Mitochondrial Cation-Hydrogen Ion Exchange

SODIUM SELECTIVE TRANSPORT BY MITOCHONDRIA AND SUBMITOCHONDRIAL PARTICLES*

(Received for publication, August 13, 1973)

MICHAEL G. DOUGLASS† and RONALD S. COCKRELL§

From the Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63104

SUMMARY

The neutral exchange between Na⁺ or K⁺ ions and protons across the mitochondrial membrane was studied by means of passive swelling in isotonic acetate salts. The selectivity for Na⁺ over K⁺ was approximately 50:1. The rate of Na⁺-dependent swelling mediated by the natural exchange system was sensitive to pH and inhibited 70% or more by 20 mM Mg²⁺. Physiological Mg²⁺ levels, however, inhibited only about 15%. According to direct flux measurements with submitochondrial particles, the endogenous cation-H⁺ exchange possessed an apparent Na⁺:K⁺ selectivity ranging from 6 to 10:1 depending upon the experimental conditions. These ratios were found to be underestimates of the actual selectivity due to translocation of endogenous Na⁺ ions. Values corrected accordingly were comparable to those obtained for intact mitochondria.

Natural mitochondrial cation-H⁺ exchange was compared to exchange mediated by the “ionophore,” nigericin. Kinetic studies with mitochondria and submitochondrial particles yielded a Na⁺:K⁺ selectivity ratio of 1.0 for nigericin. Cation discrimination was the major difference between endogenous and nigericin-dependent exchange. Differences between properties of ionophores in model systems and their kinetic behavior in biological membranes are discussed in the context of isolation-reconstitution studies.

The present studies deal with an electrically neutral cation for H⁺ exchange system of the inner mitochondrial membrane (1). Evidence for such an exchange was first provided by observations of Mitchell and Moyle (2) who examined the effects of Na⁺ and K⁺ upon mitochondrial proton ejection and passive swelling of mitochondria in isotonic weak acid salts of Na⁺ or K⁺ (3). These results were interpreted in terms of an endogenous monovalent cation for H⁺ exchange with preference for Na⁺ over K⁺ (see also Ref. 4). Further evidence for the existence of this exchange system was provided by the observation of energy-linked K⁺ uptake by beef heart submitochondrial particles (5, 6) which was stimulated by the model cation-H⁺ exchange, nigericin (7, 8). Papa et al. (9) recently reported that Mg²⁺ATP submitochondrial particles also possess an apparent nigericin-like endogenous K⁺ permeability similar to that of A-particles (5). These authors demonstrated that Na⁺ and to a lesser degree K⁺ inhibited net H⁺ uptake by these particles (9) consistent with Na⁺ over K⁺ selectivity for mitochondrial exchange (1, 2-4).

Mitchell (10) suggested that neutral cation-H⁺ exchange could be important in osmotic regulation of mitochondria, i.e. to prevent swelling and uncoupling accompanying respiration-dependent uptake of anionic metabolites. We have been interested in this exchange system since it would influence the mitochondrial pH gradient and thus could effect the distribution of anionic substrates between mitochondria and cytoplasm and might, therefore, regulate cellular metabolism (11). Although a number of observations support the existence of neutral mitochondrial cation-H⁺ ion exchange, this activity has not been treated quantitatively nor have suitable direct flux measurements been performed. These and related aspects of cation-H⁺ exchange are the subject of the present study.

Through comparative studies of mitochondria and submitochondrial particles, it has been found that the neutral cation-H⁺ exchange is highly selective for Na⁺ over K⁺. This system is quite active at physiological pH and Mg²⁺ levels although Mg²⁺ does inhibit the exchange. The natural exchange system has been compared to the ionophore, nigericin. These observations have not only shed light upon certain aspects of endogenous cation-H⁺ exchange but have revealed interesting differences between cation discrimination derived from kinetic studies with nigericin and assessments of selectivity based upon other techniques.

EXPERIMENTAL PROCEDURES

Preparations—Rat liver mitochondria were isolated by a modification of the method of Schneider (12). Submitochondrial particles (A-particles) were prepared from heavy layer rat liver mitochondria by the technique of Fessenden and Racker (13). All experiments were performed with freshly prepared mitochondria.

† This investigation was supported by funds from Public Health Service Grant No. Ca-11766 of the National Cancer Institute.

‡ Recipient of National Institutes of Health Predoctoral Fellowship GM00446-13; this work is taken from a dissertation submitted in partial fulfillment of the Ph.D. degree. Present address, Department of Biochemistry, Southwestern Medical School, Dallas, Texas.

§ To whom reprint requests should be sent.

1 The abbreviations used are: A-particles, submitochondrial particles obtained by sonication mitochondria in the presence of ammonia; FCCP, carbonyl cyanide trifluoromethoxyphenyl-hydrazone; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid.
Mitochondrial Swelling Studies: Calculated Fluxes for Comparison with Submitochondrial Particle Measurements—Mitochondrial swelling was recorded continuously with a Beckman DB spectro-photometer as absorbance changes at 600 nm. In all experiments mitochondria were pre-treated with respiratory inhibitors before addition to isotonic salt media. Swelling rates are expressed as either ΔA per min or ΔA% initial A per min. The latter was used to calculate the net flux of monovalent cations associated with passive swelling. An inverse first power relationship between changes in absorbance and mitochondrial volume was assumed (15). A representative total mitochondrial volume of 3.0 μl per mg of protein (16-18) was used in these computations.

To compare Na+ and K+ fluxes (e.g. per mg of protein values) for mitochondria and submitochondrial particles, the following assumptions were necessary: (a) cation flux was uniform across the entire inner mitochondrial membrane surface; (b) the inner mitochondrial membrane protein constituted 52% of the total (19); (c) submitochondrial particles were derived exclusively from the inner membrane; (d) the membrane protein to surface area ratio was the same for submitochondrial particles and the mitochondrial inner membrane.

Except where indicated, maximum fluxes were reported (e.g. at pH 7.0 for mitochondria and pH 7.5 plus 7.5 mM MgCl₂ for submitochondrial particles; see "Results").

Ion Transport Measurements—The suspending medium Na+ or K+ activity was measured with a Beckman 39046 Cl or 39047 ca- tion electrode, respectively, in combination with a remote junc- tion calomel reference (Corning 470052) connected to the reaction medium via an agar-KCl bridge. Electrode outputs were fed through Radiometer model 26 expanded scale pH meters to a multichannel potentiometric recorder. Proton transport was measured with a combination pH electrode (Beckman 39039). The combination pH- and cation-selective electrodes were mounted in the side of a 1.5 ml open vessel equipped with rapid overhead mixing by a modified titration assembly (Radiometer M-11).

Na+ and K+ Assays—A-particles were extracted with 0.5 N HNO₃. The extracts were analyzed for Na+ and K+ by means of a Perkin-Elmer atomic absorption spectrometer (model 303).

A-particle Resin Treatment—As described under "Results," it was necessary to remove endogenous monovalent cations from submitochondrial particles for certain measurements. This was accomplished by pretreating A-particles with Tris-Dowex 50W-X8 cation exchange resin (50 to 100 mesh, Bio-Rad Laboratories). The resin was prepared by washing on a column with 20 bed vol-umes of 1 N Tris-Cl at pH 7.2 followed by 20 volumes of distilled deionized water. A-particles (4 ml, 15 to 20 mg per ml) were treated with 2 g of Tris-Dowex resin at 0°C for 10 min immediately before use.

Reagents—NAD+, NADH, oligomycin, antimycin, rotenone, and yeast alcohol dehydrogenase (salt-free) were purchased from Sigma Chemical Co. Nigericin was supplied by Dr. D. T. Wong of Eli Lilly, Indianapolis, Ind. and Dr. R. L. Harned, Com- mercial Solvents Corporation, Terre Haute, Ind. The FCCP used in these studies was contributed by Dr. P. G. Heytler, E. I. Dupont de Nemours and Co., Wilmington, Del.

RESULTS

Mitochondrial Swelling in Isotonic Salts—One assay of electrically neutral monovalent cation exchange by mitochondria is passive swelling of metabolically inhibited mitochondria in iso tonic acetate salt media. Swelling presumably occurs by uptake of Na+ or K+ via neutral cation-H+ exchange (3). This estab-lishes a pH gradient which drives acetate uptake (most likely as the free acid) and results in osmotically induced swelling (20).

The results obtained upon addition of respiratory-inhibited mitochondria to medium containing various isotonic salts are shown in Fig. 1. In the presence of Na+ acetate, the rate of mitochondrial swelling was severalfold more rapid than swelling in K+ acetate. These results were consistent with the presence of an endogenous monovalent cation-H+ exchange system with a high Na+:K+ selectivity and agreed qualitatively with previous observations obtained with both beef heart (4) and rat liver mitochondria (3).

Additional evidence that swelling involved an electrically neutral exchange was provided by the observation that the "model" cation-H⁺-exchanger, nigericin, stimulated the rate of mitochondrial swelling in either Na+ or K+ acetate medium. Appropriate controls (e.g. no swelling in the presence of the nonpermeant anion, chloride, or substantially more rapid swelling in K+ or Na+ acetate plus nigericin) indicated that spontaneous swelling depended solely upon cation-H⁺ exchange which was rate-limiting. Acetate penetration was not rate-limiting even in the presence of nigericin since spontaneous swelling in NH₄⁺-acetate was more rapid than with the ionophore (not shown).

The influence of pH on exchange was examined since the participation of K+ and Na+ in cation-H⁺ exchange of rat liver mitochondria, according to oxygen pulse experiments, was re-portedly pH dependent (2). In contrast, no significant effect of pH upon beef heart mitochondrial exchange activity was ob-served (21). As shown in Fig. 2 (left panel), swelling was more

![Fig. 1. Passive mitochondrial swelling in acetate salts. Mitochondria (0.3 mg) pretreated with 0.2 μg antimycin per mg was added to 2.5 ml of medium containing 133 mM of the Na+ or K+ salt indicated and 15 mM glycylglycine and 0.2 mM Tris-EGTA at pH 7.0 and room temperature. When included, nigericin (0.08 μg), was added prior to mitochondria (M₀). The change in absorbance was monitored as described under "Experimental Procedures"; downward trace deflections indicate mitochondrial swelling.](http://www.jbc.org/content/5465/2/4658/F1.large.jpg)

![Fig. 2. Spontaneous and nigericin-dependent mitochondrial swelling (pH dependence). The conditions were identical with those described in the legend of Fig. 1. Left, swelling rates are expressed as changes in optical density (O.D.) per min. Right, the Na+ : K+ selectivity ratio (calculated from the ratio of initial swelling rates in the respective acetate salts) is plotted as a function of pH.](http://www.jbc.org/content/5465/2/4658/F2.large.jpg)
rapid in Na⁺ than in K⁺-acetate medium at all pH values tested although Na⁺-dependent swelling declined sharply at pH values greater than 7.0. Swelling rates for nigericin plus Na⁺ or K⁺ were virtually independent of pH except for a modest decline at pH 8.0.

The ratio of the initial swelling rates in Na⁺ and K⁺ media was employed to estimate Na⁺:K⁺ selectivity values which are plotted as a function of pH in Fig. 2 (right panel). A high Na⁺:K⁺ discrimination was evident for the natural exchange system, particularly at neutral or acid pH values. In contrast, the Na⁺:K⁺ selectivity of the ionophore, nigericin, was 1:1 throughout the pH range examined. Variation in selectivity values for endogenous exchange from one mitochondrial preparation to another resulted from small, absolute differences in the rate of K⁺-dependent swelling. The average Na⁺:K⁺ selectivity ratios for six preparations were 46, 43, 13, and 4.0 at pH values of 6.5, 7.0, 7.5, and 8.0, respectively. The normalized rates of Na⁺-dependent swelling from pH 6.5 to 8.0 were 22 ± 0.3, 22 ± 5.8, 16 ± 5.9, and 10 ± 4.3 A% initial A per min; see “Experimental Procedures.” Corresponding swelling rates in K⁺ medium at the above pH values were 0.5 ± 0.4, 0.6 ± 0.5, 1.2 ± 0.8, and 2.5 ± 1.5. Thus at acid pH the Na⁺:K⁺ selectivity could exceed 100 if K⁺-dependent swelling was especially slow. Since K⁺-dependent fluxes were extremely small and variable the selectivity values reported here must be considered as approximations of the true Na⁺:K⁺ discrimination of endogenous exchange.

Mg²⁺ influences the monovalent cation permeability of various membranes (22) and is the predominant intracellular divalent cation (23). Therefore, its effect upon endogenous exchange was examined (Fig. 3). Mg²⁺ strongly inhibited swelling in Na⁺ medium, i.e. the endogenous exchange. Maximal inhibition (70%) occurred at 20 to 25 mM Mg²⁺; half-maximal inhibition was obtained with 5.2 ± 0.4 mM Mg²⁺ (for six preparations). Mg²⁺ abolished K⁺-H⁺ exchange and also inhibited both Na⁺- and K⁺-dependent swelling meditated by nigericin (not shown). Since direct interaction between Mg²⁺ and nigericin is unlikely (24), inhibition of endogenous and nigericin-dependent exchange by Mg²⁺ may involve alteration of membrane surface charge or structure. The effect of Mg²⁺ was nonspecific since Ca²⁺ and La³⁺ also inhibited. On a concentration basis, Ca²⁺ and La³⁺ were 5 and 10 times, respectively, more effective inhibitors than magnesium.²

Inhibition of exchange by polyvalent cations could result from their influence upon the membrane surface potential by "shielding" and possible binding to fixed charges (25). Ionophore-dependent cation conductances across lipid bilayers are very sensitive to changes in surface potential (25) and stimulation of electrically neutral mitochondrial anion exchanges by various cations has been attributed to alteration of membrane surface charge (26). These latter results seemed particularly relevant since they suggested a possible reciprocal relationship between surface charge and mitochondrial exchange processes, i.e. cations facilitate neutral anion exchanges (26) whereas they inhibit neutral cation exchange. Because pH has characteristic influences upon surface charge and ionic fluxes (25, 26), the mode of action of Mg²⁺ was further probed by determining the pH profile for Mg²⁺ inhibition of cation-H⁺ exchange.

As shown in Fig. 4A, Mg²⁺ inhibition was greatest at acid pH although swelling rates in the absence of Mg²⁺ were substantially slower at alkaline pH values. The absolute inhibition by Mg²⁺ was further probed by determining the pH profile for Mg²⁺ inhibition of cation-H⁺ exchange.

² M. Douglas and R. Cockrell, manuscript in preparation.
In order to assess better the endogenous neutral exchange system, submitochondrial particles were examined since they provided the most convenient system for direct measurement of the relevant cation fluxes.

Monovalent Cation Accumulation by A-particles—A-particles (13) or EDTA-particles (see Ref. 27) were utilized because they can be "coupled" by oligomycin (9, 27-31). In sucrose plus glycyglycine medium at pH 7.5 the respiratory control indices with FCCP were 6.0 ± 0.5 for seven preparations. In the presence of high levels of Tris-NO₃ (which were optimal for K⁺ and Na⁺ ion transport measurements) the respiratory control indices were significantly lower (3.7 ± 0.3 for three preparations also at pH 7.5). The difference in respiratory control in the presence and absence of Tris-NO₃ cannot be readily explained at the present time.

Maximal cation fluxes in submitochondrial particles require the presence of a suitably permeant anion (5, 9, 31). Uncoupling of submitochondrial particles by NH₄⁺ salts can be correlated with the ability of anions to permeate as charged species, i.e. electrogeneically (31). Although SCN⁻ was the most permeable anion (Table I), NO₃⁻ was routinely employed as the permeant anion of choice since SCN⁻ has complex secondary effects (32, 33).

Representative tracings of energy-linked Na⁺ and K⁺ uptakes by A-particles are provided in Fig. 5. The initial rate and extent of Na⁺ uptake was 5- to 6-fold greater than the corresponding values for K⁺ accumulation. Cation uptake was energy-linked since it was reversed by uncoupling agent (FCCP) or abolished if uncoupler was added prior to initiation of respiration (not shown). Evidence that cation uptake was mediated by an endogenous neutral exchange was provided by the observation that nigericin stimulated both Na⁺ and K⁺ accumulation, each of which was reversed by FCCP (Table II). The initial rate and net uptake of Na⁺ promoted by nigericin was slightly less than K⁺ uptake.

Further documentation that net influx of Na⁺ (K⁺ not shown) as well as proton uptake by these submitochondrial particles was energy-linked is provided by the results summarized in Table III. Cation uptake required coupling by oligomycin and was abolished by uncoupling agent or respiratory inhibitors. Therefore, the metabolically dependent uptake of protons by submitochondrial particles (i.e. in the opposite direction of intact mitochondria, cf. Refs. 30, 34, and 35) provides the pH gradient necessary to drive monovalent cation accumulation facilitated by the endogenous cation-H⁺ exchange system or the carboxylic ionophore, nigericin.

A high Na⁺:K⁺ selectivity for endogenous exchange (Fig. 5) was also evident from the extent of energy-dependent proton uptake by A-particles in Na⁺ versus K⁺ medium (Fig. 6A). In K⁺ medium, net proton uptake was approximately one and one-half times the extent of H⁺ accumulation in Na⁺ medium. In agreement with the lack of discrimination by nigericin between Na⁺ and K⁺ (Table II), the ionophore virtually abolished proton uptake in the presence of either cation (Fig. 6A). It is noteworthy that Mg²⁺ increased the extent of H⁺ uptake in both Na⁺ and K⁺ medium to approximately the same value (Fig. 6B). This is consistent with Mg²⁺ inhibition of mitochondrial cation-H⁺ exchange (Fig. 4); however, additional studies revealed that the influence of Mg²⁺ is more complex.

Kinetic studies demonstrated saturation behavior for endogenous uptake of Cations by submitochondrial particles (Table I), as well as competitive inhibition by high concentrations of Na⁺, K⁺, and Mg²⁺ (Table II). These results indicate that the uptake system is a low-affinity mechanism, as might be expected from the lack of specific cation binding sites in the mitochondrial membrane. The apparent Km values for Na⁺ and K⁺ (Table II) are consistent with the high selectivity for Na⁺:K⁺ accumulation, as observed in intact mitochondria (37-39) and in submitochondrial particles (33).

\[ \text{Na}^+ : \text{K}^+ = 100 \mu M \]

\[ \text{Na}^+ : \text{K}^+ = 100 \mu M \]

![Fig. 5. Direct measurements of K⁺ and Na⁺ fluxes in submitochondrial particles. A-particles (4.4 mg of protein) were suspended in 1.0 ml of medium consisting of 250 mM sucrose, 50 mM glycyglycine, and 50 mM Tris-NO₃ at pH 7.0 and room temperature; oligomycin, 2.0 µg per mg of protein, 0.5 mM NAD⁺, 20 mM ethanol, and 0.3 mM KCl or NaCl were added initially. Respiration was initiated after 3 min by addition of alcohol dehydrogenase, 80 µg. The uptake of K⁺ or Na⁺ (indicated by downward deflections of the tracings) was measured with the corresponding cation selective glass electrode as described under "Experimental Procedures." Uncoupling agent (4 µM FCCP) was added as indicated.}

**Table I**

**Endogenous stimulation of A-particle respiration**

In these experiments, A-particles (1.2 to 1.8 mg of protein; pretreated with oligomycin, 2.0 to 2.5 µg per mg) were added to 3.0 ml of medium consisting of 250 mM sucrose and 10 mM Tris-glycyglycine at pH 7.0 to 7.2. Respiration was initiated by addition of 1 mM NADH. Oxygen utilization was measured polarographically with a Clark (membrane) electrode. The tabulated data is the average of three preparations (±S.E.). RCI, respiratory rate with either 10 mM NH₄⁺ salt or 4 µM FCCP divided by the basal rate.

<table>
<thead>
<tr>
<th>Anion</th>
<th>Uptake rate</th>
<th>Control</th>
<th>+NH₄⁺ salt</th>
<th>RCI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg atom 0/mg-min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td>61.5</td>
<td>107</td>
<td>1.7 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>61.6</td>
<td>124</td>
<td>2.0 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>58.8</td>
<td>184</td>
<td>3.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>SCN⁻</td>
<td>60.8</td>
<td>270</td>
<td>4.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>FCCP</td>
<td>61.5</td>
<td>396</td>
<td>6.4 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

**Table II**

**Na⁺ and K⁺ uptakes by A-particles**

All experiments were performed as described in the legend of Fig. 5; when included, nigericin (0.25 µg per mg) was added prior to initiating respiration with ADH. The uptake rates and net cation uptakes, in neq per mg or neq per mg, respectively, are averages (±S.E.) for the number of experiments given in parentheses.

<table>
<thead>
<tr>
<th>Anion</th>
<th>Uptake rate</th>
<th>Endogenous (3)</th>
<th>+Nigericin (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>67.9 ± 7.3</td>
<td>160 ± 10.3</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>11.7 ± 2.9</td>
<td>216 ± 7.8</td>
<td></td>
</tr>
<tr>
<td>Net uptake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>21.9 ± 2.0</td>
<td>48.9 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>5.2 ± 2.0</td>
<td>65.8 ± 5.5</td>
<td></td>
</tr>
</tbody>
</table>
TABLE III

Energy-linked cation uptake by submitochondrial particles

A-particles (2.6 mg treated with 2.5 μg of oligomycin per mg) were equilibrated for 2 min in 1.0 ml of 250 mM sucrose, 50 mM Tris-NO₃, 20 mM glycylglycine (pH 7.2) and 0.5 mM NaCl for monitoring sodium movements or 250 mM sucrose, 50 mM KNO₃, and 3 mM glycylglycine (pH 7.2) in the case of proton measurements. Cation flux was initiated by alcohol dehydrogenase addition as indicated in Fig. 5.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Na⁺ uptake (meq/mg)</th>
<th>H⁺ uptake (meq/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>19.7</td>
<td>33.7</td>
</tr>
<tr>
<td>Oligomycin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ FCCP (4 μM)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ Rotenone (0.4 μg/mg)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ Antimycin (0.2 μg/mg)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ KCN (1 mM)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 6. Influence of Na⁺ and K⁺ upon submitochondrial particle H⁺ ion transport. A-particles (2.2 mg) treated with oligomycin (2.0 μg per mg) were preincubated in 1.0 ml of K⁺ or Na⁺ medium containing NAD⁺ and ethanol as described in the legend of Fig. 5. The media contained final concentrations of either 112 mM KC1 or NaCl plus 75 mM sucrose and 2.0 mM glycylglycine (at pH 7.5 and ambient temperature). Proton uptakes on addition of alcohol dehydrogenase are shown in A for: 1, Na⁺ medium; 2, K⁺ medium; 3, Na⁺ medium plus nigericin (0.2 μg per mg); 4, K⁺ medium plus nigericin (0.2 μg per mg). In B, tracings 5 and 6 were obtained with particles in Na⁺ and K⁺ medium, respectively, plus 5 mM MgCl₂. Proton accumulation was completely reversed by 4 μM FCCP (indicated by arrows). Addition of FCCP prior to alcohol dehydrogenase abolished H⁺ uptake in all cases (e.g. as shown in trace 7 for KCl medium).

The influence of Mg²⁺ was studied since it effected mitochondrial exchange activity (Fig. 3) and proton uptake by submitochondrial particles (Fig. 6B). Titration with Mg²⁺ to a concentration
of 7.5 mM stimulated Na\(^+\) flux (Fig. 9) and increased Na\(^+\):K\(^+\) selectivity (Fig. 9, inset). Higher concentrations of Mg\(^{2+}\) (as in mitochondria) reduced both the rate of Na\(^+\) transport and the Na\(^+\):K\(^+\) selectivity of the endogenous exchange. Maximum Na\(^+\):K\(^+\) selectivity values of 10.6 ± 3.1 were obtained with 3.0 to 7.5 mM Mg\(^{2+}\) by similar titrations of several submitochondrial particle preparations. It should be noted that Mg\(^{2+}\) did not significantly stimulate K\(^+\) uptake in spite of its marked enhancement of Na\(^+\) transport. This could indicate that flux of these cations is mediated by separate systems.

Stimulation of submitochondrial particles Na\(^+\) flux by low Mg\(^{2+}\) concentrations is clearly different from the strictly inhibitory influence of this cation upon the exchange system in mitochondria. The more rapid rates of Na\(^+\) uptake may reflect a "coupling" influence of Mg\(^{2+}\), presumably related to the enhancement of other energy-linked reactions of submitochondrial particles by low levels of Mg\(^{2+}\) (36). This would be consistent with the observation that Mg\(^{2+}\) increases the rate and extent of proton uptake by A-particles (Table IV) at the same concentration which enhances Na\(^+\) accumulation via the endogenous exchange. On the other hand, this same Mg\(^{2+}\) level inhibited Na\(^+\) exchange mediated by nigericin (Table IV) indicating that ionophore-mediated transport may be more sensitive to this divalent cation. Higher Mg\(^{2+}\) concentrations inhibited Na\(^+\) flux mediated by endogenous exchange (Fig. 9) as well as nigericin (not shown).

Net H\(^+\) uptake remained elevated even at high Mg\(^{2+}\) levels (not shown) indicating that inhibition of Na\(^+\) flux was not a consequence of diminished H\(^+\) uptake but more likely an effect upon exchange activity per se (as in mitochondria; cf. Fig. 3).

As alluded to earlier, the influence of Mg\(^{2+}\) upon submitochondrial particles is complex. Mg\(^{2+}\) could stimulate H\(^+\) uptake by either a coupling effect or by inhibiting cation-H\(^+\) exchange. Since low levels of Mg\(^{2+}\) also stimulate Na\(^+\) accumulation (Fig. 9), the observed Na\(^+\) and H\(^+\) fluxes most likely result from a dual influence of Mg\(^{2+}\), i.e. coupling and inhibition of exchange.

The general properties of natural cation-H\(^+\) exchange are summarized in Table V for comparison with the model exchanger, nigericin. As in mitochondria (Fig. 2), the endogenous exchange system of submitochondrial particles invariably exhibited a significant preference for Na\(^+\) over K\(^+\). The Na\(^+\):K\(^+\) selectivity ratio for nigericin was 1.0 in these studies under all experimental conditions employed (cf. Fig. 2 and Table II). Under optimum conditions, the rate of Na\(^+\) uptake mediated by the natural exchange system approached that promoted by the ionophore (Table II). Saturaion kinetics were demonstrated for endogenous (Fig. 7) or nigericin-dependent exchange. The latter had an apparent \(K_m\) for Na\(^+\) or K\(^+\) of approximately 2 mM. These comparisons indicate that the natural cation-H\(^+\) exchange system although similar in certain respects to the model exchanger, nigericin, differs markedly in cation selectivity.

**DISCUSSION**

Passive mitochondrial swelling and cation uptake by submitochondrial particles have provided reliable means for assessing neutral cation-H\(^+\) exchange as indicated by comparison of flux values obtained with these two techniques. The Na\(^+\) flux calculated from passive swelling (88 neq per mg-min; see "Experimental Procedures") agrees well with the net Na\(^+\) flux in A-particles measured directly (75 neq per mg-min). Passive swelling measurements thus appear quite reliable but also offer certain advantages over other methods such as the "oxygen pulse" tech-

---

**Table IV**

*Endogenous cation-H\(^+\) exchange system of submitochondrial particles*

Representative Na\(^+\):K\(^+\) selectivity values were derived from measurements such as shown in Fig. 5. Maximum Na\(^+\):K\(^+\) discrimination quotients and cation uptake rates were obtained with Mg\(^{2+}\) concentrations that range from 3 to 7.5 mM Mg\(^{2+}\) and at pH 7.5. The pH optimum was determined as described in the legend of Fig. 8 and the apparent \(K_m\) values as detailed in Fig. 7.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental value</th>
<th>Number of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Representative Na(^+):K(^+) selectivity</td>
<td>5.8 ± 0.5</td>
<td>6</td>
</tr>
<tr>
<td>Maximum observed Na(^+):K(^+) selectivity</td>
<td>10.6 ± 3.1</td>
<td>3</td>
</tr>
<tr>
<td>Maximum observed cation flux (net/ mg-min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na(^+)</td>
<td>144.0 ± 15.3</td>
<td>3</td>
</tr>
<tr>
<td>K(^+)</td>
<td>14.7 ± 5.8</td>
<td>3</td>
</tr>
<tr>
<td>pH Optimum</td>
<td>7.25 - 7.50</td>
<td>3</td>
</tr>
<tr>
<td>Apparent (K_m) (mM)</td>
<td>1.0 ± 0.3</td>
<td>3</td>
</tr>
</tbody>
</table>

---

**Table V**

*Comparison of natural cation-H\(^+\) exchange system with model exchanger, nigericin*

<table>
<thead>
<tr>
<th>Addition(s)</th>
<th>Cation uptake rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+) uptake</td>
<td>H(^+) uptake</td>
</tr>
<tr>
<td>None</td>
<td>71</td>
</tr>
<tr>
<td>MgCl(_2) (7.5 mM)</td>
<td>138</td>
</tr>
<tr>
<td>Nigericin</td>
<td>179</td>
</tr>
<tr>
<td>Nigericin + MgCl(_2)</td>
<td>100</td>
</tr>
</tbody>
</table>

---

M. Douglas and R. Cockrell, unpublished observations.
nique of Mitchell and Moyle (2, 37) which requires extensive anaerobic equilibration of mitochondria (e.g. 20 min; see Ref. 37) during which their membrane permeability may change.

Incubation of mitochondria anaerobically for 20 min in the absence of EGTA causes uncoupling as evidenced by loss of respiratory control (38). We have observed that after 20 min, aerobic incubation without exogenous substrate, Na\(^+\), but not K\(^+\) or choline, uncouples mitochondria (11). This may be a consequence of Mg\(^{2+}\) loss (39) which can increase mitochondrial Na\(^+\) conductance (40). Na\(^+\)-dependent uncoupling would reflect Na\(^+\) conductance plus neutral Na\(^+\)-H\(^+\) exchange resulting in cyclic Na\(^+\) and proton flow. This mechanism might explain early reports of different influences of Na\(^+\) and K\(^+\) upon mitochondrial respiration and phosphorylation (41, 42). Therefore, caution must be exercised in interpreting measurements of monovalent cation-H\(^+\) exchange after extensive incubation at a relatively low energy state such as prevails in oxygen pulse experiments (3). Permeability properties may change as already mentioned and could in part account for certain apparent discrepancies between previous observations related to cation-H\(^+\) exchange, e.g. pH dependence (3, 21).

According to oxygen pulse measurements, Na\(^+\)-H\(^+\) exchange declines as the pH is increased from 6.8 to 8.0; furthermore, K\(^+\)-H\(^+\) exchange activity is extremely low except at pH values of 6.0 or below (3). Brierley et al. (21) observed that apparent endogenous Na\(^+\)-H\(^+\) exchange was relatively insensitive to pH although these results were obtained with beef heart rather than rat liver mitochondria employed in the oxygen pulse studies. The results of the present study (cf. Fig. 2) for the pH dependence of endogenous exchange agree qualitatively with those reported by Mitchell and Moyle (3) at least over the pH interval from 6.5 to 8.0. Our observations do not agree with the report of substantial K\(^+\)-H\(^+\) exchange at low pH (2, 3). The almost negligible K\(^+\)-H\(^+\) exchange described here supports Caswell's suggestion (43) that no significant K\(^+\)-H\(^+\) exchange occurs in isolated mitochondria.

In the present investigation mitochondrial- and submitochondrial particles-catalyzed K\(^+\)-H\(^+\) exchange differed significantly. The apparent K\(^+\) flux computed for submitochondrial particles, 0.2 neq per min-mg was approximated 2.5 times the calculated K\(^+\) flux derived from passive mitochondrial swelling experiments (2.4 neq per mg-min). It was pointed out that submitochondrial particles possessed significant endogenous monovalent cation (predominantly Na\(^+\)). The highly selective K\(^+\)-conducting ionophore, valinomycin (44), was found to inhibit the apparent K\(^+\) uptake of submitochondrial particles by 50% whereas FCCP inhibited completely (45). Apparently, only about one-half the observed submitochondrial particles K\(^+\) flux was actually K\(^+\) uptake and the remainder was most likely translocation of endogenous cation (presumably Na\(^+\)). If this is so, then the actual K\(^+\) flux in submitochondrial particles is nearly identical with that of mitochondria and in turn, the calculated Na\(^+\)-K\(^+\) selectivity (approximately 20:1) agrees favorably with values for mitochondria. Thus monovalent cation-H\(^+\) exchange by mitochondria and submitochondrial particles is highly Na\(^+\)-selective with a discrimination between Na\(^+\) and K\(^+\) of 50:1 or greater in mitochondria. It should be recalled that Mg\(^{2+}\) stimulated Na\(^+\) uptake by submitochondrial particles substantially but not K\(^+\) accumulation (Fig. 9). This could indicate that Na\(^+\) and K\(^+\) transport are mediated by separate exchangers with nearly absolute selectivities for the respective cations. Isolation and reconstitution studies might resolve this question and afford more reliable cation selectivity values. Results obtained with nigericin, however, underscore possible limitations of determining cation discrimination depending upon the method(s) employed.

The striking difference between K\(^+\):Na\(^+\) selectivity values for nigericin according to equilibrium measurements (e.g. cation complexation by two-phase distribution), about 70:1, and kinetic values, ranging from about 4:1 to unity, is illustrated by the tabulations of Table VI. This may be relevant to the mitochondrial cation-H\(^+\) exchange system. If endogenous mitochondrial cation-H\(^+\) exchange is mediated by a nigericin-like ionophore(s), the isolated moiety need not exhibit the same Na\(^+\)-K\(^+\) discrimination derived from kinetic studies with mitochondria and submitochondrial particles. This is a factor to be considered in any comparable reconstitution study.

The combined mitochondrial and submitochondrial particles studies described here provide strong support for the existence of a highly specific system which catalyzes electrically neutral Na\(^+\) for proton exchange. The physiological role of this process is not known. It was suggested that neutral cation-H\(^+\) exchange might perform the function of osmotic regulation in mitochondria (10). We have found that Na\(^+\) but not K\(^+\) inhibits the oxidation of various substrates by mitochondria in vitro (11) and in situ, e.g. in isolated rat liver cells (11, 47). These results are consistent with operation of Na\(^+\)-H\(^+\) exchange within the cell.

Thus Na\(^+\) could affect the distribution of anionic metabolites between mitochondrial and cytoplasmic compartments and thereby influence a variety of metabolic conversions within the cell. Now that the Na\(^+\)-H\(^+\) exchange system has been better characterized, it will be possible to explore more reliably possible relationships between mitochondrial ion transport in general and cellular metabolism.

Acknowledgment—The authors wish to thank Mr. R. B. Work who performed the swelling experiments.

REFERENCES
Mitochondrial Cation-Hydrogen Ion Exchange: SODIUM SELECTIVE TRANSPORT BY MITOCHONDRIA AND SUBMITOCHONDRIAL PARTICLES
Michael G. Douglas and Ronald S. Cockrell


Access the most updated version of this article at http://www.jbc.org/content/249/17/5464

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/17/5464.full.html#ref-list-1