Review

Strategies of antioxidant defense

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Cellular protection against the deleterious effects of reactive oxidants generated in aerobic metabolism, called oxidative stress, is organized at multiple levels. Defense strategies include three levels of protection; prevention, interception, and repair. Regulation of the antioxidant capacity includes the maintenance of adequate levels of antioxidant and the localization of antioxidant compounds and enzymes. Short-term and long-term adaptation and cell specialisation in these functions are new areas of interest. Control over the activity of prooxidant enzymes, such as NADPH oxidase and NO synthases, is crucial. Synthetic antioxidants mimic biological strategies.

Contents

The biochemistry of oxidative stress [1] and hydroperoxide metabolism in mammalian organs [2] have been a focus of research for some time. The nature of various biological oxidants was found to cover large ranges in biological lifetime, in concentration, and in the occurrence in cells and organs. Experimental studies revealed that cells and organisms require defense against oxidants, without which survival under aerobic conditions would be jeopardized. In view of the variety in oxidants, also called prooxidants, it is not surprising that nature has evolved a battery of different types of antioxidants.

This article will examine the strategies of antioxidant defense in biological systems. Emphasis will be more on identifying the types of antioxidants or the principles of defense, rather than on reviewing the available literature. For further information, the reader is referred to [3−7] which concentrate on free-radical research in the fields of biology and medicine.

Oxidative stress and the principles of protection

Aerobic metabolism entails the production of reactive oxygen species, even under basal conditions, hence there is a continuous requirement for inactivation of these reactive oxygen species. The steady-state of prooxidants and antioxidants may be disrupted. A disbalance in favor of the prooxidants and disfavoring the antioxidants, potentially leading to damage, has been called 'oxidative stress' [8, 9]. Such damage may afflict all types of biological molecules, including DNA, lipids, proteins and carbohydrates. Thus, oxidative stress may be involved in processes such as mutagenesis, carcinogenesis, membrane damage, lipid peroxidation, protein oxidation and fragmentation, as well as carbohydrate damage.

In principle, protection against such deleterious effects can be by prevention, interception and repair. All these forms of protection are realized in biology, and examples will be given below. In order to lay out the variety of problems afflicting protective measures, the nature of the prooxidants and antioxidants will first be presented.

Nature and diversity of prooxidants

Molecular oxygen can be reduced to water. The intermediate steps of oxygen reduction are formation of the superoxide anion radical, hydrogen peroxide and the hydroxyl radical, corresponding to the steps of reduction by one, two and three electrons, respectively. Further, ground-state molecular (triplet) oxygen, as a diradical, can be electronically excited to singlet molecular oxygen. Oxygen radicals, in combination with other atoms or larger molecules, can occur as $RO^{\cdot}$ or $ROO^{\cdot}$, alkyl or peroxyl radicals, e.g. in lipids. Also, there is nitric oxide, $NO^{\cdot}$, one of the gaseous radicals of biological interest.

Oxidant functions are carried out by different types of radiation, with X-irradiation generating the hydroxyl radical, and irradation with ultraviolet light generating electronically excited states with subsequent radical formation. Ultrasound

Table 1. Estimate of the half-lives of reactive oxigen species. Modified from [31, 98].

Reactive oxygen species	Half-life
	s
HO•, hydroxyl radical	10^{-9}
RO•, alkoxyl radical	10^{-6}
ROO•, peroxyl radical	7
H_2O_2, hydrogen peroxide	-(enzymic)
$O_2^{\bullet}$, superoxide anion radical	-(enzymic)
$^1O_2$, singlet oxygen	10^{-5}
$Q^{\bullet}$, semiquinone radical	days
NO•, nitric oxide radical	1–10
$ONOO^-$, peroxynitrite	0.05–1

Table 2. Antioxidant defense in biological systems. Condensed list of antioxidant compounds and enzymes. Modified from [4].

System	Remarks
Non-enzymic	
α-tocopherol (vitamin E)	radical chain-breaking
β-carotene	singlet oxygen quencher
lycopene	singlet oxygen quencher
ubiquinol-10	radical scavenger
ascorbate (vitamin C)	diverse antioxidant functions
glutathione (GSH)	diverse antioxidant functions
urate	radical scavenger
bilirubin	plasma antioxidant
flavonoids	plant antioxidants (rutin, etc.)
plasma proteins	metal binding, e.g. coeruloplasmin
chemical	food additives, drugs (see text)
Enzymic (direct)	
superoxide dismutases	CuZn enzyme, Mn enzyme, Fe enzyme
GSH peroxidases	see enzymes (GPx, PHGPx) ebselen as enzyme mimic
catalase	heme protein, peroxisomes
Enzymatic (ancillary enzymes)	
conjugation enzymes	glutathione-S-transferases UDP-glucuronosyl-transferases
NADPH-quinone oxidoreductase	two-electron reduction
GSSG reductase	maintaining GSH levels
NADPH supply	NADPH for GSSG reductase
transport systems	GSSG export thioether (S-conjugate) export
repair systems	DNA repair systems oxidized protein turnover oxidized phospholipid turnover

and microwave radiation can also generate reactive oxygen species. Even shear stress, e.g. in homogenisation, is known to generate radicals.

As shown in Table 1, the half-lives of the major reactive oxygen species are vastly different, underscoring the necessity for different types of defense mechanisms. Highest rate constants for the reaction with target molecules are found for the hydroxyl radical; its reactions are diffusion limited, i.e. they take place practically at the site of generation. In contrast, some peroxyl radicals are relatively stable, with half-lives in the range of seconds. Such molecules may diffuse away from their site of generation and thus transport the radical or oxidant function to other target sites.

In cell metabolism, clandestine oxidant functions may exist and be transported to distant target sites where they exert oxidant activity. This would include compounds or enzyme activities that are innocuous in one environment but can be activated to generate oxidants under other conditions.

The diet contains many compounds of oxidant and antioxidant nature [10]. In the present context, it is important to note that there are dietary compounds which act as potential oxidants, including a variety of quinones, capable of redox cycling [11], and substrates for enzyme systems which generate oxidants.

Nature and diversity of antioxidants

In their definition of the term antioxidant, Halliwell and Gutteridge [3] state, 'any substance that, when present at low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate'. This definition would comprise compounds of nonenzymic as well as enzymic nature. Table 2 overviews some of the antioxidants of biological interest.

Clearly, the diversity of antioxidants matches that of prooxidants. In the following, some of the principles underlying the antioxidant functions will be discussed.

Prevention

A first line of defense against reactive oxygen species is, of course, protection against their formation, i.e. prevention. There are numerous strategies in biology designed to evade oxidative stress, ranging from the plankton that descends from the surface of the seawater to lower levels of solar irradation, to the packaging of DNA in chromatin to shield the genetic material by providing alternate targets. Microbes have developed specialized strategies to prevent oxygen-de-

pendent killing by phagocytes [12]. This extends to the concept that antioxidant defense in multicellular organisms can be more efficient than that in unicellular organisms, based on the observation that catalase in *Escherichia coli* can defend colonial, but not individual cells against hydrogen peroxide [13]. Thus, group protection by the activity of enzymes that mitigate oxidative stress was proposed as a driving force in the evolution of multicellular organisms [13]. Numerous further biological, physiological and morphological examples could be given. However, here we focus on the biochemical mechanisms of prevention.

Regarding radical formation, first it should be mentioned that some of the enzymes prone to generate free radical species are ingeniously designed. Cytochrome oxidase, carrying the predominant cellular share of oxygen reduction, does not release superoxide or other radicals, even though it contains iron and copper ions. Likewise, the three-dimensional structure of the enzyme, ribonucleotide reductase, keeps the radical character of the tyrosyl function in subunit B from spreading to the environment by forming an appropriate 'cage' [14].

Further, the prevention of initiation of chain reactions includes the binding of metal ions, in particular iron and copper ions. Metal chelation is a major means of controlling lipid peroxidation and DNA fragmentation. Thus, the metal-binding proteins, ferritin, transferrin, coeruloplasmin and others, e.g. metallothionein, are of central importance in the control of potential radical-generating reactions. Another strategy to increase the resistance to metal-ion-dependent oxidation is to

modify the potential target site. An example is the stable modification of low-density lipoprotein by dehydroascorbate or decomposition products thereof to impart increased resistance to metal-ion-induced oxidation [15].

Prevention of cells against incident radiation may occur through specialized pigments, e.g. the melanins for ultraviolet radiation or the carotenoids for electronically excited states such as singlet oxygen. Alternatively, compounds acting as photosensitizers may be reductively detoxified, for example as a mechanism of fungal resistance to cercosporin [16]. However, these and other strategies are not completely preventative, because they operate by decreasing the yield of a given challenging agent with less than 100% efficiency (see below).

In this regard, there are many enzymic systems in cells and body fluids which control the level of reactive species which otherwise might generate a cascade of products which, in turn, lead to attacking oxidants. One important group of such enzymes is the glutathione S-transferases. This family of enzymes catalyzes the reaction of the major low-molecular-mass thiol, glutathione, with reactive electrophiles to form thioethers, called S-conjugates [17, 18]. Biologically reactive electrophilic intermediates can be formed in a variety of metabolic pathways, notably those involving cytochrome P-450, and are of interest in toxicology and pharmacology [19].

A strategy of preventative antioxidation could therefore be formulated as prevention by diversion, i.e. by channeling an attacking species into a less harmful product, hence lowering the risk of further damage. In the extreme, this could involve whole cells, one example being the intestinal mucosal cells. These cells are exposed to a variety of reactive intermediates and xenobiotics, and the rate of accumulation of oxidative-damage products in these cells is high. The turnover and elimination of whole cells prevents further spread of the challenging species. Again, this type of prevention overlaps in part with the concept of interception.

Interception

Nonenzymic

This is the domain of the antioxidants as defined in a more narrow sense. The basic problem is to intercept a damaging species, once formed, from further activity. This is the process of deactivation. For radical compounds, the final deactivation consists of the formation of nonradical end products. Due to the nature of the free radicals, there is a tendency towards chain reaction, i.e. a compound carrying an unpaired electron will react with another compound to generate an unpaired electron in that compound ('radicals beget radicals').

A second objective of biological importance is to transfer the radical function away from more sensitive target sites to compartments of the cell in which an oxidative challenge would be less deleterious. In general, this means transferring the oxidizing equivalents from the hydrophobic phases into the aqueous phases, e.g. from the membrane to the cytosol or from lipoproteins in the blood plasma to the aqueous phase of the plasma. Biologically, the most efficient intercepting antioxidants combine optimal properties in both these objectives; firstly, they react with initial free radicals such as lipid peroxyl radicals at suitable rates, and, secondly, they are capable of interacting with water-soluble compounds for their own regeneration. This then transfers the radical function away from further potential targets. In biological membranes, where a high-efficiency back-up system is present, there may be the need for only 1−3 antioxidant molecules/1000 potential target molecules.

Such intercepting chain-breaking antioxidants are often phenolic compounds. (R,R,R)-α-Tocopherol probably is the most efficient compound in the lipid phase [20]. This biological antioxidant [21] contains shielding methyl groups in the vicinity of the phenolic hydroxyl group of the chromane moiety, and it is optimally positioned in the membrane by its phytyl side chain.

The maintenance of a steady-state rate of peroxyl-radical reduction by tocopherol in the membrane is dependent on the reduction of the tocopheroxyl radical, once formed, by external reductants. These include ascorbate and thiols [22, 23]. Whether the reaction occurs directly or through intermediate steps is still debated (see [24] for review) but, in vitro, the reaction has been clearly demonstrated by pulse-radiolysis experiments [25] and to occur in membrane systems [22, 23].

A prerequisite for efficient interception by the phenolic antioxidants resides in the life-time of the radical to be intercepted. This predisposes the peroxyl radicals as major reaction partners, since their life-time extends into the range of seconds (Table 1). In contrast, the hydroxyl radical, with its high reactivity and extremely short life-time, cannot be intercepted with reasonable efficiency. It has been shown that up to 100 mM of an intercepting compound would be required for 90% efficiency [26], eliminating interception as a useful strategy for defense against the hydroxyl radical, if only for osmotic reasons.

Interception of oxidants by cholesterol has also been proposed [27]. The B-ring oxidized oxysterols of human blood were considered to represent past interception in vivo by cholesterol. The oxysterols are efficiently metabolized and excreted by the liver. Another example would be the function of plasmalogens, suited for the reaction with singlet molecular oxygen [28]; the oxidation products would then be replaced by intact plasmalogen molecules, and the effect would be to avoid alternate targets and decrease the biological yield of the attacking species.

Highly efficient biological polyene quenchers for singlet molecular oxygen [29], notably carotenoids and oxycarotenoids, provide a suitable defense system against this oxygen species, in spite of its reactivity and short life-time. The localized concentrations of the carotenoids are decisive in determining the efficiency of the quenching of singlet oxygen and other electronically excited states [30, 31].

Enzymic

All cells in eukaryotic organism contain powerful antioxidant enzymes (for review, see [2]). The three major antioxidant enzymes are the superoxide dismutases [32], catalase and glutathione (GSH) peroxidases. In addition, there are numerous specialized antioxidant enzymes reacting with and, in general, detoxifying oxidant compounds (Table 2). Indirect antioxidant funcions carried by enzymes are (a) the back-up function, e.g. the replenishment of GSH from glutathione disulfide (GSSG) by the flavoprotein GSSG reductase, and (b) the transport and elimination of reactive compounds, e.g. the glutathione S-transferases and the transport systems for the glutathione S-conjugates.

For the present discussion, it is of interest to consider the fact that different subcellular sites and different cell types

contain varying amounts of the antioxidant enzymes (see [2]).

Repair

Protection from the effects of oxidants can also occur by repair of damage once it has occurred. As prevention and interception processes are not completely effective, damage products are continuously formed in low yields and hence may accumulate. This refers to DNA damage, occurring as damaged bases or in the form of single-strand or double-strand breaks, to membrane damage, occurring as a variety of phospholipid oxidation products, and to proteins and other compounds as well. Correspondingly, there are multiple enzyme systems involved in DNA repair and lipolytic as well as proteolytic enzymes capable of serving the functions of restitution or replenishment. Many supportive strategies are operative, for example, in the surveillance of the building blocks for DNA synthesis, the dGTP pool is enzymically cleared from the contaminant oxidized base, 8-oxo-dG [32a, b]. This very extensive field of repair is not reviewed here in detail.

Adaption: adaptive responses

Prokaryotes

The control of antioxidant enzyme levels in cells is of key importance for survival in an aerobic environment [33, 34]. While little is known about constitutive expression of antioxidant enzymes, the adaption of cells to oxidative stress has been a topic of active research, particularly with prokaryotes such as *Salmonella typhimurium* and *E. coli*. Bacteria adapt to the lethal effects of oxidants by induced the expression of protective stress genes under the control of regulons, e.g. oxyR [35] and soxR [36]. The oxyR gene product is redox sensitive and, in its oxidized form, activates gene expression [37, 38]. It is suggested that oxidation of the oxyR protein brings about a conformational state that transduces the oxidative-stress signal to selectively activate DNA transcription.

Bacterial strains carrying deletions in oxyR exhibit significantly increased frequencies of mutagenesis [39, 40], which are pronounced under aerobic conditions. The high frequency of mutagenesis in oxyR deletion strains was suppressed by multicopy plasmids expressing high levels of catalase (katG gene), alkylhydroperoxide reductase (ahpCF gene) or superoxide dismutase (sodA gene) activities ([39]; see Table 3). These observations provide evidence that the oxyR regulon plays an important role in protecting against oxidative DNA damage that would otherwise cause mutations.

Mammalian cells

Adaptive responses to several types of challenge, including heat shock and oxidative stress, have also been identified in human cells. For example, heme oxygenase was found to be a major stress protein produced in responses to oxidative challenge [41]. Reactive oxygen species activate NF-κB, a transcriptional regulator of genes involved in inflammatory and acute-phase responses [42, 43]. Modulation of NF-κB-binding activity by oxidation/reduction has been demonstrated *in vitro* [44]. Recently, expression of a human gene encoding a protein-tyrosine phosphatase was found to be

Table 3. oxyR deletion strains have increased frequencies of spontaneous mutagenesis. The frequency of mutagenesis in the *S. typhimurium* oxyR mutant strains was assayed by the reversion of His-auxotrophy to His⁺ prototrophy (taken from [39]). pKM101 encodes mucA and mucB (analogues of the *E. coli* umuC and umuD genes) that make strains more susceptible to mutagenesis by a number of mutagens.

Strain	Number of mutants/plate
oxyR⁺ (wild type)	6
oxyRΔ2 (oxyR deletion)	76
oxyR⁺/pKM101ᵇ	57
oxyRΔ2/pKM101	3102
oxyRΔ2/pKM101/pACYC184 (vector)	946
oxyRΔ2/pKM101/pAQ5 (oxyR)	33
oxyRΔ2/pKM101/pAQ6 (sodA)	196
oxyRΔ2/pKM101/pAQ7 (katG)	47
oxyRΔ2/pKM101/pAQ8 (ahp)	31

greatly induced by oxidative stress and heat shock in skin cells [45], linking redox signaling to protein phosphorylation. Thus, there is a relationship between redox changes and regulation of receptor activity, cellular proliferation and the cell cycle. A variety of oxidative-stress models have been shown to lead to increased expression of proto-oncogenes, including c-fos, c-jun and c-myc [46].

Adaptive responses at the level of gene regulation were studied in the rat glutathione S-transferase Ya-subunit gene and the NAD(P)H:quinone reductase gene by mutation and deletion analyses [47, 48]. An antioxidant responsive element (ARE) was identified in the 5'-flanking region of both genes. The sequences,

$$5'\text{-RGTGACNNNGC-3'}$$

and

$$3'\text{-YCACTGNNNCG-5'},$$

where N is any nucleotide, represent the core sequence of the ARE required for transcriptional activation by phenolic antioxidants and metabolizable planar aromatic compounds. The observation that the ARE contains a recognition motif highly similar to the consensus binding sequence for the c-Jun/c-Fos heterodimer suggested a possible involvement of c-Jun in the ARE-regulatory-protein complex [48]. Induction of c-Jun expression in response to hydrogen peroxide has been demonstrated [49]. In addition, a redox mechanism may regulate Jun-Fos DNA-binding activity [50]. Jun-D and c-Fos were identified as two members of the ARE-protein complex in studies on the regulation of the human NAD-(P):quinone oxidoreductase gene [51].

A nuclear protein, Ref-1, has been described, that stimulates DNA binding of Fos and Jun heterodimers, identifying it as a redox factor capable of regulating the function of transcription factors [52]. The activity occurs through a conserved cysteine residue in the DNA-binding domain of Fos and Jun [53]. The oxidation state of the cysteine has not yet been identified, but it does not involve the formation of a disulfide bond [50]. The Ref-1 system probably constitutes a major switch function with regard to redox signaling.

Dietary constituents are capable of modifying the metabolism of carcinogens by the induction of antioxidant enzymes of detoxication, particularly the so-called phase-II enzymes, notably quinone reductase (DT diaphorase) and glutathione transferases [54]. Numerous epidemiological studies suggest

that high consumption of yellow and green vegetables reduces the risk of cancer development. This could be directly due to protection by the antioxidant compounds contained in these vegetables. However, alternatively, inducing effects are exerted by compounds contained in these vegetables [55]. The induction of quinone reductase was studied in particular with regard to the compound sulforaphane, an isothiocyanate derivative present in broccoli [56]. It appears possible that these dietary inducers of quinone reductase act through ARE.

Control of prooxidant enzyme activities: NADPH oxidase and nitric oxide synthase

The cellular production of reactive oxygen species by phagocytes is a well-studied phenomenon, forming the basis of an important sector of host defense. Recently, the control of the major enzymes involved in this host defense, NADPH oxidase and NO synthase, has been intensely studied. It is important to exert subtle control over the activity of these and other enzymes, because an overproduction of superoxide or nitric oxide might be harmful to the cell and the organism as a whole. Thus, the on/off switches are crucial.

NADPH oxidase

NADPH oxidase is the superoxide-forming enzyme of phagocytes and B-lymphocytes and is composed of cytosolic and membrane-associated components. The cytosolic components form a 240-kDa complex consisting of the p47phox-encoded and p67phox-encoded subunits, as well as a small GTP-binding protein, p21rac2. Subunits translocate from the cytosol to the plasma membrane where the oxidase is activated [57−59]. This signaling system provides for tight control by mediators.

It has also been shown that non-phagocytes, e.g. fibroblasts, can generate superoxide under the control of signal molecules, e.g. interleukin-1 and tumor necrosis factor [60, 61]. Interestingly, the Mn-superoxide dismutase (SOD) has been found to be induced by interleukin-1 and tumor necrosis factor and to protect against subsequent oxidant injury [62, 63], and likewise human Mn-SOD in pulmonary epithelial cells of transgenic mice conferred protection [64].

There are several further consequences of the presence of superoxide in cells and in extracellular fluids. For example, in vitro there is superoxide-dependent stimulation of leukocyte adhesion by oxidatively modified LDL [65], underlining the importance of control of superoxide production. In this regard, it is noteworthy that an adhesion protein has been found to inhibit superoxide release by human neutrophils [66], and that this adhesion protein may be considered as an antiinflammatory molecule preventing the inappropriate activation of neutrophils in the circulation [66].

Nitric oxide synthase

This family of enzymes has attracted considerable interest in biochemistry, physiology and pharmacology [67, 68]. Nitric oxide synthase is a catalytically self-sufficient cytochrome P-450 enzyme, containing both a reductase and a heme domain [69]. Whereas the enzyme in macrophages and several other cell types is only expressed following exposure of the cells to activating cytokines or microbial products and produces NO independently of added calcium and calmodulin [70], the brain enzyme is expressed constitutively and generates NO in response to calcium and calmodulin [71,

72]. The genes of the inducible [73] and the constitutively expressed forms [74] of nitric oxide synthase have been cloned and characterized. Regulatory sites of the latter were identified as phosphorylation sites and included different serines as substrates for cyclic-AMP-dependent protein kinase, protein kinase C and calcium/calmodulin protein kinase [75]. This complex regulation provides for multiple means of regulating NO levels and for cross-talk between different secondary-messenger systems. In particular, down-regulation of nitric oxide synthase activity by more than 66% was obtained by activation of protein kinase C by 50 nM phorbolester [75].

Superoxide dismutase can catalyze the reversible interconversion of nitric oxide and the nitroxyl anion, so that the redox state of the copper in superoxide dismutase can influence the metabolic fate of the generated nitric oxide [76].

Synthetic antioxidants

The strategies of antioxidant defense pursued with synthetic antioxidants basically overlap those employed by biological systems. Applications of synthetic antioxidants are similar to those of biological antioxidants but, in addition, they are of potential use in chemistry, the food industry and in medicine.

Nonenzymic

Phenolic antioxidants. There are a number of phenolic antioxidants, butylated hydroxytoluene and butylated hydroxyanisole being prominent examples. These compounds have been widely used as food antioxidants, but, because of their metabolism to potentially reactive intermediates, applications have been restricted recently [77].

Probucol, a compound containing two phenoxyl moieties, has been particularly useful in studies on the protection of low-density lipoprotein against peroxidation [78].

Modified tocopherol ascorbate and carotenoids. Natural antioxidants have been modified to generate synthetic compounds exhibiting novel properties. For example, α-tocopherol has been modified to a water-soluble derivative, trolox, by exchanging the phytyl side chain for a carboxylate group. Conversely, ascorbate has been esterified with fatty acids such as palmitate to generate a more hydrophobic derivative. Synthetic carotenoids retaining the polyene structure have been examined for their ability to quench singlet oxygen [79]. The rationale for these and other types of derivative is to exploit activities exerted at different localizations in cells or fluids due to changes in solubility properties, retaining the functional end of the antioxidant molecule. This may open new sites for protection, employing the antioxidant principle of the natural parent compound.

Thiols. Since glutathione, as the major low-molecular-mass thiol in cells, does not enter most types of cells, glutathione ethylester has been synthesized as a precursor penetrating into cells to then be hydrolyzed to glutathione [80]. Alternatively, thiazolidine derivatives or N-acetyl cysteine have been employed as precursors for cysteine supporting GSH biosynthesis by the substrate supply, but also acting as antioxidants by themselves.

Therapeutic use of synthetic racemic lipoate (thioctic acid) is based, in part, on the antioxidant function of the

dihydrolipoate/lipoate system shown by its protecion against microsomal lipid peroxidation [81, 82], against DNA damage by singlet oxygen [83], and against a decrease in membrane fluidity in hypoxia/reoxygenation [84]. Like N-acetyl cysteine [85], lipoate inhibits NF-κB activation in human T-cells [86].

Numerous other thiol compounds, notably those of aminothiol structure, were examined as radioprotectors.

Metal chelators. An important strategy of prevention is to bind metal ions [86a]. Desferrioxamine [87] and many related metal chelators were designed to bind iron or copper ions.

Miscellaneous. The targeting of compounds to membrane sites in cells may have been the strategy involved in generating compounds known as lazaroids, with the steroid ring system as a basic building block [88].

Chemical modification of structural features of flavonoids has generated a multitude of synthetic antioxidant compounds.

Enzyme mimics

Low-molecular-mass compounds exhibiting catalytic activity, i.e. operating as enzyme mimics, have been used as antioxidants. Copper diisopropyl salicylate and other copper complexes were shown to mimic superoxide dismutase activity [89]. A selenoorganic compound, ebselen, was shown to mimic the GSH peroxidase reaction [90, 91]. As discussed recently [92], the kinetic mechanism of ebselen closely resembles that of the phospholipid hydroperoxide GSH peroxidase [93] and GSH peroxidase enzymes.

Enzymic

Synthetic enzymic antioxidants have many future perspectives. One route is to generate chimeric proteins that allow for targeting. The Hb-SOD, which binds to endothelial cells and was shown to positively affect elevated blood pressure in experimental animals [94], is one example. The engineering of SOD molecules with higher catalytic rates, employing the principle of electrostatic guidance, led to recombinant enzyme preparations more active that native SOD [95].

The site-specific mutation of a crucial oxidizable methionine residue to a nonoxidizable amino acid in the elastase inhibitor provides an interesting example of a preventative strategy [96]. Elastase activation, as a consequence of oxidative stress, is considerably diminished with the oxidation-resistant inhibitor.

Only a few examples of the strategies employed in recent years to generate a multitude of potential drug antioxidants synthetically [97] have been presented here. A delicate balance exists between prooxidants and antioxidants in cells, and relationships exist between the redox state and cellular gene expression, as described briefly above. Therefore, pharmacological applications of highly efficient antioxidant compounds or enzymes may potentially interfere with important cellular functions, including changes in the enzyme activity, enzyme patterns, membrane fluidity and responses to stimuli. While this aspect deserves attention in each case, it should be mentioned that, overall, the use of antioxidant compounds

has largely been devoid of side effects and in the applications studied thoroughly, has proved predominantly beneficial.

REFERENCES

1. Sies, H. (1986) *Angew. Chem. Int. Ed. 25*, 1058–1071.
2. Chance, B., Sies, H. & Boveris, A. (1979) *Physiol. Revs. 59*, 527–605.
3. Halliwell, B. & Gutteridge, J. M. C. (1989) *Free radicals in biology and medicine* (2nd edn) Clarendon Press, Oxford.
4. Sies, H. (1985) *Oxidative stress*, Academic Press, London.
5. Sies, H. (1991) *Oxidative stress: oxidants and antioxidants*, Academic Press, London.
6. Halliwell, B. & Sies, H. (1993) *Free Radical Research Commun.*, vol. 1–18.
7. Pryor, W. A. & Davies, K. J. A. (1993) *Free Rad. Biol. Med.* 1–14.
8. Sies, H. (1985) *Oxidative stress*, pp. 1–8, Academic Press, London.
9. Sies, H. (1991) *Oxidative stress: oxidants and antioxidants*, pp. XV–XXII, Academic Press, London.
10. Ames, B. N. (1983) *Science 221*, 1256–1263.
11. Kappus, H. & Sies, H. (1981) *Experientia 37*, 1233–1241.
12. Haas, A. & Goebel, W. (1992) *Free Radical Research Commun. 16*, 137–157.
13. Ma, M. & Eaton, J. W. (1992) *Proc. Natl Acad. Sci. USA 89*, 7924–7928.
14. Reichard, P. & Ehrenberg, A. (1983) *Science 221*, 514–519.
15. Retsky, K. L., Freeman, M. W. & Frei, B. (1993) *J. Biol. Chem. 268*, 1304–1309.
16. Daub, M. E., Leisman, G. B., Clark, R. A. & Bowden, E. F. (1992) *Proc. Natl Acad. Sci. USA 89*, 9588–9592.
17. Mannervik, B. (1985) *Adv. Enzymol. 57*, 357–417.
18. Sies, H. & Ketterer, B. eds (1988) *Glutathione S-conjugation: mechanisms and biological significance*, Academic Press, London.
19. Witmer, C. M., Snyder, R. R., Jollow, D. J., Kalf, G. F., Kocsis, J. J. & Sipes, I. G. eds (1991) *Biological reactive intermediates IV*, Plenum Press, New York.
20. Burton, G. W., Joyce, A. & Ingold, K. U. (1983) *Arch. Biochem. Biophys. 221*, 281–290.
21. Tappel, A. L. (1962) *Vitam. Horm. 20*, 493–510.
22. Niki, E. (1987) *Chem. Phys. Lipids 44*, 227–253.
23. Wefers, H. & Sies, H. (1988) *Eur. J. Biochem. 174*, 353–357.
24. Sies, H. & Murphy, M. E. (1991) *J. Photochem. Photobiol. B 8*, 211–218.
25. Packer, J. E., Slater, T. F. & Willson, R. L. (1979) *Nature 278*, 737–738.
26. Czapski, G. (1984) *Isr. J. Chem. 24*, 29–32.
27. Smith, L. L. (1991) *Free Radical Biol. & Med. 11*, 47–61.
28. Morand, O. H., Zoeller, R. A. & Raetz, C. R. H. (1988) *J. Biol. Chem. 263*, 11597–11606.
29. Foote, C. S. & Denny, R. W. (1968) *J. Am. Chem. Soc. 90*, 6233–6235.
30. Krinsky, N. I. (1989) *Free Radical Biol. Med. 7*, 617–635.
31. Sies, H., Stahl, W. & Sundquist, R. A. (1992) *Ann. NY Acad. Sci. 669*, 7–20.
32. McCord, J. M. & Fridovich, I. (1969) *J. Biol. Chem. 244*, 6049–6055.
32a. Maki, H. & Sekiguchi, M. (1992) *Nature 355*, 273–275.
32b. Mo, J.-Y., Maki, H. & Sekiguchi, M. (1992) *Proc. Natl Acad. Sci. USA 89*, 11021–11025.
33. Harris, E. D. (1992) *FASEB J. 6*, 2675–2683.
34. Remacle, J., Lambert, D., Raes, M., Pigeolet, E., Michiels, C. & Toussaint, O. (1992) *Biochem. J. 286*, 41–46.

35. Christman, M. F., Morgan, R. W., Jacobson, F. S. & Ames, B. N. (1985) *Cell 41*, 753−762.
36. Greenberg, J. T., Monach, P. A., Chou, J. H., Josephy, P. D. & Demple, B. (1990) *Proc. Natl Acad. Sci. USA 87*, 6181−6185.
37. Storz, G., Tartaglia, L. A. & Ames, B. N. (1990) *Science 248*, 189−194.
38. Tartaglia, L. A., Storz, G., Farr, S. B. & Ames, B. N. (1991) in *Oxidative stress: oxidants and antioxidants* (Sies, H., ed.) pp. 155−169, Academic Press, London.
39. Storz, G., Christman, M. F., Sies, H. & Ames, B. N. (1987) *Proc. Natl Acad. Sci. USA 84*, 8917−8921.
40. Greenberg, J. T. & Demple, B. (1988) *EMBO J. 7*, 2611−2617.
41. Keyse, S. M. & Tyrrell, R. M. (1989) *Proc. Natl Acad. Sci. USA 86*, 99−103.
42. Schreck, R., Rieber, P. & Baeuerle, P. A. (1991) *EMBO J. 10*, 2247−2258.
43. Schreck, R., Albermann, K. & Baeuerle, P. A. (1992) *Free Radical Res. Comm. 17*, 221−238.
44. Toledano, M. B. & Leonard, W. J. (1991) *Proc. Natl Acad. Sci. USA 88*, 4328−4332.
45. Keyse, S. M. & Emslie, E. A. (1992) *Nature 359*, 644−647.
46. Cerutti, P., Larsson, R., Krupitzka, D., Muehlematter, D., Crawford, D. & Amstad, P. (1988) in *Oxy-radicals in molecular biology and pathology* (Cerutti, P. A., Fridovich, I. & McCord, J. M., eds) pp. 493−507, A. Liss, New York.
47. Rushmore, T. H., Morton, M. R. & Pickett, C. B. (1991) *J. Biol. Chem. 266*, 11632−11639.
48. Nguyen, T. & Pickett, C. B. (1992) *J. Biol. Chem. 267*, 13535−13539.
49. Devary, Y., Gottlieb, R. A., Lau, L. F. & Karin, M. (1991) *Mol. Cell. Biol. 11*, 2804−2811.
50. Abate, C., Patel, L., Rauscher, F. J. & Curran, T. (1990) *Science 249*, 1157−1161.
51. Li, Y. & Jaiswal, A. (1992) *J. Biol. Chem. 267*, 15097−15104.
52. Xanthoudakis, S. & Curran, T. (1991) *EMBO J. 11*, 653−665.
53. Xanthoudakis, S., Miao, G., Wang, F., Pan, Y.-C. & Curran, T. (1991) *EMBO J. 11*, 3323−3335.
54. Prochaska, H. & Talalay, P. (1991) in *Oxidative stress: oxidants and antioxidants* (Sies, H., ed.) pp. 195−211, Academic Press, London.
55. Prochaska, H., Santamaria, A. B. & Talalay, P. (1992) *Proc. Natl Acad. Sci. USA 89*, 2394−2398.
56. Zhang, Y., Talalay, P., Cho, C.-G. & Posner, G. H. (1992) *Proc. Natl Acad. Sci. USA 89*, 2399−2403.
57. Segal, A. W. (1989) *J. Clin. Invest. 83*, 1785−1793.
58. Park, J. W., Ma, M., Ruedi, J. M., Smith, R. M. & Babior, B. M. (1992) *J. Biol. Chem. 267*, 17327−17332.
59. Knaus, U. G., Heyworth, P. G., Kinsella, T., Curnutte, J. T. & Bokoch, G. M. (1992) *J. Biol. Chem. 267*, 23575−23582.
60. Meier, B., Radeke, H. H., Selle, S., Younes, M., Sies, H., Resch, K. & Habermehl, G. G. (1989) *Biochem. J. 263*, 539−545.
61. Meier, B., Radeke, H. H., Selle, S., Habermehl, G. G., Resch, K. & Sies, H. (1990) *Biol. Chem. Hoppe-Seyler 371*, 1021−1025.
62. Wong, G. H. W. & Goeddel, D. V. (1988) *Science 242*, 941−944.
63. Wong, G. H., Elwell, J. H., Oberley, L. W. & Goeddel, D. V. (1989) *Cell 58*, 923−931.
64. Wispe, J. R., Warner, B. B., Clark, J. C., Dey, C. R., Neumann, J., Glasser, S. W., Crapo, J. D., Chang, L. Y. & Whitsett, J. A. (1992) *J. Biol. Chem. 267*, 23937−23941.
65. Lehr, H. A., Becker, M., Marklund, S. L., Hubner, C., Arfors, K. E., Kohlschatter, A. & Messmer, K. (1992) *Arteriosclerosis Thromb. 12*, 824−829.
66. Wong, C. S., Gamble, J. R., Skinner, M. P., Lucas, C. M., Berndt, M. C. & Vadas, M. A. (1991) *Proc. Natl Acad. Sci. USA 88*, 2397−2401.
67. Noack, E. & Murphy, M. (1991) in *Oxidative stress: oxidants and antioxidants* (Sies, H., ed.) pp. 445−489, Academic Press, London.
68. Nathan, C. (1992) *FASEB J. 6*, 3051−3064.
69. White, K. A. & Marletta, M. A. (1992) *Biochemistry 31*, 6627−6631.
70. Stuehr, D. J., Cho, H. J., Kwon, N. S., Weise, M. F. & Nathan, C. F. (1991) *Proc. Natl Acad. Sci. USA 88*, 7773−7777.
71. Busse, R. & Mülsch, A. (1990) *FEBS Lett. 265*, 133−136.
72. Bredt, D. S. & Snyder, S. H. (1990) *Proc. Natl Acad. Sci. USA 87*, 682−685.
73. Xie, Q.-W., Cho, H. J., Calaycay, J., Mumford, R. A., Swiderek, K. M., Lee, T. D., Ding, A., Troso, T. & Nathan, C. (1992) *Science 256*, 225−228.
74. Bredt, D. S., Hwang, P. M., Glatt, C. E., Lowenstein, C., Reed, R. R. & Snyder, S. H. (1991) *Nature 351*, 714−718.
75. Bredt, D. S., Ferris, C. D. & Snyder, S. H. (1992) *J. Biol. Chem. 267*, 10976−10981.
76. Murphy, M. E. & Sies, H. (1991) *Proc. Natl Acad. Sci. USA 88*, 10860−10864.
77. Kahl, R. (1991) in *Oxidative stress: oxidants and antioxidants*, (Sies, H., ed.) pp. 245−273, Academic Press, London.
78. Carew, T. E., Schwenke, D. C. & Steinberg, D. (1987) *Proc. Natl Acad. Sci. USA 84*, 7725−7729.
79. Devasagayam, T. P. A., Werner, T., Ippendorf, H., Martin, H.-D. & Sies, H. (1992) *Photochem. Photobiol. 55*, 511−514.
80. Anderson, M. E. & Meister, A. (1989) *Anal. Biochem. 183*, 16−20.
81. Bast, A. & Haenen, G. R. M. M. (1988) *Biochim. Biophys. Acta 963*, 558−561.
82. Scholich, H., Murphy, M. E. & Sies, H. (1989) *Biochim. Biophys. Acta 1001*, 256−261.
83. Devasagayam, T. P. A., Subramanian, M., Pradhan, D. S. & Sies, H. (1993) *Chem.-Biol. Interact. 86*, 79−92.
84. Scheer, B. & Zimmer, G. (1993) *Arch. Biochem. Biophys. 302*, 385−390.
85. Staal, F. J. T., Roederer, M. & Herzenberg, L. A. (1990) *Proc. Natl Acad. Sci. USA 87*, 9943−9947.
86. Suzuki, Y. J., Aggarwal, B. A. & Packer, L. (1992) *Biochem. Biophys. Res. Commun. 189*, 1709−1715.
86a. Weglicki, W. B., Mak, I. T. (1992) *Mol. Cell. Biochem. 118*, 105−111.
87. Halliwell, B. (1989) *Free Radical Biol. Med. 7*, 645−651.
88. Hall, E. O., Yonkers, P. A., McCall, J. M., Braughler, J. M. (1988) *J. Neurosurg. 68*, 456−461.
89. Oberley, L. W., Leuthauser, S. W. C., Pasternack, R. F., Oberley, T. D., Schutt, L. & Sorenson, J. R. (1984) *Agents and Actions 15*, 536−538.
90. Müller, A., Cadenas, E., Graf, P. & Sies, H. (1984) *Biochem. Pharmacol. 33*, 3235−3240.
91. Wendel, A., Fausel, M., Safaghi, H., Tiegs, G. & Otter, R. (1984) *Biochem. Pharmacol. 33*, 3241−3245.
92. Sies, H. (1993) *Free Radicals Biol. Med. 14*, 313−323.
93. Mairoino, M., Roveri, A., Coassin, M. & Ursini, F. (1988) *Biochem. Pharmacol. 37*, 2267−2271.
94. Nakazono, K., Watanabe, N., Matsuno, K., Sasaki, J., Sato, T. & Inoue, M. (1991) *Proc. Natl Acad. Sci. USA 88*, 10045−10048.
95. Getzoff, E. D., Cabelli, D. E., Fisher, C. L., Parge, H. E., Viezzoli, M. S., Banci, L. & Hallewell, R. A. (1992) *Nature 358*, 347−351.
96. Rosenberg, S., Barr, P. J., Najarian, R. Hallewell, R. (1984) *Nature 312*, 77−79 .
97. Emerit, I., Packer, L. & Auclair, C. eds (1990) *Antioxidants in therapy and preventive medicine*, Plenum Press, New York.
98. Pryor, W. A. (1986) *Annu. Rev. Physiol. 48*, 657−667.