Free Radicals

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Free radicals are atoms, molecules or ions with one or more unpaired electrons. The most reactive ones have high reduction potentials; i.e., they readily oxidize most molecules indiscriminately. There is a common misconception that all free radicals are highly reactive, but in fact the range of those able to cause biological damage is quite narrow: it is made up principally of hydroxyl, peroxyl, alkoxyl, thiyl, phenoxyl and semiquinone free radicals, and high valence transition ions.

oxidative stress antioxidants free radicals polyphenols kinetics reaction mechanism radical adducts

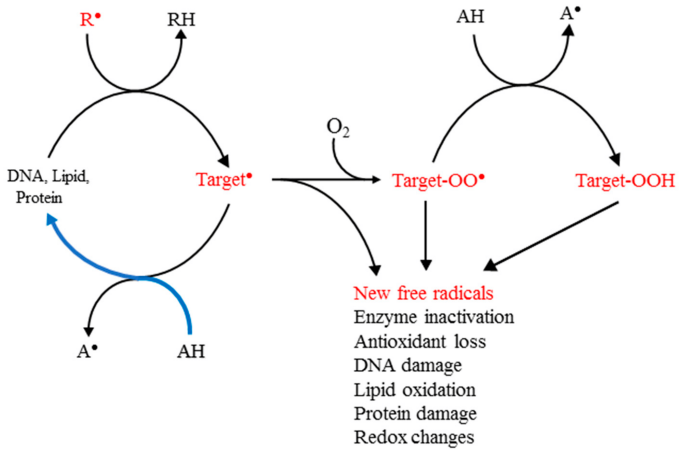
1. Introduction

Oxidative stress is commonly coupled with excessive formation of free radicals, with some researchers actually identifying free radicals as the origin and cause of OS. There is a common misconception that all free radicals are highly reactive, but in fact the range of those able to cause biological damage is quite narrow: it is made up principally of hydroxyl, peroxyl, alkoxyl, thiyl, phenoxyl and semiquinone free radicals, and high valence transition ions. The usual form of damage is abstraction of an electron or H atom from a target molecule, creating a new, less reactive secondary target free radical. In complex biological systems, the result is a chain of successive electron transfers, creating new free radicals with decreasing reactivity, as required by thermodynamics. If the chain involves critical molecules, such as DNA, proteins or lipids repaired or replaced only slowly, the result may be a permanent impairment of a vital function, which, if not reversed, can constitute the first step in the development of a disease or another form of damage. The radical chain is only terminated by reaction with another free radical, a transition metal ion or with an antioxidant, with the last creating a radical no longer able to propagate the damage.

In discussing the phenomenon of oxidative reactions of free radicals and their consequences, it has to be noted that low levels of free radicals form continuously in vivo and fulfil significant roles in tissue defence, DNA biosynthesis, redox regulation and possibly in thiol-based cell signalling [1][2][3][4][5][6]. There is therefore a clear and important distinction between the consequences of formation of low and excessive levels of free radicals; the former are essential and not a danger to the organism because its antioxidant defences can prevent or repair any collateral molecular damage, while the latter can trigger or aggravate a wide range of diseases and other undesirable conditions.

2. The Principal Reactions of Free Radicals In Vivo

The initial reaction of the primary damaging free radicals with biomolecules commonly produces carbon-centred (C-centred) free radicals. Their principal subsequent reactions result in new free radical chains, which include formation of peroxyl free radicals, ROO● , in a fast reaction with physiological oxygen (Scheme 1).



Scheme 1. Principal biological targets of the primary free radicals and damage repair. R• is the initiating free radical and AH an antioxidant. The initial reaction produces a carbon-centred free radical in the target. Reactive species are shown in red. The blue curve shows the flow of the reducing equivalents under optimal prevention of damage.

In the reducing environment of cells and tissues, peroxyl radicals are commonly converted to hydroperoxides. All these species, namely, the C-centred and other free radicals, peroxyl radicals and hydroperoxides, have the capacity to propagate the damage triggered by R● , with peroxyl radicals believed to be the main carriers of damage in living organisms; many of the commonly used assays for the formation of free radicals in cells and tissues depend on the detection of peroxyl radicals and hydroperoxides [7][8].

Defence against free radical-induced damage can be achieved by scavenging the R● or repair of the damaged target molecules. Under oxidative stress, this should be achievable in theory by enhancing the levels of the endogenous antioxidants or, if necessary, supplementation with additional antioxidants. Considerations of the mechanism of action of the most damaging free radicals, such as the hydroxyl, HO● , have shown that, because of their high reactivity and low levels in vivo, direct scavenging by added radical scavengers is not feasible, despite a commonly held contrary view [9]. This means that the earliest and most effective point for protective action is the secondary target free radical, because its repair prevents subsequent molecular damage (Scheme 1). It is important to note that the well-documented recognition of the signalling and defensive functions of ROS in vivo means that the role of antioxidants in living organisms is not to eliminate all free radicals, but rather to lower any excessive levels, so that they can be neutralised by the endogenous antioxidants present.

The practical possibility of modification of biological damage by antioxidants is based on extensive chemical knowledge of the properties of free radicals, which established that their effectiveness is largely determined by thermodynamics and kinetics [10][11]. The former is an intrinsic property of the reacting species and cannot be

altered. Importantly, however, kinetic factors can be manipulated because they depend on concentrations of the reactants:

$$\text{Rate of reaction} = k\,[AH]\,[R^{\bullet}] \quad (1)$$

In the equation, k is the bimolecular rate constant (units $M^{-1}\ s^{-1}$), AH an antioxidant and $R^{\bullet}$ a free radical, with the bracketed terms denoting molar concentrations. Since the damage-causing primary $R^{\bullet}$ cannot be scavenged in vivo and are by definition sufficiently reactive to generate C-centred free radicals in many molecules, antioxidant repair reactions must be faster than those of any competing processes. This cannot be achieved under OS by the normal levels of the endogenous antioxidants. In fact, measurements of the rate constants of reactions of ascorbate, urate and GSH with C-centred radicals have shown their reactions to be too slow to ensure effective scavenging of the radicals in tissues with a low antioxidant content [12][13][14]. The obvious defensive tactic of increasing the levels of lthe endogenous antioxidants to levels sufficient to prevent the consequences of OS is not possible: in the case of enzymes and metal-chelating proteins, their activities are tightly regulated and are not amenable to manipulation in human populations. Similarly, rates of free radical repair by the principal non-enzymatic antioxidants are not readily enhanced, because their concentrations in vivo cannot be significantly increased in humans consuming a normal healthy diet. This possibility was extensively studied for the vitamins C and E and may well be the reason for the generally disappointing benefits of treatment with vitamins and other supplements found in several long-term studies of thousands of subjects [15][16][17][18][19]. There is likely to be a limit to the effective in vivo concentrations of many added supplements: experimental evidence shows that the plasma concentration of the excellent antioxidant ascorbate reaches a limit at a modest oral intake of the vitamin. [20].

The inadequacy of the normal human antioxidant defences under OS, demonstrated by the population and theoretical results, shows that additional antioxidants are required by many individuals. These should persist in tissues and be easily administered, preferably by diet. Currently, the most promising candidates for this role are the plant-derived food flavonoids and other polyphenols, and many of their metabolites.

Polyphenols in Scavenging of Free Radicals In Vivo

The finding of high rate constants of reactions of aromatic molecules with C-centred free radicals allows an approximate estimate of the potential effectiveness of polyphenols and their aromatic metabolites in delaying, reducing or preventing biological damage under oxidative stress. Since, as already indicated, not all free radicals can or should be scavenged in vivo, the function of added antioxidants is to augment the antioxidant capacity of the organism to a level sufficient to overcome any damage caused by oxidative stress. As the damage initiating primary radicals $R^{\bullet}$ cannot be intercepted, antioxidant repair needs to be applied to the secondary $[Target]^{\bullet}$. Assuming that the two main reactions competing for the $[Target]^{\bullet}$ are with oxygen or with the polyphenol, and using the rate constants of $2 \times 10^9\ M^{-1}\ s^{-1}$ for the $[Target]^{\bullet}$ reacting with O_2 and $10^{10}\ M^{-1}\ s^{-1}$ for adduct formation, for 50% of the $[Target]^{\bullet}$ to be repaired, the concentration of the aromatic antioxidants should be 4 µM. Such levels are

easily achievable by diets containing the recommended daily amounts of fruits and vegetables and may well be sufficient to neutralise excessive levels of free radicals in oxidative stress. In contrast, a similar efficiency of reactions involving electron or H transfer would require an unachievable antioxidant concentration of ~2 mM and would include only the polyphenols and those of their metabolites retaining the phenolic groups.

3. Conclusions and Prospects

The new insight into the mechanism of the free radical–polyphenol reaction provides support for the age-old advice urging consumption of plant-derived foods. Such advice is seldom based on rigorous scientific evidence. The results provide evidence that at least part of the demonstrated health benefits of fruit and vegetable diet is likely to be derived from the capacity of the constituent polyphenols to function as antioxidants, reducing or eliminating the ability of free radicals to cause cell and tissue damage. They also identify the basis of the antioxidant properties of a large proportion of polyphenol metabolites and show that they, and other aromatic molecules, are part of the overall antioxidant arsenal of an organism. Besides the plant-derived food components, this aromatic metabolome would include other tissue constituents and many pharmaceuticals used in the treatment of pain or inflammation. New antioxidant drugs with the desirable properties of easy administration, low toxicity and persistence can be developed for individuals, especially those exposed to oxidative stress because of the particular conditions of their lives. Or, one can rely on a varied diet rich in vegetables, fruits, nuts, dark chocolate and red wine.

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