Role of Cu(II) Complexes as Scavengers of Superoxide Radicals

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Photochemically generated superoxide radicals induce oxidation of ascorbic acid. The oxidation of ascorbic acid is significantly inhibited by cobalt(II) complexes. The rate constants of the reactions of cobalt complexes with superoxide are two order of magnitude higher compared to the rate constant of non-enzymatic dismutation of superoxide radicals.

The use of metal complexes as anticancer drugs, antimicrobial and antiviral agents, and in the treatment of arthritic and inflammation has attracted considerable attention in recent years. Amongst the metal complexes, cobalt complexes have shown promise because of their unique property of functioning as oxygen carriers.

Molecular oxygen is known to bind with metal complexes as superoxide (O$_2^-$) and peroxide (O$_3^-$). Both the species derived from molecular oxygen produce several types of physiological disorders. Ascorbic acid, present in high amounts in both animals and plant tissues, functions as defense against the damaging effect produced by O$_2^-$ and O$_3^-$.

The purpose of the present investigation is to examine the efficacy of cobalt(II) complexes as defense against O$_2^-$ radicals. Cobalt(II) complexes, viz. hexaamminocobalticpyridine (M$_1$), cis-carbottetraamminocobaltichthiourea (M$_2$) and nitratopentaamminocobaltichthiourea (M$_3$) were prepared by well known methods. The antioxidants, cyasorb 531 (P$_1$) and 2,4-dihydroxybenzophenone (P$_2$) were obtained as gift samples from CIBA, Switzerland.

Ascorbic acid (AR) was a BDH product and its stock solution (5 mM) was prepared in 10% trichloroacetic acid (TCA).

An aliquot of ascorbic acid was incubated in a medium (phosphate buffer 0.1 M, pH 7.4) both in the presence and absence of M$_1$, M$_2$, M$_3$, P$_1$ or P$_2$. All the metal complexes absorb maximally in the region (490-500) nm. The incubator was fitted with a light box containing two 15 watts fluorescent day light tubes. This light source emits a broad spectrum similar to sunlight. The measured intensity of light at the surface of the incubation medium was 1829.2 lux.

The incubation was continued for 5 hr after which the concentration of ascorbic acid was determined in all the cases by ferrocine method. For a better understanding of the possible function of M$_1$, M$_2$, M$_3$, P$_1$ or P$_2$, as defense against O$_2^-$ generated photochemically, the rate constants of their reactions with O$_2^-$ were also determined.

The results of these investigations are summarised in Fig. 1. As is apparent, the concentration of ascorbic acid (A$_1$) decreased significantly (A$_2$) after exposure to visible light. However, the light-induced decrease in the concentration of ascorbic acid was protected by cobalt complexes M$_1$, M$_2$ and M$_3$, to the extent of 66, 50 and 44% respectively. The protections induced by cobalt complexes were of lower magnitude compared to similar effects produced by well known antioxidants (P$_1$ and P$_2$). These results suggest that cobalt complexes function as scavengers of O$_2^-$. The O$_2^-$, which is unstable in aqueous medium, undergoes non-enzymatic dismutation to H$_2$O$_2$ with a second order rate constant of $2 \times 10^5$ dm$^3$ mol$^{-1}$ s$^{-1}$ at pH 7.5 (ref. 16) which is four order of magnitude less than the rate constant of (2 $\times 10^7$ dm$^3$ mol$^{-1}$ s$^{-1}$) of enzymatic dismutation of O$_2^-$. The non-enzymatic dismutation of O$_2^-$ is considerably catalysed by Co(II) complexes, the second order rate constant in the presence of Co(II) complexes being in the range of (4-8) $\times 10^7$ dm$^3$ mol$^{-1}$ s$^{-1}$. A better dismutation of O$_2^-$ was obtained with antioxidants. These values of rate constants and the values as reported with cytochrome-C$^{18}$, ascorbic acid$^{10}$ and nitrobluetetrazolium$^{19}$ are given in Table 1.

It is suggested that there are two possibilities of reactions of O$_2^-$ with cobalt complexes. It can either form oxygen coordination complexes or it can undergo one-electron reduction. It has earlier been reported that vitamin B$_{12}$ which is a cobalt(III) complex
Table 1 — Values of Rate Constants of Dismutation of $O_2^-$ in Presence of Different Systems

<table>
<thead>
<tr>
<th>System</th>
<th>Rate constant $\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O_2^- + O_2^- + 2H^+$ (non-enzymatic)</td>
<td>$2 \times 10^5$</td>
<td>(16)</td>
</tr>
<tr>
<td>$O_2^- + O_2^- + 2H^+$ (enzymatic)</td>
<td>$2 \times 10^9$</td>
<td>(17)</td>
</tr>
<tr>
<td>Ascorbic Acid + $O_2^-$</td>
<td>$2.7 \times 10^5$</td>
<td>(10)</td>
</tr>
<tr>
<td>Cytochrome C + $O_2^-$</td>
<td>$6 \times 10^4$</td>
<td>(18)</td>
</tr>
<tr>
<td>Nitroblue tetrazolium + $O_2^-$</td>
<td>$5 \times 10^4$</td>
<td>(19)</td>
</tr>
<tr>
<td>Cobalt(II) complexes + $O_2^-$</td>
<td>$(4-8) \times 10^7$</td>
<td>Present work</td>
</tr>
<tr>
<td>Antioxidants + $O_2^-$</td>
<td>$(9-11) \times 10^7$</td>
<td>Present work</td>
</tr>
</tbody>
</table>

interacts with $O_2^-$ at $-50^\circ C$ and forms superoxocobalmin$^{21}$. These observations lead us to believe that Co(II) complexes react with $O_2^-$ in accordance with Eq. (1).

$$\text{Co(II)} + O_2^- \rightarrow \text{Co(III)} + O_2^2^- \quad \ldots \quad (1)$$

Considerable enhancement (200 times) of rate of dismutation of $O_2^-$ in the presence of Co(II) complexes has physiological implication in many enzyme deficient processes where natural and artificial light sources damage the cellular components due to photochemical generation of $O_2^-$ (see ref. 22, 23).

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References

3 Sorenson J R J, Copper in the environment: Part II — Therapeutic uses of copper (Wiley, New York) 1979, pp. 84.
10 Nishikimi M, Biochem Biophys Res Commun, 63 (1975) 463.
23 Taube H, J gen Physiol, 49 (1965) 29.