is maintained within the aquarium system. In this series of experiments very little iron was left at the bottom of the tanks at the end of each day and the use of the filter system to avoid iron hydroxide build up was virtually unnecessary. The very high concentrations built up over a short time period compares very favorably with iron loading by other methods, such as the use of high stability iron complexes as demonstrated by George and Coombs (1977). As such, the use of carbonyl iron is recommended as the preferred form of iron loading in metabolic studies of metal interactions in the mussel M. edulis. Such studies may well prove invaluable if we are to improve our knowledge of this organism and its role as an indicator species.

REFERENCES

Bacon BR, Tavill AS, Brittenham GM, Park CH, Recknagel RO (1983) Hepatic lipid peroxidation in vivo in rats with chronic iron overload. J Clin Invest 71:429-439

Bootsma N, Macey DJ, Webb J, Talbot V (1988) Isolation and characterisation of ferritin from the hepatopancreas of the mussel Mytilus edulis. Bio Metals 1:106-111

Cunningham PA (1979) The use of bivalve molluscs in heavy metal pollution research. In Vernberg WB, Thurberg FP, Calabrese A, Vernberg FJ (eds) Marine pollution: Functional responses, Academic Press, New York, pp 183-220

Elliot NG, Swain R, Ritz DA (1986) Metal interaction during accumulation by the mussel Mytilus edulis planulatus. Marine Biol 93:395-399

George SG, Coombs TL (1977) Effects of high stability iron-complexes on the kinetics of iron acculmulation and excretion in Mytilus edulis (L.). J Exp Mar Biol Ecol 28:133-140

George SG, Pirie BJS, Coombs TL (1976) The kinetics of acculmulation and excretion of ferric hydroxide in Mytilus edulis (L.) and its distribution in the tissues. J Exp Mar Biol Ecol 23:71-84

Hobden DJ (1969) Iron metabolism in Mytilus edulis II. Uptake and distribution of radioactive iron. J Mar Biol Assoc UK 49:661-668

Janssen HH, Ertelt-Janssen U (1983) Cytochemical demonstration of cadmium and iron in experimental blue mussels (Mytilus edulis). Mikroskopie 40:329-340

Kim K-S, Webb J, Macey DJ (1986) Properties and role of ferritin in the hemolymph of the chiton Clavarizona hirtosa. Biochim Biophys Acta 884:387-394

Lobel P (1981) Zinc in mussels from an iron pipe. Mar Poll Bull 12:410-411

Lucas MI, Newell RC, Shumway SE, Seiderer LJ, Bally R (1987) Particle clearence and yield in relation to bacterioplankton and suspended particulate availability in estuarine and open coast populations of the mussel Mytilus edulis. Mar Ecol Prog Ser 36:215-224

Pentreath RJ (1973) The accumulation from water of ^{65}Zn, ^{54}Mn, ^{58}Co and ^{59}Fe by the mussel, Mytilus edulis. J Mar Biol Assoc UK 53:127-143

Webb J, Macey DJ, Talbot V (1985) Identification of ferritin as a major high molecular weight zinc-binding protein in the tropical rock oyster, Saccostrea cucullata. Arch Environ Contam Toxicol 14:403-407

Received June 30, 1989; accepted July 26,1989.