Free Rad. Res. Comms., Vols. 12-13, pp. 161-166
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ENHANCED ELECTRON TRANSFER BY UNSATURATED FATTY ACIDS AND SUPEROXIDE DISMUTASE

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We have previously shown that unsaturated fatty acids (UFA) facilitate electron transfer between iron centers such as ferrous iron and ferricytochrome *C*. Extending this concept to a more physiologic model of fatty acids associated with proteins, we find that electron transfer is also enhanced in this model. While investigating whether free superoxide was involved in this electron transfer, we discovered that superoxide dismutase (SOD) enchanced the electron transfer. While the mechanism of electron transfer is unknown, the above findings are consistent with UFA and SOD participating in membrane redox systems.

KEY WORDS: SOD, electron transfer, unsaturated fatty acid.

INTRODUCTION

While investigating the molecular mechanism of prostaglandin formation, we earlier found that iron, unsaturated fatty acids and molecular oxygen could form a redox complex. This complex could reduce electrophilic agents like nitroblue tetrazolium (NBT), or dissociate to superoxide or to a peroxy fatty acid.[1-3] Because of its ability to transfer electrons, and in view of the necessary role of UFA in the mitochondrial electron transport chain (ETC), we speculated that a similar complex might play a role in intramembrane electron transfer. Using ferrous iron, UFA and ferricytochrome *C* (Fe^{3+} C) we found that the UFA markedly enhanced Fe^{3+} C reduction.[4] These findings were consistent with UFA functioning as electron transfer agents in membrane systems. Since these results, other pi electron-containing compounds such as DNA and the vinyl groups of heme compounds also have been found to accelerate electron transfer.[5-7]

Membranes are composed of predominantly lipid and protein. To extend the previous observations to a more physiological model, it was decided to evaluate the effect of albumin on the electron transfer of ferrous iron to Fe^{3+} C. Albumin is associated with a combination of saturated and unsaturated fatty acids[8] so that by using albumin and fatty acid-free albumin in our system one could observe the differential effects of fatty acid-associated protein and protein alone on electron transfer. In order to determine whether free superoxide was involved in this electron transfer, SOD was added to the above systems to observe its effect.

MATERIALS AND METHODS

Cytochrome *C* type III, copper/zinc SOD (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 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

Fatty acid-free albumin had no effect on the reduction of Fe^{3+} C by ferrous iron. In contrast, during the time period studied, albumin with associated fatty acid markedly increased the reduction of Fe^{3+} C. Surprisingly, SOD further enhanced the reduction of Fe^{3+} C by ferrous iron when added to either albumin or fatty acid-free albumin. This enhanced reduction was dependent on enzyme activity as DDC inactivated SOD was not effective in enhancing the reduction of Fe^{3+} C. This effect of enhanced Fe^{3+} C reduction was also not seen with MnSOD.

The mechanism for this enhanced electron transfer by CuZn SOD could be dependent on the intermediate proposed earlier:[1]

$$Fe^{2+} - O_2 - \text{pi bond} \leftrightarrow Fe^{3+} - O_2^- - \text{pi bond}$$

which could then transfer an electron to CuZn SOD and from there to cytochrome *C*. Because Cu^{2+} itself was ineffective in enhancing electron transfer in this system, it appears that the liganding of Cu in the enzyme is necessary for it to act as an electron shuttle.

DISCUSSION

Albumin-associated fatty acids significantly enhance electron transfer (Figure 1). While our previous results suggest that albumin-bound unsaturated fatty acids are responsible for this, saturated fatty acids are also associated with albumin and cannot be excluded from playing a role. These observations are consistent with the previous hypothesis regarding the necessary role of UFA in the mitochondrial electron transfer chain.

The observation of enhanced cytochrome reduction mediated by SOD is surprising (Figure 2 and 3). Active enzyme appears to be required; cupric ion and DDC-inactivated SOD are ineffective (Table I). MnSOD is also ineffective, suggesting that the two step electron transfer of the CuZn enzyme, not present with the MnSOD, is an important element in electron transfer between iron centers.[9,10] While SOD has been felt to act exclusively as a detoxifying agent for superoxide, it has recently been shown that it is not necesary for aerobic existence[11] and, in fact, excess amounts can be deleterious in the presence of an oxidant stress.[12,13]

While the amounts of SOD used in the present studies are high, they are on a similar

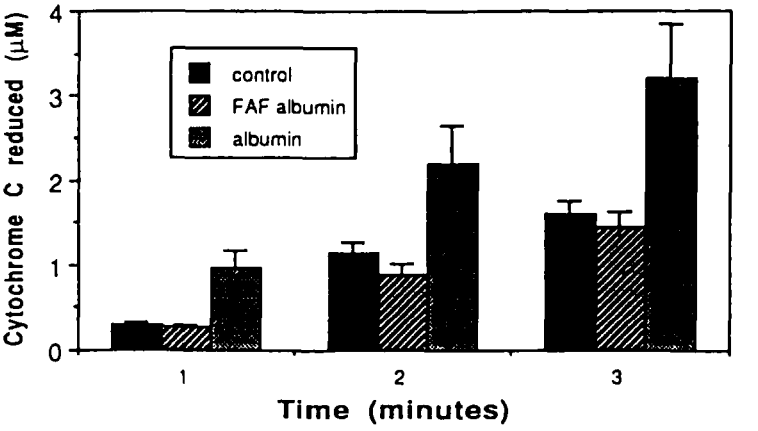

FIGURE 1 The effect of fatty acid free albumin and albumin on cytochrome *C* reduction by ferrous iron.

molar (20 μM) concentration as the cytochrome *C*, which is consistent with a direct role in electron transfer. Similar levels of SOD are also found in some tissues.[14]

Several membrane events could involve iron-mediated redox systems and could be affected by the electron transfer capability of SOD. Evidence has been presented for such involvement with the α_2 adrenergic receptor,[15] the β-adrenergic receptor,[16] the D_1 dopaminergic receptor,[17] prostaglandin activation of adenylate cyclase,[18] oncogene activation involving phosphotyrosine,[19] and the direct effect of triiodothyronine on the mitochondria.[20,21]

Other membrane systems which might be regulated by redox coupling are the calcium and sodium channels[22,23] and the regulation of pulmonary vascular tone.[24] These diverse phenomena could be affected by anything which would enhance or retard electron transfer such as UFA and SOD. It is interesting that Down syndrome patients, who have an excess of SOD, also have β-adrenergic dysfunction.[25]

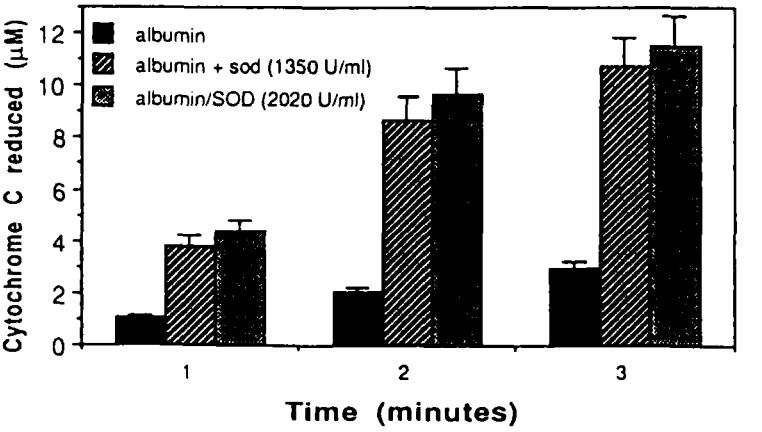

FIGURE 2 The effect of SOD on cytochrome *C* reduction by ferrous iron in the presence of albumin.

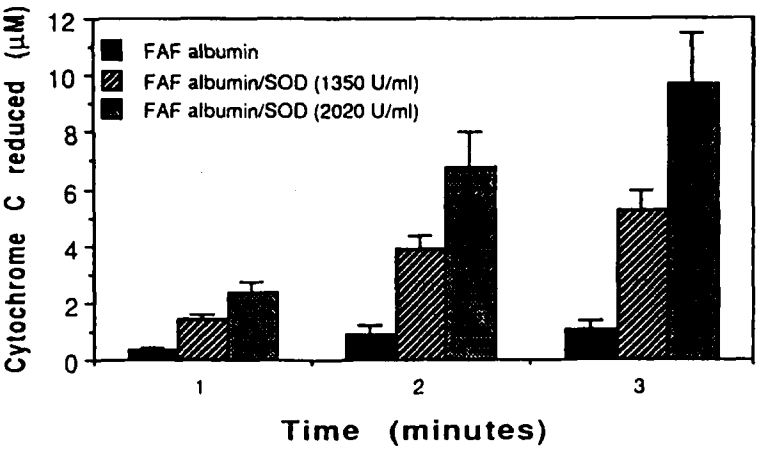

FIGURE 3 The effect of SOD on cytochrome *C* reduction by ferrous iron in the presence of fatty acid free albumin.

TABLE I

Fe^{2+}/albumin-mediated cytochrome C reduction in the presence of Cu/Zn SOD (SOD), diethyldithiocarbamate-inactivated Cu/Zn SOD(SOD/DDC), manganese SOD (MnSOD) and free copper.

Treatment	Cytochrome C reduced (μM) (/3 min)
Fe^{2+}/albumin	3.0(± 0.55)(n = 4)
+SOD (2020 U/ml) (670 μg/ml)	11.4(± 0.90)(n = 4)
+SOD/DDC (670 μg/ml)	4.4(± 0.68)(n = 4)
+Mn SOD (670 μg/m)	4.0(± 0.20)(n = 4)
Cu (1 μM)	3.4(± 0.41)(n = 4)

One area where the electron transfer capability of SOD could explain some clinical effects is in that of myocardial infarct size measurement. By enhancing reduction of "vital" electrophilic dyes in some models of coronary reperfusion, it would lead to an impression of reduced infact size compared to controls. This could explain the discrepancy with this artificial phenomenon and actual infarct size as measured by scar formation.[26,27] It is interesting that other electron transfer agents, allopurinol and coenzyme Q_{10}, have similar effects on infarct size "reduction".[28-32]

In summary, the ability of UFA and SOD to transfer electrons by as yet undefined mechanisms could play a significant part in their respective physiologic roles.

Acknowledgements

The authors wish to thank Ms. Diane Konzen for assistance in preparation of this manuscript; and B. Dylan, B. Springsteen, the Beatles, the Stones, U2, AC/DC, Van Halen, and Guns and Roses for musical background.

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Accepted by Prof. G. Czapski