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ELECTRON TRANSFER FACILITATED BY SUPEROXIDE DISMUTASE: A MODEL FOR MEMBRANE REDOX SYSTEMS?

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Membranes, which are an amalgam of proteins and lipids, effect electron transfer through largely unknown mechanisms. Using albumin with bound fatty acids as a model, we have investigated the possible role of these two membrane constituents in electron transfer. In the presence of albumin:fatty acid, there is substantial enhancement of the reduction of ferricytochrome C by ferrous iron. To assess the possible role of free superoxide in cytochrome C reduction, we added mammalian copper/zinc containing superoxide dismutase (Cu/Zn SOD), which catalyzes the transfer of electrons between superoxide anion radicals, forming oxygen and hydrogen peroxide. Surprisingly, in the presence of either albumin or fatty acid free albumin, Cu/Zn SOD actually accelerates electron transfer from ferrous iron to ferricytochrome C. By contrast, neither inactive Cu/Zn SOD nor active manganese SOD facilitates the ferrous iron-dependent reduction of cytochrome C. These results suggest that, in some circumstances, Cu/Zn SOD may transfer electrons to alternative acceptors and that such transfer depends upon the unique reduction/oxidation reaction mechanism of Cu/Zn SOD. If so, this ubiquitous enzyme could be involved in regulating cellular electron transfer reactions as well as acting as a superoxide 'detoxify-ing' agent.

We have shown that the interaction between ferrous iron, unsaturated fatty acids (UFA) and oxygen produces a species which can act as a reducing agent to electrophilic agents such as ferricytochrome C or nitroblue tetrazolium.[1,2] The ferrous iron-UFA complex can also disproportionate to superoxide or a peroxy fatty acid.[3,4]

The reduction of ferricytochrome C by a presumed fatty acid:iron complex may provide a model of how UFA could function in the mitochondrial electron transport chain.[1] While the mechanism of electron transfer is not fully understood,[5] there is evidence that pi electrons, such as those present in UFA, act to promote the transfer.[1,6-8] In the above model, UFA facilitate the exchange of electrons between iron centers (i.e., UFA:ferrous iron:O_2 and ferricytochrome C). Since this work, Purugganan et al. have found that DNA, also rich in pi electrons, can accelerate electron transfer.[6] It has also been shown that the pi electrons of heme vinyl groups are involved in electron transfer.[7,8]

The present investigations were carried out to determine whether protein-associated fatty acids - like free fatty acids - could facilitate electron transfer. Unlike the previous experimental systems involving free UFA, the UFA:albumin complex is a one-phase system. This model is probably closer to that of the electron transfer system of mitochondria (where the inner membrane is composed of protein and lipid). If an UFA-iron oxygen species is involved in electron transfer, it might involve free superoxide as an intermediate in the transfer of an electron from one reduced iron center to another. To evaluate the role of protein in the transfer of electrons from ferrous iron to ferricytochrome C, bovine serum

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albumin and defatted bovine serum albumin were added to a reaction mixture containing ferrous iron and ferricytochrome C. To determine whether free superoxide was involved in such electron transport, copper/zinc SOD (Cu/Zn SOD) was added to this model system and its effect on the rate of cytochrome C reduction observed. Unexpectedly, the results show that, rather than inhibiting electron transfer in this system, Cu/Zn SOD (but not inactive Cu/Zn SOD or manganese SOD) facilitates the reaction, suggesting an additional possible role for this ubiquitous enzyme.

MATERIALS AND METHODS

Cytochrome C type III, Cu/Zn SOD (from bovine erythrocytes), manganese SOD (Mn SOD; from *E. coli*), diethyldithiocarbamic acid (DDC), bovine serum albumin fraction V, and fatty acid-free bovine serum albumin fraction V were all obtained from Sigma Chemical Co. (St. Louis, MO). The reagents, with the exception of ferrous sulfate, were combined in 1 ml of 50 mM tris[hydroxymethyl]aminomethane (Tris), pH 7.4 @ 25°C, and a baseline absorption taken at 550 nm. Ferrous iron was added, the solution was rapidly mixed and the absorption read every 30 seconds for 3 minutes. Cu/Zn SOD was inhibited by incubation with DDC. This was done by incubating 30,000 units (ca. 10 mg) of Cu/Zn SOD in 1 ml of 50 mM Tris containing 25 mM DDC, pH 7.4 @ 25°C, for 5 hours. The excess DDC was removed by rapid gel filtration on a Pharmacia Sephadex G-15 ('PD-10') column with the same buffer. This DDC-treated enzyme was shown by direct assay[9] to have <2% of original activity.

RESULTS AND DISCUSSION

The reduction of cytochrome C by ferrous sulfate is significantly enhanced by albumin (Fig. 1). This effect is presumably caused by the fatty acids associated with the albumin; fatty acid-free albumin does not accelerate this reduction. Analyses of fatty acids removed from albumin have yielded variable results but indicate a mixture of saturated and unsaturated fatty acids (UFA).[10] Since UFA were previously shown to enhance electron transfer,[1,2] UFA bound to the bovine serum albumin might be most important in this reaction. However, the additional involvement of albumin-bound saturated fatty acids remains an untested possibility. The above findings are consistent with our earlier hypothesis that the UFA within membranes can enhance electron transfer systems by serving as catalysts for electron transfer between iron centers.[1,2]

Reduction of redox indicators such as cytochrome C and NBT does not necessarily indicate the involvement of superoxide. In order to determine whether superoxide *per se* (arising, e.g., from a dis-

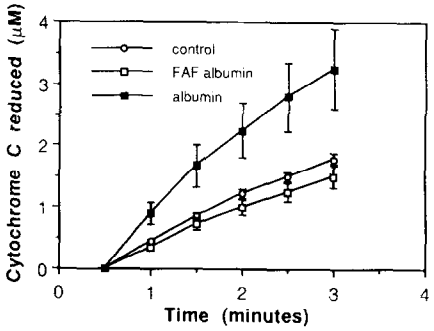

Figure 1. The effect of albumin and fatty acid-free albumin on electron transfer from ferrous iron to ferricytochrome C. Reaction mixtures contained, in a total volume of 1 ml, fatty acid-free albumin (100 μg) (❑) or albumin (100 μg) (■), ferricytochrome C (23 μM) in 50 mM Tris, pH 7.4. The blank (○) contained no added albumin. A baseline was taken at 550 nm and 180 μM $FeSO_4$ was added. In this and the following figures, values shown represent the mean ± 1 S.D. of quadruplicate determinations.

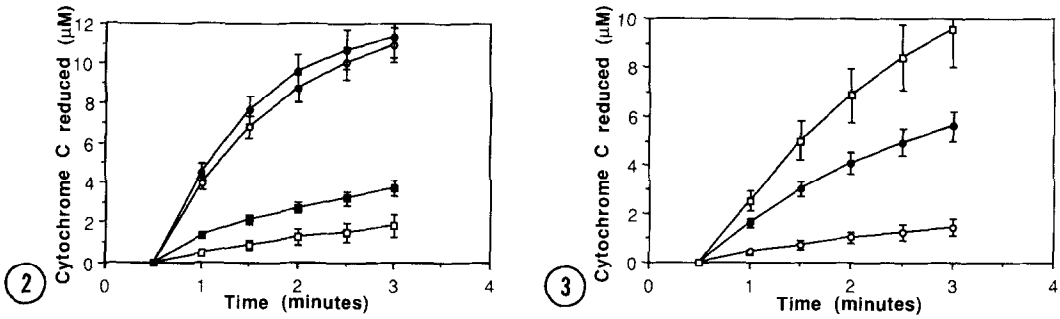

Figure 2. The effect of Cu/Zn SOD on electron transfer from ferrous iron to ferricytochrome C in the presence of albumin. Reaction mixtures contained, in a total volume of 1 ml, albumin (100 μg/ml), ferricytochrome C (23 μM) and no addition (■), 1350 U (450 μg) Cu/Zn SOD (○) or 2020 U (670 μg) Cu/Zn SOD (●) in 50 mM tris, pH 7.4. The blank (□) contained no albumin and no added SOD. A baseline reading was taken at 550 nm and 180 μM $FeSO_4$ was then added.

Figure 3. The effect of Cu/Zn SOD on electron transfer from ferrous iron to ferricytochrome C in the presence of fatty acid-free albumin. Reaction mixtures contained, in a total volume of 1 ml, fatty acid-free albumin (100 μg/ml), ferric cytochrome C (23 μM) and no addition (○), 1350 U (450 μg) Cu/Zn SOD (●) or 2020 U (670 μg) Cu/Zn SOD (□) in 50 mM Tris, pH 7.4. A baseline reading was taken at 550 nm and 180 μM $FeSO_4$ was then added.

proportionation of an iron-fatty acid-oxygen complex) might be involved in this reaction, we added Cu/Zn SOD to the reaction. In the presence of superoxide and ferricytochrome C, SOD effectively blocks the reduction of cytochrome by superoxide.[9] Surprisingly, when added to the model system of Fe^{2+}, albumin and cytochrome C, Cu/Zn SOD actually enhances cytochrome C reduction (Fig. 2). Similar SOD-dependent cytochrome C reduction is observed when fatty acid-free albumin is employed (Fig. 3). The amounts of Cu/Zn SOD added to these reactions are large (> 400 μg/ml) but approach the molar concentration of cytochrome C, suggesting a direct, possibly stoichiometric, transfer of electrons. It should also be noted that concentrations of Cu/Zn SOD of this magnitude are within the physiologic range for tissues such as cerebral grey-matter, testis, liver and renal cortex.[11]

While the mechanism involved in the enhanced reduction of cytochrome C is still uncertain, it is evidently not an artifact arising from free copper associated with the Cu/Zn enzyme, because added Cu^{2+} has little effect (Fig. 4). Catalytically active Cu/Zn SOD appears to be involved, because the

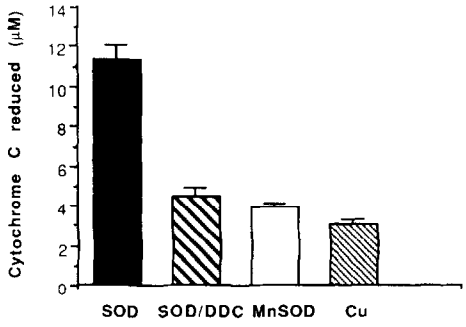

Figure 4. Comparison of Cu/Zn SOD (2020 U or 670 μg/ml), Cu/Zn SOD-treated with DDC (670 μg/ml), Mn SOD (670 μg/ml) and Cu (as $CuSO_4$; 1 μM) on electron transfer from ferrous iron to ferric cytochrome C in the presence of albumin after 3 minutes. The above reagents were combined in a 1 ml reaction mixture also containing albumin (100 μg/ml) and ferric cytochrome C (23 μM) in 50 mM Tris, pH 7.4. A baseline reading was taken at 550 nm and 180 μM $FeSO_4$ was added to start the reaction. All treatments are significantly different from Cu/Zn SOD ($p < 0.001$, Student's 't' test, unpaired; $n = 4$ in all cases).

inactive DDC-treated enzyme is almost completely ineffective (Fig. 4). Furthermore, a catalytic principle unique to Cu/Zn SOD may be necessary because MnSOD (from *E. coli*), in similar concentrations, does not have any effect on electron transfer in this system (Fig. 4).

A number of membrane electron transfer systems which may involve iron-dependent reactions have been reported, including (1) α-adrenergic receptor,[12] (2) β-adrenergic receptor,[13] (3) dopaminergic D_1 receptor[14] and (4) prostaglandin activation of adenylate cyclase.[15] It is possible that Cu/Zn SOD may play a regulatory role in such systems. For example, cells from patients with Down Syndrome (trisomy 21) not only show a (gene dosage-dependent) 50% elevation in Cu/Zn SOD activity but are also hyper-responsive to β-adrenergic agonists.[16]

While it has long been felt that SOD is an 'antioxidant' enzyme, functioning to remove superoxide, recent studies suggest that this view may be simplistic. Bacteria do not require SOD for aerobic survival and growth,[17] and the presence of increased amounts of SOD may actually cause a paradoxical increase in the sensitivity of bacteria to oxidant challenge.[18,19] If Cu/Zn SOD also participates in intracellular electron transfer reactions, such as those involved in the electron transport system and membrane-associated receptors, the effects of modified intracellular SOD levels could be quite complex and not simply interpreted with reference to acceleration of superoxide dismutation.

Thus, while mechanisms of electron transfer in biological and chemical systems remain incompletely understood, the present results suggest that protein-associated unsaturated fatty acids, in concert with transition metals and Cu/Zn SOD, could influence these important processes.

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