Biochemistry of oxidative stress

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Abstract

The terms 'antioxidant', 'oxidative stress' and 'oxidative damage' are widely used but rarely defined. This brief review attempts to define them and to examine the ways in which oxidative stress and oxidative damage can affect cell behaviour both *in vivo* and in cell culture, using cancer as an example.

Introduction: defining antioxidants

Oxygen is poisonous, and aerobic organisms survive its presence only because they contain antioxidant defences [1]. Antioxidants can be synthesized *in vivo* (e.g. GSH and superoxide dismutases) or taken in from the diet, although attempts to identify the most important diet-derived antioxidants have led to confusion rather then enlightenment, a point on which I will not elaborate here (see [1–3] for detailed discussions of my views). But what *is* an antioxidant? The term is surprisingly difficult to define clearly and comprehensively, since the hierarchy of antioxidant capacity depends to a substantial extent on the assay methodology used – change the method, and the antioxidant ability of any given compound is different [1]. Some time ago, John Gutteridge and I [4] attempted to define an antioxidant as "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate". The term oxidizable substrate includes every organic molecule found *in vivo*.

Our definition emphasizes the importance of the damage target studied and the source of RS (reactive species) used when antioxidant action is examined. We were somewhat surprised that others adopted our definition so readily, because it is clearly imperfect. For example, plasma albumin may bind copper and protect extracellular targets such as LDLs (low-density lipoproteins) against oxidative damage [5], but here the albumin is in considerable molar excess over the LDL. The definition does not take into account chaperones, repair systems (e.g. repair of oxidatively damaged DNA or of methionine sulfoxide residues in proteins) or inhibitors of RS generation; should we call these antioxidants or not? Some scientists do, others argue against. Thus we simplified the definition to "any substance that delays, prevents or removes oxidative damage to a target molecule" [1].

There is no universal 'best' antioxidant; the rank depends on the nature of the oxidative challenge. Perhaps the simplest 'antioxidant defence' (although hard to fit into the definition above, unless we rewrite it as 'any substance or action that delays') is to minimize exposure to O_2. Insects, for example, seem to open and shut their spiracles to maintain a low but constant intracorporeal P_{O_2}, subject to the need to allow CO_2 to escape [6]. Food manufacturers exploit this strategy when they seal foods under nitrogen or in vacuum packs. The human vascular system does a masterful job of delivering just the right amount of O_2 to our cells and tissues, not too little, not too much. A third example, some stem cells may lurk in hypoxic environments to prevent them from accumulating oxidative damage until they are needed. Stem cells may also be enriched in antioxidant defences [7,8].

Oxidative damage and oxidative stress

In healthy aerobes, production of RS is approximately balanced with antioxidant defence systems [1]. The balance is not perfect, however, so that some RS-mediated damage occurs continuously. In other words, antioxidant defences control levels of RS rather than eliminate them, e.g. the OxyR system in *Escherichia coli* keeps H_2O_2 levels at approx. 0.2 μM [9]. Why is this? Maintaining excess antioxidant defences has an energy cost: it could be energetically 'cheaper' to repair or replace damaged biomolecules [1]. In addition, antioxidants may simply be unable to intercept some RS. For example, hydroxyl radical ($OH^{\bullet}$) generated by homolytic fission of water due to our background exposure to ionizing radiation [10] is so highly reactive that it will react with whatever it meets first and so is virtually impossible to scavenge. Yet another factor is that RS play essential roles in *vivo*, not least in the redox regulation of gene expression and other cellular events [9,11–14].

The term 'oxidative stress' refers to a serious imbalance between RS production and antioxidant defences. Sies [15] defined it as "a disturbance in the pro-oxidant–antioxidant balance in favour of the former, leading to potential damage". Such damage is often called 'oxidative damage', another vague term. Matthew Whiteman and I have defined oxidative damage as "the biomolecular damage caused by attack of RS upon the constituents of living organisms" [16]. Increased levels of oxidative damage can result not only from oxidative stress, but also from failure of repair or replacement systems, e.g. a rise in levels of 'biomarkers' of oxidative damage need not always imply a greater level of oxidative stress [1,16]. "Caused by attack" is a phrase worthy of thought: do we mean only direct attack, or should we include secondary consequences

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Abbreviations used: Hsp, heat-shock protein; LDL, low-density lipoprotein; ROS, reactive oxygen species; RS, reactive species.
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of such attack? For example, oxidative stress can directly damage Na^+/K^+-ATPase [17,18] and modulate the actions of K^+ channels by chemical reaction with amino acid residues [18,19]. This is clearly oxidative damage. However, the changes in ion balance that result trigger many other deleterious events such as cellular volume changes [20]; do we count those as oxidative damage as well? Some of the most striking effects of oxidative stress are on cellular Ca^{2+} metabolism, tending to increase levels of intracellular 'free' Ca^{2+} [21–23]. One effect of raised Ca^{2+} is to increase cellular proteolysis by calpain activation [21]. Should we count the resulting proteolysis as oxidative damage? We prefer not to since it is not directly caused by RS [16], but the point is debatable.

'Oxidative damage' is a term frequently used to imply random, indiscriminate damage to a wide range of biomolecules, yet the targets often appear surprisingly specific. Thus, in Parkinson's disease, the increased oxidative DNA damage seems to affect only guanine [24]. In cells subjected to oxidative stress, proteomic techniques reveal that often only a small number of proteins is damaged, although the mechanisms of this selectivity remain undetermined in most cases (one must also think carefully about the accuracy of such techniques in detecting low levels of damage in low-abundance proteins). For example, in *E. coli* treated with H_2O_2, alcohol dehydrogenase E, elongation factor G, enolase, an Hsp (heat-shock protein), an outer membrane protein and oligopeptide-binding protein A were the major targets of oxidative damage [25]. In yeast, H_2O_2 treatment damaged mitochondrial enzymes, Hsp60, cytosolic fatty acid synthase, glyceraldehyde-3-phosphate dehydrogenase and peroxiredoxin [26]. During aging, only certain proteins seems to accumulate extensive oxidative damage, such as carbonic anhydrase III in rat liver [27] and aconitase and adenine nucleotide translocase in housefly muscle mitochondria [28]. Oxidative DNA damage also seems to localize preferentially in certain genes [29]. Despite this, the potentially indiscriminate nature of oxidative damage is one feature consistent with the free radical theory of aging [1]; even the genetically uniform *Caenorhabditis elegans* kept under similar laboratory conditions show a striking randomness in aging and death [30], and genetic manipulations that increase longevity seem to act, in part, by raising antioxidant defence levels [31].

Oxidative stress and cell culture

Molecular biologists often try to learn about normal cellular events by studying cells in culture. Indeed, much has been learned, but cells in culture are in an abnormal state [1,32]. Culture media are often deficient in antioxidants (e.g. vitamins C and E) and antioxidant precursors (e.g. selenium) and contain 'free' metal ions, present as contaminants or even added deliberately [e.g. Fe(III) salts are added to DMEM (Dulbecco's modified Eagle's medium) [32–33]]. Given that most animal cells are cultured as a monolayer under 95% air/5% CO_2 (approx. 152 mmHg O_2; 1 mmHg = 0.133 kPa), they are also in a grossly hyperoxic environment (most cells in the human body experience <10 mmHg O_2). This is likely to increase their rates of ROS (reactive oxygen species) formation. Given the potential rapidity of Darwinian 'natural selection' in repeatedly dividing cultured cells, it is possible that growth of such cells in the pro-oxidant environment of cell culture over many generations leads to evolution to use ROS for signalling pathways that promote proliferation and suppress cell death by mechanisms that may not normally operate *in vivo* [34,35]. The pro-oxidancy of cell culture has especially affected studies of cellular 'senescence' after repeated cell division in culture. Indeed, in mouse embryonic fibroblasts in culture, oxidative stress seems more important than telomere shortening in causing senescence. Culture under hypoxic conditions preserves the replicative potency of many cells, i.e. the 'Hayflick limit' is in part an artefact of hyperoxic culture conditions rather than a fundamental phenomenon related to aging [36–38]. Many malignant cells produce large amounts of H_2O_2 *in vitro*, but is this an adaptation to cell culture or a true feature of malignancy? A bit of both probably (the issue is discussed in detail in [35]).

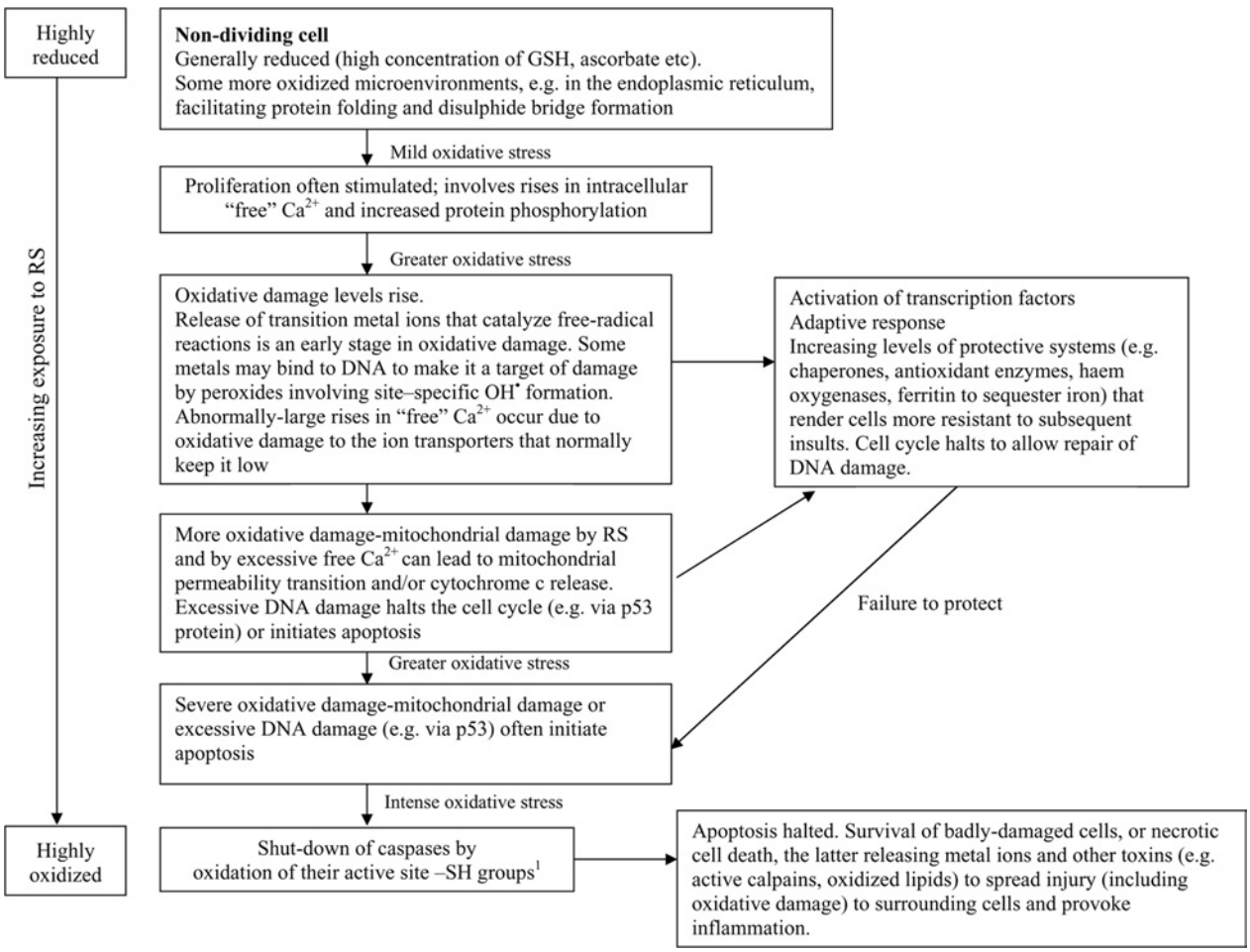
Results of cell culture studies can also be confused by oxidations in the medium, especially given the presence of iron and other transition metals [32]. This has confounded some studies of O_2 toxicity to bacteria. On exposure of bacterial cultures to O_2, media constituents can sometimes be oxidized to generate extracellular superoxide radical ($O_2^{\bullet -}$) and other ROS that can damage the bacteria, data that can be misinterpreted as direct effects of O_2 on the cells [39,40]. Oxidations in culture media have also led to fallacious results in at least some studies of the effects of ascorbate, phenolic compounds and other antioxidants on cells. The added compounds underwent oxidation in the culture media to produce H_2O_2 and other oxidation products (e.g. quinones and semiquinones from polyphenols) that were the true mediators of the effects observed [32,33,41–43].

Cellular consequences of the RS/antioxidant balance

Cells show a wide range of responses upon exposure to RS, ranging from increased proliferation, prevention of cell division, senescence, necrosis, apoptosis, or cell death mechanisms with features of both (e.g. [44]) (Figure 1). The effects are to some extent cell-type-specific, being influenced by such parameters as the presence of certain cell-surface receptors and signal transduction mechanisms, as well as antioxidant defence levels. Indeed, the mild pro-oxidancy of cell culture may be one reason why certain cells proliferate so readily in the laboratory [32,45,46]. In the context of cancer, angiogenesis, carcinogen metabolism and metastasis may also be affected by RS (reviewed in [35]). The balance of evidence indicates that oxidative stress plays a significant part in cancer development [35]. Yet oxidative stress is not always bad: formation of RS at sites of inflammation can not only destroy invading pathogens but also help modulate an overexuberant inflammatory response under certain circumstances (reviewed in [47]). This is one of many

Figure 1 | How cells respond to increasing exposure to RS

Adapted from Figure 4.1, p. 189, in 'How cells respond to oxidative stress' from *Free Radicals in Biology and Medicine* (2006) by B. Halliwell and J.M.C. Gutteridge, by permission of Oxford University Press (http://www.oup.com). Stimulation of proliferation by low levels of RS is associated with increased net phosphorylation of multiple proteins, often because RS inactivate protein phosphatase enzymes and sometimes because of increased protein kinase activity. [1]Caspase activity can also be modulated by changes in intracellular pH caused by RS.



paradoxes [1,48] in the free radical/antioxidant field that make it an exciting and stimulating arena in which to work. Indeed, all aspects of aerobic life involve free radicals and antioxidants [1,49]: you cannot escape them, nor should you wish to.

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