

TRANSITION METAL COMPLEXES AS SENSITIZERS OR PROTECTORS AGAINST O_2^- TOXICITY

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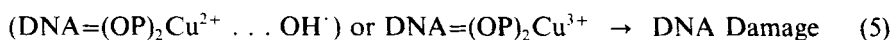
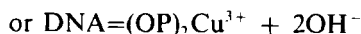
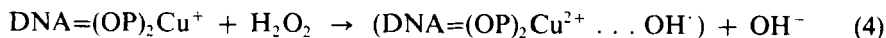
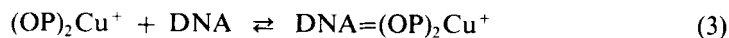
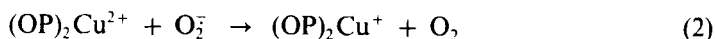
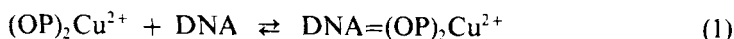
Among oxygen free radicals the OH^\cdot radical is the most reactive one. It reacts with most organic and biological molecules with rates approaching the diffusion controlled limit.¹ The O_2^- is far less reactive as compared to OH^\cdot .² Nevertheless, it seems that in vivo as well as in vitro systems, O_2^- is more harmful and destructive than OH^\cdot .³ This behaviour is especially true in the presence of transition metal ions. It has also been demonstrated that in solutions some enzymes are deactivated more efficiently by O_2^- or $(CNS)_2^-$ than by OH^\cdot , even though the rate constants of the reactions of these radicals with the enzymes are much slower than the parallel reactions with OH^\cdot .⁴

Several reasons may account for such a behaviour:

- 1) The highly oxidizing OH^\cdot radical reacts with most amino acids and it does not generally prefer any amino acid. The less reactive radicals, such as O_2^- or $(SCN)_2^-$, may react with only certain residues. If these residues happen to be at or near the active site, clearly these radicals will show a more deleterious effect than OH^\cdot .
- 2) The lower the reactivity of a radical is, the longer its half life time will be. Therefore, the unreactive O_2^- lives longer than OH^\cdot , and hence is able to diffuse longer distances and reaches the active site. In a biological environment or in an irradiated cell, where OH^\cdot and O_2^- are the main radicals being formed, the OH^\cdot radical will not react with a specific molecule or a site. However, O_2^- will diffuse large distances and thus may reach to the target, and may be more effective in causing damage than OH^\cdot .⁵
- 3) Metal ions via a site-specific mechanism may enhance the specific damage caused by free radicals.

The role of metal compounds in enhancing O_2^- damage through a site-specific mechanism may take different forms. The simplest form applies to dilute solutions where O_2^- reacts directly with DNA. However, this reaction is very slow, far too slow to account for the damage caused by this radical. It appears that metal ions play a role in expressing DNA damage initiated by O_2^- .³ The explanation for this effect assumes that O_2^- reacts with the metal bound to the target. If O_2^- reduces the metal, then the reduced metal may subsequently react with H_2O_2 , for example, forming either an OH^\cdot radical or a higher valence state of the metal both of which may cause damage. For example, $(OP)_2Cu^{2+}$ is an efficient complex in enhancing DNA damage by O_2^- .³ This feature is somewhat surprising as it is known that this copper complex catalyzes very efficiently the dismutation of O_2^- .⁶ We have offered an explanation for this apparent paradox. We have shown that $(OP)_2Cu^{2+}$ forms a ternary complex with DNA ($DNA = (OP)_2Cu^{2+}$). This ternary complex, which is in equilibrium with the free $(OP)_2Cu^{2+}$, is reduced by O_2^- through the unbound complex, and hence the rate of the reduction is much slower compared to the case where DNA is absent.⁷ Moreover, under biological conditions the reduced ternary complex will react much faster with

H_2O_2 than with O_2^- ,³ forming either an OH^\cdot radical at or near the binding site or a higher valence state of the metal ($\text{DNA}=(\text{OP})_2\text{Cu}^{3+}$). The following sequence of reactions summarizes the whole mechanism proposed:



The above mentioned mechanism shows how an OH^\cdot or a higher valence state of the metal may be formed from O_2^- or from some other reducing entity in the vicinity of the DNA through the site-specific mechanism catalyzed by a copper complex. If OH^\cdot is formed, it will not be able to diffuse away and therefore it will react with DNA and deactivate it. If a higher state of the metal is formed, then it may undergo an intramolecular electron transfer reaction causing degradation of DNA at the binding site.⁸⁻¹¹ This mechanism may apply also with macromolecules in solutions or in cells. If a macromolecule, e.g., an enzyme, has several sites which may react with O_2^- and many sites which do not, then the former sites, if they bind metal compounds, can be converted into sites reacting with O_2^- , and in some cases even reacting preferentially with O_2^- .

A more refined site specificity can be dependent on the conformation of the target. It has been found that the B form of DNA is more sensitive than A-DNA and much more than the Z-DNA towards damage induced by $(\text{OP})_2\text{Cu}^+$ and H_2O_2 .¹² It has also been shown that a given base sequence of DNA reacts preferentially with the reagent causing preferential damage. Furthermore, the various metal complexes bind differently to the double helix of DNA showing different base/complex ratios and different cleavage abilities.¹³⁻¹⁵

The above mechanism (reactions (1)–(5)) may also account for the toxicity of other reducing agents as long as we assume that the sole role of O_2^- is to reduce the metal. In cases where other reductants, such as vitamin C or glutathione, which are present in every living cell at concentrations exceeding by far that of O_2^- , cannot replace O_2^- in enhancing DNA damage, we assume that O_2^- , rather than reduces the metal does oxidize it.



In this case a higher valence state of the metal is formed, which as in the former case may cause DNA damage.⁸⁻¹¹ The idea that O_2^- may oxidize copper(II) to copper(III) is not so improbable as it may sound. Simple peptide complexes of copper(II) are readily oxidized to copper(III) not only by strong oxidants such as IrCl_6^{2-} , but also by molecular oxygen.⁸ It has also been demonstrated that O_2^- oxidizes Mn(II) compounds and that the oxidizing species thus formed are subsequently oxidizing other targets.¹⁶ It also appears that during the oxidation of NAD(P)H by O_2^- in the presence of vanadate, an oxidant is formed from vanadate and O_2^- , which then oxidizes NAD(P)H.¹⁷ This mechanism is in accordance with the site-specific mechanism,

exhibiting the sensitization effect of the metal complexes and explains the difference between the toxicity of O_2^- to that of other reductants. Nevertheless, reactions (1)–(5) are toxic and can occur as well.

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