OXYGEN: THE DARK SIDE

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Aerobes certainly gain advantages from O_2 ; in generation of useful energy, in intermediary metabolism, and in detoxication of hydrophobic xenobiotics. But, there is a down side. O_2 is a paramagnetic gas, by virtue of containing two unpaired electrons, and this predisposes it to reduction in univalent steps. Intermediates are encountered on this univalent pathway; which are reactive enough to rend the delicate fabric of living substance. These are the superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO^{\cdot}$). Furthermore, $O_2^{\cdot -}$ can give rise to H_2O_2 by dismutation, either spontaneous or catalyzed; and $O_2^{\cdot -}$ plus H_2O_2 can conspire to produce $HO^{\cdot}$. This production of $HO^{\cdot}$ occurs *in vitro* when $O_2^{\cdot -}$ reduces Fe(III) to Fe(II); which in turn reduces H_2O_2 to OH^- plus $HO^{\cdot}$. The *in vivo* process is quite different because $O_2^{\cdot -}$ then participates by oxidizing the [4Fe-4S] clusters of dehydratases, such as aconitase. The oxidized cluster is unstable and loses Fe(II) which is then able to reduce H_2O_2 to OH^- plus $HO^{\cdot}$ (1,2).

These reactive progenies of oxygen reduction are really produced under ordinary circumstances in aerobic cells, in amounts which would be intolerable were it not for multilayered defenses. These are superoxide dismutases, to convert $O_2^{\cdot -}$ into $H_2O_2 + O_2$, and catalases to convert H_2O_2 into $O_2 + H_2O$. There are also peroxidases which use cellular reductants, such as G-SH and NADPH, to reduce H_2O_2 to water, and alkyl hydroperoxides to corresponding alcohols. These defenses markedly diminish the damage which is done by reactive oxygen-derived species, but they do not entirely prevent such damage. This necessitates repair and resynthesis and there are enzymes dedicated to these tasks.

Unreliable Measures of $O_2^{\cdot -}$. Bis-acrydylm salts (lucigenin) can be univalently reduced to a monocation radical, which adds to $O_2^{\cdot -}$ to yield an unstable dioxetane, whose decomposition results in luminescence. Since this chemiluminescence depends upon $O_2^{\cdot -}$ it is inhibited by SOD and can be used to assay SOD activity, both *in vitro* (3) and within living cells (4). Lucigenin luminescence has also been used as a measure of $O_2^{\cdot -}$; but this is not advisable because the lucigenin monocation radical can autoxidize and produce $O_2^{\cdot -}$. Any system capable of the univalent reduction of lucigenin would thus appear to be producing $O_2^{\cdot -}$; even when it could not do so in the absence of lucigenin. Tetrazolium salts can similarly mediate $O_2^{\cdot -}$ production and give a false indication of $O_2^{\cdot -}$ production (5). Amino phthalhydrazide (luminol) luminescence is also a misleading indicator of $O_2^{\cdot -}$ production. In this case the luminol is oxidized univalently to a radical, which can reduce O_2 to $O_2^{\cdot -}$; or which can add $O_2^{\cdot -}$

to yield an unstable endoperoxide whose decomposition leads to luminescence (6).

Bacterial Cu,ZnSODs - It has become apparent that gram negative bacteria commonly contain a periplasmic Cu,ZnSOD. In the case of *E. coli* this SOD appears late in the growth cycle (7) and is peculiar in being monomeric; whereas most Cu,ZnSODs described to date have been homodimeric. The periplasmic localization suggests that this SOD may protect against exogenous $O_2^{\cdot -}$. It certainly contributes to survival in stationary phase (8).

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