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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.[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, 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 ($DNA{=}(OP)_2Cu^{3+}$). The following sequence of reactions summarizes the whole mechanism proposed:

$$(OP)_2Cu^{2+} + DNA \rightleftarrows DNA{=}(OP)_2Cu^{2+} \quad (1)$$

$$(OP)_2Cu^{2+} + O_2^- \rightarrow (OP)_2Cu^{+} + O_2 \quad (2)$$

$$(OP)_2Cu^{+} + DNA \rightleftarrows DNA{=}(OP)_2Cu^{+} \quad (3)$$

$$DNA{=}(OP)_2Cu^{+} + H_2O_2 \rightarrow (DNA{=}(OP)_2Cu^{2+} \ldots OH^\cdot) + OH^- \quad (4)$$

$$\text{or } DNA{=}(OP)_2Cu^{3+} + 2OH^-$$

$$(DNA{=}(OP)_2Cu^{2+} \ldots OH^\cdot) \text{ or } DNA{=}(OP)_2Cu^{3+} \rightarrow \text{DNA Damage} \quad (5)$$

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 mechansim 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 specifity 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 $(OP)_2Cu^+$ 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 gluthathione, 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.

$$DNA{=}M^{n+} + O_2^- + 2H^+ \rightarrow DNA{=}M^{(n+1)+} + H_2O_2 \quad (6)$$

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 $O_2^{\cdot-}$ to that of other reductants. Nevertheless, reactions (1)–(5) are toxic and can occur as well.

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