ACTIVATION OF Mg^{++} ACCUMULATION IN ISOLATED HEART MITOCHONDRIA BY Zn^{++} AND BY p-CHLOROMERCURIBENZENE SULFONATE*

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It is well established that isolated heart mitochondria possess the ability to accumulate massive amounts of Mg^{++} and inorganic phosphate (Pi) by an energy-dependent reaction (Brierley et al, 1962; 1963). This uptake is greatly magnified by the addition of low concentrations of Cd^{++} and other heavy metal ions under appropriate conditions (Brierley and Murer, 1964). A number of recent studies have established that mitochondrial ion transport can be induced or activated by molecules such as valinomycin (Pressman, 1965), gramicidin (Chappell and Crofts, 1965), and parathyroid hormone (Sallis et al, 1963) (Rasmussen et al, 1964). The relationship of the increase in Mg^{++} accumulation induced by heavy metal ions to the increases in ion uptake found in these studies is not yet clear. However, preliminary results indicate that certain metal ions and sulfhydryl-group reagents may mimic the effects of the above reagents on mitochondrial ion transport and may therefore prove useful in defining the mode of action of the more complicated compounds. The present communication outlines two of the conditions which result in striking increases in the accumulation of Mg^{++} by heart mitochondria.

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I. Activation of the Substrate-Supported Reaction by Zn^{++} - The oxidation of ascorbate in the presence of tetramethylphenylenediamine (TMPD) (Packer and Jacobs, 1962) is relatively insensitive to heavy metal ions. When Mg^{++} accumulation is supported by this substrate a number of cations in addition to Cd^{++} give substantial increases in the observed Mg^{++} uptake. The largest and most consistent increases were obtained in the presence of Zn^{++} at concentrations between 10^{-5} and 10^{-4} M (Table I). The accumulation of Mg^{++} and Pi in the presence of Zn^{++} is from 3 to 6 fold greater than the corresponding reaction in the absence of the activator. The Zn^{++}-dependent increment, like the reaction in the absence of Zn^{++}, was inhibited by dinitrophenol and by ADP, was insensitive to oligomycin, and was much less sensitive to ADP in the presence of oligomycin. The Zn^{++}-dependent portion of the reaction

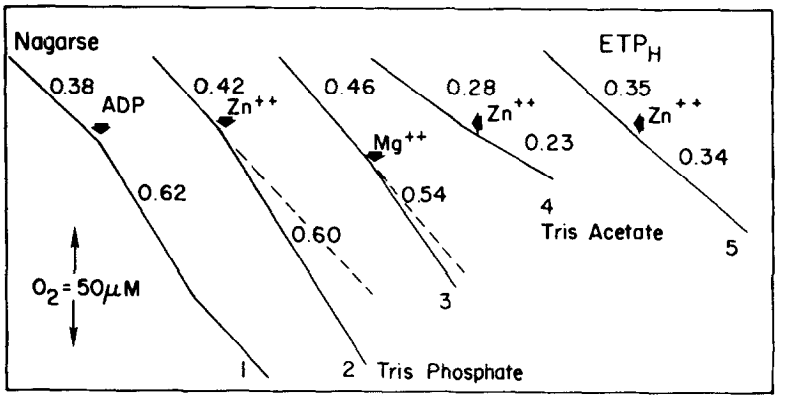

Fig. 1 - The effect of Zn^{++} on ascorbate - TMPD respiration in Nagarse mitochondria and electron transport particles (ETP_H of Linnane and Ziegler, 1958). Oxygen concentration was recorded in a closed chamber at 30° using a Beckman oxygen electrode. Mitochondria (1.2 mg of protein) were added to 5 ml of the following medium: sucrose (0.25M), $MgCl_2$ (10mM), Tris phosphate; pH 7.0 (3.3mM), Tris ascorbate (5mM), TMPD (0.1mM), and $ZnCl_2$ (when present) ($7x10^{-5}$M). Oxidation rates in μatoms O_2 per min. per mg of protein were recorded for each slope. Trace 1 shows the effect of the addition of 1.0 μmole of ADP to this system. A respiratory control ratio of 1.6 and a P/O ratio of approximately one were obtained. In the presence of Zn^{++} the same experiment (not shown) gave an initial rate of 0.54, a State 3 rate of 0.62, and no detectable decrease in P/O ratio. Trace 2 shows the effect of the addition of Zn^{++} to the complete system. Trace 3 shows the effect of Zn^{++} on the initial rate in the absence of Mg^{++} and the effect of addition of Mg^{++} to 10mM final concentration. In Trace 4 Tris phosphate was replaced by Tris acetate. Trace 5 shows the rates obtained with ETP_H.

was strongly inhibited by p-chloromercuribenzene sulfonate (p-CMS), required the presence of Pi, and was inhibited to a limited extent by both K^+ and Na^+.

The Nagarse mitochondria used in this study showed respiratory control ratios of between 1.6 and 2.0 with ascorbate-TMPD. Under conditions which result in increased Mg^{++} accumulation, Zn^{++} is nearly as effective as ADP in stimulating respiration (Fig. 1). The activation of respiration requires the presence of Pi and either Mg^{++} or K^+ and is not seen in phosphorylating submitochondrial particles (ETP_H) which do not accumulate Mg^{++}. The increased respiration is insensitive to oligomycin. Under the conditions of these experiments Zn^{++} did not significantly depress oxidative phosphorylation.

Activation of respiration by Zn^{++} under these conditions therefore appears to result from the increased energy drain on the mitochondrion associated with increased ion pumping. Such an activation of respiration

TABLE I

Activation by Zn^{++} of the Substrate-Supported Accumulation of Mg^{++}

	No Addition	Zn^{++} (4×10^{-5}M)
	(mmmoles Mg^{++}/mg of Protein)	
No Incubation	38	84
Incubated (3' at 30°)	110	490
" + dinitrophenol (10^{-4}M)	32	24
" + oligomycin (1ug/mg)	116	490
" + ADP (3mM)	28	30
" + ADP + oligomycin	60	250
" + p-CMS (7×10^{-5}M)	120	98

Nagarse mitochondria (Hatefi et al, 1961) (5 mg of protein) were shaken rapidly for 3 min. at 30° in 50 ml centrifuge tubes in 3 ml of the following medium: sucrose (0.25), $MgCl_2$ (10mM), Tris phosphate, pH 7.0 (3.3mM), Tris ascorbate (5mM), and TMPD (0.1mM). The reaction was stopped by the addition of 20 ml of ice cold 0.25 M sucrose and immediate centrifugation. The Mg^{++} content of the resulting pellets was determined by atomic absorption spectroscopy.

would not be expected if the sole effect of Zn^{++} were to increase permeability

to Mg^{++} or to reduce the outflow of accumulated ions.

II. Activation of the ATP-Dependent Reaction by p-CMS and by p-CMS Plus Zn^{++}- A second set of conditions which resulted in marked increases in the accumulated Mg^{++} was noted when ion uptake was supported by ATP in Nagarse mitochondria. These particles accumulate marginal amounts of Mg^{++} under these conditions in the absence of an activator (Table II). Accumulation is increased somewhat by the addition of Zn^{++} and quite substantially in the presence of p-CMS. Addition of both Zn^{++} and p-CMS results in a vigorous accumulation of Mg^{++} which compares in magnitude to that obtained with the Zn^{++}-activated substrate-supported reaction. The increased Mg^{++} accumulation in the presence of p-CMS and the combination of Zn^{++} and p-CMS is reflected in a marked increase in the observed ATPase. Both the accumulation of Mg^{++} and the ATPase activity induced by these reagents are sensitive to oligomycin (Table II).

TABLE II

Activation of the ATP-Supported Accumulation of Mg^{++} and ATPase Activity by p-CMS and Zn^{++}

	Mg^{++} Accumulated (mumoles/mg)	ATP Hydrolyzed (mumoles/mg)
No addition	5	130
+ Zn^{++} (6.7×10^{-5}M)	25	100
+ p-CMS (")	90	310
+ Zn^{++} + p-CMS	335	710
+ Zn^{++} + p-CMS + oligomycin (1 ug/mg)	10	40

Mitochondria (5 mg of protein) were incubated with the indicated additions in the following medium: sucrose (0.25 M), $MgCl_2$ (17mM), Tris phosphate, pH 7.0 (3mM), $Tris_2Na_2ATP$ (3mM). After 3 min. at 38° the tubes were cooled rapidly and centrifuged for 10 min. at 17,000 rpm. Immediately after centrifugation the supernatant was decanted for inorganic phosphate determination and the pellet was rinsed with 5 ml of cold 0.25 M sucrose. Results are expressed as the increase in mitochondrial Mg^{++} compared to an unincubated control and increase in Pi over the initial concentration.

The increase in ATPase, like the activation of respiration by Zn^{++}, is consistent with a greater demand for free energy to support the increased ion

uptake in the presence of p-CMS or Zn^{++} plus p-CMS. Sulfhydryl-group reagents such as Ag^{+} have previously been shown to activate ATPase in digitonin fragments (Cooper, 1959),but the observation that p-CMS activates an ATPase activity in intact mitochondria which is associated with Mg^{++} accumulation does not appear to have been reported before. Further studies on the mechanism of heavy metal activation of substrate-supported Mg^{++} accumulation and on the activation of the ATP-supported reaction by p-CMS and metals are now in progress and will be reported elsewhere. Regardless of the exact mechanism of the activation of the ATP-supported reaction by p-CMS, the fact that this reagent inhibits the substrate-supported reaction may be taken as evidence that SH groups are involved in quite different roles in the two reactions.

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