PURINE CATABOLISM AND OXIDATIVE DEFENSES IN MYTILUS EDULIS AND PLACOPECTEN MAGELLANICUS: A MODEL FOR MAMMALIAN REPERFUSION TISSUE INJURY

James A. Dykens, Megumi M. Takayama and Ross H. Wakai
Department of Biology, Grinnell College, Grinnell IA 50112

Aerobically poised animals die when denied adequate supplies of molecular oxygen. Paradoxically, in mammals there is little histological evidence of necrosis during ischemia, and only upon reintroduction of molecular oxygen during reperfusion does cellular structure begin to reveal the degeneration typical of morbibund tissues [Jennings and Ganote, Circ. Res. 34: 156-172, 1974]. According to the current paradigm of ischemia-reperfusion tissue damage [McCord, Fed. Proc. 47: 2402-2406, 1987], under normoxic conditions the native form of the cytosolic enzyme xanthine dehydrogenase (XDH) uses NAD as a cofactor. With the onset of ischemia and subsequent decline in aerobic ATP generation, the adenylate energy pool becomes depleted resulting in accumulation of inosine and xanthine. During ischemia, XDH is irreversibly converted by a protease into an oxidase form. Xanthine oxidase (XO) utilizes molecular oxygen as a cofactor and generates superoxide radicals, reactive univalent reductants of oxygen ($O_2^{\cdot-}$). Upon subsequent reperfusion of the tissue and return to normoxia, xanthine oxidase, now fueled by abundant substrate and molecular oxygen, generates superoxide radicals which mediate cell damage either directly, or indirectly *via* subsequent generation of extremely reactive hydroxyl radicals in the presence of transition metals.

This model for reperfusion tissue damage has been derived from studies of mammalian myocardium and bowel where an inadequate O_2 supply is pathological. This is not true for intertidal invertebrates which thrive in an environment characterized by alternating periods of hypoxia when the tide ebbs and normoxia when the tide returns. Because these animals are so well-adapted to environmentally imposed ischemia-reperfusion, they are ideal organisms in which to examine resistance to events underlying mammalian reperfusion injury. Because earlier phylogenetic surveys of invertebrate xanthine catabolism predate awareness that XDH is convertible into an oxidase, two years ago we began to re-examine the distribution of XDH and XO amongst the molluscs [Dykens and Shick, Comp. Biochem. Physiol., in press]. Work at MDIBL last summer expanded the initial survey and confirmed that, contrary to the earlier literature, xanthine catabolism in most normoxic invertebrates is due to xanthine dehydrogenase, not oxidase, activity (Dykens, in prep.).

Many intertidal invertebrates avoid environmentally-imposed reoxygenation injury because under anoxic conditions they reduce metabolic rates and avoid adenylate catabolism by relying on efficient anaerobic pathways. For example, during imposed anoxia, the bivalve *Mytilus edulis* undergoes metabolic arrest and reduces heat dissipation 80-90% compared to rates in aerobic conditions [Pamatmat, Mar. Biol. 53:223-230, 1979]. This bivalve survives anoxia for up to 35 days [Theede, *et al.*, Mar. Biol. 2:325-337, 1969] with no reduction in the total adenylate pool observed during at least the initial 6 h in anoxia [Brinkhoff, *et al.*, Oecologia 57:151-155, 1983]. Our work at MDIBL confirms that reoxygenation injury is also avoided because most euryoxic species have only XDH activity, eg. 44.6 mUnits x $g_w W^{-1}$ (± 7.1 SE, N = 14) in *Mytilus*. Moreover, *Mytilus* XDH resists treatment by proteases and sulfhydryl oxidants that readily convert the labile mammalian XDH into XO *in vitro*.

In contrast, adenylates are reduced in aerobically poised bivalves after even brief hypoxic exposue: total adenylates in the scallop *Placopecten magellanicus* (excluding adductor muscle) decline 30% during 90 min of hypoxia

[de Zwaan, et al., J. Comp. Physiol. 137: 105-114,1980]. Accordingly, last summer we focused our attention on Placopecten as a likely candidate for difficulty during anoxic-normoxic transition. We found that Placopecten survives 48 h of hypoxia (P_{O2} < 25mmHg, 13°C, N = 8), but dies during the ensuing 40 h of reoxygenation (N = 4). Only XDH activities were detected in normoxic Placopecten, and the only two observations of XO were both in individuals that succumbed during reoxygenation (5.2 and 4.6 mU x $g_w W^{-1}$).

Although the evidence is compelling that oxygen-derived radicals mediate reperfusion injury [McCord, op. cit.; Zweier, et al., PNAS 84: 1404-1407, 1987], XO activities in Placopecten and in mammalian tissues [Engerson, et al. J. Clin. Invest. 79:1564-1570, 1987; Parks, et al., Gastroent. 92:1124, 1987], are insufficient to account fully for observed tissue necrosis. In air-equilibrated phosphate buffer at pH 7.8, only 15% of the total oxygen flux through XO is univalently reduced to $O_2^{\cdot-}$, and this percentage is diminished by reduced intracellular P_{O2} and pH [Fridovich, J. Biol. Chem. 245: 4053-4057, 1970]. Thus, actual $O_2^{\cdot-}$ production from XO during reperfusion is > 1.0 nM x min^{-1} x $g_w W^{-1}$ in Placopecten, and > ~ 30 nM x min^{-1} x $g_w W^{-1}$ in human myocardium. It therefore seems unlikely that XO is solely responsible for the μM concentrations of oxy-radicals detected using EPR spectroscopy in mammalian myocardium during reperfusion [Zweier, op.cit.]. It should be noted in this context that oxygen-derived radicals are produced by many cellular processes such as autoxidation of NADH dehydrogenase and ubiquinone, events likely accelerated by hypoxia [Bovaris and Cadenas, pp.15-30 in Superoxide Dismutase,II. (L.W. Oberley, ed.), CRC Press, Boca Raton, FL, 1982].

Regardless of source, the cytotoxicity of radicals produced during reoxygenation would be exacerbated if cellular oxidative defenses were also impaired by hypoxia. A major cellular defense against $O_2^{\cdot-}$ is the enzyme superoxide dismutase (SOD) [Fridovich, Science 201: 875-880, 1978]. In light of the apparent inability of XO to account for reoxygenation injury, last summer we began an assessment of SOD activity in Placopecten and Mytilus during hypoxia-reoxygenation. Hypoxia (48 h) followed by normoxia (40 h) did not alter SOD activities in Mytilus, which tolerates these conditions. Conversely, SOD activity in hepatopancreas and gill from Placopecten, which succumbs to identical hypoxic-normoxic exposures, was reduced 76% and 58% respectively ($P < 0.0001$, multiway ANOVA, Scheffe's)(Dykens,in prep.).

The data suggest that resistance to reoxygenation injury in many invertebrates is imparted by several mechanisms. Animals tolerant of cyclical tidal emersion-immersion avoid xanthine accumulation by slowing metabolic rates thereby forestalling ATP depletion. These animals not only contain a form of XDH that does not become an oxidase under conditions capable of converting mammalian XDH into XO, but they also maintain oxidative defenses (= SOD activities) during hypoxia-reoxygenation. Conversely, invertebrates susceptible to reoxygenation injury show reductions in adenylate pools during hypoxia and contain a form of XDH that is convertible into the deleterious oxidase. Cytotoxicity of even low XO activities is exacerbated by concomitant impairment of oxidative defenses (= diminished SOD). Autoxidation of invertebrate ubisemiquinone and NADH dehydrogenase during hypoxia-reoxygenation, and potentiation of superoxide reactivity by transition metals, await examination. However, simultaneous assessment of both SOD and XDH/XO activities permits tentative apportioning of blame for reoxygenation injury between oxidative attack vs. failure of oxidative defenses.

Supported by a Markey Fellowship award from the Lucille Markey Charitable Trust to JAD, and by the Grinnell College Faculty Grant Board.