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Mini-Review: Oxidative stress, redox stress or redox success?

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ABSTRACT

The first life forms evolved in a highly reducing environment. This reduced state is still carried by cells today, which makes the concept of "reductive stress" somewhat redundant. When oxygen became abundant on the Earth, due to the evolution of photosynthesis, life forms had to adapt or become extinct. Living organisms did adapt, proliferated and an explosion of new life forms resulted, using reactive oxygen species (ROS) to drive their evolution. Adaptation to oxygen and its reduction intermediates necessitated the simultaneous evolution of select antioxidant defences, carefully regulated to allow ROS to perform their major roles. Clearly this "oxidative stress" did not cause a major problem to the evolution of complex life forms. Why not? Iron and oxygen share a close relationship in aerobic evolution. Iron is used in proteins to transport oxygen, promote electron transfers, and catalyse chemical reactions. In all of these functions, iron is carefully sequestered within proteins and restricted from reacting with ROS, this sequestration being one of our major antioxidant defences. Iron was abundant to life forms before the appearance of oxygen. However, oxygen caused its oxidative precipitation from solution and thereby decreased its bioavailability and thus the risk of iron-dependent oxidative damage. Microorganisms had to adapt and develop strategies involving siderophores to acquire iron from the environment and eventually their host. This battle for iron between bacteria and animal hosts continues today, and is a much greater daily threat to our survival than "oxidative stress" and "redox stress".

1. Introduction

Oxygen free radicals and other reactive oxygen species (ROS)[1] are known to be formed in all aerobic organisms and play a plethora of useful roles. However, some of them have the potential to cause damage ("oxidative damage") to biomolecules, which is thought to contribute to the development and progression of certain diseases, especially cancer and neurodegenerative diseases such as Parkinson and Alzheimer diseases [1–6]. Sadly however, the diet-derived antioxidants that have been tested to date in clinical trials have in general not proved very useful (and were sometimes harmful) in preventing the onset or even slowing the progression of these diseases [1,7–11].

In considering the role of oxidative damage and ROS in human disease, the term "oxidative stress" is frequently used. This was originally simply defined as an imbalance between ROS and antioxidant defence mechanisms, but the definition has been modified several times and become more convoluted: a recent version is *an imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signalling and control and/or molecular damage* [12]. The term "reductive stress" is now also appearing in the literature (e.g. Refs. [13,14]) although its exact definition seems unclear.

So let us go back to first principles to evaluate the meaning and likely significance of oxidative and reductive stress.

1.1. The origin of life

The first life forms evolved in a highly reducing environment, and most of those same reducing chemicals are still with us today. Living cells are generally in a highly reduced state even today and if you oxidise them they are likely to die [1]. Chemicals do not "mutate" or change their reactivity, and so any "reductive stress" experienced by cells remains essentially the same today as it did during early evolution. The fact that life forms did survive and adapt in a fearsomely reductive environment suggests that reductive stress cannot really be a problem.

A similar logic can be applied to the adaptation and

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[1] The definition of these terms is explored in detail in [1].

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development of life forms in an environment with increasing levels of molecular oxygen (O_2). When O_2 started to enrich the atmosphere some 2.2 billion years ago, due to the photosynthetic activity of the Cyanobacteria [1,15,16], adaptations to allow cells to stay mostly reduced despite an oxidative environment were initiated. Single cell organisms successfully progressed to multicellular ones, and eventually to complex vertebrate life as we know it today [1,15–17]. The use of O_2 as an oxidant to release energy from food-derived substrates inevitably means that the O_2 is reduced. The reduction intermediates (ROS) are well characterised and were present during the evolution of all aerobic life forms, and did not impede progress towards the complex life forms we know today. Indeed, the presence of O_2 facilitated an "explosion" of novel life forms, sometimes called the Cambrian explosion [16,18]. The products of O_2 reduction, namely superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and possibly even hydroxyl radical ($OH^{\bullet}$), were harnessed by cellular systems for useful purposes, such as trigger, messenger, signalling and housekeeping (phagocytosis) functions [1,15–19]. ROS are the same today as they were billions of years ago, and it seems extremely unlikely that they have suddenly become a serious threat to life forms. Otherwise, how did life evolve in such abundance? To quote Taverne et al. [15] "the ability to use ROS for cell signalling and regulation may have been the first true breakthrough in development of complex life".

2. Iron and oxygen. two free radicals that made complex life possible

Using the broad definition of a free radical [1], both O_2 and iron are free radicals. Diatomic oxygen has two unpaired electrons located in different anti-bonding orbitals. Iron ions in solution readily donate or accept a single electron, from ferrous (Fe^{2+}) to ferric (Fe^{3+}) and back again. The redox potential of Fe^{2+}/Fe^{3+} varies widely depending on the ligands around the iron, and so iron is very versatile as a catalytic centre or electron carrier in proteins [1,20].

Oxygen is the most abundant element in the Earth's crust, and iron is the fourth most abundant. During the reductive phase of the Earth's development, iron was plentiful in the great oceans of the time, and mainly in the reduced ferrous state (Fe^{2+}) [1,16,17]. Thus it became very widely used by living organisms for catalysis and electron transfer [1,17,20]. With the ever increasing release of O_2 into the atmosphere as a result of photosynthesis, ferrous ions in the Earth's waters were oxidised to the ferric state and precipitated from solution as insoluble complexes. Today, iron is only a trace constituent of sea water at around 2-3 x 10^{-9} kg/L. This solved one problem: Fe^{2+} in the presence of ROS is bad news because it can catalyse formation of much more highly-reactive species such as $OH^{\bullet}$ [1,21–25]. But it created another one: how could organisms continue to obtain the Fe^{2+} that they had become used to and dependent on? This is further addressed in Section 4 below.

2.1. Safe transport of oxygen

At concentrations greater than 21%, O_2 is demonstrably toxic to life forms. In fact, it can cause damage at any level, as demonstrated by the presence of biomarkers of oxidative damage [1,26–30], but this is easily dealt with by repair systems under normal circumstances. Probably if introduced into medicine today, O_2 would struggle to obtain a safety license from regulatory authorities. Indeed, the idea that sick patients need extra O_2 is being challenged in a range of conditions [31–33]. Nevertheless, organisms have adapted to live with the present day concentration of O_2. As the concentration increases above 21% so does the possibility of significant tissue damage; however the respiratory tract, cornea of the eye and the outer layer of skin are the only tissues exposed to this oxygen concentration [1]. Mechanisms evolved in animals to safely deliver O_2 to all tissues that require it, at concentrations well below 21%. Indeed, this may be one of the best "antioxidant defences"; minimise the exposure of most cells in the body to O_2 [1]. Oxygen is conveyed to mammalian tissues associated with the haem moiety of the proteins haemoglobin and myoglobin. In order for this association to work, iron in haem has to be in the ferrous state (Fe^{2+}). Since Fe^{2+} ions free in solution react rapidly with O_2 to form ROS [1], these proteins are a good example of the structural evolution of antioxidant protection which allows the safe transport of O_2 and safe use of Fe^{2+} [1,21–25].

2.2. Safe transport and storage of iron

A normal healthy adult male contains around 4 g of iron. Iron ions are almost entirely carefully sequestered in forms that deter their reactions with O_2, H_2O_2 etc [1,21–25], as well as greatly decreasing their availability to micro-organisms. The majority of iron is associated with the haem moiety of the O_2 transport proteins haemoglobin and myoglobin. Intracellularly, iron is mainly stored within ferritin, as well as used as a catalyst in enzymes involved in oxygen metabolism and proteins performing electron transfers [1,20,25,34–36]. Extracellularly, transferrin and lactoferrin are the major iron-containing proteins. They have the ability to bind iron with very high affinity, and are mainly involved in transporting iron around the body. In normal adult health they are around 30% loaded with iron and retain a large capacity to bind further iron ions [1,34–37]. This iron-binding capacity gives the proteins a powerful *in vitro* antioxidant activity towards iron-driven free radical reactions such as $OH^{\bullet}$ formation [1,21–25,37]. Because of the large iron-binding capacity of transferrin, there are no low molecular mass iron complexes in plasma from normal healthy adults [1,21–25,38,39]. Iron overload is a complication of certain pathological conditions such as the genetic diseases collectively called idiopathic haemochromatosis. In patients with these conditions, plasma transferrin becomes fully saturated with iron, and chelatable low molecular mass iron complexes can be detected and measured in the plasma using the bleomycin or other assays [1,21–25,38–40]. The iron has been shown to be associated with ligands such as citrate [1,41], and to be a virulence factor for the growth of certain organisms [1,35]. It is also pro-oxidant, catalysing oxidative damage, demonstrated in haemochromatosis [42,43], thalassaemia [44] and in patients with iron overload due to chemotherapy [45], among other conditions. The importance of "catalytic" iron has recently been re-illustrated by the growing literature about "ferroptosis", a form of programmed cell death driven by catalytic iron [46].

3. Antioxidant myths

For hundreds of years antioxidants have been used in the preservation of foods in order to prolong their shelf life. These can range from herbal/spice extracts (common in antiquity) to chain-breaking antioxidants to iron chelators and to techniques that limit the exposure of food to oxygen [1]. Products such as rubber, oils and plastics also require the addition of antioxidants in order to prevent their deterioration ("perishing") in an atmosphere containing O_2 [1].

Unfortunately, our own 'shelf life' cannot be prolonged in the same way, in spite of frequent claims for the opposite. The antioxidant pills and potions used to date have made little or no impact on human health or ageing [1,7,8], for the reason that antioxidant protection is an evolved strategy billions of years old that resists being easily tampered with. Indeed, there is more interest now in

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