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Sensitivity of Mitochondrial Mg^{++} Flux to Reagents Which Affect K^+ Flux[1]

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Abstract

Effects on Mg^{++} transport in rat liver mitochondria of three reagents earlier shown to affect mitochondrial K^+ transport have been examined. The sulfhydryl reactive reagent phenylarsine oxide, which activates K^+ flux into respiring mitochondria, also stimulates Mg^{++} influx. The K^+ analog Ba^{++}, when taken up into the mitochondrial matrix, inhibits influx of both K^+ and Mg^{++}. The effect on Mg^{++} influx is seen only if Mg^{++}, which blocks Ba^{++} accumulation, is added after a preincubation with Ba^{++}. Thus the inhibition of Mg^{++} influx appears to require interaction of Ba^{++} at the matrix side of the inner mitochondrial membrane. Added Ba^{++} also diminishes observed rates of Mg^{++} efflux but not K^+ efflux. This difference may relate to a higher concentration of Ba^{++} remaining in the medium in the presence of Mg^{++} under the conditions of our experiments. Pretreatment of mitochondria with dicyclohexylcarbodiimide (DCCD), under conditions which result in an increase in the apparent K_m for K^+ of the K^+ influx mechanism, results in inhibition of Mg^{++} influx from media containing approximately 0.2 mM Mg^{++}. The inhibitory effect of DCCD on Mg^{++} influx is not seen at higher external Mg^{++} (0.8 mM). This dependence on cation concentration is similar to the dependence on K^+ concentration of the inhibitory effect of DCCD on K^+ influx. Although mitochondrial Mg^{++} and K^+ transport mechanisms exhibit similar reagent sensitivities, whether Mg^{++} and K^+ share common transport catalysts remains to be established.

Key Words: Mg^{++} flux; mitochondrial; Ba^{++}; phenylarsine oxide; DCCD.

Introduction

Mitochondrial Mg^{++} and K^+ transport catalysts exhibit many similarities. Unidirectional and net fluxes of Mg^{++}, both into and out of heart and liver mitochondria, are sensitive to respiratory inhibitors and uncouplers

[1] Abbreviations used: DCCD, dicyclohexylcarbodiimide; PheAsO, phenylarsine oxide.

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(Brierley *et al.*, 1963; Johnson and Pressman, 1969; Kun, 1976; Crompton *et al.*, 1976; Hoser *et al.*, 1978; Siliprandi *et al.*, 1978a; Diwan *et al.*, 1979; Brierley *et al.*, 1987). Both influx and efflux of K^+ are also respiration dependent (Diwan and Tedeschi, 1975; Jung *et al.*, 1977; Chavez *et al.*, 1977). The respiration dependence has been explained in relation to the chemiosmotic theory by the proposal that a uniporter mediates electrophoretic K^+ entry, while the transmembrane pH gradient drives K^+ efflux via a K^+/H^+ antiporter (Mitchell and Moyle, 1969; Jung *et al.*, 1977; Chavez *et al.*, 1977). However, both the influx and efflux of K^+ increase with increasing external pH (Diwan and Lehrer, 1978; Diwan, 1981). In part on the basis of observed inhibition by nigericin, added to equilibrate transmembrane H^+ and K^+ gradients, a cation/proton antiporter has been postulated to also mediate Mg^{++} efflux (Åkerman, 1981).

Addition of ADP in the presence of P_i has been found to inhibit uptake and loss of Mg^{++} as well as K^+ (Jung *et al.*, 1977; Chavez *et al.*, 1977; Brierley *et al.*, 1987). Other studies have shown either little effect of ADP on Mg^{++} efflux in the presence of P_i (Crompton *et al.*, 1976) or activated loss of endogenous Mg^{++} under state 3 conditions (Masini *et al.*, 1983).

Unidirectional fluxes of Mg^{++} and K^+ into respiring mitochondria, measured by means of the radioisotopes ^{28}Mg and ^{42}K, exhibit saturability (Diwan and Lehrer, 1977, 1978; Jung *et al.*, 1977; Diwan *et al.*, 1979). The apparent K_m for Mg^{++} of the mechanism mediating Mg^{++} flux into rat liver mitochondria was estimated to be about 0.7 mM (Diwan *et al.*, 1979). Mg^{++} competitively inhibits K^+ influx (Jung *et al.*, 1977; Diwan *et al.*, 1979). Physiological concentrations of K^+ in the medium markedly slow Mg^{++} entry (Brierley *et al.*, 1987). The K^+ analog Tl^+ competitively inhibits influx of both Mg^{++} and K^+ (Diwan and Lehrer, 1977; Diwan *et al.*, 1979). The K^+ analog Ba^{++} is rapidly accumulated by respiring mitochondria (Vainio *et al.*, 1970). The accumulated Ba^{++} decreases the measured V_{max} of K^+ influx, while having little apparent effect on K^+ efflux (Diwan, 1985).

Ruthenium red, an inhibitor of mitochondrial Ca^{++} transport, has been found not to affect uptake or loss of Mg^{++} (Crompton *et al.*, 1976; Brierley *et al.*, 1987). However, ruthenium red has been observed to inhibit Mg^{++} flux into mitochondria treated with digitonin (Kun, 1976) and Mg^{++} efflux under conditions involving apparent linkage to cyclic Ca^{++} flux (Siliprandi *et al.*, 1978a, b). La^{+++}, which also inhibits mitochondrial Ca^{++} transport, is reported not to affect Mg^{++} or K^+ influx (Kun, 1976; Diwan *et al.*, 1979).

Mg^{++} efflux from respiring mitochondria exhibits little sensitivity to external K^+ (Brierley *et al.*, 1987). Depletion of endogenous Mg^{++} results in increased K^+ permeability, which has been attributed to release from regulation by Mg^{++} of the proposed K^+/H^+ antiporter (Azzone *et al.*, 1978;

Garlid, 1980; Jung *et al.*, 1981; Nakashima *et al.*, 1982; Bernardi and Azzone, 1983). The physiological significance of a permeability pathway which is activated by Mg^{++} depletion has been questioned (Jung *et al.*, 1984, Jung and Brierley, 1986). Measurements with isolated hepatocytes indicate the absence of a significant gradient of free Mg^{++} between the cytosolic and mitochondrial compartments (Corkey *et al.*, 1986).

Mercurial sulfhydryl reagents, including mersalyl, markedly increase rates of influx as well as efflux of K^+ (Jung *et al.*, 1977; Chavez *et al.*, 1977; Diwan *et al.*, 1977; Bogucka and Wojtczak, 1979). Stimulation of Mg^{++} entry by mersalyl has been reported (Diwan *et al.*, 1980; Brierley *et al.*, 1987). The stimulatory effect of mersalyl on Mg^{++} flux into respiring liver mitochondria is less than the effect on K^+ influx (Diwan *et al.*, 1980). Partial inhibition by mersalyl of net Mg^{++} efflux from heart mitochondria has been observed (Brierley *et al.*, 1987).

Other sulfhydryl-reactive reagents which activate K^+ flux into respiring mitochondria include Cd^{++} (Rasheed *et al.*, 1984) and PheAsO (Diwan *et al.*, 1986). PheAsO can form stable adducts with vicinal dithiols, such as that which has been identified in the ATP synthase subunit, Coupling Factor B (Fluharty and Sanadi, 1960; Stiggall *et al.*, 1979; Joshi and Hughes, 1981; Sanadi, 1982). However, the stimulation of K^+ influx by PheAsO was found to be blocked by subsequent treatment with the monothiol 2-mercaptoethanol; thus it was concluded that the reactive group involved in activation of K^+ influx by PheAsO is a monothiol, not a dithiol (Diwan *et al.*, 1986).

Quinine inhibits various mitochondrial K^+ transport activities which have been attributed to the proposed K^+/H^+ antiporter. These include the loss of endogenous K^+ activated by Mg^{++} depletion, the respiration-dependent contraction of mitochondria swollen in K^+ nitrate, and the swelling of Mg^{++}-depleted mitochondria in K^+ acetate (Nakashima and Garlid, 1982; Jung *et al.*, 1984; Garlid *et al.*, 1986). Quinine also partially inhibits influx, and at high concentrations fully blocks efflux, of Mg^{++} as well as K^+, in mitochondria not treated to deplete endogenous Mg^{++} (Diwan, 1986).

Pretreatment with DCCD results in inhibition of K^+ influx (Gauthier and Diwan, 1979; Jung *et al.*, 1980). DCCD increases the apparent K_m for K^+ of the K^+ influx mechanism, while having little effect on the measured V_{max} of K^+ influx; thus DCCD has little effect on K^+ influx at a high external K^+ (Gauthier and Diwan, 1979). Inhibition of apparent K^+/H^+ antiport activity is not observed (Jung *et al.*, 1980), unless the mitochondria are depleted of endogenous Mg^{++} prior to DCCD treatment (Martin *et al.*, 1984, 1986). An 82,000 dalton protein, postulated to have a role in mediating K^+/H^+ exchange, has been identified on the basis of the Mg^{++} and quinine sensitivity of its reactivity with [^{14}C]DCCD (Martin *et al.*, 1984). DCCD pretreatment results in inhibition of net Mg^{++} uptake by heart mitochondria respiring

in the presence of P_i, while having no apparent effect on Mg^{++} efflux (Brierley *et al.*, 1987).

The present studies have examined effects on unidirectional Mg^{++} flux of three reagents which affect mitochondrial K^+ flux, Ba^{++}, PheAsO, and DCCD.

Materials and Methods

Rat liver mitochondria were isolated by standard procedures. The 0.25 M sucrose isolation medium was supplemented with 0.4 mM Tris EGTA in the initial stages of preparation, except that in experiments involving Ba^{++} sucrose alone was used. Mitochondria (4–7 mg protein per ml) were incubated at 20°C in media (unless specified otherwise) containing 200 mM sucrose, 3 mM succinic acid, 16 mM Tris, and 0.8 mM $MgCl_2$, with the pH adjusted to 7.5 with HCl, plus ^{28}Mg (approximately 0.2 μCi/ml, obtained from Brookhaven National Laboratory), ^3H_2O (approximately 3 μCi/ml, from NEN Research Products), and in some experiments [^{14}C]sucrose (approximately 0.8 μCi/ml, from NEN Research Products). When included, PheAsO or $BaCl_2$ were obtained from Aldrich Chemical Co. DCCD-treated mitochondria were preincubated at 0°C for at least 40 min with 30 nmol DCCD (from Sigma Chemical Co.) per mg protein.

Mitochondrial samples were separated from incubation media by rapid centrifugation through silicone (Harris and VanDam, 1968). The silicone used (SF1154) was a generous gift of the General Electric Co. ^{28}Mg was assayed by counting of the Cerenkov radiation in aqueous dilutions of mitochondrial and supernatant samples, and the counts were corrected for decay. Following decay of the ^{28}Mg, total Mg^{++} levels were assayed by atomic absorption spectroscopy, and ^3H and ^{14}C were counted using a standard liquid scintillation cocktail. Protein was measured by the biuret technique (Layne, 1957). Fluid compartments, and mitochondrial contents of total and labeled Mg^{++}, were calculated as in earlier studies (Johnson and Pressman, 1969; Diwan *et al.*, 1979). Unless stated otherwise, unidirectional Mg^{++} influx is calculated from the change in labeled Mg^{++} content between samples taken after 0.5 and 7.0 min of incubation. The net Mg^{++} flux is the change in total Mg^{++} content during the same time period. Unidirectional Mg^{++} efflux is calculated as the difference between influx and net flux rates.

Results

Figure 1 shows the effect on ^{28}Mg uptake by respiring rat liver mitochondria of 8.5 nmol PheAsO per mg protein, a concentration found to

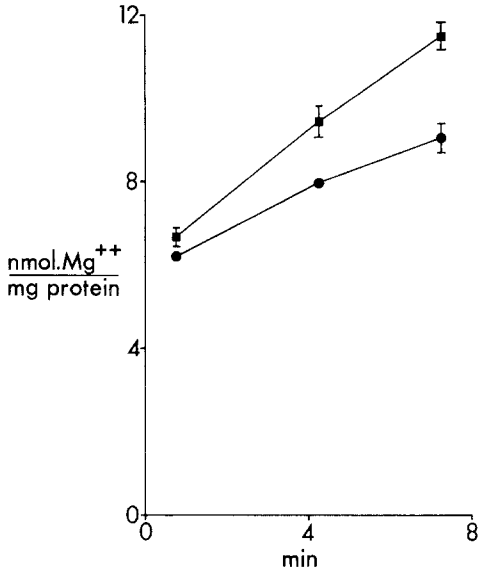

Fig. 1. Effect of PheAsO on the time course of ^{28}Mg uptake. The mitochondrial content of labeled Mg^{++}, in units of nmol per mg protein, is plotted against the incubation time in min. Mg^{++} contents are corrected for contaminating external Mg^{++} sedimented with the mitochondria, estimated from the product of the [^{14}C]sucrose distribution space and the supernantant Mg^{++} concentration (0.25 mM). The incubation medium included 8 mM succinic acid, with the pH adjusted to 7.5 with Tris. Shown are means of three measurements. Standard deviations are indicated when in excess of the symbol height. Symbols: ●, control samples; ■, the medium included 8.5 nmol PheAsO per mg protein.

substantially activate K^+ influx (Diwan *et al.*, 1986). PheAsO increases the unidirectional Mg^{++} influx rate, corresponding to the slope of the uptake curve, without appreciably affecting the extrapolated zero time ^{28}Mg uptake. The rapid ^{28}Mg binding, which occurs before the initial sample is taken, has been attributed to adsorption (Johnson and Pressman, 1969). This component of Mg^{++} binding is not dependent on respiration, while the progressive increase in ^{28}Mg content, assumed to reflect transport into the mitochondrial matrix, is respiration dependent (Johnson and Pressman, 1969; Diwan *et al.*, 1979; Brierley *et al.*, 1987).

Unidirectional flux rates calculated from average values determined in five similar experiments are summarized in Table I, A. Both the influx and efflux of Mg^{++} are activated by PheAsO. The observed percent stimulation of Mg^{++} influx varied in these experiments from 31 to 68%, with the average stimulation being 46%. In two experiments carried out at a higher Mg^{++} concentration (0.8 mM), stimulation of Mg^{++} influx by PheAsO was also observed. There was more variability in the percent stimulation of Mg^{++}

Table I. Effects of PheAsO and Ba^{++} on Mg^{++} Flux Rates[a]

	Additions	nmol Mg^{++}/mg protein (min)	
		Mg^{++} influx	Mg^{++} efflux
A	None	0.48 ± 0.10	0.59 ± 0.12
	PheAsO	0.70 ± 0.11	0.75 ± 0.05
B	None	0.94 ± 0.12	0.75 ± 0.16
	Ba^{++}	0.96 ± 0.08	0.34 ± 0.10
C	None	0.98 ± 0.11	0.61 ± 0.13
	Ba^{++}, 1 min preincubation	0.55 ± 0.12	0.26 ± 0.07

[a]In A, Mg^{++} flux rates are means of average values determined in five experiments ± SEM. PheAsO when present was at 8.5 nmol per mg protein (51–60 μM in individual experiments). The medium included 8 mM succinic acid, with the pH adjusted to 7.5 with Tris. The Mg^{++} concentration in the medium was 0.19–0.26 mM in individual experiments. In B, Mg^{++} flux rates, determined between 1 and 7 min of incubation, are means of nine measurements pooled from two experiments ± SD. Ba^{++} when present was at 150 μM (23 nmol per mg protein). The medium included 0.81 mM Mg^{++}. In C, Mg^{++} flux rates, determined from 1.5 to 7 min of incubation, are means of average values obtained in four separate experiments ± SEM. Ba^{++} when present was at 150 μM (29–38 nmol per mg protein in individual experiments). $MgCl_2$ was added with the ^{28}Mg at 1 min to a total concentration of 0.80 mM.

efflux from one experiment to another. Although the pooled data show a significant effect, stimulation of Mg^{++} efflux was not observed in every experiment. Generally efflux values vary more than influx values, because values of Mg^{++} efflux are based in part on atomic absorption assays of total Mg^{++}, which are less precise than the radioisotope counts.

Initial studies of effects of Ba^{++} on mitochondrial Mg^{++} flux were carried out under conditions similar to those used in published studies of effects of Ba^{++} on K^+ flux (Diwan, 1985). However, 0.8 mM Mg^{++} was included in the medium with the ^{28}Mg, in place of the 3–4 mM K^+ present during the ^{42}K flux studies. Flux rates were determined between 1 and 7 min of incubation. The results, summarized in Table I, B, indicate that 150 μM Ba^{++} (23 nmol per mg protein) partially inhibited Mg^{++} efflux, but not Mg^{++} influx. Approximately the same concentration of Ba^{++} was earlier found to cause partial inhibition of K^+ influx, while within the error level no effect on K^+ efflux was discernible (Diwan, 1985).

A possible basis for this difference is apparent in observations of ^{133}Ba uptake. In equivalent media containing 3–4 mM K^+ with no added Mg^{++}, it was earlier determined that Ba^{++} rapidly enters the mitochondria, approaching within 1–2 min a steady-state distribution in which most of the Ba^{++} is within the mitochondria (Diwan, 1985). Similar results are shown in Fig. 2 for mitochondria in the K^+-containing medium. Even more rapid Ba^{++} uptake was earlier found at lower K^+ concentration (1 mM) (Diwan, 1985). The results shown in Fig. 2 indicate that Ba^{++} uptake by mitochondria

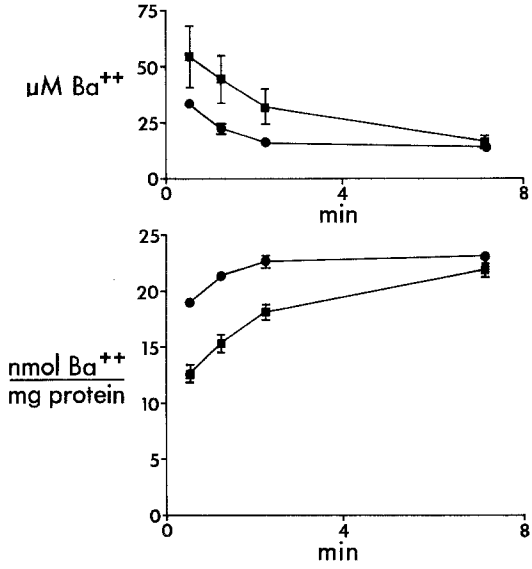

Fig. 2. ^{133}Ba uptake by respiring mitochondria. The upper graph is a plot of the ^{133}Ba content of the incubation medium as a function of incubation time. The initial Ba^{++} concentration was 150 μM. The lower graph is a plot of the ^{133}Ba content of the mitochondria, in units of nmol per mg protein, as a function of incubation time in min. The incubation medium was as indicated in Materials and Methods except for the presence of Ba^{++} and the following: ●, the medium included 3.2 mM KCl (no added Mg^{++}); ■, the medium included 0.8 mM Mg^{++} (no added K^+). Values are means of three (K^+ medium) or five (Mg^{++} medium) determinations, with standard deviations shown when in excess of the height of the symbols.

is substantially slower when Mg^{++}, at the level present during ^{28}Mg flux measurements, is substituted for the K^+ level present during earlier studies of effects of Ba^{++} on K^+ flux. Therefore, additional experiments were carried out in which the mitochondria were preincubated with Ba^{++} for 1 min prior to addition of the ^{28}Mg with unlabeled Mg^{++}, and flux rates were measured between 1.5 and 7 min of incubation. Table I, C, summarizes data from four such experiments. The data show significant inhibition by Ba^{++} of influx as well as efflux of Mg^{++}, when Ba^{++} uptake is promoted by omitting Mg^{++} during the first minute of incubation.

The experiment depicted in Table II examines the effect of pretreatment with DCCD on Mg^{++} flux into respiring mitochondria. The conditions of DCCD treatment used are the same as those previously shown to increase the apparent K_m for K^+ of the K^+ influx mechanism (Gauthier and Diwan, 1979). The results show partial inhibition of Mg^{++} influx by DCCD when the Mg^{++} concentration in the medium is 0.22 mM. When the medium contains 0.78 mM Mg^{++}, there is no detectable effect of the DCCD pretreatment on

Table II. Effect of DCCD on Mg^{++} Influx[a]

mM Mg^{++}	Mg^{++} influx, nmol/mg protein (min)	
	Control mitochondria	DCCD-treated mitochondria
0.22	0.38 ± 0.04	0.24 ± 0.04
0.78	0.82 ± 0.09	0.81 ± 0.10

[a] Mg^{++} flux values are means of four determinations ± SD. The medium included 8 mM succinic acid, with the pH adjusted to pH 7.5 with Tris. The Mg^{++} concentration was varied as indicated.

Mg^{++} influx. In four such experiments, DCCD consistently had no significant effect on Mg^{++} influx from media containing approximately 0.8 mM Mg^{++}. The extent of inhibition observed at low external Mg^{++} was somewhat variable. In a total of 12 experiments carried out, the inhibition by DCCD in the presence of approximately 0.2 mM Mg^{++} was 20 ± 13% (mean ± SEM). In these experiments there was not any consistent effect of DCCD on Mg^{++} efflux.

Discussion

The stimulatory effect of PheAsO on mitochondrial Mg^{++} influx is similar to the effect of this reagent on K^+ influx. The physiological significance of the activation of mitochondrial K^+ and Mg^{++} transport by sulfhydryl modification is unclear. Perhaps the effect of the artificial activating reagents reflects the triggering of some as yet undefined regulatory mechanism.

On the basis of the observed sensitivity of Ba^{++} uptake to inhibitors of Ca^{++} uptake, it has been proposed that Ba^{++} enters mitochondria at least in part via the Ca^{++} influx mechanism (Vainio *et al.*, 1970; Lukacs and Fonyo, 1985). That uptake of Ba^{++} involved transport into the matrix compartment is indicated by the respiration dependence of Ba^{++} uptake (Vainio *et al.*, 1970; Diwan, 1985; Lukacs and Fonyo, 1985). The observed sensitivity to Mg^{++} of Ba^{++} uptake is consistent with earlier observations (Vainio *et al.*, 1970).

As indicated by the data in Table I, C, Ba^{++}, when taken up into the mitochondria, inhibits Mg^{++} influx. This is similar to the effect of accumulated Ba^{++} on K^+ flux into respiring mitochondria (Diwan, 1985). Inhibition of Mg^{++} influx is not observed when the uptake of externally added Ba^{++} is diminished by the presence of Mg^{++} in the medium. Thus inhibition of Mg^{++} influx appears to require interaction of Ba^{++} at the

matrix side of the inner mitochondrial membrane. Under the conditions of these experiments, Ba^{++} also causes some inhibition of Mg^{++} efflux. Such an effect of Ba^{++} on K^+ efflux was not observed (Diwan, 1985). However, a higher concentration of Ba^{++} remaining in the medium during the incubations in the presence of Mg^{++} may account for this difference. Specificity with regard to the side of the membrane at which Ba^{++} inhibition is observable has been reported for other cation transport mechanisms. For example, Ca^{++} efflux from rat liver mitochondria, which appears to be mediated at least in part by a Na^+-independent Ca^{++}/H^+ antiporter, is inhibited by Ba^{++} only when Ba^{++} is present in the matrix compartment (Lukacs and Fonyo, 1985). In contrast, the Na^+-dependent Ca^{++} efflux mechanism in heart mitochondria is inhibited by Ba^{++} at the cytoplasmic surface of the mitochondria (Lukacs and Fonyo, 1986).

The evidence indicating that Mg^{++} influx, like K^+ influx, is inhibited only at low concentration of the cationic substrate suggests a similar interaction of DCCD with the K^+ and Mg^{++} influx catalyst(s). While inhibition by DCCD of Mg^{++} flux into rat liver mitochondria was not observed when the Mg^{++} concentration was increased to 0.8 mM (see Table II), substantial inhibition by DCCD of Mg^{++} uptake by heart mitochondria was earlier observed in the presence of 3 mM Mg^{++} (Brierley *et al.*, 1987). In addition to the different tissue source of the mitochondria, the experiments with heart mitochondria differed from the present studies in focusing on the effect of DCCD on the large net uptake of Mg^{++} observed in the presence of added P_i. Whether there is a dependence on Mg^{++} concentration of the effect of DCCD on Mg^{++} flux into heart mitochondria, in the presence or absence of P_i, was not reported.

Although the processes of transport of Mg^{++} and K^+ into and out of mitochondria exhibit many similarities, whether these cations, which differ in charge and ionic size, share common transport mechanisms is unclear. The transport mechanisms for Mg^{++} and K^+ might be distinct but very similar, e.g., because of common evolutionary origins. Several nonmitochondrial ATPases involved in cation transport are known to be similar to one another in structure and mechanism and share some sequence homology (Shull *et al.*, 1985; MacLennan *et al.*, 1985). Evaluating the commonality of mitochondrial transport mechanisms for Mg^{++} and K^+ ultimately may require purification and reconstitution of the individual transporters involved in catalyzing influx and efflux of these cations. Recently a DCCD-reactive protein of approximately 53,000 daltons molecular weight has been purified from mitochondrial membranes via affinity chromatography on immobilized quinine, an inhibitor of K^+ and Mg^{++} translocation (Diwan *et al.*, 1988). The transport capabilities of this isolated protein have not yet been determined.

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References

Åkerman, K. E. O. (1981). *J. Bioenerg. Biomembr.* **13**, 133–139.
Azzone, G. F., Bortolotto, F., and Zanotti, A. (1978). *FEBS Lett.* **96**, 135–140.
Bernardi, P., and Azzone, G. F. (1983). *Biochim. Biophys. Acta* **742**, 212–223.
Bogucka, K., and Wojtczak, L. (1979). *FEBS Lett.* **100**, 301–304.
Brierley, G., Murer, E., Bachmann, E., and Green, D. E. (1963). *J. Biol. Chem.* **238**, 3482–3489.
Brierley, G., Davis, M., and Jung, D. W. (1987). *Arch. Biochem. Biophys.* **253**, 322–332.
Chavez, E., Jung, D. W., and Brierley, G. P. (1977). *Arch. Biochem. Biophys.* **183**, 460–470.
Corkey, B. E., Duszynski, J., Rich, T. L., Matschinsky, B., and Williamson, J. R. (1986). *J. Biol. Chem.* **261**, 2567–2574.
Crompton, M., Capano, M., and Carafoli, E. (1976). *Biochem. J.* **154**, 735–742.
Diwan, J. J. (1981). *Biochem. Soc. Trans.* **9**, 153–154.
Diwan, J. J. (1985). *J. Membr. Biol.* **84**, 165–171.
Diwan, J. J. (1986). *Biochem. Biophys. Res. Commun.* **135**, 830–836.
Diwan, J. J. and Lehrer, P. H. (1977). *Biochem. Soc. Trans.* **5**, 203–205.
Diwan, J. J., and Lehrer, P. H. (1978). *Membr. Biochem.* **1**, 43–60.
Diwan, J. J., and Tedeschi, H. (1975). *FEBS Lett.* **60**, 176–179.
Diwan, J. J., Markoff, M., and Lehrer, P. H. (1977). *Indian J. Biochem. Biophys.* **14**, 342–346.
Diwan, J. J., Dazé, M., Richardson, R., and Aronson, D. (1979). *Biochemistry* **18**, 2590–2595.
Diwan, J. J., Aronson, D., and Gonsalves, N. O. (1980). *J. Bioenerg. Biomembr.* **12**, 205–212.
Diwan, J. J., Srivastava, J., Moore, C., and Haley, T. (1986). *J. Bioenerg. Biomembr.* **18**, 123–134.
Diwan, J. J., Moore, C., Haley, T., Herbrandson, H. F., and Sanadi, D. R. (1988). In *Advances in Membrane Biochemistry and Bioenergetics* (Kim, C., Tedeschi, H., Diwan, J., and Salerno, J., eds.), Plenum Press, New York.
Fluharty, A., and Sanadi, D. R. (1960). *Proc. Natl. Acad. Sci. USA* **46**, 608–616.
Garlid, K. D. (1980). *J. Biol. Chem.* **255**, 11273–11279.
Garlid, K. D., DiResta, D. J., Beavis, A. D., and Martin, W. H. (1986). *J. Biol. Chem.* **261**, 1529–1535.
Gauthier, L. M., and Diwan, J. J. (1979). *Biochem. Biophys. Res. Commun.* **87**, 1072–1079.
Harris, E. J., and VanDam, K. (1968). *Biochem. J.* **106**, 759–766.
Hoser, N., Dawczynski, H., Winnefeld, K., and Dargel, R. (1978). *Acta Biol. Med. Ger.* **37**, 19–29.
Johnson, J. H., and Pressman, B. C. (1969). *Arch. Biochem. Biophys.* **132**, 139–145.
Joshi, S., and Hughes, J. B. (1981). *J. Biol. Chem.* **256**, 11112–11116.
Jung, D. W., and Brierley, G. P. (1986). *J. Biol. Chem.* **261**, 6408–6415.
Jung, D. W., Chavez, E., and Brierley, G. P. (1977). *Arch. Biochem. Biophys.* **183**, 452–459.
Jung, D. W., Shi, G.-Y., and Brierley, G. P. (1980). *J. Biol. Chem.* **255**, 408–412.
Jung, D. W., Shi, G.-Y., and Brierley, G. P. (1981). *Arch. Biochem. Biophys.* **209**, 356–361.
Jung, D. W., Farooqui, T., Utz, E., and Brierley, G. P. (1984). *J. Bioenerg. Biomembr.* **16**, 379–390.
Kun, E. (1976). *Biochemistry* **15**, 2328–2336.
Layne, E. (1957). *Methods Enzymol.* **3**, 447–454.
Lukacs, G. L., and Fonyo, A. (1985). *Biochim. Biophys. Acta* **809**, 160–166.
Lukacs, G. L., and Fonyo, A. (1986). *Biochim. Biophys. Acta* **858**, 125–134.
MacLennan, D. H., Brandl, C. J., Korczak, B., and Green, N. M. (1985). *Nature (London)* **316**, 696–700.
Martin, W. H., Beavis, A. D., and Garlid, K. D. (1984). *J. Biol. Chem.* **259**, 2062–2065.
Martin, W. H., DiResta, D. J., and Garlid, K. D. (1986). *J. Biol. Chem.* **261**, 12300–12305.

Masini, A., Ceccarelli-Stanzani, D., and Muscatello, U. (1983). *J. Bioenerg. Biomembr.* **15**, 217–234.
Mitchell, P., and Moyle, J. (1969). *Eur. J. Biochem.* **9**, 149–155.
Nakashima, R. A., and Garlid, K. D. (1982). *J. Biol. Chem.* **257**, 9252–9254.
Nakashima, R. A., Dordick, R. S., and Garlid, K. D. (1982). *J. Biol. Chem.* **257**, 12540–12545.
Rasheed, B. K. A., Diwan, J. J., and Sanadi, D. R. (1984). *Eur. J. Biochem.* **144**, 643–647.
Sanadi, D. R. (1982). *Biochim. Biophys. Acta* **683**, 39–56.
Shull, G. E., Shwartz, A., and Lingrel, J. B. (1985). *Nature* (*London*) **316**, 691–695.
Siliprandi, N., Siliprandi, D., Toninello, A., Rugolo, M., and Zoccarato, F. (1978a). In *The Proton and Calcium Pumps* (Azzone, G. F., Avron, M., Metcalfe, J. C., Quagliariello, E., and Siliprandi, N., eds.), Elsevier/North Holland, Amsterdam, pp. 263–271.
Siliprandi, D., Toninello, A., Zoccarato, R., Rugolo, M., and Siliprandi, N. (1978b). *J. Bioenerg. Biomembr.* **10**, 1–11.
Stiggall, D. L., Galante, Y. M., Kiehl, R., and Hatefi, Y. (1979). *Arch Biochem. Biophys.* **196**, 638–644.
Vainio, H., Mela, L., and Chance, B. (1970). *Eur. J. Biochem.* **12**, 387–391.