ON THE INHIBITION OF MITOCHONDRIAL ELECTRON TRANSPORT BY Zn^{2+} IONS

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1. Introduction

On the basis of the crossover theorem Zn^{2+} ions have been postulated to inhibit mitochondrial electron transport between the cytochromes *b* and *c* [1, 2]. We have extended these studies and found a crossover between *b* and *c* in coupled mitochondria only at zinc concentrations below 10 μM. At 25 μM $Zn^{2+}$and in uncoupled mitochondria the site of inhibition was found to be located between ubiquinone and cytochrome *b*.

2. Materials and methods

Rat liver mitochondria were prepared and used in 0.25 M sucrose + 0.02 M Tris-HCl at pH 7.4. Spectra of the cytochromes were recorded with a split-beam spectrophotometer, designed and constructed for the measurements of small turbid samples by M. Klingenberg. The redox state of ubiquinone was determined by the method of Kröger and Klingenberg [3]. Double-beam records were taken with the Perkin-Elmer 356 two-wavelength double beam spectrophotometer. Mitochondrial O_2 consumption was measured polarographically.

3. Results

Fig. 1 shows difference spectra of isolated rat liver mitochondria at liquid N_2-temperature. After the addition of 25 μM Zn^{2+} to uncoupled respiring mitochondria the cytochromes *b* and *c* are more oxidized (C) than in the steady state (B). Respiration under

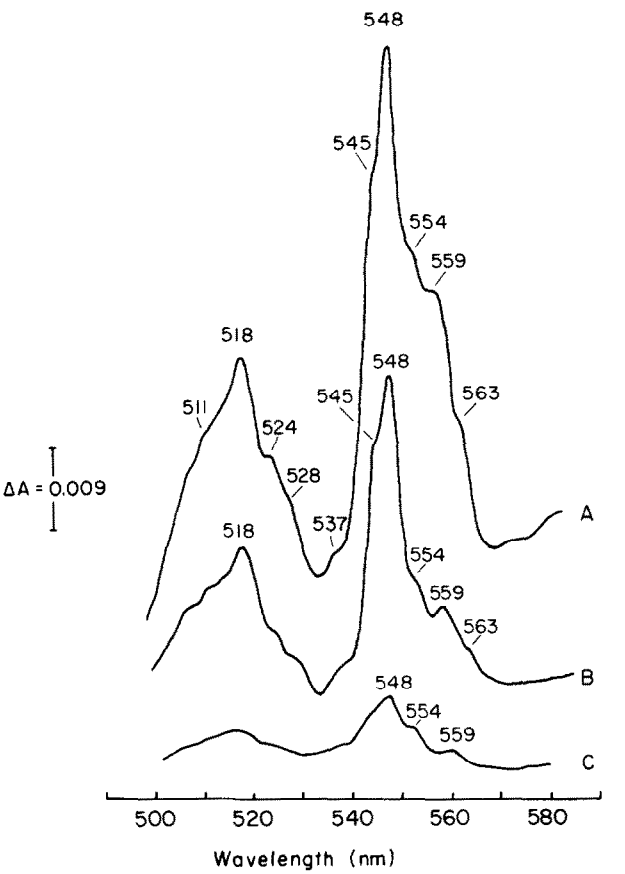

Fig. 1. Split-beam spectra of cytochromes in different states. Reference cuvette: aerobic state. Sample cuvette is : A = anaerobic state, B = uncoupled steady state, C = like B + 25 μM Zn^{2+}. The cuvettes contained 1.5 mg mitochondrial protein at 25° (reaction temp). Measurement temp = – 190°; cuvette volume = 0.11 ml; light path = 0.1 cm. The sample cuvette in addition contained 20 mM succinate and 0.1 nmole S_{13}/mg protein as uncoupler.

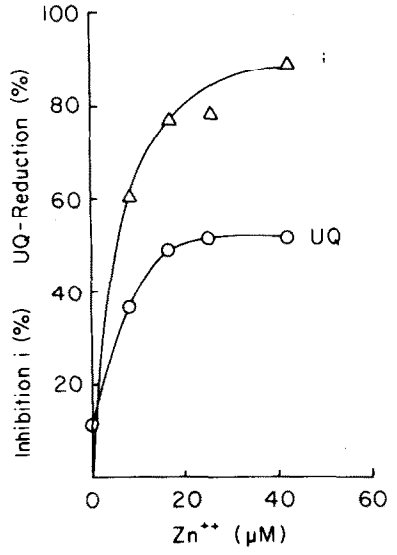

Fig. 2. Change of the redox state of ubiquinone by addition of Zn^{2+} as compared to the degree of inhibition. 2.0 mg of protein were used. Conditions as in fig. 1. (Δ–Δ–Δ): Inhibition of respiration; (o–o–o): % ubiquinone reduced.

these conditions is inhibited by about 70%.

Fig. 2 shows the dependency of the mitochondrial respiratory activity and the degree of reduction of ubiquinone on the zinc ion concentration. The degree of ubiquinone reduction increases parallel to the inhibition of the respiration. At a zinc concentration of 25 μM the ubiquinone is reduced to 50% in contrast to 11% reduction without zinc ions.

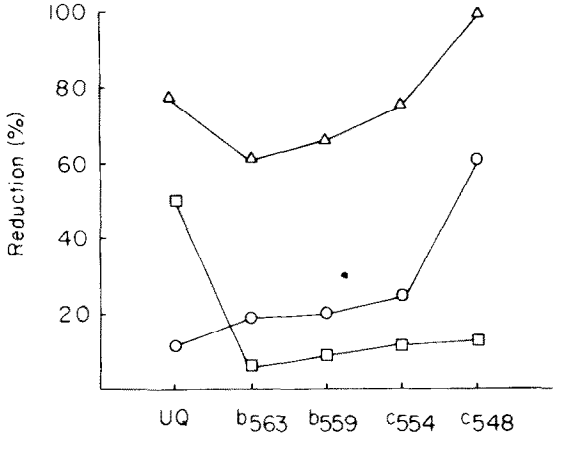

Fig. 3. The effect of Zn^{2+} on the steady state levels of respiratory carriers. Values are from fig. 1 and 2. The reduction was referred to the reduction by dithionite. (Δ–Δ–Δ): Anaerobic state, (o–o–o): steady state, (□–□–□): steady state + 25 μM Zn^{2+}.

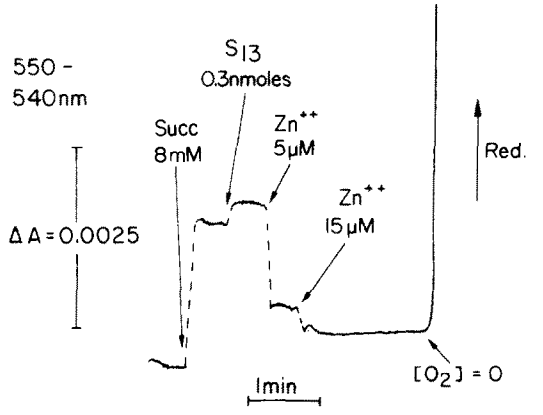

Fig. 4. Change of the redox state of cytochrome *c* in the uncoupled state by addition of Zn^{2+}. 2.9 mg protein + 5 nmoles rotenone + 0.1 nmole S_{13}/mg in 1.0 ml; temp = 25°; light path = 0.5 cm.

A plot of the redox pattern of the respiratory chain in the uninhibited state and in the inhibited state (fig. 3) shows a cross-over between ubiquinone and cytochrome *b*. This indicates, contrary to previous reports, that the point of attack of zinc ions is located on the substrate side of cytrochrome *b*. Zn^{2+} titration curves of the cytochromes *b* and *c* were recorded using the double beam technique. The redox changes of cytochrome *c* induced by Zn^{2+} inhibition are shown in the uncoupled (fig. 4) and in the energy controlled state (fig. 5). In both cases oxidation increases with zinc ion concentrations.

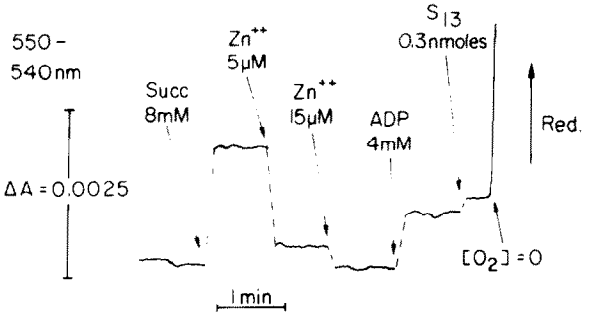

Fig. 5. Change of the redox state of cytochrome *c* in the controlled state by addition of Zn^{2+}. Conditions as in fig. 4.

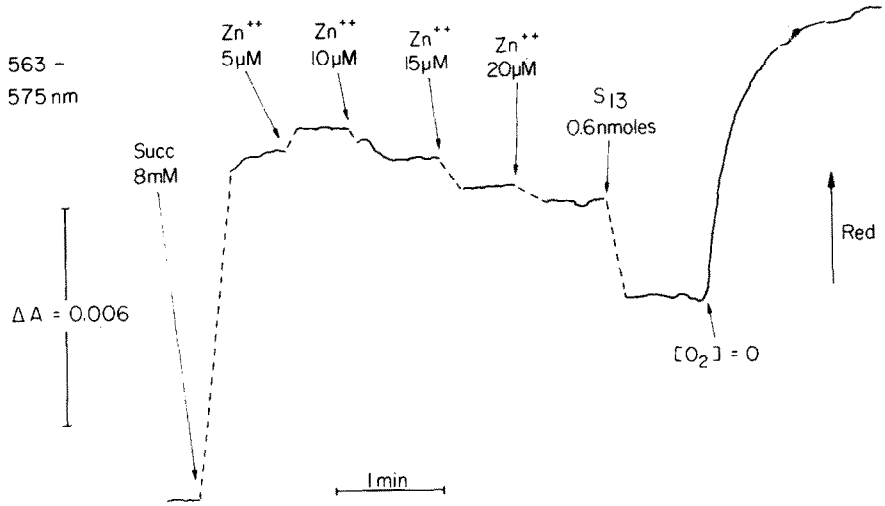

Fig. 6. Change of the redox state of cytochrome *b* in the uncoupled state by Zn^{2+}. Conditions as in fig. 4, except the protein content was 5.2 mg.

The reversing effect of ADP is shown in fig. 5. It forms a complex with Zn^{2+} [4], and thus diminishes the free zinc ion concentration. The effect of zinc ions on the redox state of cytochrome *b* is more complex. As reported before [2], we find an initial small increase in reduction followed by an oxidation of cytochrome *b* at higher zinc concentrations in the controlled state (fig. 6). However, in the uncoupled state an oxidation takes place also at low zinc levels (fig. 7). The reversing effect of inorganic phosphate due to complexation of zinc ions [5] is also shown.

4. Discussion

The addition of 25 μM Zn^{2+} to respiring mitochondria results in an oxidation of cytochromes *b* and

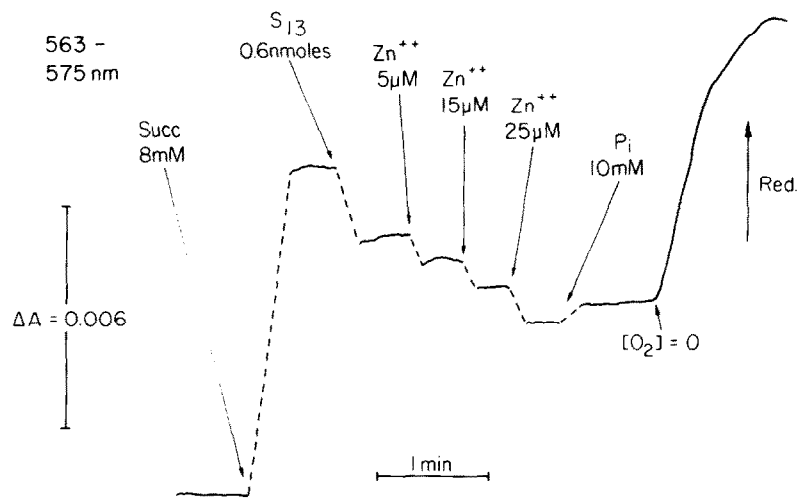

Fig. 7. Change of the redox state of cytochrome *b* in the controlled state by Zn^{2+}. Conditions as in fig. 6.

c and in a reduction of ubiquinone. This indicates that the site of inhibition is located between ubiquinone and cytochrome *b* and not between cytochrome *b* and *c* as reported earlier [1, 2]. Those conclusions were based on the fact that small additions of Zn^{2+} cause a reduction of cytochrome *b* which was replaced by a reoxidation at higher Zn^{2+} concentrations. We found that this effect is only observed in coupled mitochondria. It is remarkable that Nicholls and Malviya [2] found a strong increase in the absorption of cytochrome *b* already at 0.3 μM Zn^{2+} in 60 mM phosphate buffer. Our experiments on the reversion of Zn^{2+} inhibition by various compounds indicate that in 60 mM phosphate buffer the addition of 0.3 μM Zn^{2+} causes a decrease of respiration by less than 3%. The reduction of cytochrome *b* at lower Zn^{2+} concentrations seems to be connected with an active transport of zinc ions across the mitochondrial membrane (D. Kleiner and M. Klingenberg, in preparation).

References

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