The Role of Superoxide Anion Radical in the Reduction of Ferritin Iron by Xanthine Oxidase

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ABSTRACT Superoxide dismutase exerted a pronounced inhibitory effect upon xanthine oxidase-mediated reduction of iron in ferritin, ferric chloride, or ferric ADP. Maximal inhibition was observed when the superoxide dismutase concentration was only about 1% of that found in normal porcine liver. These observations indicate that superoxide anion radical is an intermediate in the reduction of iron by xanthine oxidase in vitro but not in vivo.

INTRODUCTION

Two hypotheses have been proposed to explain how iron is mobilized from ferritin (1, 2). Although reactions central to both hypotheses can occur in vitro, the in vivo mechanism remains unknown. According to one hypothesis, iron mobilization depends upon formation of hypothetical low molecular weight iron chelates without prior reduction. This theory will not be considered here. The other hypothesis holds that mobilization of iron depends on its prior reduction to the ferrous form.

Of the possible intracellular redox enzymes, xanthine oxidase is the one that has been most frequently implicated in ferritin iron release. Mazur, Green, Saha, and Carleton demonstrated that iron could be reduced and mobilized from ferritin in vitro during the xanthine oxidase-catalyzed oxidation of xanthine (3, 4). Furthermore, xanthine oxidase as well as other flavin enzymes are able to reduce inorganic iron (5). Initially, it was assumed that in these reactions, iron accepted an electron from xanthine oxidase directly (Fig. 1A). However, later studies of the enzymatic properties of xanthine oxidase have established that many of its reactions depend upon the generation of superoxide anion radical ($O_2^{\cdot-}$), and that one such reaction is the reduction of iron in cytochrome C (6, 7). In such reactions, xanthine oxidase first brings about the reduction of oxygen to superoxide anion radical, which in turn serves as an electron donor for the reduction of the iron in cytochrome C. It seemed possible that superoxide anion might play a similar role in the xanthine oxidase-mediated reduction of other iron compounds, including ferritin. If so, these reactions should be inhibited by superoxide dismutase (erythrocuprein) in vitro (8, 9), and perhaps in vivo as well (Fig. 1B).

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METHODS

Superoxide dismutase was prepared from porcine erythrocytes by the method of McCord and Fridovich (8), except that the final chromatographic purification step was omitted. The preparation contained 1,120 U/mg protein. Xanthine oxidase was prepared from fresh raw cream by a method that avoids the use of proteolytic enzymes (10). Ferric ADP (Fe ADP) was prepared in acid solution by the method of Goucher and Taylor (11). Cadmium-free, twice-crystallized equine spleen ferritin was obtained from Calbiochem, San Diego, Calif.

Reduction of iron was measured as the rate of development of the Fe II-orthophenanthroline complex at a wavelength of 510 nm at 25°C in a Cary model 15 recording spectrophotometer (Cary Instruments, Monrovia, Calif.). Orthophenanthroline (0.8 mM), xanthine, and the iron substrate were equilibrated for 30 min; then xanthine oxidase was added in amounts adjusted to produce a linear reaction rate. For each iron substrate, conditions were selected that provided maximal reaction rates. $FeCl_3$ (120 μM) and FeADP (200 μM) were studied at a xanthine concentration of 60 μM in 5 mM phosphate buffer, pH 7.8. Ferritin (8 mM) was studied at a xanthine concentration of 100 μM in 5 mM carbonate buffer, pH 10.5. Varying amounts of superoxide dismutase were added and the effect on reaction rate was measured. To determine hepatic superoxide dismutase activity, whole liver slices were homogenized in 0.25 M sucrose and the homogenates were assayed by the method of McCord and Fridovich (8).

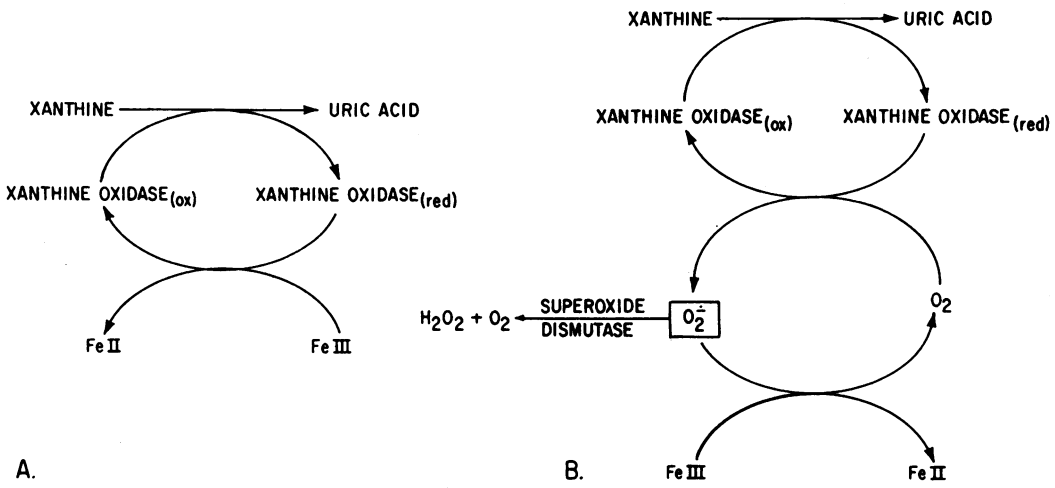

FIGURE 1 Alternative hypotheses for the reduction of iron by xanthine oxidase. A. Iron accepts an electron directly from xanthine oxidase. B. The superoxide anion radical serves as an intermediate electron carrier. Superoxide dismutase would be expected to inhibit the latter reaction but not the former.

RESULTS

Xanthine oxidase-mediated reduction of iron and subsequent chelation by orthophenanthroline was demonstrated with all three iron substrates, $FeCl_3$, FeADP, and ferritin. In each case, the reaction could be markedly inhibited by the addition of superoxide dismutase (Fig. 2). At a superoxide dismutase concentration of 18 U/ml, reduction of $FeCl_3$ was inhibited 85%; of FeADP, 100%; and of ferritin, 92%.

These concentrations of superoxide dismutase are considerably less than those found in normal liver. The activity in liver specimens from 20 normal pigs was 1,863±524 (mean±SD) U/g of tissue (wet weight).

DISCUSSION

The inhibition by superoxide dismutase of the xanthine oxidase-mediated reduction of the iron in ferric chloride, FeADP, and ferritin indicates that the superoxide anion radical ($O_2\cdot^-$) is an intermediate in the reaction (8, 9). Although it is hazardous to extrapolate from in vitro studies to the in vivo situation, an important possible physiologic implication of this observation should be noted. The hepatic concentration of superoxide dismutase (hepatocuprein) is very high, about 100 times greater than that required to exert a maximal inhibitory effect on superoxide-mediated iron reduction in vitro. Thus, the xanthine oxidase reaction is unlikely

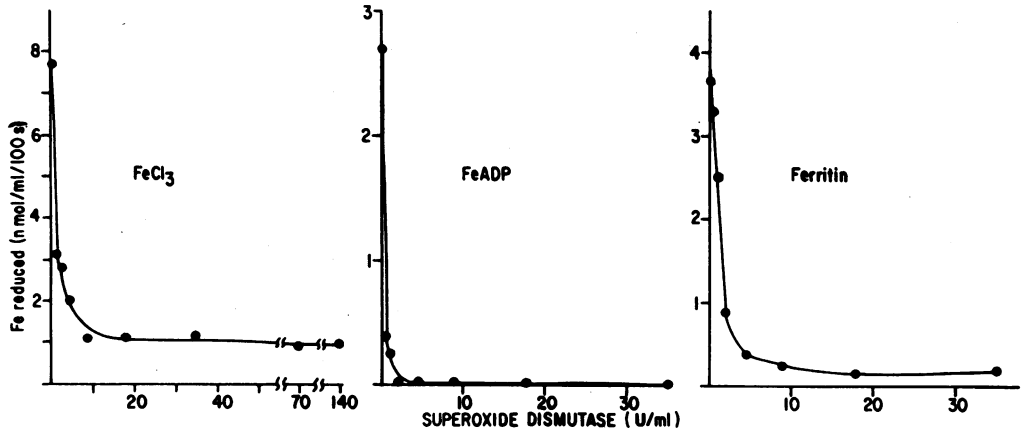

FIGURE 2 The effect of superoxide dismutase in varying concentration on the rate of iron reduction in three different iron substrates: ferric chloride (left), Fe ADP (center), and ferritin (right).

to be an important physiologic mechanism for mobilization of iron from hepatic stores.

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