Journal of Bioenergetics and Biomembranes, Vol. 12, Nos. 3/4, 1980

Induction of Na^+/K^+ Exchange in Swollen Heart Mitochondria

Guey-Yueh Shi, Dennis W. Jung, and Gerald P. Brierley[1]

Department of Physiological Chemistry
College of Medicine
Ohio State University, Columbus, Ohio 43210

Received February 4, 1980; revised April 7, 1980

Abstract

Heart mitochondria swollen passively in nitrate salts contract in a respiration-dependent reaction which can be attributed to an endogenous cation/H^+ exchange component (or components). The rate of contraction increases with increased extent of passive swelling in both Na^+ and K^+ salts. Since nearly constant internal cation concentrations are maintained during osmotic swelling, this result suggests that both Na^+/H^+ and K^+/H^+ exchange is enhanced by increased matrix volume. Endogenous Mg^{2+} is also lost with increased matrix volume, and this observation, in conjunction with other evidence available in the literature, suggests that monovalent cation/H^+ exchanges may be regulated by divalent cations. Passive exchange of Na^+/K^+, $^{42}K^+/K^+$, and $^{24}Na^+/Na^+$ can be readily demonstrated in mitochondria swollen in nitrate. All these exchanges are low or not detectable in unswollen control mitochondria, and it appears that they are manifestations of the activated cation/H^+ component (or components) functioning in the absence of ΔpH.

Introduction

Heart mitochondria which have been swollen passively in nitrate salts contract and extrude the accumluated ions when respiration is initiated [1–4]. This reaction has been ascribed to the activity of an endogenous cation/H^+ exchanger [5] which permits a net extrusion of internal cations at the expense of the ΔpH component of the respiratory pmf[2] [2]. In this reaction Na^+ is extruded more rapidly, completely, and with greater

[1]Address correspondence to G. P. B.

[2]The abbreviations used are as follows: pmf, proton motive force [5]; ΔpH, pH gradient across the membrane; $\Delta\psi$, mitochondrial membrane potential; CCP, *m*-chlorocarbonylcyanidephenylhydrazone; cyt *c*, cytochrome *c*; TEA, tetraethylammonium.

0145-479X/80/0800-0233$03.00/0

efficiency ($\Delta Na^+/O_2$) than is K^+. However, since unswollen mitochondria show little indication of K^+/H^+ exchange activity (see [6] for a review), the question arises as to why such an exchange would proceed so vigorously during contraction in K^+ nitrate (see [7] and [8] for discussions of this issue). Several lines of evidence indicate that, unlike the readily apparent Na^+/H^+ exchange reaction, K^+/H^+ exchange in mitochondria may be modulated by reversible interaction with a matrix solute such as Mg^{2+} [9–12]. Such a regulated exchange would provide a mechanism for extrusion of excess matrix K^+ [5], and osmotic volume control while preventing excessive loss of matrix K^+ under normal metabolic conditions.

In the present communication we report that the rate of respiration-dependent contraction increases markedly with the extent of passive swelling in Na^+ and K^+ nitrates and that these increases in cation/H^+ exchange activity parallel a loss of mitochondrial Mg^{2+}. In addition, passive K^+/Na^+, $^{42}K^+/K^+$, and $^{24}Na^+/Na^+$ exchange activities are activated by swelling in nitrate salts. These passive exchanges can be viewed as expressions of the activated cation/H^+ exchange components operating in the absence of a ΔpH.

Methods

Beef heart mitochondria were prepared in Nagarse-EGTA as previously described [13]. Swelling and contraction were followed by changes in absorbance at 540 nm with simultaneous recording of oxygen uptake and ΔpH (see [2] for details). Membrane potential by safranine distribution was monitored at 511–533 nm in an Aminco-Chance spectrophotometer [14]. The cation content of centrifuged or filtered mitochondria was estimated by atomic absorption spectroscopy (Varian AA-6) of acid extracts. Total water and sucrose-permeable water space were determined by distribution of ^3H_2O and ^{14}C-sucrose [15]. $^{42}K^+$ and $^{24}Na^+$ (New England Nuclear) were counted in aqueous solution in a Beckman scintillation counter. Other details are included with the individual experiments described.

Results

Two-Stage Incubation Experiments for Swelling and Contraction in Nitrate Salts

The records in Fig. 1A show the previously reported cycle of passive swelling at alkaline pH in Na^+ nitrate followed by succinate-dependent contraction at pH 7.1 [2]. Since the experimental variables which can be

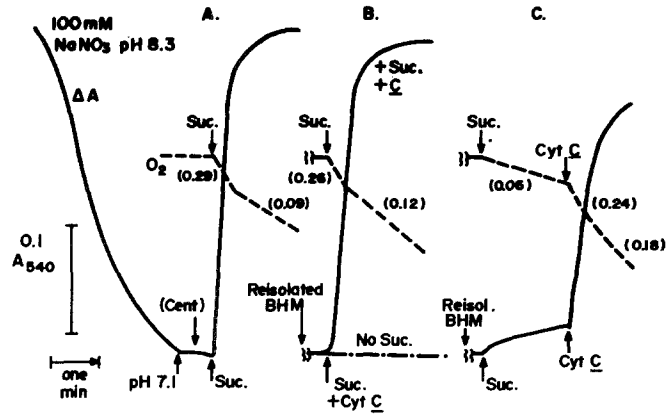

Fig. 1. Passive swelling and respiration-dependent contraction of heart mitochondria suspended in Na^+ nitrate. A. Single-stage incubation. Beef heart mitochondria (0.5 mg/ml) suspended at 37°C in Na^+ nitrate (100 mM) containing Tris (2 mM, pH 8.3), EGTA (30 uM), rotenone (3 μg/ml), and sucrose (5 mM, added with the mitochondria) were allowed to swell (as shown by the record of A_{540}). At the indicated point the pH was adjusted to 7.1 by addition of nitric acid and contraction initiated by adding Tris succinate (5 mM). The response of the oxygen electrode is shown as a dashed trace with rates of respiration (μg-atom $O_2 \cdot min^{-1} \cdot mg^{-1}$) in parentheses. B. Two-stage incubation. The swelling step was carried out as described for A and the mitochondria isolated by centrifugation (cent. in figure) just after pH adjustment to 7.1. The mitochondria were suspended carefully in a fresh charge of the swelling medium (pH 7.1) and returned to the cuvette. Succinate and cyt *c* (3.2 μM) were added to produce the contraction and respiration shown. C. Two-state incubation (as described for B) with cyt *c* omitted initally and added at the point shown.

introduced in these single-stage incubations are somewhat limited (elevated temperature is necessary to obtain passive swelling, for example), a two-stage incubation protocol was introduced. In these experiments, the swollen mitochondria are reisolated after adjusting the pH to 7.1 to minimize further swelling (point designated in Fig. 1A) and resuspended in a fresh charge of the identical medium. The resuspended mitochondria maintain nearly the same volume for several minutes after reisolation by absorbance criteria (Fig. 1B) and by Na^+ and ^3H_2O content (data not shown). However, these reisolated mitochondria show only low rates of contraction and low respiration on addition of succinate until supplemented with cytochrome *c* (Fig. 1C). In the presence of cytochrome *c* the rates of contraction and respiration approach those of the single-stage experiments (Fig. 1B) and show the respiratory control associated with the contraction reaction [2, 4]. A similar picture is obtained for two-stage swelling–contraction cycles in K^+ nitrate. In each case the low rates of respiration in the absence of cyt *c* support

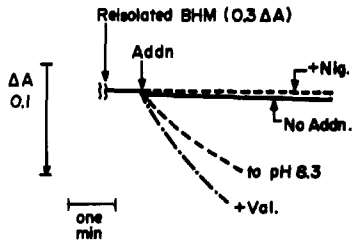

Fig. 2. Passive swelling of mitochondria swollen in K^+ nitrate. Heart mitochondria were swollen as for Fig. 1A with K^+ nitrate replacing the Na^+ salt, reisolated, and resuspended in the same medium. The effect of addition of KOH to pH 8.3 and an addition of valinomycin (10^{-7} M) or nigericin (2×10^{-7} M) on swelling (ΔA_{540}) is shown.

contraction with the same efficiency ($\Delta A/O_2$ consumed) as the more rapid rates in its presence, a result which suggests that added cyt *c* is acting solely as a component of electron transport in the reaction.[3]

Mitochondria swollen in KNO_3 and resuspended in this salt at pH 7.1 maintain a constant volume for 5 min or more and undergo further swelling only if supplemented with valinomycin or returned to pH 8.3 (Fig. 2). The cation/H^+ exchanger nigericin produces no volume change under these conditions (Fig. 2).

Alteration of the temperature of the second incubation in two-stage protocols shows that the efficiency of respiration-dependent contraction does not vary significantly in the range from 20–37°C (Fig. 3).

Loss of Endogenous Mg^{2+} and Increased Rate of Contraction as a Function of Extent of Swelling in Nitrate Salts

Analysis of mitochondria swollen to various extents in Na^+ or K^+ nitrate shows a progressive loss of endogenous Mg^{2+} with increased swelling (Fig. 4B). It has been established that mitochondria swelling in Na^+ nitrate lose endogenous K^+ immediately after swelling commences under these conditions and that increases in matrix Na^+ and matrix water content consistent with osmotic swelling are observed [2]. Swelling in K^+ nitrate results in an appropriate increase in matrix K^+ and water. The rate of contraction of heart mitochondria swollen in Na^+ or K^+ nitrate increases with the extent of swelling prior to initiating contraction by adjustment of pH and addition of

[3]The two-stage incubations reveal that the requirement for exogenous cyt *c* becomes more acute as the concentration of Na^+ or K^+ nitrate is increased above 100 mM. Earlier studies from this laboratory ([2], Fig. 9) showed that contraction is nearly abolished at 140 mM K^+ and 180 mM Na^+ nitrate and this inhibition was interpreted in terms of competition between cation and proton on the external sites of the exchange component. A re-assessment of the effect of increasing K^+ and Na^+ on succinate-dependent contraction (conditions of Fig. 1A) when optimal levels of cyt *c* are present shows that the reaction is indeed sensitive to increasing external salt concentration, but that inhibition is still incomplete at 240 mM K^+ or Na^+. Respiration associated with contraction declines linearly above 160 mM K^+ or Na^+ and the respiratory control of contraction is lost above this concentration of salt.

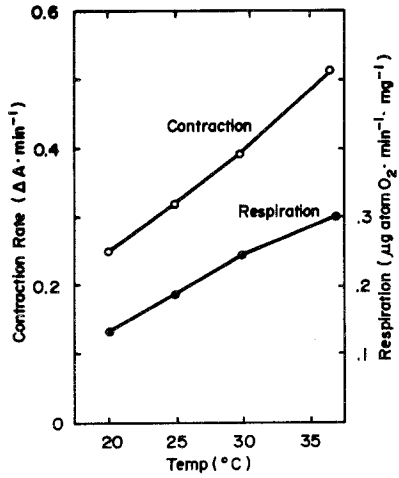

Fig. 3. Temperature dependence of respiration-dependent contraction in Na^+ nitrate. Two-stage incubations under the conditions of Fig. 1B with temperature adjusted as shown.

succinate. This effect is also seen clearly in two-stage experiments such as those summarized in Fig. 4A. In this case mitochondria were swollen in Na^+ nitrate and the rate of succinate-dependent contraction was assessed in both Na^+ and K^+ nitrates in the second incubation. The contraction rate was less for each extent of swelling in K^+ than in Na^+ (Fig. 4A), but both contraction reactions were strongly dependent on the initial extent. Nearly identical

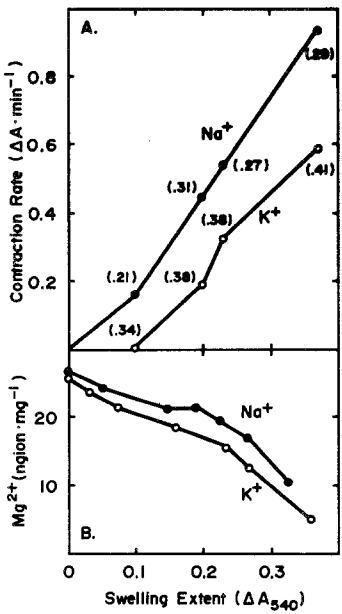

Fig. 4. Increased rate of respiration-dependent contraction (A) and loss of endogenous Mg^{2+} (B) as a function of extent of passive swelling. Heart mitochondria were swollen in Na^+ nitrate (conditions of Fig. 1A) to varying extents and the pH adjusted to 7.1. The mitochondria were reisolated by centrifugation and analyzed for matrix water [15], Mg^{2+}, and Na^+ (atomic absorption). The concentration of matrix Na^+ did not vary significantly with extent of swelling (85 ± 5 mM) at this point The rates of contraction and respiration (values in parentheses) of these mitochondria suspended in a fresh charge of Na^+ or K^+ nitrate at pH 7.1 (other components as in Fig. 1) are shown in A. The Mg^{2+} content of mitochondria swollen passively in Na^+ and in K^+ nitrate is shown in B.

responses are seen when mitochondria are swollen in K^+ nitrate and then contraction in the two salts is compared as in Fig. 4A. The rate of respiration is slightly higher in the K^+ salt, even when little contraction occurs, and this has been taken to mean that the K^+ contraction is more sensitive to influx–efflux uncoupling than is Na^+ under these conditions [4]. Records of the membrane potential ($\Delta\psi$) of swollen mitochondria contracting in Na^+as opposed to K^+ nitrate support this concept (Fig. 5). Swollen mitochondria contracting in Na^+ nitrate establish a membrane potential during contraction which is considerably smaller than that produced in unswollen, control mitochondria (Fig. 5A). The $\Delta\psi$ is maintained for only about 1 min before fading, whereas continued respiration maintains a steady state of contraction and respiration. In contrast, mitochondria contracting in K^+ nitrate show little, if any, $\Delta\psi$ during or following contraction (Fig. 5B).

Passive Exchange of K^+ for Na^+

An unexpected finding from nonrespiring control experiments for protocols such as those of Fig. 4A was that the swollen mitochondria promote a rapid, passive exchange of Na^+ and K^+ across the membrane (Fig. 6A). The replacement of accumulated Na^+ by K^+ occurs with virtually no change in total ion content (Fig. 6A) or in volume as determined by either absorbance change or tritiated water content. The reverse reaction also occurs, since the K^+ in mitochondria swollen in K^+ nitrate is rapidly replaced by Na^+, again with no significant changes in total cation or mitochondrial volume (Fig. 6B). The exchange of Na^+ for K^+ is inhibited to a limited extent by added Mn^{2+} (Fig. 6B), but reagents which provide effective control of the reaction have not yet been found. The reaction shows a sensitivity to temperature, but quite significant exchange rates persist at 0°C (Fig. 7).

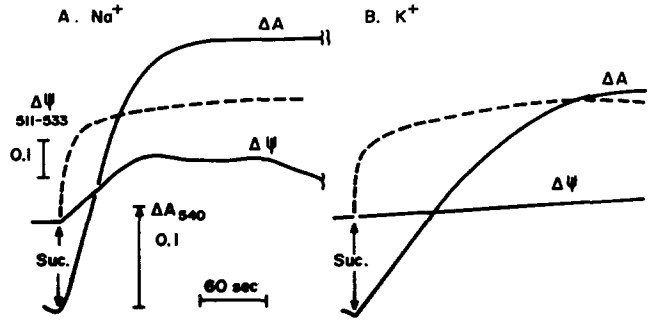

Fig. 5. Membrane potential of swollen mitochondria contracting in Na^+ (A) or K^+ (B) nitrates. Experimental conditions as in Fig. 1 with parallel determination of $\Delta\psi$ by safranine distribution [14] at 511–533 in an Aminco-Chance spectrophotometer. The dashed traces are those from unswollen control mitochondria in the same medium at pH 7.1.

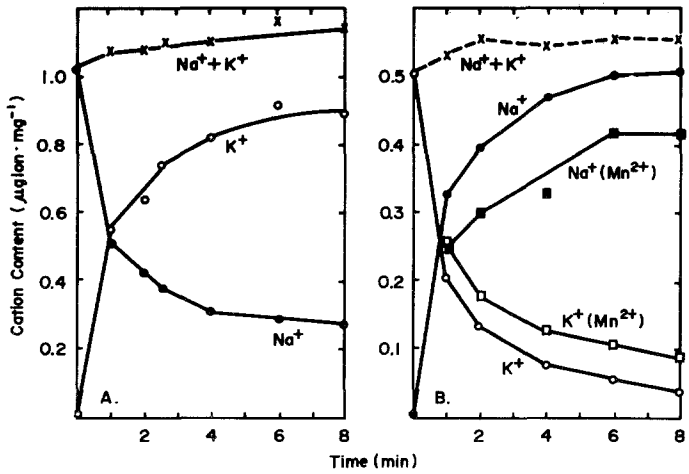

Fig. 6. Passive exchange of Na^+ for K^+ in mitochondria swollen in nitrate salts. A. Mitochondria swollen in Na^+ nitrate (as in Fig. 1) and resuspended in KNO_3 (100 mM) containing Tris (2 mM, pH 7.1) rotenone (3 μg/ml), and EGTA (30 μM) at 0°C. B. Identical to A but swollen in K^+ nitrate and resuspended in Na^+ nitrate. The effect of including $MnCl_2$ (1 mM) is also shown.

The comparisons shown in Fig. 4A were originally designed to show the rate of Na^+ efflux to an external medium containing K^+ as opposed to Na^+. However, the rapid passive exchange of these cations (Fig. 6) means that most of the internal Na^+ has been replaced by K^+ before respiration is initiated and that the rates shown in Fig. 4A are essentially those for the extrusion of K^+ to a K^+ nitrate medium and Na^+ to one containing Na^+.

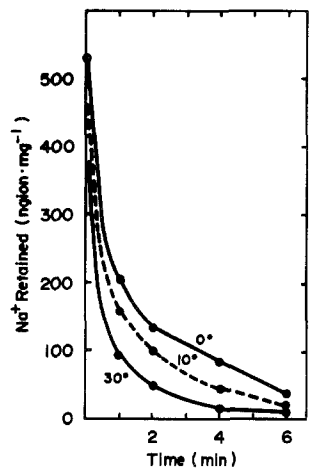

Fig. 7. Effect of temperature on passive exchange of internal Na^+ for K^+. Conditions as for Fig. 6A.

Since Na^+/K^+ exchange is not detected in unswollen mitochondria [16, 17], this activity appears to be induced as a function of passive swelling in nitrate salts. The studies in Fig. 4 suggest that K^+/H^+ exchange is activated under these conditions, and it seems likely that the K^+/H^+ exchange is a reflection of the activity of the Na^+/H^+ and K^+/H^+ exchange components (or a single component with altered selectivity) in the absence of ΔpH. Support for this concept comes from the studies shown in Fig. 8. Here mitochondria swollen in K^+ nitrate are resuspended in choline chloride, a salt in which both the cation and anion are poorly permeable, and a limited net loss of internal K^+ (accompanied by contraction, records not shown) is observed (Fig. 8A). This must be assumed to result from a leak of internal solutes in response to the newly imposed ion activity gradients and is to be contrasted with the rapid and nearly complete replacement of K^+ by Na^+ which occurs under these conditions (Fig. 8A). This result strongly supports the concept that the membrane has a limited electrophoretic or diffusional permeability to K^+, but the ability to exchange Na^+ for K^+. The experiment shown in Fig. 8B establishes that mitochondria loaded with $^{42}K^+$ in the initial incubation exchange the label with external K^+ at a rate only slightly slower than the K^+/Na^+ exchange. It should be noted that passive $^{42}K^+/K^+$ exchange is very low in unswollen mitochondria suspended in K^+ nitrate [17].

Mitochondria loaded with equal parts Na^+ and K^+ by swelling in 50 mM of each of these nitrate salts lose the two cations to a choline chloride medium at nearly equal rates (Fig. 9A). This net loss of cation is accompanied by contraction (Fig. 9A), and both cation efflux and contraction are accelerated by uncouplers such as CCP. The model shown in the inset of Fig. 9B provides a rationale for these responses. Net loss of Na^+ and K^+ to the choline chloride medium can be taken as an indication of the transmembrane permeability of these cations and the nitrate anion in response to the ion activity gradients now present. If it is assumed that choline$^+$ cannot react with the exchanger (cf. [8]), then loss of cations by the exchange pathway can occur only by Na^+ or K^+/H^+ exchange as shown. This reaction would produce a limiting ΔpH which the uncoupler would alleviate. Addition of

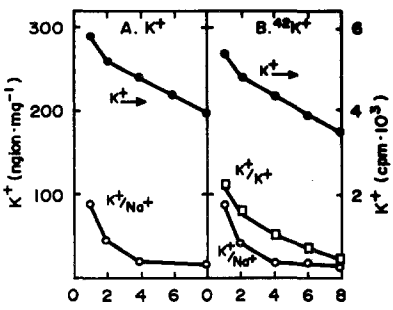

Fig. 8. Comparison of K^+ permeability, K^+ for Na^+ exchange, and K^+ for K^+ exchange. Mitochondria were swollen in K^+ nitrate labeled with $^{42}K^+$ (conditions of Fig. 1) and reisolated. The loss of K^+ (A) and of label (B) to choline chloride (100 mM) can be taken as an indication of permeability to cation ($K^+ \rightarrow$). The loss to 100 mM NaCl is indicative of the exchange of K^+/Na^+. The loss of $^{42}K^+$ radioactivity to 100 mM K^+ nitrate in B is an indication of K^+/K^+ exchange. In each case the second incubation was carried out as in Fig. 6A.

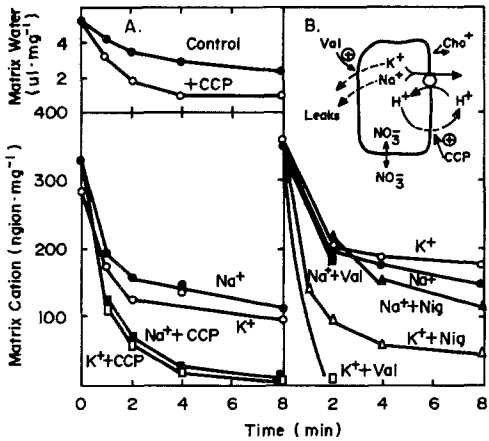

Fig. 9. Permeability and exchange activity of mitochondria swollen in Na^+ and K^+ (50 mM each). Mitochondria were swollen in equal parts Na^+ and K^+ as for Fig. 1, reisolated, and the loss of cations to a medium of choline chloride (100 mM) containing Tris (2 mM, pH 7.1), rotenone (3 μg/ml), and EGTA (30 μM) is reported. The extent of osmotic contraction produced by the passive net loss of ions is also shown in A as matrix water values. The effect of CCP (2 μM) on Na^+ and K^+ loss is shown in A. The effect of valinomycin (val, 2×10^{-7} M) and nigericin (nig, 4×10^{-7} M) is shown in B. The inset shows a model to explain these observations in which passive leak pathways and uncoupler-stimulated exchanges both contribute.

valinomycin to mitochondria containing K^+ and Na^+ accelerates the loss of K^+ with retention of Na^+ (Fig. 9B). The exogenous exchanger nigericin ($K^+ > Na^+$) also increases K^+ loss with Na^+ retention under these conditions (Fig. 9B), but the loss of K^+ in the presence of nigericin is incomplete until an uncoupler is also added (not shown). These studies suggest that the outward exchange of cations for protons does not greatly favor Na^+ over K^+. The uncoupler-dependent loss of Na^+ to a choline chloride medium increases with increased extent of swelling (Table I) in much the same way as net loss of Mg^{2+} and increased rate of respiration-dependent contraction (Fig. 4). It should be noted that the Na^+ concentration gradient does not change radically with the extent of swelling in these experiments (Table I).

$^{24}Na^+/Na^+$ Exchange in Heart Mitochondria

Attempts to load mitochondria with $^{24}Na^+$ in order to compare the efflux of this cation to the well-characterized $^{42}K^+$ efflux process [17] have been

Table I. Rate of Loss of Mitochondrial Na^+ to Choline Chloride as a Function of the Extent of Swelling[a]

Extent of swelling in $NaNO_3$ (ΔA_{540})	Matrix Na^+ concentration (mM)	Mitochondrial Na^+ = (ng-ion · mg^{-1})			Rate of CCP-dependent Na^+ loss (ng-ion · min^{-1} · mg^{-1})
			1 min in choline Cl^-		
		Initial	− CCP	+ CCP	
0.12	68	250	159	133	26
0.22	70	355	203	107	96
0.31	75	582	304	145	156

[a]Heart mitochondria were swollen in Na^+ nitrate, the pH adjusted to 7.1, and the mitochondria reisolated (as described for Fig. 1A) after swelling to the indicated extent. The matrix Na^+ and water content was established by atomic absorption and distribution of ^{14}C-sucrose and ^3H-water respectively [15]. Pellets of reisolated mitochondria were then suspended in choline chloride (100 mM) containing rotenone, EGTA (30 μM), and Tris (5 mM, pH 7.1) at 0°C for 1 min in the presence and absence of CCP (2 μM). The Na^+ content of these mitochondria after centrifugation (Sorvall SE-12 rotor) is tabulated.

unsuccessful. It appears that membrane alterations (such as the removal of membrane Mg^{2+} by chelators) used to increase matrix Na^+ are not sufficiently reversible to permit retention of the cation through such protocols. The study shown in Fig. 10 establishes that even during the rapid, net extrusion of accumulated Na^+ nitrate (supported by succinate at pH 7.1) the influx of $^{24}Na^+$ is sufficient to equilibrate internal Na^+ in a few seconds at 25°C. The time resolution and precision of these Millipore filter experiments are not completely satisfactory, but it seems clear from several repetitions of this experiment that the matrix Na^+ reaches isotopic equilibrum in contracting mitochondria at rates which are not significantly different from those of nonrespiring, swollen mitochondria (Fig. 10) and that these rates are much faster than those for unswollen control mitochondria [16].

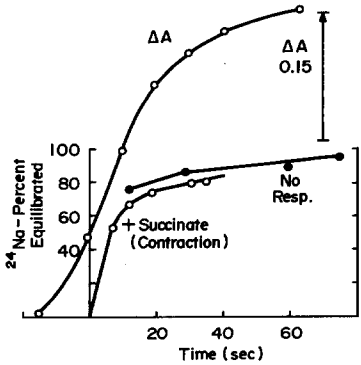

Fig. 10. $^{24}Na^+/Na^+$ exchange in heart mitochondria swollen in Na^+ nitrate. Mitochondria were swollen at 37°C and the pH brought to 7.1 as for Fig. 1. The temperature was then rapidly shifted to 25°C and contraction was initiated by addition of succinate. After 15 sec (when contraction has reached a steady state) a pulse of $^{24}Na^+$ was added and mitochondria rapidly filtered through Millipore filters (0.8 μm) held on a sampling manifold. Within 5 sec after filtration the filters were washed with a charge of the suspending medium (0°C). The filters were extracted and the specific activity of Na^+ determined. The filled circles show the response of swollen mitochondria (identical conditions with succinate omitted).

Discussion

The passive swelling and respiration-dependent contraction cycle in nitrate salts has been explained in terms of two opposing pathways for cations across the membrane [18], a uniport (or leak) which permits electrophoretic or diffusional flow of cations and an electroneutral exchange component. The exchanger brings about cation extrusion at the expense of the ΔpH component of pmf. The present studies offer additional support for this two-pathway model and also reveal several complexities which will require further clarification.

Swollen heart mitochondria show no sign of a membrane potential during contraction at pH 7.1 in K^+, whereas a $\Delta\psi$ of some magnitude can be detected transiently in the Na^+ salt (Fig. 5). This observation, in conjunction with the slower and less complete contraction in K^+ and the fact that respiration in K^+ does not return to a controlled rate as it does in Na^+ [2], strongly suggests that the mitochondria have become more permeable to K^+ than to Na^+ under these conditions. Passive swelling at pH 8.3 is initiated more rapidly in Na^+ than K^+ nitrate [2], but the swelling rates in the two cation salts are quite similar. Respiration-dependent contraction is more rapid and efficient in Na^+ than in K^+ at pH 8.3 [4] and, since the uniport pathway for both cations seems to be open at this pH, this may indicate that efflux by Na^+/H^+ exchange is more effective than K^+/H^+ exchange. It is clear that comparisons of exchange activity using the net efflux of ions (contraction) are complicated by cation permeability considerations.

Activation of Cation/H^+ Exchange and the Loss of Mitochondrial Mg^{2+}

The present studies establish that heart mitochondria lose endogenous Mg^{2+} as a function of the extent of swelling (Fig. 4B) and that the rate of succinate-dependent contraction in both Na^+ and K^+ increases as the matrix volume increases (Fig. 4A). Since swelling occurs with uptake of nearly iso-osmolar salts from the suspending medium [2], this increased rate cannot be referred to increased internal Na^+ or K^+ concentration. It has been suggested that an increased matrix volume may favor the dissociation of a regulatory substance (such as Mg^{2+}) from the K^+/H^+ exchange component so that exchange activity increases with matrix volume [10]. Such a role for divalent cations in the regulation of the electroneutral K^+/H^+ exchange has been supported by the swelling studies of Duszynski and Wojtczak [11] and of Azzone et al. [12], by the properties of a respiration-dependent extrusion of endogenous K^+ in Mg^{2+}- and Ca^{2+}-depleted heart mitochondria, [19] and by steady-state K^+ fluxes in liver mitochondria [20]. Other factors such as

membrane stretching [12] or release of fatty acids which may act as ionophores [11] have not been ruled out, however.

It should be noted that added Mg^{2+} has little, if any, direct inhibitory effect on either respiration-dependent contraction in nitrate salts or passive cation/H^+ exchange reactions [1, 21]. The cation/cation exchanges reported here are only marginally inhibited by Mg^{2+} (data not presented). At first glance these responses would appear to argue against a regulatory role for Mg^{2+} on the cation/H^+ exchanges process. However, it is well known that added Mg^{2+} does not distribute into the mitochondrial matrix rapidly and the ineffectiveness of external Mg^{2+} on monovalent cation exchange reactions may simply reflect the need for the regulatory cation to react from the matrix side of the exchange component. In support of this interpretation we have noted that Mn^{2+}, a divalent cation which is readily taken up by mitochondria, strongly inhibits both passive cation/H^+ exchanges [8, 22] and respiration-dependent contraction in Na^+ and K^+ nitrates (D. W. Jung, unpublished studies).

Induction of Cation/Cation Exchange Reactions

The analyses shown in Fig. 6 establish that mitochondria swollen in one nitrate salt rapidly exchange K^+ for Na^+ when suspended in the salt of the other cation. The reaction has no energy requirement and can be viewed as an alternative expression of an activated or deregulated cation/H^+ exchanger operating in the presence of an ion gradient, but no ΔpH. Alternatively the combination of Na^+/H^+ and H^+/K^+ exchanges on separate components would produce the same effect.

A trivial explanation for these exchanges would be that the mitochondria are retaining a constant amount of nitrate and that the cations are merely leaking across the membrane in the direction of their concentration gradients. There are a number of indications, however, that mitochondria are quite permeable to nitrate at pH 7.1 [23–26]. If the mitochondria are permeable to nitrate, the failure to continue passive osmotic swelling at neutral pH (Figs. 1 and 2) can be taken as an indication of low transmembrane permeability to monovalent cations [1, 2]. In addition, the stimulation of sucrose-dependent contraction by cation-translocating ionophores suggests that net extrusion of Na^+ or K^+ is limited by low cation permeability under these conditions [2]. Mitochondria reisolated after swelling in K^+ nitrate show no further volume change when resuspended in this salt until valinomycin is added or the pH is returned to 8.3, and no volume change is produced by nigericin under these conditions (Fig. 2). The additional swelling produced by valinomycin (Fig. 2) establishes that solute concentration gradients are still present to support ion uptake and swelling in the swollen, reisolated mitochondria, but that the

reaction appears to be limited by low cation permeability under these conditions.

The experiments on the loss of accumulated nitrate salts to choline chloride salts offer support for the presence of both leak pathways and cation/H^+ exchanges as discussed above (Fig. 9). The uncoupler-dependent efflux of cations in this system increases with increasing extent of initial swelling (Table I) in much the same way as the rate of respiration-dependent contraction (Fig. 4A), and it seems that this efflux is another indication of the activation of exchange activity under these conditions.

K^+/K^+ and Na^+/Na^+ Exchanges

These exchanges are low or absent in unswollen mitochondria [16, 17, 27] but become quite apparent in parallel with increased cation/H^+ exchange activity as the mitochondria swell in nitrate salts. The exchange of $^{42}K^+/K^+$ parallels K^+/Na^+ activity (Fig 8). Other recent studies show that a rapid K^+/K^+ exchange develops along with K^+/H^+ activity when unswollen heart mitochondria are depleted of Mg^{2+} and Ca^{2+} [19]. The early experiments of Gamble [16] showed little net uptake or $^{22}Na^+$ exchange in unswollen mitochondria, but heart mitochondria swollen in either acetate or chloride salts have high rates of $^{22}Na^+/Na^+$ exchange [28, 29]. The present studies (Fig. 10) confirm that high rates of Na^+/Na^+ exchange occur in swollen heart mitochondria even during the very efficient respiration-dependent net extrusion of Na^+. This result is not predicted by our model for Na^+/H^+-dependent net ion extrusion [2, 18], since under these conditions as many as $3Na^+/2e^-$ are extruded per site and there is little indication of uniport-type back flux during the reaction [2]. A possible explanation would be that the turnover of the exchange component is rapid compared to the ability of the mitochondrion to generate ΔpH, so that high rates of $^{24}Na^+/Na^+$ exchange could occur even during efficient Na^+/H^+-dependent net ion extrusion.

Are Separate Exchange Components Present for Na^+ and K^+?

The exchange of Na^+/H^+ is readily apparent in mitochondria from most sources as passive swelling in Na^+ acetate [23, 30–32] as well as other characteristic reactions [6]. The K^+/H^+ exchange activity is low or absent in most of these cases, but appears to be stimulated by depleting mitochondria of Mg^{2+} and Ca^{2+} with A23187 [11, 12, 19–21] by hypotonic swelling [9], and by osmotic swelling in TEA salts [33]. It should be noted that Dordick et al. [20] have recently established that A23187 does not, itself, promote K^+/H^+ exchange in these protocols as has been suggested in the past [34]. In

agreement with the present results (Fig. 4, Table I), Azzone et al. [35] observed considerable enhancement of Na^+/H^+ exchange activity under conditions which probably resulted in divalent cation depletion. Douglas and Cockrell [31] reported that Mg^{2+} inhibits Na^+/H^+ exchange, but Wehrle et al. [21] found no effect of Mg^{2+} depletion or addition on Na^+/H^+ exchange in heart mitochondria. Interpretation of these swelling studies is complicated by the now well-established effects of membrane Mg^{2+} on Na^+ uniport pathways [21, 22]. In the presence of Mg^{2+}, electrophoretic entry of K^+ is approximately equal to that of Na^+ whereas, in EDTA-treated mitochondria, Na^+ uptake far exceeds K^+ influx.

Mitochondria swollen in acetate exchange Na^+ rapidly with external Na^+ but show little $^{42}K^+/K^+$ exchange [29], passive Na^+ acetate accumulation occurs with retention of matrix K^+ [17], and osmotic contraction can extrude Na^+ with retention of K^+ from liver mitochondria swollen in acetate [36]. All of these observations suggest that mitochondrial cation/H^+ exchange components can discriminate between Na^+ and K^+ in acetate salts. The lack of competition between Na^+ and K^+ seen in the present study, plus the high rates of exchanges involving both Na^+ and K^+, indicate that swelling in nitrate has altered this situation. Two alternative explanations appear possible. The first is that a single component promotes exchange of Na^+, K^+, and H^+ and its specificity is regulated by a component (such as Mg^{2+}) which is removed as the matrix is diluted in certain salts. The de-controlled exchanger would be assumed to show increased overall activity and much increased K^+/H^+ exchange. The second alternative is that the Na^+/H^+ exchange remains relatively constant and that a second, regulated exchanger (favoring K^+/H^+) can be brought into play by removal of the "brake" (cf. [10]). Further work will be necessary to permit a choice between these alternatives.

Acknowledgments

These studies were supported in part by United States Public Health Services Grant HL09364.

References

1. G. P. Brierley and M. Jurkowitz, *Biochem, Biophys. Res. Commun.*, **68** (1976) 82–88.
2. G. P. Brierley, M. Jurkowitz, E. Chavez, and D. W. Jung, *J. Biol. Chem.*, **252** (1977) 7932–7939.
3. G. P. Brierley and D. W. Jung, in *The Proton and Calcium Pumps*, G. F. Azzone, M. Avron, J. C. Metcalfe, E. Quagliariello, and N. Siliprandi, eds., Elsevier/North Holland, Amsterdam, (1978), pp. 185–192.

4. D. W. Jung and G. P. Brierley, *Arch. Biochem. Biophys.*, **193** (1979) 76–87.
5. P. Mitchell, *Chemiosomotic Coupling and Energy Transduction,* Glynn Research, Bodmin, Cornwall (1968).
6. G. P. Brierley, *Mol. Cell. Biochem.*, **10** (1976) 41–62.
7. G. F. Azzone, T. Pozzan, S. Massari, and M. Bragadin, in *The Proton and Calcium Pumps,* G. F. Azzone, M. Avron, J. C. Metcalfe, E. Quagliariello, and N. Siliprandi, eds., Elsevier/North Holland, Amsterdam (1978), p. 193–200.
8. G. P. Brierley, M. Jurkowitz, and E. Chavez, *Biochem. Biophys. Res. Commun.*, **74** (1977) 235–241.
9. K. D. Garlid, *Biochem. Biophys. Res Commun.*, **83** (1978) 1450–1455.
10. K. D. Garlid, *Fed. Proc.*, **39** (1980) 1706 abs.
11. J. Duszynski and L. Wojtczak, *Biochem, Biophys, Res. Commun.*, **74** (1977) 417–424.
12. G. F. Azzone, F. Bortolotto, and A. Zanotti, *FEBS Lett.*, **96** (1978) 135–140.
13. D. W. Jung, E. Chavez, and G. P. Brierley, *Arch. Biochem. Biophys.*, **183** (1977) 452–459.
14. K. E. O. Akerman and M. K. F. Wikstrom, *FEBS Lett.*, **68** (1976) 191–197.
15. G. R. Hunter and G. P. Brierley, *Biochem. Biophys. Acta,* **180** (1969) 68–80.
16. J. L. Gamble, Jr., *Biochem. Biophys. Acta,* **66** (1963) 158–163.
17. E. Chavez, D. W. Jung, and G. P. Brierley, *Arch. Biochem. Biophys.*, **183** (1977) 460–470.
18. G. P. Brierley, in *The Molecular Biology of Membranes,* S. Fleischer, Y. Hatefi, D. H. MacLennan, and A. Tzagaloff, eds., Plenum Press, New York (1978), pp. 295–308.
19. G-Y. Shi, D. W. Jung, K. D. Garlid, and G. P. Brierley, *J. Biol. Chem.*, (1980) in press.
20. R. S. Dordick, G. P. Brierley, and K. D. Garlid, *J. Biol. Chem.*, (1980) in press.
21. J. P. Wehrle, J. Jurkowitz, K. M. Scott, and G. P. Brierley, *Arch. Biochem. Biophys.*, **174** (1976) 312–323.
22. G. P. Brierley, M. Jurkowitz, and D. W. Jung, *Arch. Biochem. Biophys.*, **190** (1978) 181–192.
23. G. P. Brierley, M. Jurkowitz, K. M. Scott, and A. J. Merola, *J. Biol. Chem.*, **245** (1970) 5404–5411.
24. R. S. Cockrell, *J. Biol. Chem.*, **248** (1973) 6828–6833.
25. K. Van Dam, F. J. R. M. Nieuwenhuis, and J. H. W. L. Steins, *FEBS Sympos.*, **28** 5404–5411.
26. S. Papa, F. Guerrieri, M. Lorusso, and E. Quagliariello, *FEBS Lett.*, **10** (1970) 295–298.
27. J. J. Diwan, *Biochem. Biophys. Res. Commun.*, **50** (1973) 384–391.
28. G. A. Blondin and D. E. Green, *J. Bioenerg.*, **1** (1970) 193–213.
29. G. A. Blondin and D. E. Green, *J. Bioenerg.*, **1** (1970) 479–492.
30. P. Mitchell and J. Moyle, *Eur. J. Biochem.*, **9** (1969) 149–155.
31. M. G. Douglas and R. S. Cockrell, *J. Biol. Chem.*, **249** (1974) 5464–5471.
32. M. Crompton, R. Moser, H. Ludi, and E. Carofoli, *Eur. J. Biochem.*, **82** (1978) 25–31.
33. K. D. Garlid, *Biochem. Biophys. Res. Commun.*, **87** (1979) 842–847.
34. D. R. Pfeiffer and H. A. Lardy, *Biochemistry,* **15** (1976) 935–943.
35. G. F. Azzone, A. Zanotti, and R. Colonna, *FEBS Lett.*, **96** (1978) 141–147.
36. J. L. Gamble, Jr. and C. R. Hackenbrock, *Fed. Proc.*, **28** (1969) 283 abs.