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Desferrioxamine: a scavenger of superoxide radicals?

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The iron chelating agent desferrioxamine is frequently used to inhibit the iron-catalyzed production of hydroxyl radicals ($OH^{\cdot}$) by superoxide generating systems [1–5]. This effect of desferrioxamine can be related either to iron chelation or to the intervention of an iron-independent mechanism, such as a direct scavenging effect on reactive oxygen radicals. Whereas desferrioxamine was shown to scavenge $OH^{\cdot}$ in certain conditions [6], the present study was performed to test a possible reactivity of desferrioxamine on superoxide radicals ($O_2^{\cdot}$).

Materials and methods. Desferrioxamine methane sulfonate (Desferal) was from Ciba-France. Xanthine oxidase (EC 1.2.3.1), horseradish peroxidase (EC 1.11.1.7), nitroblue tetrazolium (NBT), ferricytochrome c, xanthine sodium salt, phenol red, diethylenetriaminepentaacetic acid (Detapac) were obtained from Sigma Chemical Co., St Louis, MO, U.S.A. Other reagent grade chemicals were from Merck, Darmstadt, F.R.G.

The ability of desferrioxamine at different concentrations to scavenge $O_2^{\cdot}$ and thereby inhibit reactions mediated by $O_2^{\cdot}$ was assayed by measuring the inhibition of the reduction of ferricytochrome *c* or NBT mediated by the aerobic action of xanthine oxidase on xanthine [7, 8]. Reductions of ferricytochrome *c* to ferrocytochrome *c* and of NBT to formazan were measured at 550 nm and 560 nm, respectively, at 25°C, in an Uvikon model 820 recording spectrophotometer. Xanthine oxidase was itself assayed by following the conversion of xanthine to urate at 292 nm. Detapac was added, when quoted, to the assay mixture in order to chelate trace amounts of iron and prevent thereby the production of $OH^{\cdot}$ radicals by the xanthine–xanthine oxidase which occurs in presence of traces of iron [9].

H_2O_2 production in the xanthine–xanthine oxidase system was measured in presence and in absence of desferrioxamine. After incubation during 15 min, 100 μl of reactive media were removed and H_2O_2 measured according to [10] after addition of a buffered solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.28 mM phenol red and 8.5 U/ml of horseradish peroxidase). The samples were incubated for 5 min at room temperature and brought to pH 12.5 by addition of NaOH. Absorbance was measured at 610 nm and compared to H_2O_2 standards and appropriate reagent banks.

Results and discussion. We studied the ability of desferrioxamine to compete with ferricytochrome *c* for a flux of $O_2^{\cdot}$ generated by the action of xanthine oxidase on xanthine. Figure 1 shows that desferrioxamine inhibited in a concentration-dependent fashion this reduction of ferricytochrome *c* mediated by $O_2^{\cdot}$. Further experiments using NBT, a substrate involving no iron, instead of ferricytochrome *c* showed that desferrioxamine inhibits NBT reduction by $O_2^{\cdot}$ in the same way as ferricytochrome *c* reduction (results not shown). This finding excludes the intervention of iron chelation in the observed effects.

A direct inhibitory action of desferrioxamine on xanthine oxidase can be excluded as desferrioxamine does not modify the rate of urate formation from xanthine as shown previously by Gutteridge *et al.* [1] and confirmed in our experimental conditions (results not shown).

As the results were consistent with desferrioxamine acting as a scavenger of $O_2^{\cdot}$ like ferricytochrome *c* itself, we studied the competition between desferrioxamine and cytochrome *c* for $O_2^{\cdot}$. As it has been shown recently that the familiar Lineweaver–Burk plot can be used to describe the trapping by ferricytochrome *c* of $O_2^{\cdot}$ generated by the xanthine–xanthine oxidase system [11], we used this plot to interpret the results with desferrioxamine addition. As

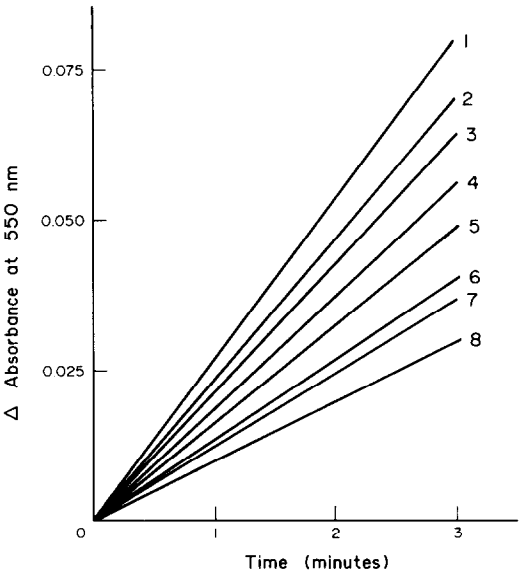

Fig. 1. Effect of desferrioxamine on the ferricytochrome *c* reduction mediated by the aerobic action of xanthine oxidase on xanthine. Reaction mixtures contained 0.1 mU/ml of xanthine oxidase, 1×10^{-5} M ferricytochrome *c* and 5×10^{-5} M xanthine in 3.0 ml of 0.05 M potassium phosphate buffer, pH 7.8, containing 1 mM detapac. Results are expressed as the change in absorbance at 550 nm vs time either in the absence (1) of desferrioxamine or with increasing concentrations of this compound (2, 3, 4, 5, 6, 7, 8 = 0.2, 0.5, 1, 1.5, 2.25, 4.12, 5.5 mM, respectively). Each reaction was carried out four-fold giving almost identical results.

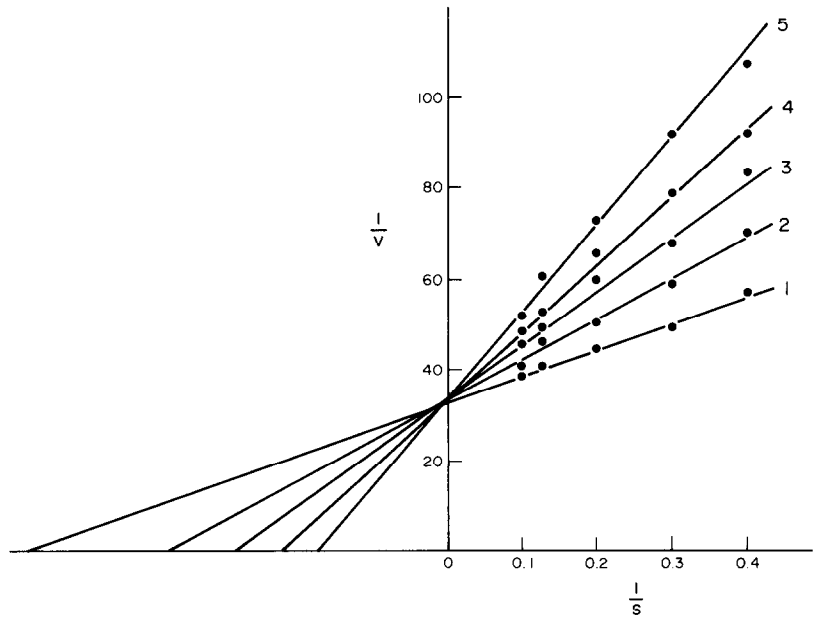

Fig. 2. Lineweaver–Burk plots of the inhibition by desferrioxamine of the ferricytochrome *c* reduction mediated by $O_2^{\cdot}$ with ferricytochrome *c* as the variable substrate. Reaction mixtures contained 0.1 mU/ml of xanthine oxidase and 5×10^{-5} M xanthine in 3.0 ml of 0.05 M potassium phosphate buffer, pH 7.8 containing 1 mM detapac. The abscissa gives the reciprocal of the micromolarity of ferricytochrome *c* and the ordinate the reciprocal of the initial rate expressed in absorbance/min/cm. Concentrations of desferrioxamine: (1) none, (2) 0.25 mM; (3) 0.5 mM; (4) 0.75 mM, (5) 1 mM. Each reaction was carried out twice, giving almost identical results.

shown in Fig. 2, desferrioxamine addition decreased the initial rate of ferricytochrome *c* reduction but was without apparent effect on its V_m value. At the same time it increased the $K_{0.5}$ value. These results suggest that desferrioxamine is reacting with superoxide radicals as competitor with respect to ferricytochrome *c*.

In order to determine whether desferrioxamine acts as superoxide dismutases which scavenge $O_2^{\cdot}$ and induce $O_2^{\cdot}$ dismutation into O_2 and H_2O_2 [7], we measured the generation of H_2O_2 by the xanthine–xanthine oxidase system in the presence and absence of desferrioxamine. Yields of H_2O_2 in the presence of desferrioxamine were significantly lower than those measured in its absence (0.259 ± 0.023 vs 0.534 ± 0.048 μM/15 min). These results show that desferrioxamine while scavenging $O_2^{\cdot}$ does not have dismutase activity.

As the data described above are consistent with an ability of desferrioxamine to scavenge $O_2^{\cdot}$, it was interesting to test whether this ability is also shared by ferrioxamine (the desferrioxamine–Fe^{III} complex). We studied therefore the effects of the addition of Fe^{III} on the inhibition of ferricytochrome *c* or NBT reduction mediated by desferrioxamine. It appears that this inhibition is suppressed when desferrioxamine (1 mM) is converted into ferrioxamine by addition of Fe^{III} (1 mM). The lack of action of ferrioxamine on $O_2^{\cdot}$ has been also previously recorded by Butler and Halliwell [12]. On the other hand Hoe *et al.* [6] have shown that ferrioxamine had a much weaker scavenging property on $OH^{\cdot}$ as compared to desferrioxamine in the aerobic hypoxanthine–xanthine oxidase system, although ferrioxamine and desferrioxamine are equally potent as $OH^{\cdot}$ scavengers in an aerobic system. It can therefore be suggested that the difference found by these authors between desferrioxamine and ferrioxamine depending on O_2 supply may be related mainly to the $O_2^{\cdot}$ scavenging effect of desferrioxamine. The scavenging effect of desferrioxamine on $O_2^{\cdot}$ together with its action on $OH^{\cdot}$ may play a role in the cellular defense against oxygen radicals.

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