CREATINE AND ARGININE PHOSPHOKINASE ARE SELECTIVELY INACTIVATED BY SUPEROXIDE RADICAL EXPOSURE: IMPLICATIONS FOR REOXYGENATION INJURY

James A. Dykens and John Rogers
Biology Department, Grinnell College, Grinnell, IA 50112

Aerobically poised mammalian tissues such as heart and brain survive hypoxia for several minutes without apparent harm. However, necrosis becomes inevitable after 10-20 minutes of hypoxia either from prolonged hypoxia, or paradoxically, from O_2 resupply. Such reoxygenation tissue damage (RTD) is mediated by cytotoxic superoxide (O_2^-) and hydroxyl radicals (·OH) produced during reoxygenation [McCord, N. Engl. J. Med. 312: 159-163, 1985].

Although the source of these radicals remains unresolved (see Dykens et al., this issue), even slight increases in radical formation could be injurious if key regulatory enzymes were particularly susceptible to radical exposure. It has been reported that O_2^- selectively inactivates creatine phosphokinase (CPK) during rat myocardial reperfusion (McCord and Russell, pp. 27-35 in Oxy-Radicals in Molecular Biology and Pathology, eds. Cerutti, Fridovich, and McCord, Alan Liss Inc., 1988). If so, the ability to access and recharge the phosphagen energy pool could be impaired during reperfusion.

The evidence for selective CPK inactivation by O_2^- is suggestive, but the susceptibility of other metabolic enzymes has not been examined. Moreover, the buffers in the initial report [McCord and Russell, op. cit.] contained EDTA, a chelator that fails to block Fe-catalyzed ·OH production.

Accordingly, we examined the susceptibility of mammalian CPK, lactate dehydrogenase (LDH), and glucose-6-phosphate dehydrogenase (G6PDH), to O_2^- and ·OH inactivation. We also examined arginine phosphokinase (APK) and octopine dehydrogenase (ODH) from two stenoxic, aerobically-poised invertebrates (lobster and scallop respectively). Superoxide was generated using 1.4 mU commercial XOD in the presence of 50 μM hypoxanthine and diethylenetriamine-pentaacetic acid (DETAPAC), a chelator which ligands all five iron orbitals thereby preventing ·OH production [Halliwell and Gutteridge, op. cit.]. Hydroxyl radicals were generated using 0.18 μM H_2O_2 in the presence of 1 mM Fe^{3+}-EDTA.

In accord with its extreme reactivity, exposure to ~90 nmoles of ·OH substantially impairs the activities of almost all the enzymes examined, although to varying degrees: CPK activity declined 54%, APK by 50%, LDH by 24%, and G6PDH by 65%. Intriguingly, ODH was unaffected by ·OH. Exposure to over 200 nmoles O_2^- reduces LDH, ODH, and G6PDH activities by less than 5%. Conversely, CPK and APK are extraordinarily susceptible to O_2^-; exposure to less than 50 nmoles O_2^- diminishes CPK activity by 85%, and APK by 40%.

RTD is mediated by a variety of oxy-radicals that kill cells via multiple mechanisms, such as membrane lipid peroxidation and impairment of oxidative phosphorylation. The observed radical-induced differential impairment of metabolic enzymes, which could disrupt normal metabolic flux, plus selective O_2^- inactivation of phosphagen kinases, which could impede reestablishment of normal cellular energy status during reoxygenation, should be considered not only as additional causes of RTD, but also as proximate determinants of stenoxia in aerobically-poised mammalian and molluscan tissues.

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