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Respiration-Dependent Contraction of Swollen Heart Mitochondria: Participation of the K^+/H^+ Antiporter

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Abstract

Respiration-dependent contraction of heart mitochondria swollen passively in K^+ nitrate is activated by the ionophore A23187 and inhibited by Mg^{2+}. Ion extrusion and osmotic contraction under these conditions are strongly inhibited by quinine, a known inhibitor of the mitochondrial K^+/H^+ antiporter, as measured in other systems. The inhibition by quinine is relieved by the exogenous antiporter nigericin. Respiration-dependent contraction is also inhibited by dicyclohexylcarbodiimide (DCCD) when reacted under conditions known to inhibit K^+/H^+ antiport (Martin *et al., J. Biol. Chem.* **259**, 2062–2065, 1984). These studies strongly support the concept that K^+ is extruded from the matrix by the endogenous K^+/H^+ antiporter and that inhibition of this component by quinine or DCCD inhibits respiration-dependent contraction. The extrusion of K^+ nitrate is accompanied by a respiration-dependent efflux of a considerable portion of the endogenous Mg^{2+}. This Mg^{2+} efflux does not occur in the presence of nigericin or when the mitochondrial Na^+/H^+ antiporter is active. Mg^{2+} efflux may take place on the K^+/H^+ antiporter. DCCD, reacted under conditions that do not result in inhibition of the K^+/H^+ antiporter, blocks a monovalent cation uniport pathway. This uniport contributes to futile cation cycling at elevated pH, and its inhibition by DCCD stimulates respiration-dependent contraction.

Key Words: K^+/H^+ antiport; mitochondria; mitochondrial contraction; dicyclohexylcarbodiimide.

Introduction

Heart mitochondria swollen passively in Na^+ or K^+ nitrate extrude accumulated ions and contract when respiration is initiated (Brierley *et al.*, 1977). The reaction has been explained in terms of two factors: the extrusion of nitrate ion as the membrane potential (negative interior) is established by

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respiration, and the outward exchange of the accumulated cation on a cation$^+$/H^+ antiport (Mitchell, 1968, 1970). Contraction in Na^+ nitrate is more rapid and efficient than the corresponding reaction in the K^+ salt (Brierley *et al.*, 1977). Extrusion of Na^+ nitrate proceeds to completion and a low, or resting, rate of respiration is established after the salt extrusion. The extrusion of K^+ is seldom complete and elevated rates of respiration are seen which indicate that influx–efflux cycling of ions may persist under these conditions (Brierley *et al.*, 1977).

There is considerable evidence that mitochondria contain an overt Na^+/H^+ antiport (see Brierley and Jung, 1987a, for a recent review). There are also numerous indications that a second cation$^+$/H^+ antiport, capable of extruding both K^+ and Na^+, is present in a latent form that can be activated by Mg^{2+} depletion or by osmotic swelling (see Garlid, 1980; Brierley, 1983; Brierley *et al.*, 1984; Garlid *et al.*, 1986; or Brierley and Jung, 1987b, for example). The properties of this antiporter, hereafter referred to as the K^+/H^+ antiport, have been deduced from studies of a respiration-dependent extrusion of endogenous K^+ (Dordick *et al.*, 1980; Shi *et al.*, 1980a; Bernardi and Azzone, 1983), from passive swelling in K^+ acetate (Nakashima and Garlid, 1982; Brierley *et al.*, 1984; Martin *et al.*, 1984; Garlid *et al.*, 1986), and from a passive $^{42}K^+$/cation exchange reaction (Jung *et al.*, 1981; Brierley *et al.*, 1984). These studies agree that the putative K^+/H^+ antiport is activated by Mg^{2+} depletion, by elevated pH, and by a hypotonic medium. The data are also consistent with this antiporter being sensitive to inhibition by added divalent cations, organic amines such as quinine and quinacrine, and DCCD[2] when this reagent is reacted with the mitochondrion under conditions that produce an activation of the antiport. This latter reaction has been used to label an 82,000-Dalton component that may represent the K^+/H^+ antiporter (Martin *et al.*, 1984, 1986).

One of the postulated roles of the K^+/H^+ antiport is to protect mitochondria against excessive K^+ influx and the consequent osmotic swelling (Garlid, 1980). In the present work we examine the respiration-dependent contraction of mitochondria, swollen passively in Na^+ or K^+ nitrate, in terms of the properties of the K^+/H^+ antiport as established in other systems. It is concluded that the respiration-dependent contraction of these mitochondria shows properties consistent with the participation of the K^+/H^+ antiport. It was also noted that considerable extrusion of Mg^{2+} accompanies the respiration-dependent contraction in K^+, but not in Na^+, a finding that may relate to the observed ion cycling in the K^+ salt and the incomplete ion extrusion under these conditions.

[2]The abbreviations used are as follows: DCCD, dicyclohexylcarbodiimide; TEA^+, tetraethylammonium ion; TES, *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid.

Methods

Beef heart mitochondria and heart mitochondria depleted of Mg^{2+} in a TEA^+ medium were prepared as described by Brierley *et al.* (1984). Swelling and contraction were monitored at 520 or 540 nm using a Brinkman PC801 probe colorimeter or a Gilford spectrophotometer and a strip chart recorder. Mitochondria were incubated in a water-jacketed glass chamber containing the colorimeter probe and a combination glass electrode to record pH. The composition of the suspending medium and other experimental details are given with the individual experiments reported. Magnesium was estimated by atomic absorption using perchloric acid extracts of mitochondria or direct reading of mitochondrial supernates after centrifugation in an Eppendorf microfuge.

Results

Effect of Inhibitors and Activators of K^+ H^+ Antiport on Respiration-Dependent Contraction in K^+ Nitrate

Nonrespiring mitochondria swell passively in K^+ nitrate, pH 8.3, at 37°C (Brierley *et al.*, 1977; Jung *et al.*, 1980). Swelling under these conditions is inhibited by exogenous Mg^{2+} and delayed by quinine (Fig. 1A). Passive swelling is also accelerated by A23187, an ionophore that rapidly depletes matrix Mg^{2+}. Respiration-dependent contraction of mitochondria swollen in K^+ nitrate is inhibited to some extent by Mg^{2+} and markedly accelerated by A23187 (Fig. 1B). The contraction reaction is strongly inhibited by quinine, both at pH 7.1 where contraction is optimal (Fig. 1B) and at pH 8.3 (Fig. 1C), the pH used for passive swelling. The inhibition of respiration-dependent contraction by quinine is removed to a large extent by addition of nigericin, an exogenous K^+/H^+ antiporter (Figs. 1B and 1C). Quinine inhibits respiration-dependent contraction in Na^+ nitrate as well as in the K^+ salt (Jung *et al.*, 1984) and addition of nigericin also relieves the inhibition in the Na^+ salt (not shown).

Effect of DCCD on Respiration-Dependent Contraction

Jung *et al.* (1980) reported that DCCD, reacted with otherwise untreated heart mitchondria, inhibits passive swelling in Na^+ nitrate, but stimulates respiration-dependent contraction at pH 8.3. The identical response to DCCD is seen when passive swelling and respiration-dependent contraction in K^+ nitrate at pH 8.3 are examined (Fig. 2). Contraction in the presence of DCCD is more rapid than in the controls (Fig. 2), goes to completion, and

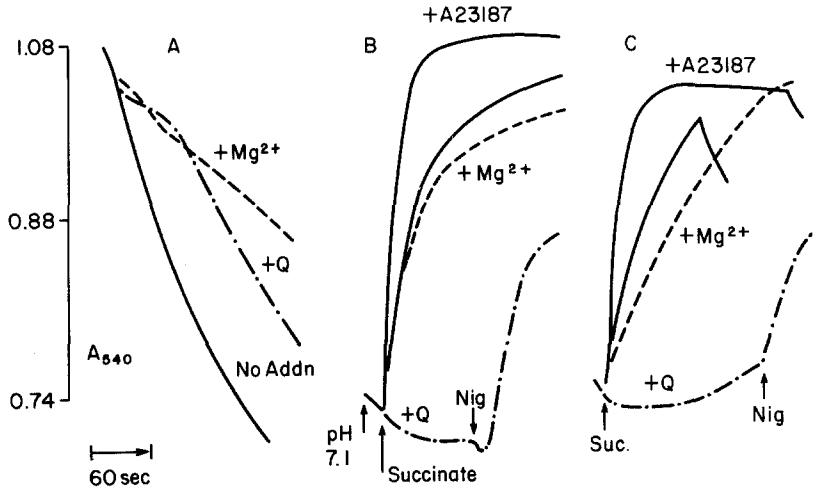

Fig. 1. Passive swelling (A) and respiration-dependent contraction at neutral pH (B) and pH 8.3 (C) of beef heart mitochondria suspended in K^+ nitrate. Mitochondria (0.5 mg protein/ml) were added to a medium of KNO_3 (100 mM) containing Tris (2 mM, pH 8.3), rotenone (3 μg/ml), and EGTA (30 μM) and the swelling monitored at 37°C using a Brinkman PC801 probe colorimeter at 540 nm. The passive swelling in the absence of further addition, in the presence of $MgCl_2$ (1.5 mM), of quinine (Q, 1 mM) and of A23187 (1 μM) is compared in (A). The records reproduced in (B) were obtained after different times of swelling (to the indicated extent) at which point the pH was adjusted to 7.1 with nitric acid and succinate added to initiate respiration. Where indicated, nigericin (0.2 μM) was also added. The records in (C) were obtained when succinate was added to the suspension at pH 8.3. The inflection in the control trace corresponds to the anaerobic point (see Brierley *et al.*, 1977).

establishes a low rate of respiration that indicates that the expenditure of energy in futile ion cycling has decreased markedly (see Brierley *et al.*, 1977).

Heart mitochondria depleted of Mg^{2+} by treatment with A23187 in a TEA^+ medium swell very rapidly when suspended in K^+ nitrate at 37°C. Swelling is so rapid and extensive at pH 7.5 and above that no respiration-dependent contraction can be detected on addition of succinate (not shown). The swelling reaction in Na^+ or K^+ nitrate is slowed to more manageable rates at pH 7.2 (Fig. 3). Swelling is also slowed by lowering the temperature to 25°C, but in this case the lower rate of respiration produces only a slow contraction (not shown). Addition of succinate to Mg^{2+}-depleted mitochondria swelling at 37°C in Na^+ (Fig. 3A) or K^+ nitrate (Fig. 3B) results in continued swelling for about 30 sec, followed by respiration-dependent contraction. The rate of contraction is slower than that seen in untreated mitochondria respiring in the same medium at pH 7.2 (Fig. 3A).

DCCD reacted with these Mg^{2+}-depleted mitochondria decreases the rate of passive swelling in Na^+ nitrate and also decreases succinate-dependent contraction considerably in this medium (Fig. 3A). DCCD at 100 nmol · mg^{-1}

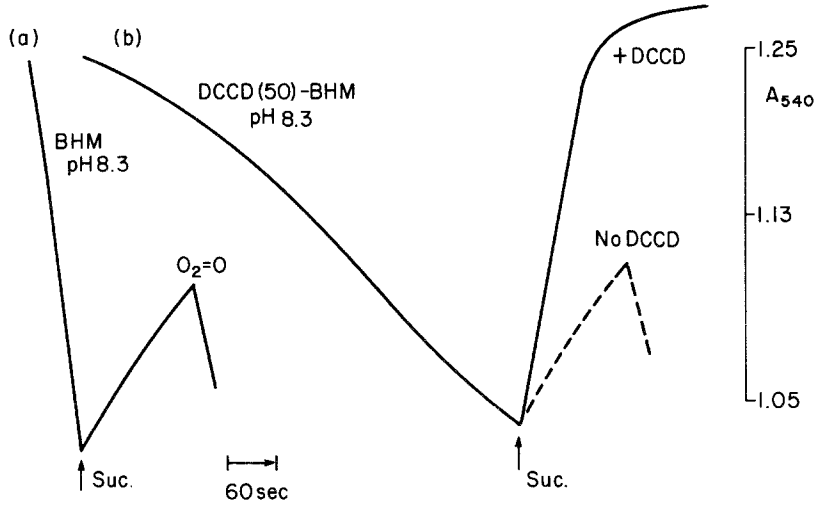

Fig. 2. DCCD reacted with otherwise untreated heart mitochondria inhibits passive swelling in K^+ nitrate but stimulates respiration-dependent contraction at pH 8.3. DCCD-treated mitochondria were reacted with 50 nmol DCCD/mg protein for 30 min on ice. Control beef heart mitochondria (BHM) were incubated under identical conditions without DCCD. Passive swelling in KNO_3 (100 mM) was monitored as described in Fig. 1. The records for untreated BHM (a) are retraced (dotted line) in (b) to allow comparison of the respiration-dependent contraction initiated by addition of succinate (3 mM).

is no more effective than is 50 nmol · mg^{-1} in the Na^+ salt, and no further contraction is seen when nigericin is added to DCCD-treated mitochondria (Fig. 3A). In contrast, DCCD virtually abolishes respiration-dependent contraction in K^+ nitrate (Fig. 3B), and addition of nigericin stimulates contraction of the DCCD-treated mitochondria. The rate and extent of contraction elicited by nigericin depends on the amount of DCCD reacted with the mitochondria (Fig. 3B).

The passive swelling of heart mitochondria in Na^+ or K^+ nitrate is accompanied by the loss of a significant portion of the endogenous Mg^{2+} (Shi *et al.*, 1980b). When DCCD is reacted with otherwise untreated mitochondria swollen in the nitrate salts, the respiration-dependent contraction in Na^+ nitrate at pH 7.3 shows little effect (Fig. 4A). In contrast, the succinate-supported contraction in K^+ nitrate is strongly inhibited by DCCD reacted under these conditions (Fig. 4B). The DCCD-treated swollen mitochondria contract in K^+ nitrate when supplemented with nigericin (Fig. 4B).

Extrusion of Endogenous Mg^{2+} During Respiration-Dependent Contraction in K^+ Nitrate

As mentioned above, heart mitochondria lose a portion of their endogenous Mg^{2+} when they swell passively in nitrate salts. There is considerable

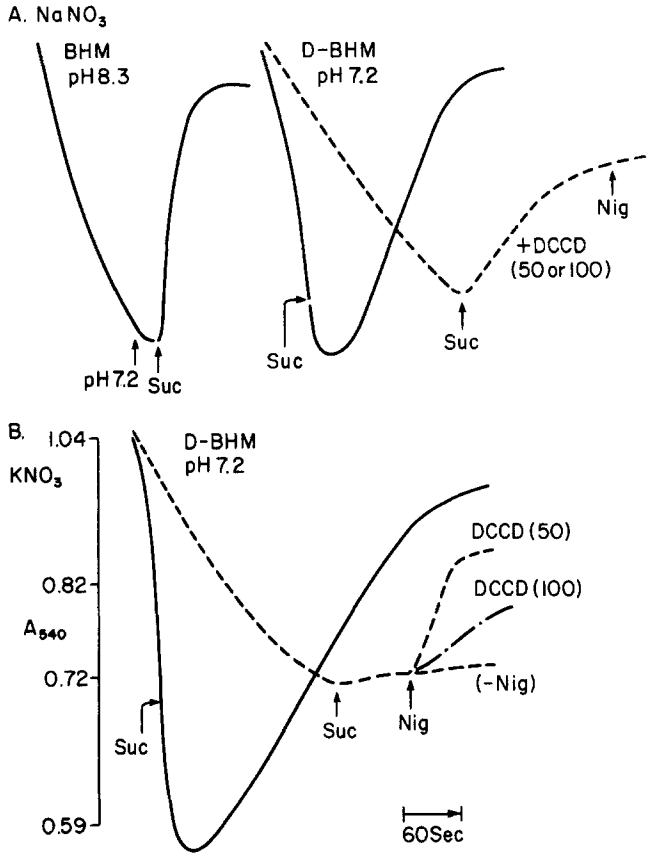

Fig. 3. DCCD reacted with Mg^{2+}-depleted heart mitochondria inhibits both passive swelling and respiration-dependent contraction in Na^+ nitrate (A) and K^+ nitrate (B). Passive swelling and succinate (3 mM) dependent contraction of untreated beef heart mitochondria (BHM, 0.5 mg/ml) in Na^+ nitrate (100 mM) containing TES (10 mM, pH 8.3), rotenone (3 μg/ml), and EGTA (30 μM) at 37°C is shown in (A). These mitochondria contained 30 nmol $Mg^{2+} \cdot mg^{-1}$. Depletion of Mg^{2+} to 3.0 nmol $\cdot$ mg^{-1} with A23187 (see Methods) results in mitochondria that swell rapidly at pH 7.2 in this medium. Depleted BHM reacted with either 50 or 100 nmol DCCD $\cdot$ mg^{-1} for 30 min on ice give the response shown by the dashed trace under these conditions. Nigericin was added to 0.4 μM where indicated. The records reproduced in (B) were obtained under identical conditions in a K^+ nitrate (100 mM) medium.

further loss of Mg^{2+} during respiration-dependent contraction in the K^+ medium (Fig. 5). Mitochondrial Mg^{2+} decreases from about 28 nmol/mg to 18.6 during passive swelling at pH 8.3 in the experiment reported (Fig. 5). The passive loss of Mg^{2+} is slowed by lowering the pH to 7.3 (2.1 nmol $\cdot$ mg^{-1} lost in 2.5 min, Fig. 5B), but the contracting mitochondria respiring with succinate lose an average of 5.7 nmol $\cdot$ mg^{-1} during this period. In the

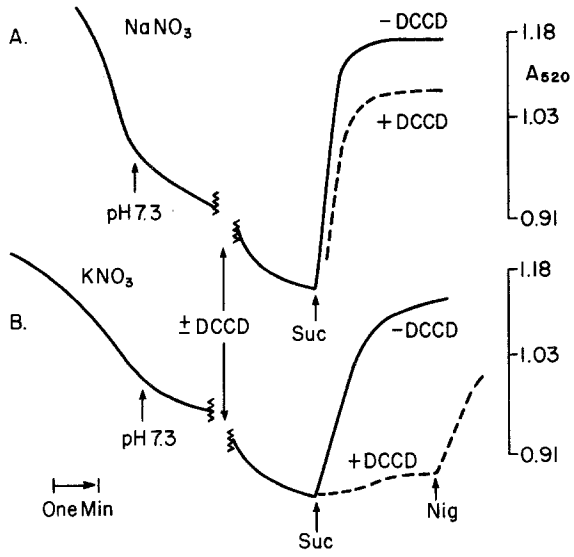

Fig. 4. DCCD reacted with swollen mitochondria inhibits respiration-dependent contraction in Na^+ nitrate (A) to a lesser extent than in K^+ nitrate (B). Untreated heart mitochondria (0.5 mg/ml) were added to a medium of either $NaNO_3$ (100 mM) or KNO_3 (100 mM) containing 2 mM Tris (pH 8.3), rotenone (2 μg/ml), and EGTA (30 μM). After swelling for the indicated time at 37°C the pH was adjusted to 7.3 with nitric acid. The mitochondrial suspension was then removed to an ice bath and one portion treated with DCCD (30 nmol/mg) for 30 min while the other served as a control. The suspensions were then returned to the colorimeter water bath in which they rapidly warmed to 37°C and contraction was initiated by addition of succinate (suc, 3 mM). Where indicated, nigericin (0.4 μM) was also added.

presence of A23187, almost all of the available Mg^{2+} is lost (Fig. 5B), but the mitochondria contract effectively (see Fig. 1B). When valinomycin is added, respiration promotes further swelling, instead of contraction (Fig. 5A), and additional Mg^{2+} is lost (7.6 nmol · mg^{-1}). However, nigericin, which strongly accentuates respiration-dependent contraction (Fig. 5A), virtually eliminates the loss of Mg^{2+} under these conditions (0.2 nmol · mg^{-1}, Fig. 5B). The quantities of Mg^{2+} lost in protocols such as that shown in Fig. 5 vary somewhat from preparation to preparation, but more than a dozen such replications agree that a significant, respiration-dependent and nigericin-sensitive loss of endogenous Mg^{2+} accompanies the contraction in K^+ nitrate. The loss of Mg^{2+} is as high as 60% of that released by A23187 in some preparations.

In contrast, when the respiration-dependent contraction of mitochondria swollen in Na^+ nitrate is examined (Fig. 6), there is virtually no loss of Mg^{2+} during respiration-dependent contraction (and no effect of nigericin on Mg^{2+} retention). It should be recalled that the passive loss of Mg^{2+} is only slightly less in Na^+ nitrate than in the K^+ medium (Shi *et al.*, 1980b).

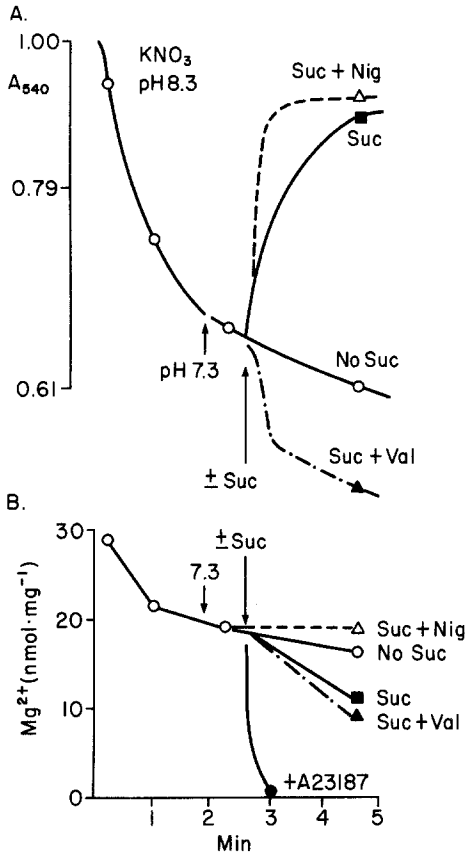

Fig. 5. Endogenous Mg^{2+} is lost during respiration-dependent contraction in K^+ nitrate. Mitochondria were swollen passively under the conditions given in Fig. 1 at pH 8.3 in K^+ nitrate. Where indicated, the pH was adjusted to 7.3 by addition of nitric acid. Contraction was initiated with succinate (3 mM). Valinomycin (1 μM), nigericin (0.4 μM), and A23187 (1 μM) were added just prior to succinate. Where indicated, duplicate samples were removed for Mg^{2+} analysis. The mitochondria were removed by rapid centrifugation (Eppendorf microfuge) and Mg^{2+} determined by atomic absorption. The Mg^{2+} content at each point along the swelling and contraction record (A) is plotted in (B).

Effect of DCCD on the Permeability of Heart Mitochondria to Nitrate

Heart mitochondria are quite permeable to nitrate at neutral pH (Brierley *et al.*, 1970). Nonrespiring heart mitochondria swell rapidly when suspended in K^+ nitrate at pH 7.2 (37°C) and supplemented with valinomycin to permit rapid entry of the cation (Fig. 7A). Swelling under these conditions is limited by nitrate uniport and is inhibited, but by no means abolished, when the mitochondria are treated with DCCD (Fig. 7A). At pH 8.3, passive

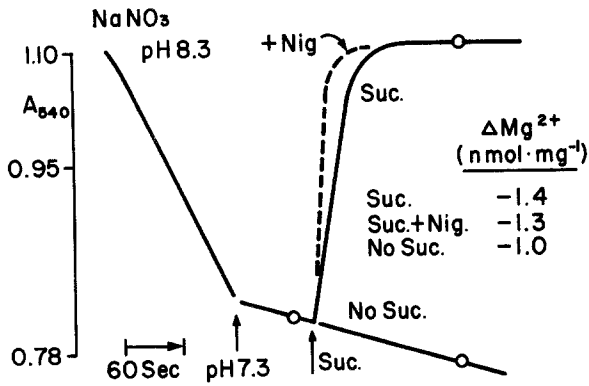

Fig. 6. Respiration-dependent contraction of heart mitochondria swollen in Na^+ nitrate is not accompanied by Mg^{2+} loss. The experimental conditions were identical to those in Fig. 5 except that $NaNO_3$ replaced KNO_3. Mg^{2+} loss is reported as the decrease in Mg^{2+} between the indicated points.

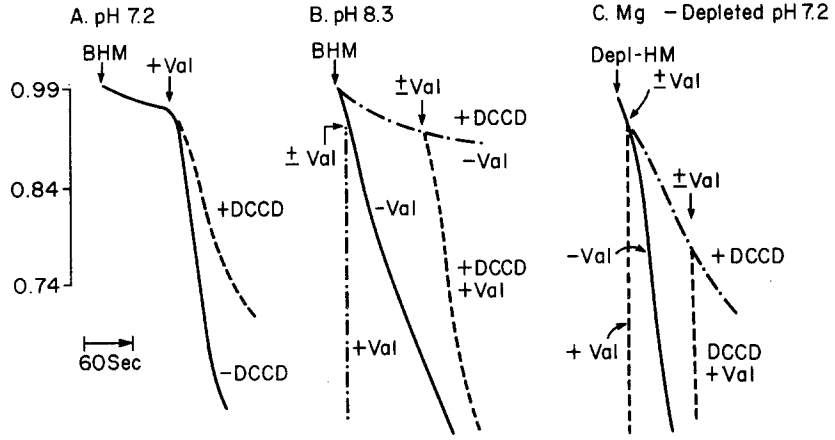

Fig. 7. Effect of DCCD on nitrate permeability of heart mitochondria. (A) Passive swelling (37°C) of heart mitochondria (0.5 mg/ml) in K^+ nitrate (100 mM), TES (10 mM, pH 7.2), and EGTA (30 μM) containing rotenone (3 μg/ml) and antimycin. Where indicated, valinomycin (1 μM) was added. The dashed trace is the response of mitochondria reacted with DCCD (50 nmol/mg for 30 min on ice). (B) Passive swelling in K^+ nitrate, pH 8.3. All other conditions described for (A). (C) Passive swelling of Mg^{2+}-depleted heart mitochondria under the conditions of (A) at pH 7.2. Values on the ordinate are for absorbance at 540 nm.

swelling of heart mitochondria in K^+ nitrate becomes much more rapid than at pH 7.2 (Fig. 7B). Swelling is still limited by cation permeability under these conditions, because addition of valinomycin elicits a very much more rapid swelling (Fig. 7B). Swelling in K^+ nitrate at pH 8.3 is strongly inhibited by reacting the mitochondria with DCCD (50 nmol · mg^{-1} for 30 min on ice). Addition of valinomycin to DCCD-treated mitochondria induces a rapid

swelling (Fig. 7B), a result that establishes that these mitochondria remain very permeable to nitrate ion. DCCD does not inhibit K^+/H^+ when reacted under these conditions (Martin *et al.*, 1984), so it appears that the principal effect of DCCD is on K^+ uniport.

Mg^{2+}-depleted mitochondria swell rapidly at pH 7.2 in K^+ nitrate and still more rapidly if supplemented with valinomycin (Fig. 7C). DCCD reacted with these mitochondria inhibits passive swelling in K^+ nitrate, but again the inhibition does not appear to result from restricted nitrate permeability, because valinomycin produces a very rapid swelling in DCCD-treated, Mg^{2+}-depleted mitochondria (Fig. 7C).

Discussion

These studies have established that the respiration-dependent contraction of heart mitochondria swollen in K^+ nitrate is activated by A23187 and inhibited by added Mg^{2+} and by quinine (Fig. 1). The latent K^+/H^+ antiporter shows these same properties when assayed in other systems (see Garlid *et al.*, 1986). The very marked inhibition of contraction by quinine can be relieved by addition of nigericin, an exogenous K^+/H^+ antiporter (Fig. 1B, 1C). This strongly supports the concept that quinine blocks the endogenous K^+/H^+ antiporter as proposed by Nakashima and Garlid (1982) and indicates that K^+/H^+ antiport is essential for respiration-dependent contraction.

Additional support for the direct and necessary participation of the mitochondrial K^+/H^+ antiporter in respiration-dependent contraction comes from studies with DCCD. This reagent, reacted with Mg^{2+}-depleted mitochondria (A23187-treated, Fig. 3), strongly inhibits respiration-dependent contraction whereas the same reagent, reacted under identical conditions with Mg^{2+}-containing mitochondria, does not inhibit and actually enhances the contraction reaction (Fig. 2). These effects of DCCD are consistent with the concept that DCCD inactivates the K^+/H^+ antiporter when reacted in the absence of Mg^{2+}, but not when Mg^{2+} is present (Martin *et al.*, 1984). The inhibition of contraction is largely removed when nigericin is added to provide an exogenous pathway for K^+/H^+ exchange (Fig. 3), a result that establishes that DCCD has not abolished the ability of the mitochondria to generate a protonmotive force, and that a monovalent cation/H^+ is required for contraction.

Most of the properties of the putative K^+/H^+ antiporter have been established in mitochondria depleted of Mg^{2+} with A23187. The observation that DCCD inhibits K^+/H^+ antiport (as indicated by contraction) in passively swollen mitochondria (Fig. 4) established that A23187 is not necessary for the

activation of the antiporter. Garlid (1978, 1979, 1980) has also reported conditions for unmasking the K^+/H^+ antiport that do not require A23187. The studies in Fig. 4 also establish that DCCD sensitivity of contraction develops as the mitochondria swell (compare with Fig. 2), a result in line with the unmasking of K^+/H^+ antiport.

The failure of DCCD to completely inhibit contraction in Na^+ nitrate under conditions in which it abolishes K^+/H^+ antiport activity (Figs. 3 and 4) suggest that Na^+ can be extruded by another, less-sensitive, pathway, presumably the Na^+/H^+ antiport. The Na^+/H^+ antiport (assayed by passive swelling in Na^+ acetate) is not sensitive to DCCD, reacted with Mg^{2+}-containing mitochondria (Jung *et al.*, 1980), or with Mg^{2+}-depleted mitchondria (not shown).

The present studies have also established that considerable depletion of endogenous Mg^{2+} occurs during respiration-dependent contraction in K^+ nitrate (Fig. 5). The loss of Mg^{2+} closely resembles the efflux reaction seen when unswollen heart mitochondria are suspended in Mg^{2+}-free media (Crompton *et al.*, 1976; Brierley *et al.*, 1987). This extrusion of Mg^{2+}, like that seen during the contraction in K^+ nitrate, is slow (1–2 nmol $\cdot$ min^{-1} $\cdot$ mg^{-1}) and is strongly inhibited by nigericin (Akerman, 1981). The reaction is best explained in terms of a $Mg^{2+}/2H^+$ antiport, perhaps on the same component responsible for K^+/H^+ antiport (Akerman, 1981; Brierley *et al.*, 1987). In support of this suggestion, the efflux of Mg^{2+} is strongly inhibited by quinine (Brierley *et al.*, 1987; Diwan, 1986), and $^{28}Mg^{2+}/Mg^{2+}$ exchange in mitochondria shares many of the features of K^+/K^+ exchange (Diwan *et al.*, 1979).

The failure to detect Mg^{2+} efflux during contraction in K^+ nitrate when nigericin is present (Fig. 5) or when the reaction is carried out in Na^+ nitrate (Fig. 6) may be explained by the failure of Mg^{2+} to react with either nigericin or the Na^+/H^+ antiporter. It seems possible that the rapid exchange of cations for external H^+ seen under these two conditions would lower matrix pH sufficiently to inhibit the Mg^{2+}-extruding component. Inhibition of the K^+/H^+ antiport by matrix H^+ has been reported (Martin *et al.*, 1984).

DCCD has been shown to inhibit $^{42}K^+/K^+$ exchange in respiring liver mitochondria (Gauthier and Diwan, 1979), and this reagent strongly inhibits passive swelling of heart mitochondria in nitrate salts at pH 8.3 and 37°C (see Fig. 2 and Jung *et al.*, 1980). Since both of these effects are seen in Mg^{2+}-containing mitochondria, they presumbaly cannot be ascribed to inhibition of K^+/H^+ antiport by DCCD (Martin *et al.*, 1984, 1986; Garlid *et al.*, 1986). These results, along with the stimulation of contraction at elevated pH (Fig. 2), have been explained as an inhibition of a cation unport pathway by DCCD (Jung *et al.*, 1980). Such a uniport would permit passive swelling by providing a pathway for Na^+ or K^+ entry with the permeant nitrate ion (see Fig. 7) and would contribute to the futile cation cycling

presumed to undermine respiration-dependent contraction at elevated pH (see Brierley *et al.*, 1977). Inhibition of this uniport by DCCD would therefore decrease passive swelling and stimulate contraction at elevated pH as seen in Fig. 2. Since the cation uniport seems to be closed at neutral pH (Brierley *et al.*, 1977), no enhancement of contraction by DCCD would be expected under these conditions.

In contrast, Garlid *et al.* (1986) view such effects of DCCD as inhibition of an anion uniport pathway. It is well established that permeability of the mitochondrial membrane to anions increases at elevated pH (Azzi and Azzone, 1967; Brierley *et al.*, 1970; Selwyn *et al.*, 1979). Anion permeability is also increased by depletion of mitochondrial Mg^{2+} (Beavis and Garlid, 1983; Brierley *et al.*, 1984; Garlid *et al.*, 1986). It has been reported that DCCD inhibits the anion conducting pathway of mitochondria (Warhurst *et al.*, 1982), a result that is confirmed by the present studies (Fig. 7). However, our studies establish that nitrate permeability does not limit swelling under these conditions and that DCCD is preventing cation uniport at pH 8.3 with Mg^{2+}-containing mitochondria and presumably also at neutral pH with Mg^{2+}-depleted mitochondria (Fig. 7). In each case, addition of the exogeneous K^+ uniporter valinomycin elicits rapid swelling in K^+ nitrate, a response that indicates that the DCCD-treated mitochondria are quite permeable to nitrate and that a low permeability to cations is limiting swelling. The observation that DCCD-treated mitochondria retain good permeability to nitrate (Fig. 7) also explains why respiration-dependent contraction can be detected (and is actually stimulated under some conditions, Fig. 2) in DCCD-treated mitochondria. If DCCD were preventing nitrate flux as proposed by Garlid *et al.* (1986), respiration-dependent contraction should be inhibited by this reagent.

It is also clear from the studies shown in Fig. 7 that both, the permeability to K^+ and to nitrate are substantially increased by Mg^{2+}-depletion. In the absence of valinomycin the high rate of swelling of Mg^{2+}-depleted mitochondria indicates considerable K^+ permeability. When valinomycin is added to provide unlimited K^+ uniport, the rate of swelling is faster in Mg^{2+}-depleted than in control mitochondria (Fig. 7C vs. 7A) at 37°C and pH 7.2. Swelling in K^+ nitrate at pH 8.3 of mitochondria containing Mg^{2+} (Fig. 7B) can be presumed to represent simultaneous K^+ and nitrate uniport, because K^+/H^+ antiport should be inactive and the alternative route for K^+ permeability, K^+/H^+ antiport plus H^+ uniport, should not be available. The rather small effect of quinine (Fig. 1A) on passive swelling under these conditions is in line with K^+ entry that is largely quinine-insensitive (i.e., the putative K^+ uniport pathway). The Mg^{2+}-depleted mitochondria show a substantial rate of swelling, even in the presence of DCCD (Fig. 7C), and this reagent should inhibit K^+/H^+ antiport when reacted with the depleted

mitochondria. It is concluded that the Mg^{2+}-depleted mitochondria also contain a DCCD-insensitive pathway for K^+ influx and that conditions of Mg^{2+} depletion that produce maximum activation of K^+/H^+ antiport also produce a membrane that is "leaky" to both anions and cations.

The extrusion of Mg^{2+} during respiration-dependent contraction in K^+ nitrate may have the dual effect of increasing K^+ conductance by uniport pathways and activating the K^+/H^+ antiport. The additional K^+ conductance would be expected to contribute to the ion cycling that seems to undermine the efficiency of contraction in K^+, as opposed to Na^+ nitrate. Because nitrate is a nonphysiological anion, it remains to be established whether the ion extrusion and contraction seen in the K^+ nitrate system, as described here, has relevance to the maintenance of volume homeostasis by mitochondria *in situ*. These issues are discussed in more detail in the preceding review (Brierley and Jung, 1987b).

Acknowledgments

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