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On the regulation of Na^+/H^+ and K^+/H^+ antiport in yeast mitochondria: evidence for the absence of an Na^+-selective Na^+/H^+ antiporter

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Unlike mammalian mitochondria, yeast mitochondria swell spontaneously in both NaOAc and KOAc. This swelling reflects the activity of an electroneutral cation/H^+ antiport pathway. Transport of neither salt is stimulated by depletion of endogenous divalent cations; however, it can be inhibited by addition of exogenous divalent cations (Mg^{2+} $IC_{50} = 2.08$ mM, Ca^{2+} $IC_{50} = 0.82$ mM). Transport of both Na^+ and K^+ can be completely inhibited by the amphiphilic amines propranolol ($IC_{50} = 71$ μM) and quinine ($IC_{50} = 199$ μM) with indistinguishable IC_{50} values. Dicyclohexylcarbodiimide inhibits with a second-order rate constant of $1.6 \cdot 10^{-4}$ (nmol DCCD/mg)$^{-1}$ min^{-1} at 0°C; however, with both Na^+ and K^+ inhibition reaches a maximum of about 46%. The remaining transport can still be inhibited by propranolol. Transport of both cations is sensitive to pH; yielding linear Hill plots and Dixon plots with a pIC_{50} value of 7.7 for both Na^+ and K^+. These properties are qualitatively the same as those of the non-selective K^+/H^+ antiporter of mammalian mitochondria. However, the remarkable similarity between the data obtained in Na^+ and K^+ media suggests that an antiporter akin to the Na^+-selective Na^+/H^+ antiporter of mammalian mitochondria, which is inhibited by none of these agents, is absent in yeast. In an attempt to reveal the activity of a propranolol-insensitive Na^+-selective antiporter, we compared the rates of Na^+/H^+ and K^+/H^+ antiport in the presence of sufficient propranolol to block the K^+/H^+ antiporter. Between pH 4.6 and 8.8 no difference could be detected. Consequently, we conclude that yeast mitochondria lack the typical Na^+-selective Na^+/H^+ antiporter of mammalian mitochondria.

Introduction

Since Mitchell proposed the existence of cation/proton antiporters for effecting mitochondrial volume homeostasis [1], the existence of two monovalent cation/proton antiporters has been demonstrated in mammalian mitochondria [2], namely the Na^+-selective Na^+/H^+ antiporter and the non-selective Na^+/H^+ antiporter. These are commonly known as Na^+/H^+ antiporter and K^+/H^+ antiporter, respectively. The Na^+/H^+ antiporter is believed to be involved in the regulation of mitochondrial Ca^{2+} [3], while the K^+/H^+ antiporter is involved in volume homeostasis [4]. These carriers are similar in that both catalyze electroneutral exchange of Na^+ for H^+ but differ in ion selectivity, Mg^{2+} sensitivity, pH profile and inhibitor profile. The K^+/H^+ antiporter is not cation selective. It transports Na^+, K^+, Li^+, Rb^+ and Cs^+ at similar rates and is inhibited by quinine [2], DCCD [5] and propranolol [6]. Another distinct property of the K^+/H^+ antiporter is that it is allosterically regulated by matrix Mg^{2+} [4,5,7]. In contrast, the Na^+/H^+ antiporter is selective for Na^+ and Li^+ [8], and Li^+ inhibits Na^+ transport competitively [8]. It is not inhibited by quinine [2], DCCD [5] or matrix Mg^{2+} [9]. At present there is no indication that this carrier is regulated. The K^+/H^+ antiporter has been identified as an 82 kDa protein in rat liver mitochondria [5], and successfully reconstituted into proteoliposomes with its known native properties intact [10]. The Na^+/H^+ antiporter has also been partially purified and reconstituted in an active form and has been tentatively identified as a 59 kDa protein [11].

To date very little is known about Na^+/H^+ antiporters in yeast mitochondria. However, the existence

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Abbreviations: EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; Tes, *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid; LS, light scattering; DCCD, dicyclohexylcarbodiimide; RLM, rat liver mitochondria.

of K^+/H^+ exchange has been demonstrated by Villalobo et al. [12], who showed that yeast mitochondria swell passively in K^+ acetate. More recently, Manon and Guerin [13] have presented evidence for the existence of a K^+/H^+ antiporter in yeast. In view of the postulated role of the Na^+/H^+ antiporter [3], and the report that yeast mitochondria lack a high-affinity uptake system for Ca^{2+} [14,15], it would not be surprising if they also lack the Na^+/H^+ and the $Ca^{2+}/2Na^+$ antiporters. The object of the present study was to determine whether yeast contain both types of Na^+/H^+ antiporter, and whether their properties differ from those of their mammalian counterparts. If they are significantly different, use of yeast as a host for the expression mammalian Na^+/H^+ antiporter(s) will be greatly facilitated, since it may be possible to select conditions where the endogenous yeast protein(s) do not interfere with functional detection of the foreign protein(s).

In this paper, evidence is presented that yeast mitochondria possess an electroneutral K^+/H^+ antiporter which has most of the properties of the K^+/H^+ antiporter from mammals; however, we could find no evidence for the existence of the Na^+/H^+ antiporter in yeast mitochondria.

Materials and Methods

Mitochondrial preparations. Saccharomyces cerevisiae diploid wild-type strain C276 was grown in 1% yeast extract, 2% peptone, 2% lactic acid and 0.1% glucose medium (pH 6.5; adjusted with NaOH) at 30°C. Cells were harvested at mid-exponential growth phase and mitochondria were isolated as described by Daum et al. [16]. The final mitochondrial pellet was resuspended in 0.25 M sucrose to yield a stock suspension of about 15–25 mg/ml protein. Using this procedure, we routinely obtained preparations with RCR values between 2.5–3.0.

Mitochondrial pretreatment. In order to diminish the initial lag in swelling observed in ion transport assays, the mitochondrial stock suspension was mixed with an equal volume of a pretreatment medium containing the K^+ salts of glucuronate (200 mM) and Tes (5 mM) at pH 7.8 and incubated on ice for a minimum of 15 min prior to use. Where indicated, mitochondria were also pretreated with DCCD in this medium. Methanol was used as the solvent for the stock solution of DCCD. To deplete matrix Mg^{2+}, mitochondria were pretreated with A23187 as described by Beavis and Garlid [17].

Light scattering (LS) measurements. Unless otherwise mentioned, all transport assays were carried out at pH 7.8 and 25°C in assay medium containing either K^+ or Na^+ salts of acetate (200 mM) Tes (5 mM) EGTA (0.1 mM) and EDTA (0.1 mM). Cation transport was assayed by following swelling which accompanies net salt transport, using the light-scattering technique described in detail for RLM elsewhere [18,19].

Using this technique, a light scattering variable, β, is generated which normalizes reciprocal absorbance for mitochondrial protein concentration P (mg/ml), according to the formula

$$\beta = (P/P_s)(A^{-1} - a)$$

where P_s is a constant introduced to make β dimensionless and a is a machine constant.

Experiments were carried out to determine the most appropriate osmolality for the assay medium by examining the relationship between β and reciprocal osmolality in sorbitol which is essentially impermeant. This osmotic curve revealed that β increases linearly with increase in matrix volume as the sorbitol concentration is decreased from 333 mM to 125 mM which represents a 2.7-fold increase in volume. Hence, we utilized 200 mM acetate salts for our standard assay medium in order to exploit the entire linear range.

Determination of Mg^{2+}. Mg^{2+} content of the mitochondria was determined by analysis of extracts using atomic absorption spectroscopy as describe elsewhere [17].

Media drugs and reagents. Microbiological media were obtained from Difco, drugs and reagents were obtained from Sigma.

Results

Yeast mitochondria swell spontaneously in both Na^+ and K^+ acetate

Rat liver and other mammalian mitochondria swell spontaneously when suspended in NaOAc [9], but do not swell in KOAc unless the endogenous Mg^{2+} is depleted [17]. This behavior reflects the existence of two antiporters, the Na^+-selective Na^+/H^+ antiporter and the Mg^{2+}-regulated non-selective K^+/H^+ antiporter. In contrast, as shown by traces a and b contained in Fig. 1, yeast mitochondria swell spontaneously in both NaOAc and KOAc without depletion of endogenous divalent cations. That this swelling is dependent on electroneutral cation/H^+ antiport and not electrophoretic cation uniport is demonstrated by the finding that the rate of swelling is much lower when OAc^- is replaced by SCN^- which crosses the membrane electrophoretically and not as the acid (Fig. 1, trace c).

As is evident from the traces in Fig. 1, swelling in both NaOAc and KOAc exhibits an acceleration phase or lag. This contrasts with the behavior of RLM in NaOAc [20], but is similar to their behavior in KOAc [14]. In RLM the lag has been attributed to inhibition of the K^+/H^+ antiporter by matrix H^+ [17]. That this conclusion may also be true for yeast mitochondria is supported by the finding that the lag decreases as the medium pH is raised (not shown, [13]), and the finding that pretreatment of the mitochondria at pH 7.8 in K^+ glucuronate reduces the length of the lag (not shown).

These findings, together with the data recently published by Manon and Guerin [13], suggest that yeast mitochondria contain a K^+/H^+ antiporter similar to the one in mammalian mitochondria. However, a question which remains to be answered is whether they also contain a separate Na^+/H^+ antiporter. To answer this question, we have examined the effects of a number of agents which inhibit the K^+/H^+ antiporter but not the Na^+/H^+ antiporter.

Effect of divalent cations on K^+/H^+ and Na^+/H^+ antiport

Since yeast mitochondria swell spontaneously in KOAc without depleting endogenous Mg^{2+}, the question arises as to whether the K^+/H^+ antiporter of yeast is regulated by divalent cations. In agreement with the finding of Manon and Guerin [13], we find that A23187 has virtually no effect on swelling in KOAc medium (data not shown). Moreover, the same result was obtained when mitochondria were pretreated with A23187 and EDTA to deplete endogenous divalent cations (data not shown). Consequently, we went on to investigate whether exogenous divalent cations could inhibit K^+/H^+ antiport in the presence and absence of A23187. Mg^{2+}, Mn^{2+} and Ca^{2+} (0.1 mM) were found to inhibit swelling by 10%, 19% and 24%, respectively, in the presence of A23187. However, in the absence of A23187 the same concentrations of these cations failed to show any effect (data not shown) [13]. Sr^{2+} and Zn^{2+} had no effect on swelling in the presence or absence of A23187 (data not shown). Typical dose-response curves are contained in Fig. 2. The IC_{50} values are 2.08 mM for Mg^{2+} and 0.82 mM for Ca^{2+}. These values are significantly higher than the IC_{50} values for the rat liver K^+/H^+ antiporter, which are 50–65 μM for Mg^{2+} and 12–18 μM for Ca^{2+} [4]. Inhibition by Mg^{2+} in the presence of A23187 was also observed in NaOAc (data not shown). In a similar study, Manon and Guerin [13] also found no inhibition of K^+/H^+ antiport in this concentration range in the absence of A23187; however, they did not investigate the effect in the presence of A23187.

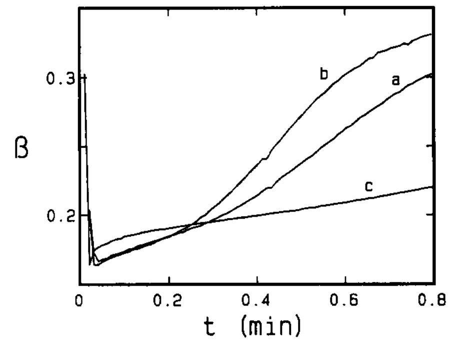

Fig. 1. Yeast mitochondria swell spontaneously in both Na^+ and K^+ acetate. LS kinetics of mitochondria (0.06 mg/ml) suspended in different assay media are shown. Trace a, NaOAc medium; trace b, KOAc medium; trace c, KSCN medium. Mitochondria were added at zero time. The assay media contained K^+ or Na^+ salts of acetate (200 mM) or thiocyanate (200 mM), Tes (5 mM), EGTA (0.1 mM), EDTA (0.1 mM) and antimycin (50 nM) and were adjusted to pH 7.8 and maintained at 25°C.

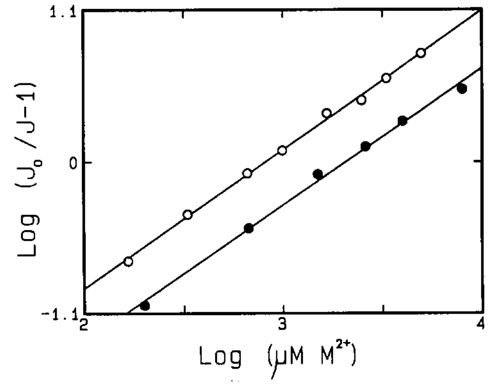

Fig. 2. Divalent cations inhibit K^+/H^+ antiport in yeast mitochondria. Hill plots for inhibition of K^+/H^+ antiport by Mg^{2+} and Ca^{2+} are shown. Mitochondria were suspended in KOAc assay medium supplemented with A23187 (10 nmol/mg) and various doses of Mg^{2+} or Ca^{2+} and the relative rate of K^+/H^+ antiport determined from LS kinetics similar to those shown in Fig. 1. ●, Mg^{2+} data IC_{50} = 2.08 mM, Hill coefficient = 1.0. ○, Ca^{2+} data, IC_{50} 0.82 mM, Hill coefficient = 1.0. The assay medium contained K^+ salts of acetate (200 mM) and Tes (5 mM) and antimycin (50 nM) and was adjusted to pH 7.8 and maintained at 25°C.

Quinine and propranolol inhibit both K^+ and Na^+ antiport

In mammalian mitochondria, quinine is a reversible inhibitor of the K^+/H^+ antiporter [2]; however, it does not inhibit the Na^+/H^+ antiporter [2]. The spontaneous swelling of yeast mitochondria in both KOAc and NaOAc is also blocked by quinine. Typical data obtained in NaOAc are shown in Fig. 3A. Note that the inhibition of the maximum rate of swelling is associated with an increase in the length of the lag. In fact, by simply adjusting the time scale, the traces can be superimposed (data not shown). This suggests that the lag is related to the activity of the antiporter. In rat liver mitochondria, a similar lag or acceleration phase has been observed and shown to be related to a change in the matrix pH which occurs as the mitochondria swell [17]. Results obtained in KOAc medium were essentially identical (not shown). Fig. 3B and 3C contain dose-response curves for quinine and propranolol, respectively. For both drugs in all preparations examined, the data obtained in NaOAc could be superimposed on the KOAc curve. This is significant because

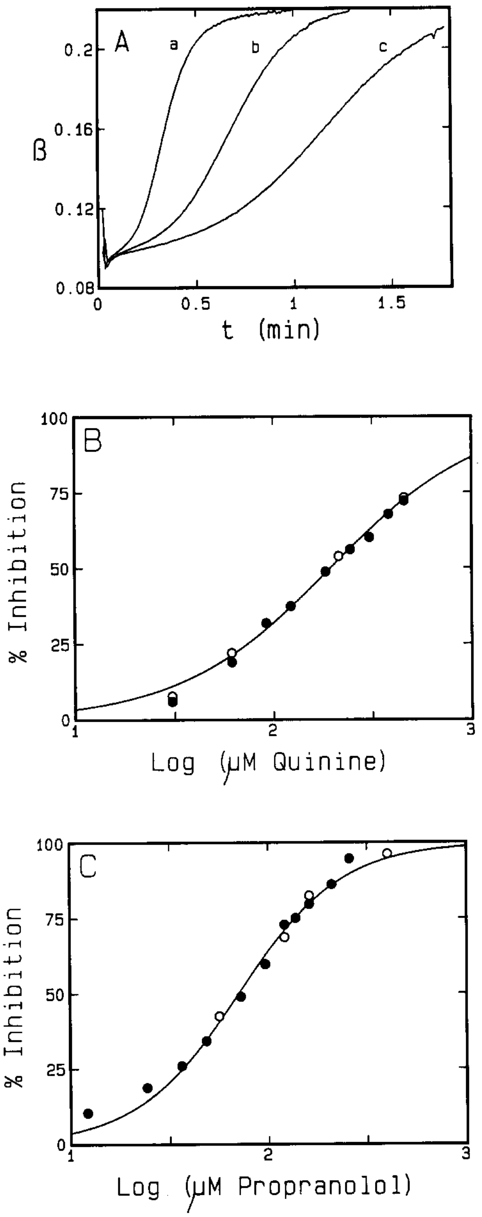

Fig. 3. Quinine and propranolol inhibit K^+/H^+ and Na^+/H^+ antiport in yeast mitochondria. (A) Typical LS traces of mitochondria (0.07 mg/ml) suspended in NaOAc medium containing various doses of quinine are shown. The traces were initiated by the addition of mitochondria and quinine was included in the medium from zero time. Trace a, control; trace b, + quinine (214 μM); trace c, + quinine (459 μM). (B) Dose-response curves for inhibition of K^+/H^+ and Na^+/H^+ antiport by quinine are shown. The relative rates of transport were determined by measuring the maximum rate of swelling in each trace. ●, KOAc medium, quinine $IC_{50} = 199$ μM, Hill coefficient = 1.1. ○, NaOAc medium. (C) Dose-response curves for inhibition of K^+/H^+ and Na^+/H^+ antiport by propranolol. ●, KOAc medium, propranolol $IC_{50} = 71$ μM, Hill coefficient = 1.67. ○, NaOAc medium. The composition of the assay media is as described in the legend to Fig. 1.

the curves are drawn (Fig. 3B,C) on the basis of a model in which the drugs can inhibit transport by 100%. Thus, the data provide no evidence for the existence of a quinine- and propranolol-insensitive Na^+/H^+ antiport pathway.

The IC_{50} values obtained from these data are 199 μM and 71 μM for quinine and propranolol, respectively. In other preparations we obtained values which differed by no more than 10% from these values. These values are significantly lower than the values which may be estimated from the data of Manon and Guerin [13]; however, these authors assayed transport at pH 6.8, where it is already significantly inhibited by protons and the IC_{50} may be different.

N,N'-dicyclohexylcarbodiimide (DCCD) inhibits K^+/H^+ and Na^+/H^+ antiport

DCCD which alkylates carboxyl groups in a hydrophobic environment is a potent inhibitor of the K^+/H^+ antiporter in RLM [5], in which inhibition is essentially complete after pretreatment of mitochondria with DCCD at 50 nmol/mg for 45 min. In contrast, DCCD has no effect on the Na^+/H^+ antiporter. In yeast mitochondria, these pretreatment conditions did not yield any detectable inhibition of K^+/H^+ antiport; however, when the dose was increased to 240 nmol/mg, inhibition could be detected in both Na^+ and K^+ acetate media (see Fig. 4). In contrast with findings in RLM, inhibition was incomplete not only for Na^+ transport but also for K^+ transport. Since the residual transport may have reflected the activity of another transport pathway, we compared the magnitude of inhibition by DCCD at pH 7.8 and pH 8.8. However, even though the rate of K^+/H^+ antiport is much higher at pH 8.8 (see below), the maximum percent inhibition did not change. Moreover, the remaining flux of both Na^+ and K^+ could be blocked by propranolol (see Fig. 4A,B). These findings suggest that DCCD is only a partial inhibitor of the K^+/H^+ antiporter in yeast. We examined this inhibition in several different preparations and in no case did the inhibition go beyond 50%. Typical data for the time-course for inhibition are shown in Fig. 4C. In this case, inhibition reaches a maximum of about 46% at infinite time, and may be described by pseudo-first-order kinetics with a second order rate constant of $1.6 \cdot 10^{-4}$ (nmol DCCD/mg)$^{-1}$min^{-1}. This may be compared with a value of $1.2 \cdot 10^{-3}$ (nmol DCCD/mg)$^{-1}$min^{-1} reported for the rat liver K^+/H^+ antiporter [21].

pH dependence of K^+/H^+ and Na^+/H^+ antiport

Another feature which distinguishes the Na^+/H^+ antiporter from the K^+/H^+ antiporter is the pH profile. Consequently, we have compared the effect of pH on the transport of these ions. As expected for the K^+/H^+ antiporter, we found that the rate of K^+/H^+ antiport increases markedly as the pH of the assay medium is raised (see Fig. 5A). This finding is in agreement with the report of Manon and Guerin [13]. To determine the pIC_{50}, we first constructed a Dixon plot (data not shown) to determine the value of J_o ($[H^+] \rightarrow 0$) and then used this value to construct a Hill plot (Fig. 5B). The linearity of both these plots suggests that the effect of pH may be explained by the existence of a single inhibitory site for protons with a pIC_{50} value of 7.7. The data obtained with Na^+ in same experiment gave indistinguishable results. In fact, as shown in Fig. 5 (open symbols) the data may be described by the same relationship. In another experiment, a pIC_{50} of 7.5 was obtained; however, the results obtained with K^+ and Na^+ were still indistinguishable.

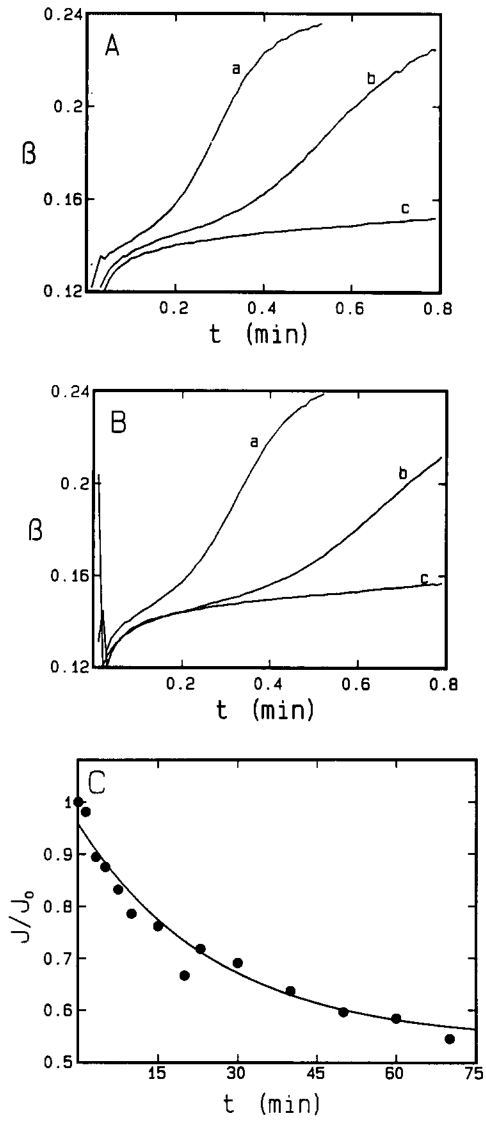

Fig. 4. DCCD inhibits K^+/H^+ and Na^+/H^+ antiport in yeast mitochondria. LS kinetics of DCCD-pretreated mitochondria suspended in KOAc assay medium (panel A) and NaOAc assay medium (panel B) are as shown. Trace a, control mitochondria pretreated with methanol only (DCCD solvent); trace b, mitochondria pretreated with DCCD (240 nmol/mg); trace c, same as trace b with propranolol (240 mM) added to the assay medium. Panel C; J/J_o, the ratio of the K^+ flux at time t to the flux at $t = 0$ is plotted versus time of pretreatment with DCCD (240 nmol/mg) determined from LS traces similar to those shown in panel A. The assay media were as described in the legend to Fig. 1, except the pH was adjusted to 8.8.

The studies presented so far are fully consistent with existence of a single pathway for K^+/H^+ and Na^+/H^+ antiport in yeast mitochondria. However, in RLM the activity of Na^+/H^+ antiporter is lower than the K^+/H^+ antiporter, and it has a bell-shaped pH profile with a peak between pH 7.2 and 7.5 [2,20]. Consequently, we could not rule out the possibility that the activity of the Na^+/H^+ antiporter was masked by that of the K^+/H^+ antiporter. Since, a more detailed examination of the pH profile of Na^+/H^+ antiporter revealed no irregularities (data not shown), we adopted the strategy used by Nakashima and Garlid [2] to discriminate between the K^+/H^+ and Na^+/H^+ antiporters in rat liver mitochondria. This approach utilizes the selective inhibition of the K^+/H^+ antiporter by amphiphilic amines to reveal the Na^+-selective transport pathway. Thus, we compared the pH profiles of transport for Na^+ and K^+ in the presence of sufficient propranolol to block the K^+/H^+ antiporter by 98%. The data contained in Fig. 5A show that the transport of both Na^+ and K^+ was blocked over the entire pH range examined. In fact, for Na^+ we examined swelling traces at pH values down to 4.6, yet no transport was observed.

Discussion

In this paper, we have presented evidence that yeast mitochondria contain only one electroneutral cation/H^+ antiporter. This antiporter, which carries both K^+ and Na^+ has many properties which are similar to the so-called K^+/H^+ antiporter of mammalian mitochondria. We could find no evidence for the existence of an Na^+ and Li^+-selective antiporter akin to the so-called Na^+/H^+ antiporter of mammalian mitochondria.

Although the properties of the yeast K^+/H^+ antiporter are similar to those of the K^+/H^+ antiporter which has been characterized in RLM and beef heart mitochondria, they do differ in a number of respects. The most significant difference is that it is not blocked by endogenous divalent cations. Thus, full activity is observed in the absence of A23187. This does not appear to reflect the absence of endogenous Mg^{2+}, since atomic absorption measurements made by us (data not shown) and those reported by Manon and Guerin [13] reveal that yeast mitochondria contain amounts of Mg^{2+} and K^+ equal to those of RLM. Dose-response curves for Mg^{2+} and Ca^{2+}, carried out in the presence of A23187, to allow access of the divalent cations to the matrix compartment, demonstrate that these cations do inhibit, but the IC_{50} values are much higher than in mammalian mitochondria [4]. As in RLM [22], in the absence of A23187, no inhibition is observed over this dose range, indicating that the inhibitory site is located in the matrix. Manon and Guerin [13] reported inhibition by Mg^{2+} in the absence of A23187, however, 50% inhibition required about 75 mM $MgCl_2$. Since the osmotic effect of this very high concentration would by itself substantially decrease swelling rates, without appropriate controls, this result can only emphasize the insensitivity of K^+/H^+ antiport to external Mg^{2+}. Manon and Guerin [13] also reported that low concentrations of Zn^{2+} could inhibit K^+/H^+ antiport; however, we could not reproduce this finding.

As in RLM, K^+/H^+ antiport in yeast mitochondria is inhibited by drugs such as propranolol and quinine. The data are fully consistent with complete inhibition by interaction at a single site. Yeast mitochondria differ, however, in that Na^+ transport is inhibited in an identical way. The data provide no evidence for the existence of a propranolol-insensitive Na^+/H^+ exchange.

Like propranolol, DCCD also blocks K^+/H^+ antiport and Na^+/H^+ antiport to the same extent; however, unlike in RLM, in yeast mitochondria inhibition is incomplete. This does not appear to reflect the existence of two pathways or induction of a second pathway or leakiness, since the residual flux remains sensitive to both propranolol and pH. These results are consistent with work by Manon and Guerin [13] who showed that transport in both mitochondria and in proteoliposomes containing proteins extracted from mitochondria could be inhibited by DCCD.

The finding that both Na^+/H^+ and K^+/H^+ antiport exhibit a pH dependence, which is completely consistent with inhibition of the antiporter by H^+ binding to a single allosteric site, provides further evidence that the antiporter in yeast is similar to that in RLM. Moreover, the finding that the rate of transport accelerates as swelling in the acetate salt proceeds suggests that, as with the RLM antiporter [17], this site is located in the matrix.

Thus, the lack of selectivity, and inhibition by Mg^{2+}, propranolol, DCCD and protons provide strong evidence that yeast mitochondria contain a K^+/H^+ antiporter. The findings that Na^+ and K^+ behave almost identically and that at no pH could Na^+ transport not be blocked by propranolol led us to conclude that yeast mitochondria lack an active Na^+/H^+ antiporter.

This conclusion is consistent with the proposed role of the Na^+/H^+ antiporter. Mitochondria from vertebrates possess a high-affinity Ca^{2+} uniporter, which mediates the influx of Ca^{2+} driven by the membrane potential, generated by the proton pumps of the respiratory chain [23]. This influx is believed to be important in the regulation of several matrix enzymes including 2-oxoglutarate dehydrogenase and NAD^+-isocitrate dehydrogenase [24]. To allow regulation, a separate efflux pathway exists which employs an electroneutral $Ca^{2+}/2Na^+$ antiporter, to allow Ca^{2+} efflux, and the Na^+/H^+ antiporter to allow subsequent Na^+ efflux and complete the cycle [3]. Consistent with this mechanism, it has been shown previously by others [24], that enzymes in mitochondria from invertebrates and plants are not regulated by Ca^{2+}, and that the uptake pathway is absent [14,24]. Thus, the absence of the Na^+/H^+ in yeast mitochondria may be taken as further evidence for its role in regulation of mitochondrial Ca^{2+} levels. Interestingly, Haworth et al. [26] have suggested that the cytoplasmic membrane of *Saccharomyces cerevisiae* may also lack a Na^+/H^+ antiporter, while Jia et al. [27] have obtained evidence for the existence of one in *Schizosaccharomyces pombe*. The existence of the $Ca^{2+}/2Na^+$ antiporter in yeast mitochondria has not

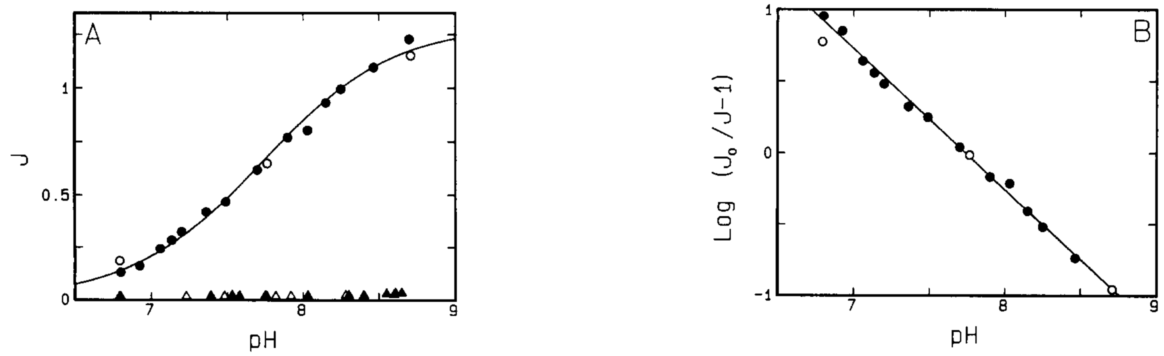

Fig. 5. pH dependence of K^+/H^+ and Na^+/H^+ antiport in yeast mitochondria. (A) The rate of swelling, J (arbitrary units), in KOAc and NaOAc assay media is plotted versus the pH of the assay medium. ●, KOAc; ○, NaOAc; ▲, KOAc+propranolol (0.5 mM); △, NaOAc+propranolol (0.5 mM). (B) Hill plots of the pH dependence of K^+/H^+ (●) and Na^+/H^+ (○) antiport. The values for J_0 were determined from the y-intercept of Dixon plots, which were linear over the entire pH range studied. Rates were determined from LS kinetics similar to those shown in Fig. 1. Except for the pH value, the composition of the assay medium was as described in the legend to Fig. 1.

been established; however, antibodies made against the $Ca^{2+}/2Na^+$ antiporter of beef heart mitochondria, which react with a protein with similar molecular weight from rat tissues, do not react with any protein from yeast mitochondria, suggesting that it also may be absent [25].

Since the K^+/H^+ antiporter is believed to be important for mitochondrial volume homeostasis, one may expect it to be present in all mitochondria and, to date, no mitochondria have been identified which do not contain this antiporter. Regulatory properties do, however, differ, with the most notable being the absence of inhibition by matrix Mg^{2+} in yeast. This may reflect different metabolic requirements and suggests that in yeast pH may be the most important physiological regulator of the K^+/H^+ antiporter.

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