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PHYSIOLOGICAL SOCIETY SYMPOSIUM: IMPAIRED ENDOTHELIAL AND SMOOTH MUSCLE CELL FUNCTION IN OXIDATIVE STRESS

OXIDATIVE STRESS: OXIDANTS AND ANTIOXIDANTS

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SUMMARY

An imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage, is termed 'oxidative stress'. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. Antioxidant defense involves several strategies, both enzymatic and non-enzymatic. In the lipid phase, tocopherols and carotenes as well as oxy-carotenoids are of interest, as are vitamin A and ubiquinols. In the aqueous phase, there are ascorbate, glutathione and other compounds. In addition to the cytosol, the nuclear and mitochondrial matrices and extracellular fluids are protected. Overall, these low molecular mass antioxidant molecules add significantly to the defense provided by the enzymes superoxide dismutase, catalase and glutathione peroxidases.

INTRODUCTION

An imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage, is termed 'oxidative stress' (Sies, 1985, 1986, 1991). Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. A quasi-steady state is maintained by an intricate pattern of antioxidants. The antioxidant defense is, in part, capable of adapting to changing needs. This brief overview discusses selected topics from work in the author's laboratory and is not intended to cover the whole field, which is progressing rapidly (see Sies, 1993, 1995).

OXIDANTS

Molecular oxygen can be reduced to water. The intermediate steps of oxygen reduction are the 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 can occur as alkyl or peroxyl radicals, e.g. in lipids. Also, there is nitric oxide, one of the gaseous radicals of biological interest. Peroxynitrite, a non-radical reactive species, is formed from the nitric oxide and superoxide anion radicals.

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Oxidants are also generated by different types of radiation, with X-irradiation generating the hydroxyl radical and irradiation with ultraviolet light generating electronically excited states with subsequent radical formation. Ultrasound and microwave radiation can also generate reactive oxygen species. Even shear stress, e.g. in homogenization, is known to generate radicals.

The half-lives of the major reactive oxygen species are vastly different, underscoring the necessity for different types of defense mechanisms (see Sies, 1993). 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 oxidants may exist and may be transported to distant target sites where they exert oxidant activity. This would include compounds or enzymes with activities that are innocuous in one environment but can be activated to generate oxidants under other conditions.

The human diet contains many compounds of an oxidant and antioxidant nature (Ames, 1983). 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, and substrates for enzyme systems which generate oxidants.

ANTIOXIDANTS

In their definition of the term, Halliwell & Gutteridge (1989) state that an antioxidant is 'any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate'. This definition includes compounds of a non-enzymatic as well as an enzymatic nature. Clearly, the diversity of anti-oxidants matches that of pro-oxidants. The principles underlying the antioxidant functions have been discussed (Sies, 1993).

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 alternative targets. Microbes have developed specialized strategies to prevent oxygen-dependent killing by phagocytes.

Regarding radical formation, first it should be mentioned that some of the enzymes prone to generate free radical species are ingeniously designed. Cytochrome oxidase, which carries out most of the cellular 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'.

Furthermore, 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. metallothioncin, 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.

Protection of cells from 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. 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.

In this regard, there are many enzymatic systems in cells and body fluids to control the level of reactive species which otherwise might generate a cascade of products which, in turn, would lead to attacking oxidants. One important group of such enzymes is the glutathione S-transferases. This family of enzymes catalyses the reaction of the major low molecular mass thiol, glutathione, with reactive electrophiles to form thioethers, called S-conjugates. Biologically reactive electrophilic intermediates can be formed in a variety of metabolic pathways, notably those involving cytochrome P450, and are of interest in toxicology and pharmacology (see, for example, recent work on NAD(P)H : quinone oxidoreductase; Schulz, Eickelmann & Sies, 1996).

A strategy of preventative antioxidation could therefore be formulated as prevention by diversion, i.e. by channelling 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 products of oxidative damage in these cells is high. The turnover and elimination of whole cells prevents further spread of the challenging species. This type of prevention overlaps in part with the concept of interception.

Interception

Non-enzymatic antioxidants. 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, to prevent it from further deleterious reactions. This is the process of deactivation. For radical compounds, the final deactivation consists of the formation of non-radical and non-reactive 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 leave 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 to the aqueous phase of the plasma. Biologically, the most efficient intercepting antioxidants combine optimal properties for both these objectives: first, they react with initial free radicals, such as lipid peroxyl radicals, at suitable rates; and second, they are capable of interacting with water-soluble compounds for their own regeneration. This combined action 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 one to three antioxidant molecules per 1000 potential target molecules.

Such intercepting chain-breaking antioxidants are often phenolic compounds. (R,R,R)-α-Tocopherol is probably the most efficient compound in the lipid phase (for recent review, see Traber & Sies, 1996). This biological antioxidant 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 (for review, see Briviba & Sies, 1994).

A prerequisite for efficient interception by the phenolic antioxidants is that the lifetime of the radical to be intercepted must not be too short. The peroxyl radicals are therefore major reaction partners, since their lifetime extends into the range of seconds. In contrast, the hydroxyl radical, with its high reactivity and extremely short lifetime, 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, eliminating interception as a useful strategy for defense against the hydroxyl radical, if only for osmotic reasons. Highly efficient biological polyene quenchers for singlet molecular oxygen, notably carotenoids and oxy-carotenoids, provide a suitable defense system against this oxygen species, in spite of its reactivity and short lifetime (for recent reviews, see Sies & Stahl, 1995; Stahl & Sies, 1996). The local concentrations of the carotenoids are decisive in determining the efficiency of the quenching of singlet oxygen and other electronically excited states.

Enzymatic antioxidants. All cells in eukaryotic organisms contain powerful antioxidant enzymes. The three major classes of antioxidant enzymes are the superoxide dismutases, catalases and glutathione (GSH) peroxidases. In addition, there are numerous specialized antioxidant enzymes reacting with and, in general, detoxifying oxidant compounds. Indirect antioxidant functions carried out by enzymes are: (a) the back-up function, e.g. the replenishment of GSH from glutathione disulphide (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. Different subcellular sites and different cell types may contain varying amounts of the antioxidant enzymes (see Soboll, Gründel, Harris, Kolb-Bachofen, Sies & Ketterer, 1995).

Repair

Protection from the effects of oxidants can also occur by repair of damage once it has occurred. Since prevention and interception processes are not completely effective, products of damage are continuously formed in low yields and hence may accumulate. This causes DNA damage, in the form of damaged bases or as single-strand or double-strand breaks, membrane damage, in the form of a variety of phospholipid oxidation products, and damage 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.

RECENT TOPICS OF INTEREST

Our recent work has addressed the potential defenses against peroxynitrite, which is formed from nitric oxide and superoxide. It was observed that a seleno-organic compound reacts very efficiently with peroxynitrite (Masumoto & Sies, 1996; Sies & Masumoto, 1997) and that seleno-organic compounds, such as selenomethionine or selenocystine, protect against peroxynitrite-induced DNA strand breaks (Roussyn, Briviba, Masumoto & Sies, 1996) or against nitration reactions (Briviba, Roussyn, Sharov & Sies, 1996). We have obtained

preliminary evidence that such protective action may be exerted by selenoproteins, identifying a novel function of selenoproteins in defense against peroxynitrite.

Another topic addressed is the modification of proteins by mixed disulphide formation with glutathione, called glutathiolation (see Thomas & Sies, 1991). Dafré, Sies & Akerboom (1996) examined the properties of protein S-thiolation and regulation of microsomal glutathione transferase activity by the glutathione redox couple.

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REFERENCES

AMES, B. N. (1983). Dietary carcinogens and anticarcinogens. *Science* **221**, 1256–1263.

BRIVIBA, K., ROUSSYN, I., SHAROV, V. S. & SIES, H. (1996). Attenuation of oxidation and nitration reactions of peroxynitrite by selenomethionine, selenocystine and Ebselen. *Biochemical Journal* **319**, 13–15.

BRIVIBA, K. & SIES, H. (1994). Nonenzymatic antioxidant defense systems. In *Natural Antioxidants in Human Health and Disease*, ed. FREI, B., pp. 107–128. Academic Press, Orlando, FL, USA.

DAFRÉ, A. L., SIES, H. & AKERBOOM, T. (1996). Protein S-thiolation and regulation of microsomal glutathione transferase activity by the glutathione redox couple. *Archives of Biochemistry and Biophysics* **332**, 288–294.

HALLIWELL, B. & GUTTERIDGE, J. M. C. (1989). *Free Radicals in Biology and Medicine*, 2nd edn. Clarendon Press, Oxford, UK.

MASUMOTO, H. & SIES, H. (1996). The reaction of Ebselen with peroxynitrite. *Chemical Research in Toxicology* **9**, 262–267.

ROUSSYN, I., BRIVIBA, K., MASUMOTO, H. & SIES, H. (1996). Selenium-containing compounds protect DNA from single-strand breaks caused by peroxynitrite. *Archives of Biochemistry and Biophysics* **330**, 216–218.

SCHULZ, W. A., EICKELMANN, P. & SIES, H. (1996). Free radicals in toxicology: redox cycling and NAD(P)H : quinone oxidoreductase. *Archives of Toxicology*, suppl. 18, 217–222.

SIES, H. (1985). Oxidative stress: introductory remarks. In *Oxidative Stress*, ed. SIES, H., pp. 1–8. Academic Press, London.

SIES, H. (1986). Biochemistry of oxidative stress. *Angewaudte Chemie*, International Edition in English **25**, 1058–1071.

SIES, H. (1991). Oxidative stress: introduction. In *Oxidative Stress*: *Oxidants and Antioxidants*, ed. SIES, H., pp. xv–xxii. Academic Press, London.

SIES, H. (1993). Strategies of antioxidant defense. *European Journal of Biochemistry* **215**, 213–219.

SIES, H. (1995). Strategies of antioxidant defense: relations to oxidative stress. In *Signalling Mechanisms from Transcription Factors to Oxidative Stress*, ed. PACKER, L. & WIRTZ, K., pp. 165–186. Springer Verlag, Berlin.

SIES, H. & MASUMOTO, H. (1997). Ebselen as a glutathione peroxidase mimic and as a reactant with peroxynitrite. *Advances in Pharmacology* **38**, 229–246.

SIES, H. & STAHL, W. (1995). Antioxidant functions of vitamins E and C, β-carotene and other carotenoids. *American Journal of Clinical Nutrition* **62**, 1315–1321S.

SOBOLL, S., GRÜNDEL, S., HARRIS, J., KOLB-BACHOFEN, V., SIES, H. & KETTERER, B. (1995). The content of glutathione and glutathione S-transferases and the glutathione peroxidase activity in rat liver nuclei determined by a non-aqueous technique of liver cell fractionation. *Biochemical Journal* **311**, 889–894.

STAHL, W. & SIES, H. (1996). Lycopene: a biologically important carotenoid for the human? *Archives of Biochemistry and Biophysics* **336**, 1–9.

THOMAS, J. A. & SIES, H. (1991). Protein S-thiolation and dethiolation. In *The Post-translational Modification of Proteins*: *Roles in Molecular and Cellular Biology*, ed. TSUBOI, S., TANIGUCHI, N. & KATUNUMA, N., pp. 239–255. Japan Scientific Societies Press, Tokyo.

TRABER, M. G. & SIES, H. (1996). Vitamin E in humans: demand and delivery. *Annual Reviews in Nutrition* **16**, 321–347.