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Kinetics of Mg^{2+} Flux into Rat Liver Mitochondria†

Joyce Johnson Diwan,* Michel Dazé, Ronald Richardson, and David Aronson

ABSTRACT: Unidirectional fluxes of Mg^{2+} across the limiting membranes of rat liver mitochondria have been measured in the presence of the respiratory substrate succinate by means of the radioisotope ^{28}Mg. Rates of both influx and efflux of Mg^{2+} are decreased when respiration is inhibited. A linear dependence of the reciprocal of the Mg^{2+} influx rate on the reciprocal of the external Mg^{2+} concentration is observed. The apparent K_m for Mg^{2+} averages about 0.7 mM. *N*-Ethylmaleimide, an inhibitor of transmembrane phosphate–hydroxyl exchanges, enhances the observed pH dependence of Mg^{2+} influx. In the presence of MalNEt, the apparent V_{max} of Mg^{2+} influx is greater at pH 8 than at pH 7, and there is a linear dependence of the Mg^{2+} influx rate on the external OH^- concentration. The K^+ analogue Tl^+ inhibits Mg^{2+} influx, while La^{3+}, an inhibitor of mitochondrial Ca^{2+} transport, has no effect on Mg^{2+} influx. Mg^{2+} competitively inhibits the flux of K^+ into rat liver mitochondria. The mechanism(s) mediating mitochondrial Mg^{2+} and K^+ fluxes appear to be similar in their energy dependence, pH dependence, sensitivity to Tl^+, and insensitivity to La^{3+}.

Rat liver mitochondria take up Mg^{2+} by an energy-dependent mechanism (Judah et al., 1965; Johnson & Pressman, 1969). It has been suggested that Mg^{2+} and K^+ may be transported into the mitochondria by a common mechanism (Judah et al., 1965). Depletion of endogenous Mg^{2+} has been shown to result in stimulation of K^+ uptake by both liver and heart mitochondria (Duszynsky & Wojtczak, 1977; Wehrle et al., 1976). Competitive inhibition by Mg^{2+} of K^+ flux into heart mitochondria has been reported (Jung et al., 1977). Other data suggest competitive inhibition by Mg^{2+} of Ca^{2+} transport in both liver and heart mitochondria (Hutson et al., 1976; Parr & Harris, 1976). Energy-dependent net Mg^{2+} efflux from rat liver mitochondria associated with Ca^{2+} uptake has been observed in the presence of added phosphate (Siliprandi et al., 1977).

Kun (1976a,b) has examined the kinetics of net Mg^{2+} uptake by mitochondria which have been treated with digitonin to remove lysosomal contaminants. The data fit a proposed kinetic model which assumes an active uptake of Mg^{2+} (Kun, 1976a).

Measurements of unidirectional K^+ flux into rat liver mitochondria have been carried out under conditions of approximately steady-state K^+ content, in which requirements for secondary counterion fluxes may be assumed to be minimal (Diwan, 1973; Diwan & Lehrer, 1977, 1978; Diwan et al., 1977). The reciprocal of the K^+ influx rate is a linear function of the reciprocal of the external K^+ concentration. K^+ influx is competitively inhibited by the K^+ analogue Tl^+. The V_{max} of K^+ influx increases when the pH of the medium is increased from 7 to 8, while the apparent K_m for K^+ remains approximately constant at about 5 mM. The pH dependence of the V_{max} of K^+ influx is increased in the presence of MalNEt[1] or mersalyl, each of which is known (Meijer et al., 1970) to block transmembrane phosphate–hydroxyl exchange. In the presence of MalNEt or mersalyl, a linear dependence of K^+ influx on external OH^- concentration is observed. On the basis of these results and evidence indicating lack of involvement of a membrane potential in driving K^+ influx (Diwan & Tedeschi, 1975), it has been postulated that K^+ may enter the mitochondria by a nonelectrogenic mechanism involving cotransport with OH^- (Diwan, 1973; Diwan et al., 1977; Diwan & Lehrer, 1978).

Brierley and co-workers have measured the dependence of unidirectional K^+ flux into beef heart mitochondria on external K^+ concentration (Jung et al., 1977). Linear Lineweaver–Burk plots are observed in agreement with the data obtained with rat liver mitochondria. However, the experiments with beef heart mitochondria indicate a higher K_m for K^+ of about 12 mM (Jung et al., 1977).

Steady-state Ca^{2+} fluxes, estimated indirectly from measurements of respiration by rat liver mitochondria in the

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[1] Abbreviations used: MalNEt, *N*-ethylmaleimide; EDTA, ethylenediaminetetraacetate.

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presence of the ionophore A23187, indicate a sigmoidal dependence on the external free Ca^{2+} concentration (Hutson et al., 1976; Heaton & Nicholls, 1976). A sigmoidal relationship between the initial rate of net Ca^{2+} uptake by rat liver mitochondria and the external Ca^{2+} concentration has also been reported (Vinogradov & Scarpa, 1973; Reed & Bygrave, 1975). Sigmoidal kinetics have also been observed for Ca^{2+} transport in heart mitochondria (Noack & Heinen, 1977). However, the sigmoidal kinetics are not observed under all conditions (Crompton et al., 1976). Harris (1977) has suggested that chelation of Ca^{2+} by materials present in reaction media and changes in internal concentrations of respiratory substrates accompanying Ca^{2+} uptake may alter the apparent kinetic relationship.

The present studies have examined the dependence of unidirectional Mg^{2+} influx on external pH and Mg^{2+} concentration, in the presence of the respiratory substrate succinate. Effects of some reagents on Mg^{2+} influx and efflux rates have also been investigated.

Materials and Methods

Rat liver mitochondria were isolated by standard procedures (Johnson & Lardy, 1967). The 0.25 M sucrose isolation medium was supplemented with 0.4 mM Tris–EDTA, pH 7.4, in the initial stages of preparation. The mitochondria were washed twice by centrifugation and resuspension in 0.25 M sucrose. Mitochondrial respiration was monitored with a Clark-type, membrane-covered oxygen electrode connected to a potentiometric recorder. Mitochondrial protein was assayed by the biuret procedure (Layne, 1957).

Mitochondria were incubated at 20 °C in media containing ^{28}Mg or ^{42}K, $^{3}H_2O$, and [^{14}C]sucrose (see figure legends for details). At timed intervals, mitochondrial samples were separated from incubation media by rapid centrifugation through silicone (Harris & VanDam, 1968). ^{28}Mg or ^{42}K counts were assayed by means of the Cerenkov radiation in aqueous dilutions of mitochondrial and supernatant samples, by using a liquid scintillation counter, and the results were corrected for decay. Following decay of the ^{28}Mg or ^{42}K, total Mg^{2+} or K^+ levels were assayed by atomic absorption, and ^{3}H and ^{14}C counts were measured by using a standard liquid scintillation counting cocktail.

$^{3}H_2O$ and [^{14}C]sucrose distribution spaces and values of total and labeled Mg^{2+} or K^+ were calculated from the data as in previous studies (Johnson & Pressman, 1969). The mitochondrial content of labeled Mg^{2+} or K^+ was determined from the ^{28}Mg or ^{42}K counts sedimented with the mitochondria and the initial (0.75- or 1-min) supernatant specific activity. The contaminating extra-mitochondrial Mg^{2+} or K^+ was calculated as the product of the [^{14}C]sucrose distribution space and the Mg^{2+} or K^+ concentration in the supernatant. Unidirectional Mg^{2+} or K^+ influx rates were calculated as the difference in mitochondrial content of labeled cation between an initial sample taken after 0.75 or 1 min of incubation and a sample taken after 7 or 8 min of incubation. For these calculations, the mitochondrial contents of labeled cation were not corrected for the contaminating extra-mitochondrial Mg^{2+} or K^+, which was estimated from the measured [^{14}C]sucrose spaces to be essentially constant during the 7- or 8-min incubations. Values of net Mg^{2+} flux were calculated similarly as the difference in total mitochondrial Mg^{2+} content between an initial (0.75-min) sample and one taken after 8 min of incubation. Values of Mg^{2+} efflux rate were calculated as the difference between influx and net flux rates.

^{28}Mg was obtained from Brookhaven National Laboratory. All other isotopes were obtained from New England Nuclear.

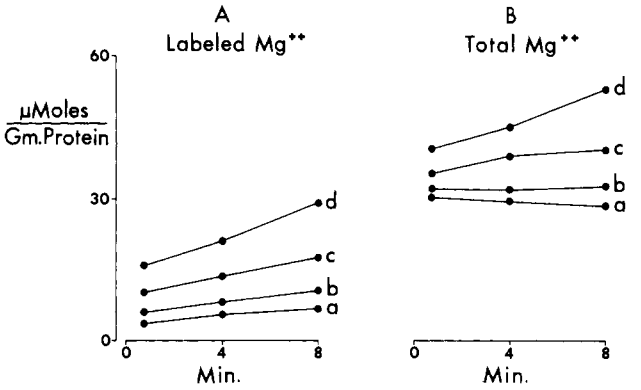

FIGURE 1: Uptake of Mg^{2+} as a function of incubation time. The mitochondria (5.0 mg of protein per mL) were incubated in medium (adjusted to pH 8.0 with HCl) containing 200 mM sucrose, 30 mM Tris, 7.5 mM succinate, ^{28}Mg (approximately 0.05 μCi/mL), [^{14}C]sucrose (approximately 0.4 μCi/mL), $^{3}H_2O$ (approximately 2.6 μCi/mL), and the following (measured) Mg^{2+} concentrations: (a) 0.14; (b) 0.32; (c) 1.09; (d) 5.27 mM. The mitochondrial contents of labeled Mg^{2+} (graph A) and total Mg^{2+} (graph B), in units of micromoles per gram of protein per minute, are plotted against the incubation time in minutes. The values shown are corrected for contaminating extra-mitochondrial Mg^{2+}.

The silicone used (SF-1154) was a gift of the General Electric Co.

Results

The mitochondrial content of labeled Mg^{2+}, at four concentrations of external Mg^{2+}, is plotted as a function of incubation time in Figure 1A. The values shown are corrected for Mg^{2+} in the external, sucrose-penetrated space. There is a rapid binding of ^{28}Mg, followed by a slower increase in the content of labeled Mg^{2+} during the 8-min incubations. It was previously shown that the initial rapid Mg^{2+} binding is not sensitive to metabolic inhibitors and hence probably corresponds to a passive adsorption process (Johnson & Pressman, 1969). The amount of rapid Mg^{2+} binding varies with the concentration of Mg^{2+} in the medium, within the concentration range studied, in a manner consistent with the existence of saturable binding sites. The slower uptake of labeled Mg^{2+} that proceeds during the incubation period is inhibited when respiration is blocked [see Johnson & Pressman (1969) and below] and is thus assumed to represent the energy-linked flux of Mg^{2+} into the mitochondria. The results in Figure 1A show that the rates of uptake of labeled Mg^{2+} are approximately linear during the incubation period.

Values of total mitochondrial Mg^{2+} content, determined from atomic absorption measurements and corrected for contaminating external Mg^{2+}, are depicted in Figure 1B. As in the case of the values of labeled Mg^{2+}, the initial values of total Mg^{2+} vary with the external Mg^{2+} concentration, presumably because of varied Mg^{2+} adsorption. The initial value of approximately 30 μmol of Mg^{2+} per g of protein for the sample at the lowest external Mg^{2+} concentration tested (curve a) agrees well with previous estimates of the endogenous Mg^{2+} content of isolated rat liver mitochondria (Johnson & Pressman, 1969; Bogucka & Wojtczak, 1971; Kun, 1976a).

The data of Figure 1B show that the mitochondria are nearly in the steady state with respect to total Mg^{2+} content under the conditions of these experiments. However, a significant net uptake of Mg^{2+} occurs in samples exposed to relatively high external Mg^{2+} concentrations. This is because the Mg^{2+} influx rate varies with the external Mg^{2+} concentration, while the Mg^{2+} efflux rate is little affected by varied external Mg^{2+} concentration. Pooling data for several ex-

Table I: Apparent Kinetic Constants[a]

additions	K_m (mM)		V_{max} (μmol per g of protein per min)	
	pH 7	pH 8	pH 7	pH 8
none	0.77 ± 0.31 (14)	0.65 ± 0.22 (23)	0.93 ± 0.26 (14)	1.23 ± 0.32 (23)
MalNEt	0.56 ± 0.17 (5)	0.97 ± 0.14 (8)	0.30 ± 0.05 (5)	2.29 ± 0.53 (8)

[a] Lines relating the reciprocal of the Mg^{2+} influx rate to the reciprocal of the external Mg^{2+} concentration were fitted by the method of least squares to the data of several individual experiments similar to that depicted in Figure 2. Values of the apparent K_m and V_{max} were calculated from these lines by the usual relationships: V_{max}^{-1} equals (Mg influx)$^{-1}$ when $[Mg^{2+}]^{-1}$ equals zero; $-K_m^{-1}$ equals $[Mg^{2+}]^{-1}$ when $(K^+ \text{ influx})^{-1}$ equals zero. Conditions were the same as indicated in the legend to Figure 1 except that in individual experiments the mitochondrial protein concentration varied from 4.3 to 9.6 mg/mL, the external Mg^{2+} concentration varied from 0.1 to 7.0 mM, and the pH was adjusted to 7.0 or 8.0 with HCl as indicated. MalNEt, when present, was at 500 μM. All data are expressed as the means of values obtained in several experiments ± standard deviations, followed by the number of experiments in parentheses.

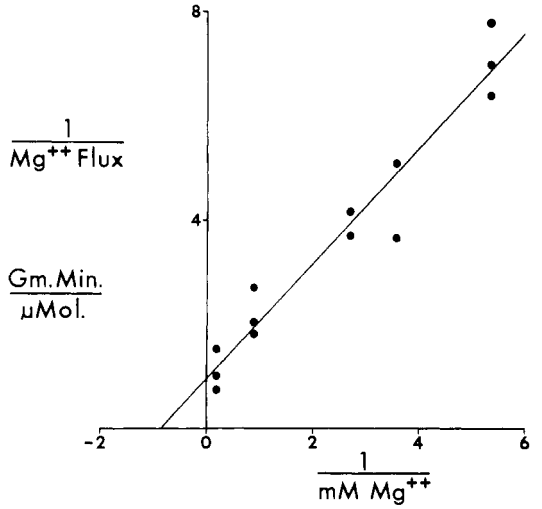

FIGURE 2: Dependence of Mg^{2+} influx on external Mg^{2+} concentration at pH 7.0. All conditions were the same as those indicated in the legend to Figure 1 except that the pH of the medium was 7.0 and the Mg^{2+} concentration of the medium varied from 0.19 to 5.18 mM. The reciprocal of the Mg^{2+} influx rate, in units of grams of protein times minutes per micromole of Mg^{2+}, is plotted against the reciprocal of the external millimolar Mg^{2+} concentration. The line drawn was calculated by the method of least squares.

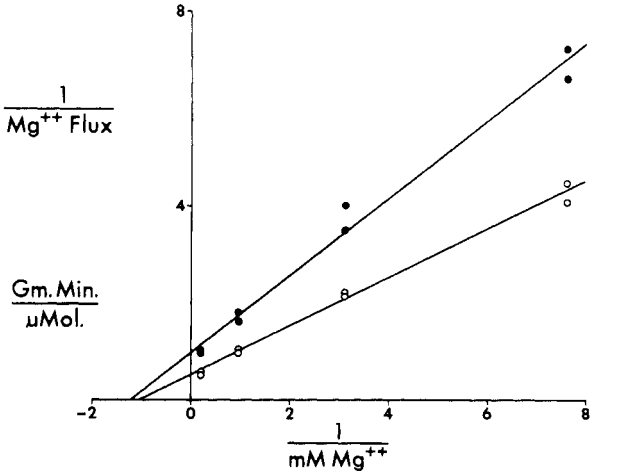

FIGURE 3: Effect of MalNEt on the kinetics of Mg^{2+} influx at pH 8.0. All conditions were the same as those indicated in the legend to Figure 1 except for the mitochondrial protein concentration (9.6 mg/mL) and the Mg^{2+} concentration which varied from 0.13 to 4.95 mM. The reciprocal of the Mg^{2+} influx rate, in units of grams of protein times minutes per micromole of Mg^{2+}, is plotted against the reciprocal of the external millimolar Mg^{2+} concentration. The lines drawn were calculated by the method of least squares. Symbols: (●) control samples; (○) the medium included 500 μM MalNEt.

periments in which conditions were similar to those of Figure 1, we found unidirectional Mg^{2+} efflux rates, in units of micromoles per gram of protein per minute, to be 0.3 ± 0.2 (13) [average ± standard deviation (number of samples)] in pH 7 media containing 0.12–0.23 mM Mg^{2+}; 0.5 ± 0.2 (18) in pH 8 media with 0.12–0.23 mM Mg^{2+}; 0.5 ± 0.2 (13) in pH 7 media with 4.7–5.5 mM Mg^{2+}; and 0.3 ± 0.1 (18) in pH 8 media with 4.7–5.5 mM Mg^{2+}. In contrast, unidirectional Mg^{2+} influx rates vary with external pH and Mg^{2+} concentration (see Figure 1A and below).

An experiment which examines the dependence on external Mg^{2+} concentration of Mg^{2+} influx from a medium buffered at pH 7.0 is depicted in Figure 2. There is an essentially linear relationship between the reciprocal of the Mg^{2+} influx rate and the reciprocal of the external Mg^{2+} concentration. Similar results are obtained when the medium is buffered at pH 8.0, as indicated by the control samples in Figure 3. Apparent kinetic constants determined in a large number of such experiments are summarized in Table I.

Kinetic constants determined in different experiments vary considerably as is evident from the values listed in Table I. Nevertheless, some conclusions are justified by the data. The apparent K_m for Mg^{2+} averages about 0.7 mM at both pH 7.0 and 8.0. The average value of V_{max} is higher at the alkaline pH than at neutral pH; however, the standard deviations about the mean values of V_{max} at the two pH values tested overlap. More significant differences between values of V_{max} at pH 7.0 and 8.0 are observed in the presence of MalNEt.

Table II: Effect of Some Reagents on Mg^{2+} Influx[a]

additions	Mg^{2+} influx [(μmol/g)/min]
none	1.01
	1.10
	0.83
MalNEt	1.46
	1.71
	1.43
antimycin A	0.27
	0.16
	0.15
MalNEt + antimycin A	0.33
	0.45
	0.62

[a] Conditions were the same as those indicated in the legend to Figure 1 except that the concentration of mitochondrial protein was 3.9 mg/mL, the pH of the medium was 8.0, and the Mg^{2+} concentration was 2.3 mM. When present, antimycin A was at 0.25 μg/mL and MalNEt was at 500 μM concentration. The samples containing antimycin A were preincubated for 2 min prior to the addition of the ^{28}Mg at zero time to allow for depletion of endogenous ATP.

In Figure 3, Mg^{2+} influx from a pH 8.0 medium is compared in the presence and absence of MalNEt. The Lineweaver–Burk plot remains linear in the presence of MalNEt. However, Mg^{2+} influx rates from the alkaline medium are higher in the presence of MalNEt. The energy dependence

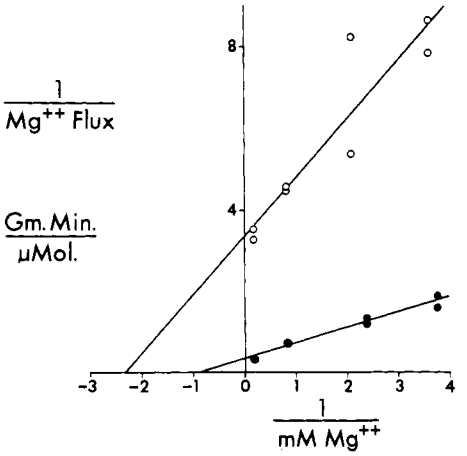

FIGURE 4: Effect of pH on the kinetics of Mg^{2+} influx in the presence of MalNEt. Conditions were the same as those indicated in the legend to Figure 1 except that the mitochondrial protein concentration was 8.7 mg/mL, the Mg^{2+} concentration in the medium varied from 0.26 to 5.4 mM, and all samples contained 500 μM MalNEt. The reciprocal of the Mg^{2+} influx rate, in units of grams of protein times minutes per micromole of Mg^{2+}, protein (min) (μmoles Mg^{2+})$^{-1}$, is plotted against the reciprocal of the external millimolar Mg^{2+} concentration. The lines drawn were calculated by the method of least squares. Symbols: (O) the pH of the medium was 7.0; (●) the pH of the medium was 8.0.

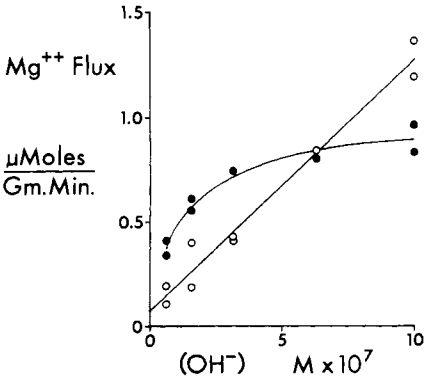

FIGURE 5: Effect of pH on Mg^{2+} influx in the presence and absence of MalNEt. All conditions were the same as those indicated in the legend to Figure 1, except for the mitochondrial protein concentration (5.7 mg of protein per mL), the Mg^{2+} concentration (1.3 mM), and the pH of the medium, which was varied from 6.8 to 8.0. The Mg^{2+} influx rate, in units of micromoles per gram of protein per minute, is plotted against the molar OH^- concentration. Symbols: (●) control samples; (O) the medium included 500 μM MalNEt.

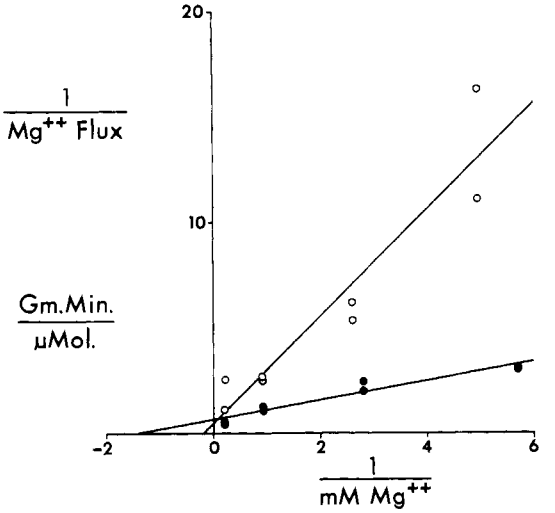

FIGURE 6: Effects of Tl^+ on the kinetics of Mg^{2+} influx. Conditions were the same as those indicated in the legend to Figure 1 except that the pH of the medium was adjusted to 8.0 with H_2SO_4, the mitochondrial concentration was 6.7 mg of protein per mL, and the Mg^{2+} concentration varied from 0.18 to 5.0 mM. The reciprocal of the Mg^{2+} influx rate, in units of grams of protein times minutes per micromole of Mg^{2+}, is plotted against the reciprocal of the external millimolar Mg^{2+} concentration. The lines drawn are calculated by the method of least squares. Symbols: (●) control samples; (O) the medium included 5.0 mM thallous sulfate.

of the effect of MalNEt on Mg^{2+} influx at pH 8.0 is examined in Table II. Consistent with the previously reported effect of rotenone on Mg^{2+} influx supported by glutamate plus malate (Johnson & Pressman, 1969), the respiratory inhibitor antimycin A inhibits the succinate-supported Mg^{2+} influx. Furthermore, antimycin A largely blocks the stimulated Mg^{2+} influx in the presence of MalNEt.

As indicated in Table I, MalNEt increases the apparent V_{max} of Mg^{2+} influx at pH 8. In contrast, MalNEt causes a decrease in the measured V_{max} at pH 7. Thus, MalNEt increases the pH dependence of the V_{max} of Mg^{2+} influx. An experiment comparing the dependence of Mg^{2+} influx on Mg^{2+} concentration at pH 7 and 8 in the presence of MalNEt is depicted in Figure 4. The pattern of the Lineweaver–Burk plots at the two pH values intersecting to the left of the vertical axis is similar to that observed for similar plots of K^+ flux data (Diwan & Lehrer, 1978). The lines intersect below the horizontal axis, resulting in slightly different values of apparent K_m for Mg^{2+} at pH 7 and 8 in the presence of MalNEt, as indicated in Table I.

The pH dependence of Mg^{2+} influx is examined further in the experiment of Figure 5, in which the pH of the medium was varied with the Mg^{2+} concentration held constant. The relationship between the Mg^{2+} influx rate and the external OH^- concentration becomes linear in the presence of MalNEt. This pattern resembles the previously described linear relationship between K^+ influx and OH^- concentration in the presence of MalNEt (Diwan & Lehrer, 1978).

The lack of effect on Mg^{2+} influx of La^{3+}, an inhibitor of mitochondrial Ca^{2+} transport (Mela, 1969), has been demonstrated in three experiments, in which the concentration of EDTA in the initial homogenization medium was decreased from 400 to 20 μM. Oxygen electrode measurements had indicated that this was necessary to prevent residual EDTA in the mitochondrial stock suspensions from blocking interaction of La^{3+} with the Ca^{2+} transport mechanism. Oxygen electrode recordings have confirmed that 2.4 μM La^{3+} (0.8 μmol/g of protein) markedly inhibits the stimulation of respiration by Ca^{2+} in mitochondria prepared in the medium of lower EDTA concentration. In one representative experiment in which the Mg^{2+} concentration in the medium was 1.0 mM and the pH of the medium was 7.5, Mg^{2+} influx rates in the presence of 5 μM La^{3+} (0.8 μmol/g of protein) ranged from 0.52 to 0.58, as compared to control values of 0.34–0.52 μmol of Mg^{2+} per g of protein per min. Other experiments have shown no effect on Mg^{2+} influx of atractyloside, an inhibitor of adenine nucleotide translocation (Klingenberg, 1970), at concentrations found sufficient to completely block stimulation of respiration by ADP plus P_i under equivalent conditions. For example, in one experiment in which the pH of the medium was 7.5 and the Mg^{2+} concentration was 0.9 mM, rates of Mg^{2+} influx in the absence and presence of 10 μM atractyloside ranged from 0.91 to 1.01 and 0.90 to 1.02 μmol of Mg^{2+} per g of protein per min, respectively.

Figure 6 demonstrates inhibition of Mg^{2+} influx from a pH 8 medium by the K^+ analogue Tl^+. Mg^{2+} influx rates in the presence of Tl^+ are low relative to the precision of the measurements. Values of apparent K_m for Mg^{2+} estimated

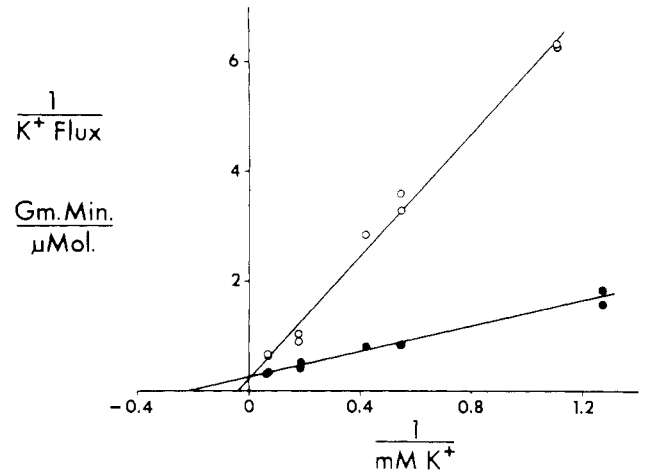

FIGURE 7: Effect of Mg^{2+} on the kinetics of K^+ influx. Conditions were the same as those indicated in the legend to Figure 1 except that ^{28}Mg was omitted and ^{42}K (approximately 0.6 μCi/mL) was included in the medium, the mitochondrial protein concentration was 3.9 mg/mL, and Mg^{2+} was omitted from the medium while the K^+ concentration was varied from 0.8 to 14.9 mM. The reciprocal of the K^+ influx rate, in units of grams of protein times minutes per micromoles of K^+, is plotted against the reciprocal of the external millimolar K^+ concentration. The lines drawn were calculated by the method of least squares. Symbols: (●) control samples; (○) the medium included 4 mM $MgCl_2$.

from Lineweaver–Burk plots of data obtained in the presence of 5 mM Tl^+ ranged from 1.1 to 11.8 mM in seven different experiments (5.0 ± 4.7 mM, average ± standard deviation). Estimated values of V_{max} in these experiments averaged 1.9 ± 1.4 μmol per g of protein per min in the presence of Tl^+ (compare to the control values in Table I). The experiment shown in Figure 6 may be considered representative, although intersection of the double-reciprocal plots on the vertical axis was not observed in every experiment. The estimated K_m values were, however, consistently greater in the presence than in the absence of Tl^+, and no consistent difference between V_{max} values was noted in the presence and absence of Tl^+. The data are at least suggestive of competitive inhibition. Values of K_i estimated in six experiments from the change in the slope of the double-reciprocal plots averaged 1.2 ± 0.3 mM Tl^+. The K_i for inhibition by Tl^+ of K^+ influx has been estimated to be about 2 mM Tl^+ (Diwan & Lehrer, 1977).

In Figure 7 the effect of Mg^{2+} on the kinetics of K^+ influx from a pH 8 medium is examined. The intersection of the double-reciprocal plots near the vertical axis is indicative of competitive inhibition by Mg^{2+} of K^+ influx. The apparent K_m for K^+ in these experiments was found to increase with the addition of 4 mM Mg^{2+} from 5.5 ± 1.9 (3) to 19.8 ± 9.1 (4) mM [average ± SD (number of experiments)]. The estimated V_{max} remained more constant at 4.4 ± 1.0 (3) and 3.8 ± 1.6 (4) μmol of K^+ per g of protein per min in the absence and presence of Mg^{2+}.

Other experiments have examined the effect of 1 mM NaCN on unidirectional Mg^{2+} influx and efflux rates. For these experiments, control samples contained 1 mM NaCl. Conditions were otherwise similar to those indicated in the legend to Figure 1 except that the pH of the medium was 7.5. In one representative experiment in which the medium contained 0.9 mM Mg^{2+}, control influx and efflux values were 1.02 ± 0.08 and 0.84 ± 0.12 μmol of Mg^{2+} per g of protein per min, respectively. Corresponding influx and efflux values for the cyanide-treated mitochondria were 0.05 ± 0.03 and 0.22 ± 0.12 μmol of Mg^{2+} per g of protein per min, respectively (means of six determinations ± SD).

Discussion

It is difficult to reconcile the evidence for inhibition of Mg^{2+} influx by antimycin A and cyanide with the suggestion (Kun, 1976a) that ATP, but not respiration, can supply energy for Mg^{2+} uptake by rat liver mitochondria. Kun's suggestion is based on the finding that Mg^{2+} uptake by digitonin-treated mitochondria proceeds in the presence of oligomycin, which blocks respiration approximately 95%. Yet the small rate of respiration which continues in the presence of oligomycin cannot be considered insignificant relative to the observed slow rates of Mg^{2+} flux. For example, in two experiments in the present investigations, succinate-supported respiration rates of 7.5 and 9.1 μmol of O_2 per g of protein per min were measured in the absence of added ADP or phosphate. Mg^{2+} influx rates measured in these experiments varied from 0.3 to 0.9 μmol of Mg^{2+} per g of protein per min. Assuming four ATP equivalents of conserved energy per O_2 with succinate as electron donor and assuming any stoichiometry from one to several Mg^{2+} per ATP equivalent, it is apparent to us that only a small fraction of available respiratory energy would be required to support the observed rates of Mg^{2+} flux.

The experiments in which effects of cyanide were tested indicate decreases in Mg^{2+} influx and efflux rates when respiration is inhibited. Analysis of similar K^+ flux measurements in relation to the classical formulations of Ussing, Goldman, and Hodgkin has led to the conclusion that K^+ does not diffuse passively across the mitochondrial membrane driven by a metabolism-dependent membrane potential (Diwan & Tedeschi, 1975). An energy-linked carrier mechanism was thus postulated to mediate mitochondrial K^+ flux. A similarly rigorous analysis of Mg^{2+} flux data cannot readily be carried out because of the difficulty in estimating the internal Mg^{2+} activity. Nevertheless it is interesting to note that the data show metabolism dependence for both influx and efflux of Mg^{2+} as well as K^+.

The linear dependence of the reciprocal of the Mg^{2+} influx rate on the reciprocal of the external Mg^{2+} concentration is evidence for involvement of a saturable transport mechanism. The results do not indicate a sigmoidal relationship such as has been described for the dependence of Ca^{2+} flux on external Ca^{2+} concentration (Hutson et al., 1976; Heaton & Nicholls, 1976). The linear Lineweaver–Burk plots instead resemble the previously reported relationship between K^+ influx and external K^+ concentration (Diwan & Lehrer, 1977, 1978).

The kinetic constants estimated from these data must be considered approximate, since values of Mg^{2+} concentration rather than Mg^{2+} activity were used for the calculations. Activity values would tend to be lower than the measured Mg^{2+} concentrations if complexation of Mg^{2+} is significant. Thus, the actual K_m values would be lower than those reported. Such a possible source of error could not account for the discrepancy between the average apparent K_m value of approximately 0.7 mM Mg^{2+} determined in the present experiments and the K_m value of 12 mM Mg^{2+} estimated by Kun (1976b). The value reported by Kun was determined from measurements of net Mg^{2+} flux into digitonin-treated mitochondria in the presence of added ATP. Thus, the mitochondrial preparations, experimental conditions, and parameters measured were different in the two studies.

It has been proposed that MalNEt affects mitochondrial cation flux secondarily as a result of its inhibitory effect on phosphate–hydroxyl exchange (Diwan, 1973; Diwan & Lehrer, 1978). Recently, a stimulatory effect of MalNEt on net Ca^{2+} and phosphate efflux from calcium-loaded mitochondria has been described which suggests a direct effect of MalNEt on

the Ca^{2+} transport mechanism or on the mechanism of energization of Ca^{2+} transport (Lofrumento & Zanotti, 1978). The conditions of Ca^{2+} loading may cause changes in the mitochondrial membrane which make accessible otherwise unreactive sulfhydryl groups. Measurements carried out under conditions similar to those of the present experiments have shown no stimulation by MalNEt of efflux of endogenous mitochondrial phosphate (Diwan, 1972a). Observations that both Mg^{2+} and K^+ fluxes remain sensitive to respiratory inhibitors in the presence of MalNEt [see Table II and Diwan (1973)] indicate that MalNEt does not interfere with the availability of energy to support cation influx under the conditions of these experiments.

The finding that the kinetics of influx of both Mg^{2+} and K^+ remain essentially unchanged in the presence of MalNEt, except for an increased pH dependence, supports the view that MalNEt indirectly affects the Mg^{2+} and K^+ fluxes by blocking transmembrane exchanges of endogenous phosphate. The concentration of MalNEt tested in the present experiments (500 μM or 52–117 μmol/g of protein in individual experiments) is sufficient to cause nearly complete inhibition of the phosphate–hydroxyl translocator (Meijer et al., 1970). It is proposed that the pH dependence of Mg^{2+} influx is more accurately observed in the presence of MalNEt, since dissipation of experimentally manipulated pH gradients via phosphate–hydroxyl exchange is prevented.

In the presence of MalNEt, pH affects primarily the V_{max} of Mg^{2+} influx. Lineweaver–Burk plots of data obtained at pH 7 and 8 intersect to the left of the vertical axis. A linear dependence of the Mg^{2+} influx rate on external OH^- concentration is observed. On the basis of a similar pattern of pH dependence of K^+ influx in the presence of MalNEt, a sequential bisubstrate reaction mechanism was earlier postulated to couple uptake of K^+ and OH^- by rat liver mitochondria (Diwan & Lehrer, 1978). The present results are at least consistent with a similar mechanism for magnesium transport involving a cosubstrate role for OH^-.

The lack of effect of atractyloside on Mg^{2+} influx indicates that the adenine nucleotide translocator is not significantly involved in mediating Mg^{2+} uptake under the conditions of these experiments. La^{3+}, at a concentration which inhibits mitochondrial Ca^{2+} transport, does not block Mg^{2+} influx. It was previously reported that La^{3+} has no effect on net Mg^{2+} flux (Kun, 1976a) or on unidirectional K^+ flux (Diwan, 1972b) into rat liver mitochondria. These observations plus the apparent differences in shape of the concentration-dependence curves for Mg^{2+} and Ca^{2+} fluxes support the conclusion that Mg^{2+} and Ca^{2+} are transported into liver mitochondria by mechanisms which are at least partially separate. In contrast, the K^+ analogue Tl^+ markedly inhibits Mg^{2+} influx, and Mg^{2+} competitively inhibits K^+ influx. The mechanism(s) mediating mitochondrial Mg^{2+} and K^+ fluxes appear to be similar in their kinetic patterns, pH dependence, and energy requirements. Whether the same mechanism is involved in transport of Mg^{2+} and K^+ across the limiting membranes of rat liver mitochondria remains to be determined.

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