similar to that suggested for deoxyribose can be invoked. The OH· scavengers would bind the metal and compete for it with the detector molecule. This would result in the decreased site-specific generation of OH· on deoxyribose, in the formation of the OH· scavenger radical and in its successive oxidation by the Fe^{3+} generated by the Fenton reaction. This mechanism explains the apparent reaction of thiourea with H_2O_2 reported by Cederbaum *et al.* (1979), as in that paper H_2O_2 concentration was measured by the thiocyanate method, i.e. measuring Fe^{3+} produced by Fenton reaction. The lack of correlation observed in the presence of formate may be due to the insufficient reactivity to induce Fe^{3+} of the secondary radical produced by OH· attack on this scavenger. This agrees with the known inertness of carbonyl radicals to oxidation by Fe^{3+} (Walling, 1975). The effects of OH· scavengers on deoxyribose damage and Fe^{2+} oxidation are both pH-dependent. When the reactions are conducted in Mops buffer, pH 7 or 7.4, a parallel decreased inhibition, by all OH· scavengers, of the two phenomena is observed. However the dependence of the effects of the various OH· scavengers on the pH greatly differs. A rough order of sensitivity is ethanol, butan-1-ol, methanol, formate > thiourea > mannitol.

In summary, our results indicate that the ability of classical OH· scavengers to inhibit deoxyribose degradation correlates with their ability to decrease Fe^{2+} oxidation by H_2O_2. If the mechanism that can explain the present findings is that proposed by Haber & Willstätter (1931) and by Walling (1975), some conclusions can be drawn. Not only mannitol and thiourea but other classical OH· scavengers can affect OH·-dependent damage to detector molecules by binding Fe^{2+}. All these OH· scavengers act predominantly by this mechanism, as they are practically unable to protect against the damage produced by OH· radical generated at specific sites (Gutteridge, 1984) in experimental conditions where they do not interact with Fe^{2+}. As it appears more and more evident that OH· generation by the Fenton reaction in biological systems is site-specific, the suggested use of OH· scavengers to indicate the involvement of OH· in such systems (Halliwell & Gutteridge, 1985) becomes questionable.

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Received 14 January 1988

The deoxyribose assay: an assay both for 'free' hydroxyl radical and for site-specific hydroxyl radical production

The interaction of iron ions with hydrogen peroxide in biological systems can lead to formation of a highly-reactive tissue-damaging species that is thought to be the hydroxyl radical, ·OH [1,2]. Various reactive iron–oxygen complexes may also exist, such as ferryl, perferryl and $Fe^{2+}/Fe^{3+}/O_2$ species (reviewed in [3–5]). There has thus been considerable interest in the development of methods for assaying ·OH and related species in biological systems (reviewed in [6]).

The pentose sugar 2-deoxyribose is attacked by ·OH radicals to yield a mixture of products (reviewed in [7]). On heating with thiobarbituric acid at low pH, some or all of these products react to form a pink chromogen that can be measured by its absorbance at 532 nm; this chromogen is indistinguishable from a thiobarbituric acid–malondialdehyde (TBA–MDA) adduct [8]. Generation of a TBA–MDA adduct from deoxyribose was thus introduced in 1981 [8,9] as a simple assay for ·OH generation in biological systems, provided that suitable control experiments are performed. The assay has been widely used [8–10].

If deoxyribose is incubated with H_2O_2 and an Fe^{2+}–EDTA complex (or an Fe^{3+}–EDTA complex in the presence of a reducing agent such as ascorbate or superoxide, O_2^-), the resulting deoxyribose degradation is inhibited by any added scavenger of ·OH to an extent that depends only on the concentration of scavenger relative to deoxyribose, and on the scavenger's second-order rate constant for reaction with ·OH [11–13]. It seems that, when ·OH is generated by reaction of Fe^{2+}–EDTA with H_2O_2, any ·OH that escapes scavenging by the EDTA itself [14] enters 'free solution' and is equally accessible to deoxyribose and to any added scavenger. Indeed, the deoxyribose assay in the presence of Fe^{3+}–EDTA, H_2O_2 and a reducing agent has been proposed as a simple 'test-tube' method for determining rate constants for the reaction of substrates with ·OH [11,12].

If deoxyribose is incubated with H_2O_2 and Fe^{2+} (or Fe^{3+} plus a reductant) in the absence of EDTA, it is still degraded into products that can react to form a TBA–MDA chromogen [6,8,9]. However, some ·OH scavengers (such as ethanol, formate, dimethyl sulphoxide and Hepes) no longer inhibit the deoxyribose degradation whereas others, such as mannitol, thiourea and hydroxychloroquine, still do [11,13,15]. Two possible explanations of this observation have been advanced.

One is that the deoxyribose-degrading species is not 'OH but is some other oxidant, such as ferryl [10]. It is known that oxidants other than 'OH can degrade deoxyribose to a TBA-reactive material; such a deoxyribose-degrading oxidant is produced by reaction of human oxyhaemoglobin with equimolar H_2O_2, for example [16]. An alternative explanation, which the authors prefer [5], is that unchelated iron ions added to deoxyribose-containing reaction mixtures can become weakly associated with deoxyribose. When the bound iron ions react with H_2O_2, any 'OH formed would be expected to attack the deoxyribose immediately and scavengers could not easily prevent this 'site-specific' attack. This explanation was advanced by Gutteridge in 1984 [15]. Indeed, it is likely that most 'OH formation *in vivo* occurs by site-specific mechanisms [17–19].

Evidence for a weak binding of both Fe^{3+} [20] and Fe^{2+} (M. Grootveld, unpublished work) ions to deoxyribose at physiological pH values has been obtained.

If the explanation of Gutteridge [15] is correct, why do some 'OH scavengers (e.g. thiourea and mannitol) still inhibit the deoxyribose degradation? It seems unlikely that they do so by scavenging 'OH generated site-specifically. Gutteridge [15] further proposed that those scavengers that inhibit do so because they themselves have metal-binding capacity, and they act by removing iron ions from the deoxyribose and directing damage to themselves. Two recent studies [11,13] confirm that the metal-binding ability of a compound is a major determinant of its ability to inhibit deoxyribose degradation in the presence of H_2O_2 and Fe^{2+} (or Fe^{3+} and a reducing agent). For example, citrate is a poor scavenger of 'OH but is a good inhibitor of deoxyribose degradation in the presence of H_2O_2, Fe^{3+} and ascorbate [13].

The overall conclusion of Tadolini & Cabrini [22], that deoxyribose degradation under their reaction conditions is mediated by site-specific 'OH formation by iron ions bound to deoxyribose, is one with which we would agree fully, since it confirms our published work [15,20,12]. Binding of Fe^{2+} to deoxyribose or to any other metal-binding 'scavenger' is likely to alter the rate of autoxidation of Fe^{2+}; part of our evidence for the binding of Fe^{2+} to deoxyribose is the ability of this sugar to decrease Fe^{2+} oxidation over a range of pH values (M. Grootveld, unpublished work). Thus a correlation between the ability of scavengers to block deoxyribose degradation and to diminish Fe^{2+} oxidation is explicable in terms of an iron-binding mechanism.

Some other aspects of the work of Tadolini & Cabrini [22] deserve comment, however. Deoxyribose reacts with 'OH with a rate constant of 3.1×10^9 $M^{-1} \cdot s^{-1}$, whereas Mes and Mops have values of $(2.0–3.0) \times 10^9$ and $(2.0–2.6) \times 10^9$ $M^{-1} \cdot s^{-1}$ respectively [12]. Thus the comment "the concentration of the buffer was kept rather low (5 mM) to limit the possible interference due to its reaction with $OH^{\bullet}$" is chemically illogical; over 50% of any free 'OH generated would react with the buffers! We would suggest that Mes and Mops do not inhibit in their experiments simply because, having little or no metal-binding capacity, these buffers cannot interfere with site-specific 'OH generation and deoxyribose degradation involving iron ions bound to the sugar. Secondly, Tadolini & Cabrini [22] misquote the work of Moorhouse *et al.* [21]. Moorhouse *et al.* [21] did not specifically attribute the effect of thiourea in the cobalt/H_2O_2/deoxyribose system to a reaction of thiourea with H_2O_2, as it stated by Tadolini and Cabrini. Moorhouse *et al.* (p. 226 in [21]) stated that "thiourea is having effects in addition to radical scavenging. Thiourea is known to react directly with H_2O_2 and *it may also bind metals*".

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Received 7 April 1988