Ion Transport by Heart Mitochondria

IX. INDUCTION OF THE ENERGY-LINKED UPTAKE OF K+ BY ZINC ION*

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SUMMARY

The accumulation of K+ by isolated heart mitochondria suspended in isotonic sucrose is markedly stimulated by the addition of Zn++. The accumulation requires a source of energy, the presence of a permeant anion such as acetate, and the presence of either inorganic phosphate or arsenate. The reaction is inhibited and reversed by uncouplers of oxidative phosphorylation and by anaerobiosis. It is also inhibited by Mg++ and Na+ ions and by ethylenediaminetetraacetate and other Zn++ chelators. The Zn++-dependent accumulation is accompanied by increased respiration and extensive reversible swelling of the mitochondria. The extent of the observed swelling and the time course of swelling are dependent on the concentration of K+ and of Zn++. In the absence of added K+, Zn++ induces a rapid energy-linked expulsion of the endogenous K+ of the mitochondrion. These studies indicate that the addition of Zn++ under carefully defined experimental conditions is sufficient to induce the transport of K+ by heart mitochondria. This transport of K+ closely resembles that reported by other investigators in the presence of valinomycin, gramicidin, and parathyroid hormone.

Zn++ and certain other heavy metal ions markedly increase the energy-linked accumulation of Mg++ by isolated heart mitochondria under the appropriate experimental conditions (1-4). The relationship between the increased ion accumulation in the presence of Zn++ and other examples of induced ion uptake is not yet clear. The effects of valinomycin (5-8) and gramicidin (5, 8, 9) appear to be limited to increased transport of monovalent cations while parathyroid hormone (10, 11) increases both K+ and Mg++ transport. In the present communication, studies are presented which indicate that Zn++ shares with parathyroid hormone the ability to induce K+ transport as well as Mg++ transport in heart mitochondria.

MATERIALS AND METHODS

Beef heart mitochondria were prepared by the Nagarse procedure as previously described (4). The uptake and release of K+ was followed with a Beckman model 39047 electrode essentially as described by Pressman (5). Oxygen consumption was measured with either the Beckman oxygen electrode as previously described (2) or the YSI 5331 electrode. Mitochondrial swelling and shrinking was monitored by recording the changes in absorbance at 540 nm with the use of a Beckman DK-2 spectrophotometer or at 546 nm with an Eppendorf photometer. The Eppendorf chamber was stirred with a magnetic stirrer; no stirring was used with the DK-2. The two measuring systems give qualitatively identical results. In a number of experiments three parameters (absorbance, oxygen uptake, and K+ concentration) were recorded simultaneously with an apparatus consisting of a standard YSI electrode chamber mounted on the Eppendorf photometer and equipped with a small side arm to accept the K+ and reference electrodes. Exact experimental conditions and the composition of the suspending medium are detailed with the individual experiments reported.

RESULTS

Heart mitochondria respiring with ascorbate and N,N',N'-tetramethylphenylenediamine as substrate and suspended in 0.25 M sucrose buffered with Tris-acetate and Tris-phosphate show little tendency to swell or to accumulate K+ in short term experiments at 25°. The addition of low concentrations of Zn++ under these conditions results in a rapid and extensive uptake of K+ by the mitochondria, extensive reversible swelling, and activation of respiration. The series of experiments shown in Fig. 1 documents this response and shows some of the requirements of the system. Fig. 1A shows the response of the K+ electrode, optical density trace, and oxygen electrode in a control experiment in which no Zn++ was added. It also shows the response to the addition of 67 μM Zn++ as the acetate salt.

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With this addition to a suspension containing 2 mM K$^+$ a total of about 190 mmoles of K$^+$ per mg of protein enters the mitochondrial phase at a rate of about 210 mmoles per min per mg. Respiration is increased from a rate of 0.24 to a rate of 0.32 μmole of O$_2$ per min per mg of protein, and the optical density of the suspension decreases by nearly 0.3 optical density unit. At the anaerobic point both the K$^+$ uptake and the swelling reverse, and a rapid loss of K$^+$ to the medium and contraction of the mitochondria occur. The uptake of K$^+$ and the simultaneous changes in volume and respiration are prevented by the addition of dinitrophenol and other uncouplers, EDTA and other Zn$^{++}$ chelators, or cyanide before the addition of Zn$^{++}$. The traces for K$^+$ and swelling obtained under these conditions are virtually identical with the control traces shown in Fig. 1A. Zn$^{++}$ added after the cuvette has gone anaerobic also produces no response. Oligomycin does not inhibit the reaction. The uptake of K$^+$ and the swelling are rapidly reversed by the addition of an uncoupler after the accumulation has begun and before the anaerobic point (Fig. 1B). Substitution of Cl$^-$ for acetate in the suspending medium results in much less accumulation of K$^+$ and less swelling. Omission of P$_i$ from the medium prevents the uptake of K$^+$, the swelling, and the activation of respiration (Fig. 1C). Addition of P$_i$ (or arsenate) after the Zn$^{++}$ results in stimulation of K$^+$ accumulation and the expected response with the other parameters. It should be noted that Zn$^{++}$ produces a noticeable effect on the K$^+$ electrode in the absence of P$_i$.

**Fig. 1.** The effect of Zn$^{++}$ on mitochondrial K$^+$ content, volume, and rate of oxidation. The measurements were carried out simultaneously in the apparatus described in the text with the use of a Beckman cation-sensitive electrode for K$^+$ concentration, a Clark electrode for O$_2$, and the change in optical density at 546 nm as the measure of swelling. Mitochondria (7.5 mg of protein) were added to 9 ml of the following medium: sucrose (0.25 M), Tris-acetate (20 mM, pH 7.0), Tris-phosphate (2 mM), Tris-ascorbate (5 mM), N,N,N',N'-tetramethylphenylenediamine (0.1 mM), and rotenone (7 μM). The suspension was stirred at 25°C. With the exception of D, the uptake of K$^+$ was initiated by the addition of Zn$^{++}$-acetate (100 μM). In D, the inducer was gramicidin (0.3 μM). The dotted traces in A show the results of a control incubation in which the addition of Zn$^{++}$ was omitted. B shows the effect of the addition of the uncoupler m-chlorocarbonyl cyanide phenylhydrazone (CCP, 10 μM) after the accumulation has proceeded for 1 min. In C, Tris-phosphate was omitted from the original suspending medium and added to the same final concentration at the indicated point. E shows the effect of the addition of Tris-EDTA (200 μM) 1 min after the Zn$^{++}$.
above. The uptake of K\(^+\) and the swelling both proceed at a much greater rate than that observed in the presence of Zn\(^{++}\), but the total amount of uptake and extent of swelling are roughly the same.

The activation of K\(^+\) uptake by Zn\(^{++}\) can be reversed to a large extent by the addition of EDTA after the accumulation has begun. Fig. 1E shows the effect of the addition of 200 \(\mu\)M EDTA 1 min after the accumulation of K\(^+\) had been induced by the addition of 100 \(\mu\)M Zn\(^{++}\). Another series of experiments (12) has shown that this treatment rapidly removes bound Zn\(^{++}\) from the mitochondrion as well as Zn\(^{++}\) in solution. Zn\(^{++}\) removal results in a rapid extrusion of K\(^+\) and contraction of the mitochondria although this contraction is followed rapidly by an irreversible swelling phase with no indication of K\(^+\) uptake with the K\(^+\) electrode (Fig. 1E). EDTA addition before the addition of Zn\(^{++}\) does not result in swelling under these conditions.

Titration of the reaction with increasing concentrations of Zn\(^{++}\) revealed that the optimal response occurred at between 67 and 100 \(\mu\)M Zn\(^{++}\). The study shown in Fig. 2A establishes that these two concentrations of Zn\(^{++}\) resulted in identical rates of swelling but that the extent was greater at the higher concentration. Concentrations of Zn\(^{++}\) over 100 \(\mu\)M gave similar responses to that shown for 100 \(\mu\)M. The traces reported in Fig. 2A also show a decided tendency to rebound, i.e. undergo a cycle of swelling, contraction, and swelling again. The second inflection point corresponds to the anaerobic point. This incipient oscillation is not obtained in all preparations of mitochondria under these conditions (6 mM K\(^+\)). However, when prep-

fig. 3. The swelling of mitochondria in the presence of Zn\(^{++}\) and various monovalent cations. In each case the dotted trace represents a control incubation in which Zn\(^{++}\) was omitted; the solid trace is the response to the addition of Zn\(^{++}\) (100 \(\mu\)M). In each case the incubation medium consisted of sucrose (0.25 M), Tris-acetate (20 mM, pH 7.0), Tris-phosphate (2 mM), Tris-ascorbate (5 mM), N, N, N', N'-tetramethylphenylenediamine (0.1 mM), rotenone (2 \(\mu\)M), and mitochondria (5 mg of protein). The total volume was 6 ml. At the indicated point the monovalent cation was added as the chloride salt to a final concentration of 6 mM. Runs 1 and 2 show the response to K\(^+\) addition before and after Zn\(^{++}\); run 3 shows the response to Na\(^+\); run 4 to Li\(^+\); run 5 to NH\(_4^+\); run 6 to choline; and run 7 to additional Tris. Run 8 gives the response to Zn\(^{++}\) in the presence of Rh\(^+\) and the reversal by m-chlorocarbonyl cyanide phenyl hydrazine (CCP); run 9 shows the response in the presence of Cs\(^+\).

fig. 4. Inhibition of Zn\(^{++}\)-induced, K\(^+\)-dependent swelling by Na\(^+\). Swelling was monitored at 546 mg with the Eppendorf photometer. The medium described in the legend for Fig. 1 was employed with the addition of 2.5 mg of mitochondrial protein to 6 ml of the medium. The K\(^+\) concentration was 6 mM; Zn\(^{++}\), 67 \(\mu\)M.

fig. 5. Inhibition of Zn\(^{++}\)-induced, K\(^+\)-dependent swelling by Mg\(^{++}\). Swelling was monitored at 540 mg on the DK-2 spectrophotometer with 3 ml of the medium described in the legend for Fig. 1. The concentration of K\(^+\) was 0 \(\mu\)M; that of Zn\(^{++}\), 100 \(\mu\)M.
12 mM. Studies with the K⁺ electrode (not shown) verify that the rate and extent of K⁺ uptake are much greater from 6 mM K⁺ solutions than from solutions containing 2 mM K⁺ such as those reported in Fig. 1. In the experiment presented in Fig. 1 of Reference 1, over 500 μmole of K⁺ were taken up at a rate of 550 μmole of K⁺ per mg of protein per min.

The specificity of the induction of monovalent ion transport by Zn²⁺ was also investigated. Cd⁺⁺ substitutes rather well for Zn²⁺ in the role of inducer, and some slight response was obtained with Cu⁺⁺. Other divalent ions tested did not induce K⁺ uptake.

The specificity for the monovalent ion is shown in Fig. 3. This study establishes that mitochondria swell to the extent of about 0.5 optical density unit in the presence of 6 mM K⁺ when 100 μM Zn⁺⁺ is added. Addition of K⁺ after the Zn⁺⁺ is only slightly less effective (trace 2). Substitution of 6 mM Na⁺, Li⁺, choline⁺, or Tris for K⁺ results in a slight Zn⁺⁺-dependent swelling which reverses on anaerobiosis. Substitution of 6 mM NH₄⁺ results in a slight contraction under these conditions (trace 5) while Rb⁺ and Cs⁺ are only slightly less effective than K⁺ in supporting the Zn⁺⁺-dependent swelling.

The Zn⁺⁺-dependent uptake of K⁺ and the associate swelling are inhibited by Na⁺ (Fig. 4) and more strongly by Mg⁺⁺ (Fig. 5). The addition of Zn⁺⁺ to suspensions of mitochondria in the absence of added K⁺ results in a rapid release of endogenous K⁺. The study shown in Fig. 6 establishes that the release of K⁺ is accompanied by activation of respiration requires a source of energy (in this case provided by ascorbate-N,N,N,N'-tetramethylphenylenediamine respiration as shown in Fig. 6B), and requires the presence of P₁ (Fig. 6C). Shrinkage of the mitochondria is frequently observed to parallel the release of endogenous K⁺ (Fig. 6A). The amplitude of this change is small, and a secondary swelling usually follows as shown in Fig. 6A. The secondary swelling reverses when the system becomes anaerobic. Since Tris is the only cation added under these conditions, it appears possible that the swelling trace may reflect movements of this cation (cf. Fig. 3 also).

**DISCUSSION**

The results of this study establish that Zn⁺⁺ (and Cd⁺⁺) induces a massive energy-linked uptake of K⁺ under conditions in which respiration is maintained. The response of the mitochondrion to the addition of Zn⁺⁺ closely resembles that previously reported to the addition of the toxic antibiotics valinomycin (5–8) and gramicidin (5, 8, 9) and to parathyroid hormone (10, 11). Each of these reagents causes the rapid uptake of K⁺ which is closely associated with swelling and contraction cycles, and each activates respiration. In each case the reaction requires respiration and is prevented or reversed by anaerobiosis and uncouplers of phosphorylation. There is also some indication that Zn⁺⁺, like valinomycin (13, 14) and certain other antibiotics (15) can produce oscillations in the K⁺ content and the volume of the mitochondrion under the proper conditions. In the absence of external K⁺, Zn⁺⁺ induces an energy-linked extrusion of the endogenous K⁺ of the mitochondrion. Under these conditions, valinomycin (6) and certain histones (10) have been reported to produce a similar effect. The ability of Zn⁺⁺ to increase the energy-linked uptake of Mg⁺⁺ as well as K⁺ is a property which has been reported for parathyroid hormone (10) whereas the effects of the antibiotics appear limited to the monovalent cations. The ability of the membrane to discriminate between Na⁺ and K⁺ in the presence of Zn⁺⁺ supports the argument that the effects of the heavy metal are rather specific and do not result merely in a generalized increase in permeability to ions.

A major difference in the requirements for the induction of K⁺ transport by Zn⁺⁺ as compared to other inducers appears to be the necessity for added P₁ or arsenate. Acceleration of K⁺ transport by P₁ in the presence of valinomycin has been reported (6), but the requirement appears much more absolute in the case of Zn⁺⁺ induction. Our most recent experiments indicate that phosphate may be involved in the uptake of Zn⁺⁺ by the mitochondria membrane.

In the case of the activation of Mg⁺⁺ accumulation by Zn⁺⁺ it is possible to advance the rather trivial explanation that the very insoluble heavy metal phosphate permits more effective precipitation of Mg⁺⁺-phosphate and therefore that the activation of ion accumulation does not occur at the level of the membrane (4). Since the accumulation of K⁺ does not appear to involve precipitation of insoluble salts, the present study would appear to establish that Zn⁺⁺ is effective in stimulating ion accumulation at the level of the membrane.

Harris, Cockrell, and Pressman (6) have observed that under some circumstances the effect of valinomycin is seen only in an increased turnover of bound K⁺ as indicated by tracer studies. Scott and Gamble (17) have previously reported that a number of heavy metal ions including Zn⁺⁺ and Cd⁺⁺ cause increased exchange but a net loss of bound K⁺ from mitochondria. Since it is well established (18) that these ions inhibit respiration with physiological substrates, it appears that the observations of Scott and Gamble (17) could be explained by the combination of increased K⁺ uptake induced by the heavy metal in conjunction with secondary degenerative changes which account for the net loss.

1 G. P. Brierley and V. A. Knight, unpublished observations.
The number of available reagents which affect the flux of ions across the mitochondrial membrane is increasing rapidly as the phenomena of induced ion transport receives more attention. It cannot be assumed that all of these reagents are reactive through a common mechanism, but the use of Zn^{++} as a probe into the mechanism of ion transport appears to offer certain experimental advantages. From a chemical standpoint it represents the simplest inducer yet reported. It is available as an isotope for labeling studies and can be readily estimated chemically. In addition it can be quantitatively removed from the membrane by chelation with EDTA (12). Further studies on the binding of Zn^{++} to the mitochondrion and its relation to the mechanism responsible for the uptake of K^{+} and Mg^{++} are currently in progress in our laboratory.

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