INDUCTION OF K^+ TRANSPORT IN ISOLATED HEART MITOCHONDRIA BY ZINC IONS

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The energy-linked accumulation of Mg^{++} and inorganic phosphate (Pi) by isolated heart mitochondria is markedly increased by the addition of Zn^{++} or Cd^{++} ions (Brierley and Murer, 1964; Brierley and Bhattacharyya, 1966) when the particles oxidize ascorbate in the presence of tetramethylphenylenediamine (TMPD). Respiration with this substrate (Packer and Jacobs, 1962), in contrast to more physiological sources of reducing equivalents, is not inhibited by heavy metals and, in fact, is activated by Zn^{++} under conditions which result in increased Mg^{++} accumulation (Brierley and Bhattacharyya, 1966). Since it was noted that this increased respiration in the presence of Zn^{++} was also obtained when K^+ was substituted for Mg^{++}, it seemed of interest to investigate the effects of Zn^{++} and other heavy metals on the energy-linked transport of K^+ in heart mitochondria. In the present communication, we wish to report that zinc ions induce a rapid and extensive uptake of K^+ by an energy-linked process under these conditions. This effect of Zn^{++} on the accumulation of K^+ resembles effects previously reported for the antibiotics valinomycin (Pressman, 1965) and gramicidin (Chappell and Crofts, 1965) and for parathyroid hormone (Rasmussen et al, 1964).

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Results - Figure 1A shows a typical set of experiments obtained when the K^+ concentration of the medium is monitored with a K^+-sensitive electrode (Pressman, 1965; Moore and Pressman, 1964). In the absence of Zn^{++} only small apparent changes in K^+ are observed. When 60 µM Zn^{++} is added there is a rapid and extensive uptake of K^+ by the mitochondria. In the experiment shown (Figure 1A) over 500 mumoles of K^+ per mg of protein were accumulated at a maximum rate of about 550 mumoles of K^+ per mg of protein per minute. The Zn^{++}-dependent uptake of K^+ requires substrate and the presence of phosphate. It is abolished by anaerobiosis and by the presence of uncouplers but is insensitive to oligomycin. The accumulated K^+ is rapidly released when the incubation chamber becomes anaerobic or when an uncoupler of oxidative phosphorylation is added (Figure 1A).

Under the conditions which result in increased K^+ accumulation, respiration is stimulated almost two fold by Zn^{++} (Figure 1B). The increased

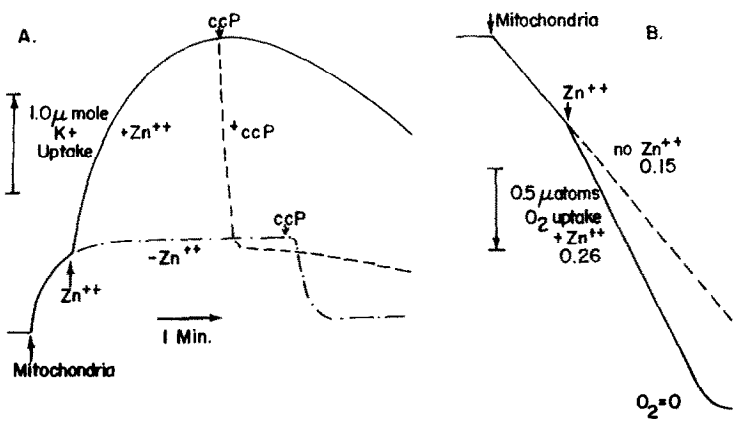

Fig. 1A - The uptake of K^+ by heart mitochondria as recorded with a Beckman 39137 electrode. Mitochondria prepared by the method of Hatefi et al. (1961). (3.7 mg of protein) were added to 5 ml of a medium containing sucrose (0.25M), Tris acetate (20 mM, pH 7.0), Tris phosphate (2 mM, pH 7.0) potassium acetate (6 mM), Tris ascorbate (5 mM), and TMPD (0.1 mM), at 25°. Where indicated, zinc acetate was added to a final concentration of 60 uM and m-chlorocarbonylcyanide phenylhydrazone (CCP) to a final concentration of 5 uM.

Fig. 1B - Activation of respiration by Zn^{++} as recorded with a Beckman oxygen sensor in a closed chamber at 25°. Other conditions were identical to those of Figure 1A. The figures given are the rates of oxygen consumption in uatoms O_2 per mg of protein per minute.

oxygen uptake also requires the presence of phosphate and is insensitive to oligomycin. The increase in respiration is consistent with an increased energy demand on the mitochondrion to support the increased K^+ transport.

When K^+ uptake is initiated by the addition of Zn^{++} in acetate medium, a rapid and extensive swelling of the mitochondrion takes place. This swelling reflects the uptake of K^+ as recorded with the K^+ electrode (Figure 2). The increase in volume requires the presence of K^+, inorganic phosphate, and the ascorbate-TMPD substrate system. The swelling does not take place in the presence of dinitrophenol or other uncouplers and reverses spontaneously when the cuvette approaches anaerobiosis. Addition of an uncoupler to the swollen mitochondria results in rapid shrinking of the mitochondria (Figure 2). It is apparent, therefore, that the observed swelling and K^+ accumulation, while measured under slightly different experimental conditions, respond in such a way as to indicate a close relationship between the two processes.

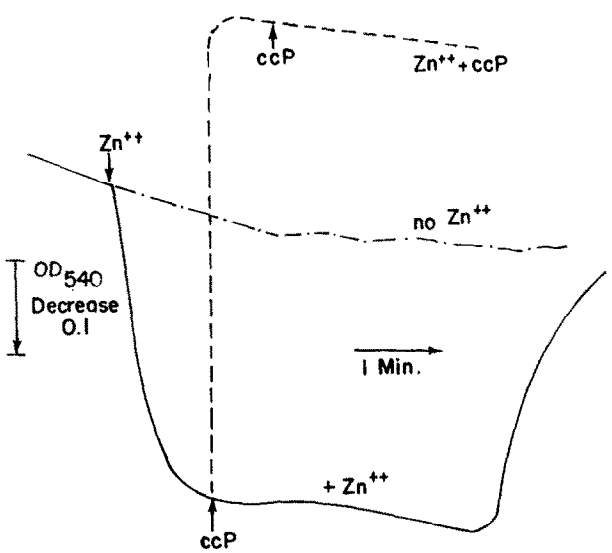

Fig. 2 - Swelling and shrinking of heart mitochondria in the presence of K^+ and Zn^{++} as recorded with a Beckman DK-2 spectrophotometer. Mitochondria (1.2 mg of protein) were added to 3 ml. of the medium described in the legend for Figure 1A. The optical density at 540 mu was recorded as a function of time.

Discussion - Induction of an energy-linked transport of K^+ in the presence of valinomycin, gramicidin, or parathyroid hormone has been well established in a number of recent reports. The present study establishes that the presence of Zn^{++} is sufficient to initiate a vigorous uptake of K^+ in heart mitochondria provided an energy source which is not inhibited by the metal ion is available. The Zn^{++}-dependent K^+ accumulation, like the reaction induced by more complicated molecules, is accompanied by increased O_2 uptake and is closely related to a reversible swelling of the mitochondrion. The precise relationship of the Zn^{++}-induced transport of K^+ to other induced ion movements in the mitochondrion is not yet clear and is currently under investigation. Preliminary studies indicate that gramicidin induces a more rapid movement of K^+ than does Zn^{++} under these conditions, but that about the same total K^+ accumulation is observed with the two reagents. It should also be noted that Zn^{++} shares with parathyroid hormone the ability to activate both K^+ and Mg^{++} uptake.

The fact that mitochondrial ion transport can be induced by a simple metal ion such as Zn^{++} may permit a more complete description of the induction process. Studies relating the effects of Zn^{++} to specific mitochondrial sulfhydryl groups and to the net charge of the membrane are currently in progress and will be presented for publication elsewhere.

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