INHIBITION OF THE RESPIRATORY CHAIN BY ZINC IONS

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Among metal ions influencing the mitochondrial functions, zinc and cadmium are active at the lowest concentrations. Cadmium ions uncouple oxidation from phosphorylation at concentrations of about 10^{-6}M (Fletcher et al., 1962). Zinc ions inhibit mitochondrial respiration at concentrations of about 10^{-5}M, as demonstrated by Hunter and Ford (1955). Even smaller concentrations of Zn^{2+} induce swelling of mitochondria (Cach and Gardy, 1965). Further investigation of the mechanism of zinc ion action would be interesting for a number of reasons. First of all, the amount of zinc in tissues is relatively high as compared with that of other heavy metals. Only iron is present in a higher quantity (Parr and Taylor, 1964). Hence, inhibition of the respiration by low zinc ion concentrations may be of some physiological importance. Secondly, some effects of chelating agents upon mitochondria could be explained in terms of removal of traces of zinc ions normally presented in mitochondria or absorbed from other components of homogenate (or from reagents) during isolation procedure. Lastly, zinc could be used as an effective inhibitor in the studies of the mechanism of electron and energy transfer.

We investigated the effect of zinc ions upon the respira-

tory chain and found it to be a complicated, concentration-dependent character. The most sensitive sites appeard to be the reduction of NAD^+ with succinate and the electron transfer between cytochromes b and c_I.

RESULTS AND DISCUSSION

The respiration of phosphorylating rabbit heart mitochondria was strongly inhibited by zinc ions (Fig.I). The most sensitive state appeared to be the active respiration in the presence of phosphate acceptor or uncoupling agent (States 3 and 3u). Higher concentrations of Zn^{2+} were required to inhibit the respiration in the absence of the acceptor (States 2 and 4).

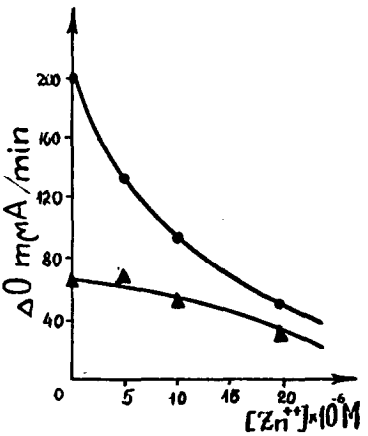


Fig. I. The effect of Zn^{2+} on the respiration of rabbit-heart mitochondria.
Mitochondria were isolated and suspended in the medium containing 0.3 M sucrose and 0,02M. Tris-HCl. Incubation mixture: 0,3 sucrose, 0,01M KCl, 0,01M potassium phosphate, 5mM potassium malate, 5mM potassium glutamate, I mg/ml mitochondrial protein. The concentration of ADP was 0,2mM. pH of the reaction mixture here and throughout 7.5; respiration was measured polarographically using platinum electrode. t=25°C. ●—● +ADP, ▲—▲ without ADP.

For comparison, a number of other metal ions were investigated. Some of them also inhibited mitochondrial respiration, but none was as effective as Zn^{2+}. Cd^{2+}, Ag^+, Hg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+},

Fe^{3+} inhibited the process at concentrations 50-1000 times greater than that of Zn^{2+}.

Fig.2 illustrates the effect of Zn^{2+} upon electron transfer in sonicated mitochondria and in purified respiratory enzyme complexes. It is seen, that the activity of succinate-cytochrome c - oxidoreductase complex, and especially that of mitochondrial NADH and succinate oxidase, is inhibited at much lo-

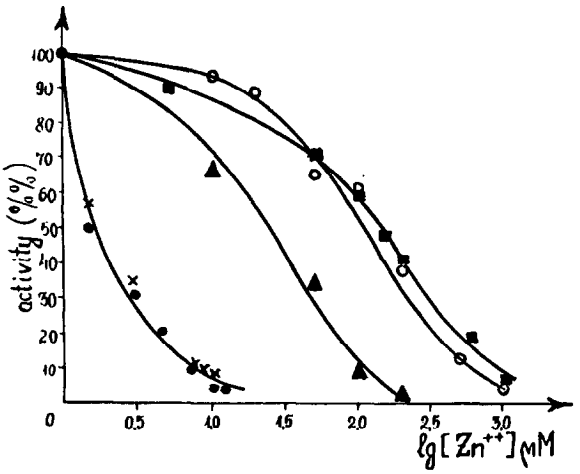


Fig.2. The effect of Zn^{2+} on the different steps of electron transfer in the respiratory chain.
NADH-oxidase activity of rabbit-heart mitochondria sonicated for 2 min. at 20Kc, was measured polarographically in the reaction mixture, containing 0.03M sucrose, 2mM KCl, 0.05M Tris-HCl, I mM NADH and 0.7mg/ml of mitochondrial protein. Succinate oxidase (X—X) was measured in the same system with 10mM of succinate as substrate. The activity of soluble succinate-cytochrome-oxidoreductase(▲—▲), isolated after Hatefi et al. (1961), in our modification, was measured spectrophotometrically in the reaction mixture containing 0.05M Tris-HCl, 5 x 10^{-5}M cytochrome c, 0.01 mg/ml enzyme protein, 10mM succinate and 0.2mM NaN_3. Succinate phenasine metosulfate (PMS) oxidoreductase (o—o) of the same preparation was measured in the presence of 8x10^{-4} M PMS and 7x10^{-5}M dichlorophenol indophenol as a terminal electron acceptor. The activity of soluble beef-heart mitochondria cytochrome c - oxidase (■—■) Fowler et al., 1962) was measured polarographically, incubation mixture contained 0.05M Tris-HCl, 0.01M ascorbate, 2 x 10^{-5}M cytochrome c, 0.06mg/ml enzyme protein. Temperature of incubation was 25°C and 18°C in polarographic and spectrophotometric experiments respectively. (•—•) NADH-oxidase.

wer concentrations of Zn^{2+} than those needed to inhibit the succinate dehydrogenase of the same complex or cytochrome oxidase activity of complex IV.

In intact mitochondria zinc ions at very low concentrations 10^{-6}M, induced oxidation of nicotinamide nucleotides, which had been reduced by succinate. (Fig.3a). Addition of ascorbate and TMPD to the inhibited system caused the reduction of nicotinamide nucleotides. Subsequent addition of ADP or of the uncoupling

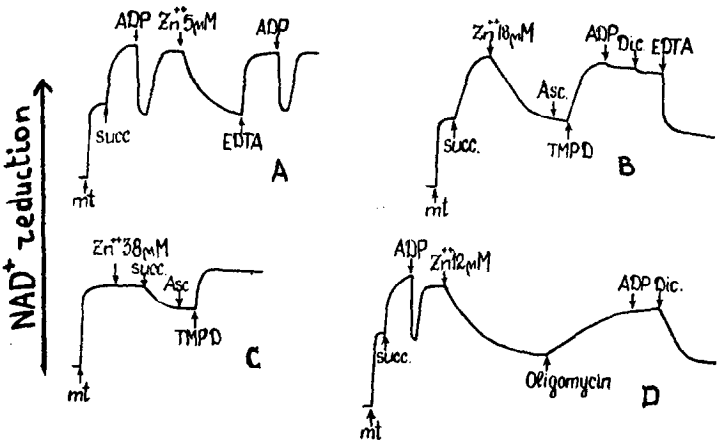


Fig.3. The effect of Zn^{2+} on the reverse electron transfer. Rabbit-heart mitochondria were isolated in 0.3M sucrose, 0.02 M Tris-HCl, 0.1mM EDTA and suspended in 0.3M sucrose + 0.02 mM Tris-HCl. Incubation mixture as in Fig.I but without glutamate and malate. NADH was measured fluorimetrically at I8°C. Additions: I.7 mg mitochondrial protein, 0.0I5 M succinate, 0.2mM ADP, 0.5mM EDTA, 0.0IM ascorbate, 0.2mM TMPD, 50mM Dicoumarol, Img/ml oligomycin.

agent Dicoumarol does not result in oxidation of nicotinamide nucleotides. It should be mentioned, that the inhibition of the oxidizing effect of ADP and dicoumarol by zinc ions was most effective in the presence of ascorbate and TMPD. Addition of EDTA removed the inhibition, and nicotinamide nucleotides were rapidly reduced in the former (Fig.3a) and oxidized in the latter (Fig.3b) cases. If Zn^{2+} was added before succinate, the addition of succinate did not result in the reduction of NAD^+.Treatment

with ascorbate and TMPD allowed to overcome the inhibition of reversed electron transport (Fig.3c). The conditions for the reverse electron transfer in the presence of Zn^{2+} might also be improved by the addition of oligomycin (Fig.3d). Oligomycin functioned presumably as an inhibitor of ATPase reactions competing with the reverse electron transfer reaction for energy.

One may conclude from the results of the above experiments that low concentrations of Zn^{2+} inhibit the electron transfer in the middle part of the respiratory chain, and have no effect on electron transfer and energy transformation at the beginning and at the end of the respiratory chain.

Fig.4. illustrates the effect of Zn^{2+} upon the degree of reduction of respiratory carriers in sonicated mitochondria oxidizing glutamate and malate. It is seen, that the addition

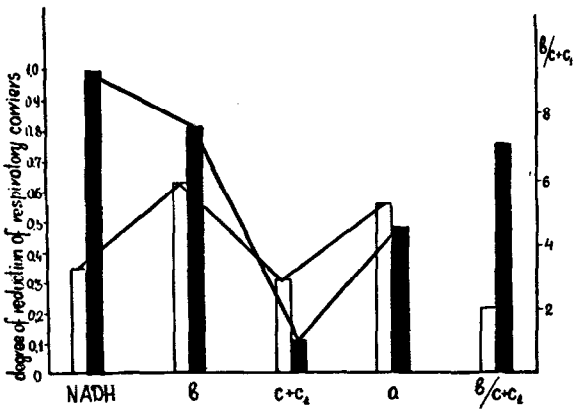
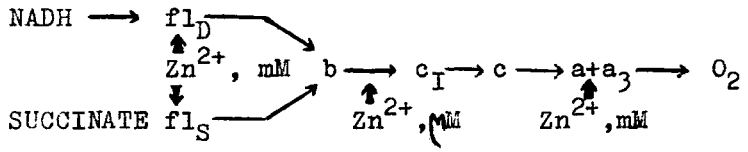


Fig. 4. The effect of Zn^{2+} on the steady-state levels of respiratory carriers.
Sonicated rabbit-heart mitochondria were incubated in the presence of 0.03M sucrose, 2mM KCl, 0.05M Tris-HCl, 1mM NAD^+, 5mM potassium glutamate, 5mM potassium malate and 0.3mM Na_3N. Measurements were made with double-beem spectrophotometer. ▭ control, ▬ +10mM Zn^{2+}.

of small amounts of Zn^{2+} increases the extent of reduction of nicotinamide uncleotides and of cytochrome b and decreases the

reduction of cytochrome c ($+c_1$) and a. The same results was obtained with succinate as substrate.

It is important that the inhibitory effect of Zn^{2+} between cytochrome b and c_I may be observed after the addition of very small amounts of the inhibitor, particularly at a dilutions of I:I00 millions. High concentrations of Zn^{2+} cause a further inhibition at the levels of flavine and cytochrome oxidase. The results described above may be represented by the scheme:



REFERENCES

Cash W.D. and Gardy M. J.Biol.Chem., 240, PC3450, (I965).

Fletcher M.J.,Fluharthy A.L. and Sanadi D.R. Biochim.Biophys. Acta, 60, 425, (I962).

Fowler L.R.,Richardson S.H. and Hatefi G. Biochim.Biophys. Acta, 64, I70, (I962).

Hatefi I.,Haavik A.G. and Jurtshuk P. Biocim.Biophys.Acta, 52, I06, (I96I).

Hunter F.E. and Ford L. J.Biol.Chem., 2I6, 357, (I955).

Parr R.M. and Taylor D.M. Biochem.J., 9I, 424, (I964).