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Kinetic characterization of the reconstituted ornithine carrier from rat liver mitochondria

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Abstract

The ornithine carrier was purified from rat liver mitochondria and reconstituted into liposomes by removing the detergent from mixed micelles by hydrophobic chromatography on Amberlite XAD-2. The efficiency of reconstitution was optimized with respect to the concentration of protein and phospholipid, the Triton X-100/phospholipid ratio, the Amberlite/detergent ratio and the number of passages through a single Amberlite column. The activity of the carrier was influenced by the phospholipid composition of the liposomes, increasing in the presence of acidic phospholipids and decreasing in the presence of dioleoylphosphatidylcholine. In the reconstituted system the incorporated ornithine carrier catalyzed a first-order reaction of ornithine/ornithine or ornithine/citrulline exchange. The maximum transport rate of external [^{14}C]ornithine was 3.2 mmol/min per g protein at 25°C. This value was independent of the type of substrate present at the external or internal space of the liposomes (ornithine, citrulline and lysine). The half-saturation constant (K_m) was 0.16 mM for ornithine, 1.2 mM for lysine and 3.6 mM for citrulline. The activation energy of the ornithine/ornithine exchange reaction was 89 kJ/mol. The rate of exchange had a pH optimum at 8 and was inhibited by cations.

Keywords: Ornithine carrier; Reconstitution; Kinetics; Liposome; Mitochondrion; (Rat liver)

1. Introduction

The inner mitochondrial membrane contains at least 12 transport systems for metabolites (for reviews see Refs. [1–3]). One of these is the ornithine carrier, which plays an important role in urea cycle by catalyzing the entry of ornithine into the mitochondrial matrix in exchange for citrulline. This carrier has recently been isolated from rat liver mitochondria by hydroxyapatite, PD-10, DEAE-Sephacel and Celite chromatography [4]. In SDS-containing gels, the purified fraction consists of a single band with an apparent molecular mass of 33.5 kDa. After incorporation into liposomes it catalyzes an ornithine/ornithine exchange. Besides L-ornithine, also L-citrulline, L-lysine and L-arginine are actively transported by an antiport mechanism. The carrier is inhibited by several sulphydryl reagents, by pyridoxal 5'-phosphate and by $PrCl_3$ [4].

The kinetic properties of the ornithine carrier have not yet been characterized either in intact mitochondria or in the reconstituted system with the purified protein. In this paper, the conditions for optimal reconstitution of the purified mitochondrial ornithine carrier are described. In addition, the values of transport rate and related kinetic parameters of the exchanges between ornithine, citrulline and lysine in reconstituted liposomes are reported.

2. Materials and methods

2.1. Materials

Hydroxyapatite (Bio-Gel HTP) was purchased from Bio-Rad, Celite 535 from Roth, Amberlite XAD-2 from Fluka, DEAE-Sephacel, Sephadex PD-10, Sephadex G-50 and G-75 from Pharmacia, L-[U-^{14}C]ornithine and L-[U-^{14}C]lysine from Amersham, L-

Abbreviations: EYPL, egg yolk phospholipids; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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[ureido-^{14}C]citrulline from Du Pont NEN, egg yolk phospholipids (L-α-phosphatidylcholine from fresh turkey egg yolk), phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, cardiolipin, Hepes and Triton X-100 from Sigma. All other reagents were of analytical grade.

2.2. Isolation and reconstitution of the ornithine carrier

The ornithine carrier of rat liver mitochondria was purified in Triton X-100 as described previously [4]. Reconstitution of the ornithine carrier into liposomes was performed by removing the detergent with a hydrophobic ion-exchange column [5,6]. In this procedure, the mixed micelles containing detergent protein and phospholipids were repeatedly passed through Amberlite XAD-2 columns. The composition of the starting mixture used for reconstitution was: 400 μl of the purified protein (Celite eluate, about 3 μg protein), 100 μl of 10% egg yolk phospholipids in the form of sonicated liposomes prepared as described in Ref. [7], 30 mM L-ornithine or 50 mM L-citrulline and 20 mM Hepes (pH 8), in a final volume of 680 μl. After vortexing, this mixture was passed 14 times through the same Amberlite column (0.5 × 2.5 cm), preequilibrated with a buffer containing 20 mM Hepes (pH 8), and 30 or 50 mM of the substrate present in the starting mixture. All the operations were performed at 4°C, except the passages through Amberlite that were performed at room temperature.

2.3. Transport measurements

The external substrate was removed by passing 550 μl proteoliposomes through a Sephadex G-75 column (0.7 × 15 cm) preequilibrated with 60 mM sucrose and 10 mM Hepes-NaOH (pH 8 unless otherwise indicated). The first 600 μl of the turbid eluate from the Sephadex column were collected, transferred to reaction vessels (100 μl each), incubated at 25°C for 4 min, and then used for transport measurements by the inhibitor stop method [8]. Transport was initiated by adding 10 μl of labelled substrate [^{14}C]ornithine, [^{14}C]citrulline or [^{14}C]lysine at the final concentrations indicated in the legends to tables and figures, and stopped, after the desired time interval, by the addition of 10 μl 330 mM pyridoxal 5'-phosphate. In control samples, the inhibitor was added together with the labelled substrate at time zero. The assay temperature was 25°C, unless otherwise specified. The external radioactivity was removed by passing the samples (100 μl) through a Sephadex G-50 column (0.6 × 8 cm). The liposomes eluted with 1.2 ml of 40 mM NaCl were collected in 4 ml of scintillation mixture, vortexed and counted. The experimental values were corrected by subtracting the respective control. The pyridoxal 5'-phosphate-insensitive radioactivity associated to the control samples was always less than 10% with respect to the pyridoxal 5'-phosphate-sensitive radioactivity taken up during the transport assay. The transport rate was evaluated from the radioactivity taken up by the proteoliposomes within 2 min, i.e., within the initial linear range of labelled substrate uptake into the proteoliposomes. K_m and V_{max} values were determined by a computer-fitting program based on linear regression analysis. Control experiments showed that the rate of ornithine, citrulline or lysine uptake was maximal when the proteoliposomes were loaded with 30 mM ornithine.

2.4. Other methods

Protein was determined by the Lowry method modified for the presence of Triton [9]. The samples used

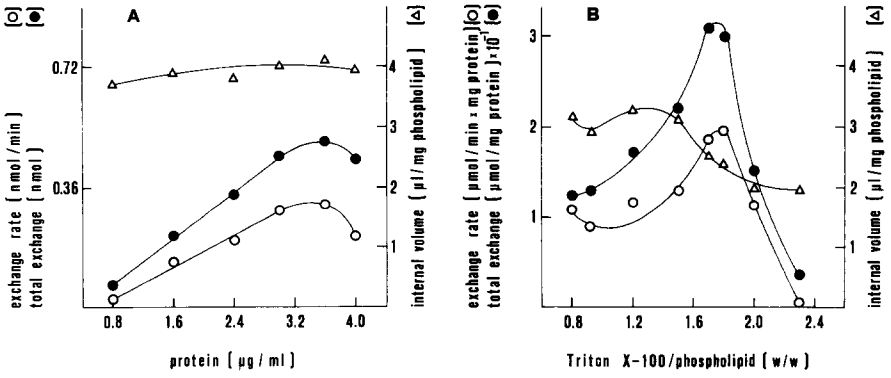

Fig. 1. Dependence of the efficiency of reconstitution of the ornithine carrier on protein concentration (A) and on the detergent/phospholipid ratio (B). The reconstitution was performed as described in Section 2 except that increasing concentrations of protein (A) or detergent (B) were used. 1 mM [^{14}C]ornithine was added to proteoliposomes containing 30 mM ornithine. The exchange rate (○), the total exchange calculated from the exchange equilibrium after 40 min (●) and the intraliposomal volume (△) were determined as described in Section 2. In this figure and all the following ones, data from representative experiments are reported. Similar results were obtained in at least three independent experiments.

for protein determination were subjected to acetone precipitation and redissolved in 1% SDS [10]. The internal volume of total liposomes (i.e., liposomes with and without incorporated carrier protein) was determined as described in Ref. [5].

3. Results

3.1. Optimal conditions of reconstitution

In a previous paper the [^{14}C]ornithine/ornithine exchange was measured in reconstituted liposomes to monitor the presence of the ornithine carrier during the purification procedure [4]. The aim of the present work is to determine the kinetic parameters of the transport catalyzed by the purified ornithine carrier. For this purpose, an accurate measurement of the initial rate of transport was necessary. Therefore, the reconstitution procedure was optimized by adjusting the parameters that influence the efficiency of carrier incorporation into the liposomes. In these experiments we chose three appropriate criteria to describe the state of the reconstituted protein and/or the liposomes: (a) the initial transport rate, that gives information on the specific activity of the carrier, (b) the total exchange, i.e., the amount of labelled substrate taken up after reaching equilibrium (40 min after addition of label), that is correlated to the number (and/or size) of the liposomes loaded with active carrier, and (c) the intraliposomal volume.

Fig. 1A shows the dependence of these three parameters on the protein concentration. The exchange rate and the total exchange increased linearly with the protein until about 3 μg/ml of purified protein. Above the optimal protein concentration of 3.5 μg/ml both parameters decreased. The internal volume of the proteoliposomes, on the other hand, was more or less constant in the whole range of protein concentration, indicating that the formation of liposomes was not influenced by the added carrier protein.

The transport rate and the total transport remained practically constant by varying the phospholipid concentration from 9 to 14 mg/ml (data not shown); therefore 11.5 mg/ml phospholipids were used in all the experiments. The dependence of the exchange on the detergent/phospholipid ratio is shown in Fig. 1B. Both the exchange rate and the total exchange increased on raising the detergent/phospholipid ratio until a value of 1.7–1.8 was reached after which both decreased markedly. The intraliposomal volume was virtually constant from a detergent/phospholipid ratio of 0.8 to 1.5 and then decreased gradually. Due to this behavior a detergent/lipid ratio of 1.6 was chosen as a compromise between the activity and the formation of the proteoliposomes.

Other critical parameters for the method of reconstitution used in this work are the Amberlite/detergent ratio and the number of passages of the reconstitution mixture through the Amberlite column. As shown in Fig. 2A, while the internal volume of the liposomes was practically constant above an Amberlite/detergent ratio of 32.5, the transport rate and the total transport showed an optimum at an Amberlite/detergent ratio of 30. Consequently we chose to use an Amberlite/detergent ratio of 31 in all the experiments. The ornithine/ornithine exchange was also strongly influenced by the number of passages through Amberlite showing an optimum after 14 passages (Fig. 2B). The internal volume markedly increased from 6 to about 15 passages. As an optimal compromise, 14 passages were applied in all the experiments.

It has been reported that lipids modulate the activity of reconstituted mitochondrial carriers [11–18]. The influence of various phospholipids on the activity of the ornithine carrier when added to EYPL (egg yolk phos-

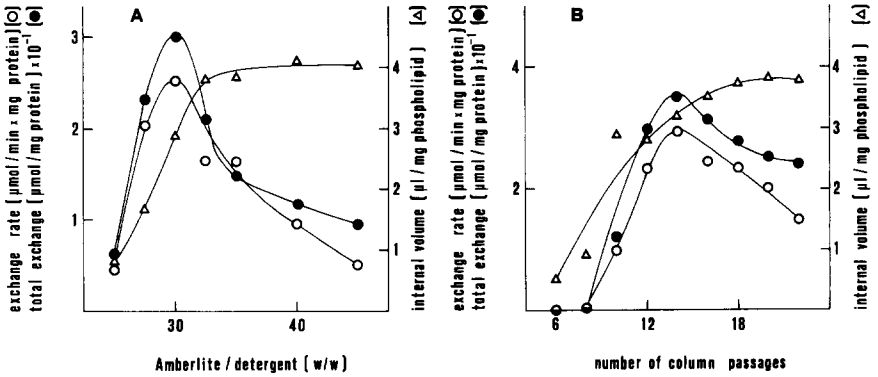

Fig. 2. Dependence of the efficiency of reconstitution of the ornithine carrier on the Amberlite/detergent ratio (A) and on the number of the Amberlite column passages (B). The reconstitution was performed as described in Section 2 except that the amount of Amberlite XAD-2 (A) or the number of passages through the same Amberlite column (B) were varied as indicated. 1 mM [^{14}C]ornithine was added to proteoliposomes containing 30 mM ornithine. The exchange rate (○), the total exchange calculated from the exchange equilibrium after 40 min (●) and the intraliposomal volume (△) were determined as described in Section 2.

Table 1
Dependence of the reconstituted ornithine/ornithine exchange activity on the phospholipid composition of liposomes

Phospholipid composition	Expt. 1 (with 4% phospholipid added to EYPL)			Expt. 2 (with 10% phospholipid added to EYPL)		
	S.A.	T.E.	V_i	S.A.	T.E.	V_i
EYPL	220	1600	2.8	212	1530	2.7
+DOPC	175	1570	2.7	169	1410	2.6
+DPPC	213	1610	2.9	157	1370	2.8
+DSPC	210	1580	2.7	156	1380	2.8
+PE	201	1240	3.0	161	980	2.9
+PI	270	1750	2.2	182	1440	2.1
+PS	338	2050	2.9	240	1680	2.6
+DPG	345	1990	2.9	225	1580	2.9

Reconstitution was performed with the PD-10 eluate, instead of purified ornithine carrier, and with liposomes prepared from EYPL or a mixture of EYPL and the indicated phospholipids present at a concentration of 4% or 10%. 1 mM [^{14}C]ornithine was added to the proteoliposomes loaded with 30 mM ornithine. Abbreviations: EYPL, egg-yolk phospholipids (L-α-phosphatidylcholine from turkey eggs, Sigma); DOPC, dioleylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; DPG, cardiolipin; S.A., specific activity expressed in μmol/min × g protein; T.E., total exchange expressed in μmol/40 min × g protein; V_i, total internal volume expressed in ml/mg phospholipid. The data are from representative experiments. Similar results were obtained in three different experiments for each phospholipid concentration.

pholipids) during reconstitution is shown in Table 1. In these experiments the effect of lipids was investigated using the enriched carrier preparation obtained after the PD-10 chromatography step, since cardiolipin is needed for the elution of the ornithine carrier from DEAE and is therefore always present in the purified preparation of this carrier protein [4]. At a concentration of 4%, the ornithine/ornithine exchange activity was increased by the acidic phospholipids phosphatidylinositol, phosphatidylserine and cardiolipin, decreased by dioleoylphosphatidylcholine and not significantly influenced by all the other phospholipids tested. When the concentration of the phospholipids added to EYPL in the reconstitution mixture was increased to 10%, the rate of exchange was not significantly affected by the acidic phospholipids and slightly decreased by all the other phospholipids tested. Only phosphatidylethanolamine markedly decreased the total exchange, indicating a reduction in the carrier molecules incorporated.

3.2. Reaction order and temperature dependence

The reaction order of the ornithine/ornithine and ornithine/citrulline exchanges was investigated by plotting the ln of maximum ornithine uptake minus ornithine uptake after a given period against time [19]. As shown in Fig. 3, straight lines were obtained, demonstrating that the two exchange reactions follow first-order kinetics. The first-order rate constants, k, extrapolated from the slopes of the logarithmic plots, were 0.10 min^{-1} for the ornithine/ornithine homoexchange and 0.08 min^{-1} for the ornithine/citrulline heteroexchange.

Fig. 4 shows the temperature dependence of the rate of ornithine/ornithine exchange. In an Arrhenius

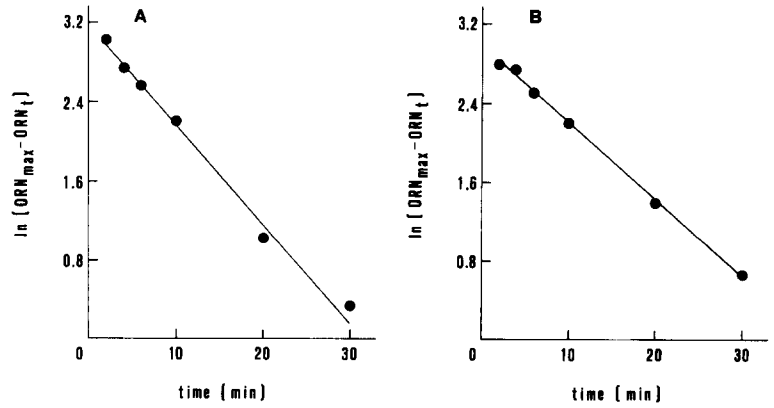

Fig. 3. Logarithmic plot of reconstituted ornithine/ornithine (A) and ornithine/citrulline (B) exchange, demonstrating first-order kinetics: ln $(ORN_{max} - ORN_t)/ORN_{max} = -kt$. 1 mM [^{14}C]ornithine was added to proteoliposomes containing 30 mM ornithine (A) or 30 mM citrulline (B). ORN_{max} is the maximum ornithine exchange/g protein and ORN_t is the ornithine exchange at time t. The values of ORN_{max} were extrapolated at infinite time by a computer non-linear regression analysis; they were 23.8 and 20.0 mmol/g protein for the ornithine/ornithine exchange and the ornithine/citrulline exchange, respectively.

plot a straight line was obtained in the range from 11.5°C to 35°C. The activation energy as derived from the slope was 87 kJ/mol (89 ± 5.5 kJ/mol in four experiments).

3.3. K_m and V_{max} values

In order to obtain the basic kinetic data of the ornithine/citrulline carrier, the dependence of the exchange rate on substrate concentration was studied by changing the concentration of externally added [^{14}C]ornithine, [^{14}C]citrulline or [^{14}C]lysine at constant internal concentration of 30 mM ornithine. The data from a typical experiment are shown in Fig. 5 as a Lineweaver–Burk plot. In the presence of all three substrates linear functions were obtained, whose intersections with the ordinate were very close. This means that V_{max} for the rate of ornithine, citrulline and lysine transport is the same. The slopes, however, were different, i.e., the K_m is lower for ornithine than for lysine and much lower than for citrulline. Table 2 reports mean values and standard errors of K_m and V_{max} for the uptake of ornithine, citrulline and lysine in ornithine-loaded liposomes, measured in several experiments under the conditions of Fig. 5. It should be noted that the standard error of the V_{max} values was rather high when comparing different experiments, presumably due to variations in the amount of active carrier molecules present in each preparation of the purified carrier. Nevertheless, the V_{max} values for the three substrates as compared in one experiment were not significantly different. Virtually the same K_m and V_{max} values for external [^{14}C]ornithine were found when the rate of ornithine uptake was measured as a function of substrate concentration in liposomes loaded with 50 mM citrulline, instead of ornithine.

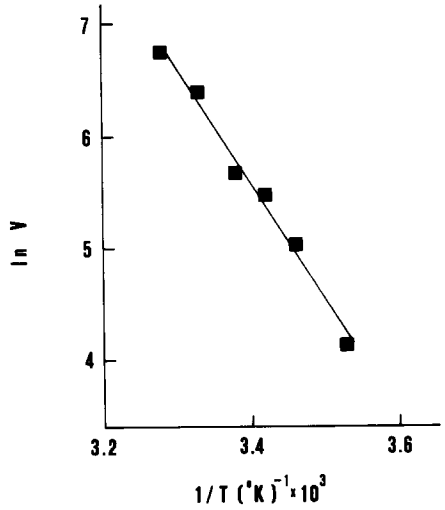

Fig. 4. Temperature dependence of the rate of the reconstituted ornithine/ornithine exchange. 0.1 mM [^{14}C]ornithine was added to proteoliposomes which contained 30 mM ornithine and were incubated at the indicated temperatures. The exchange activity, V, is expressed in μmol/min per g protein.

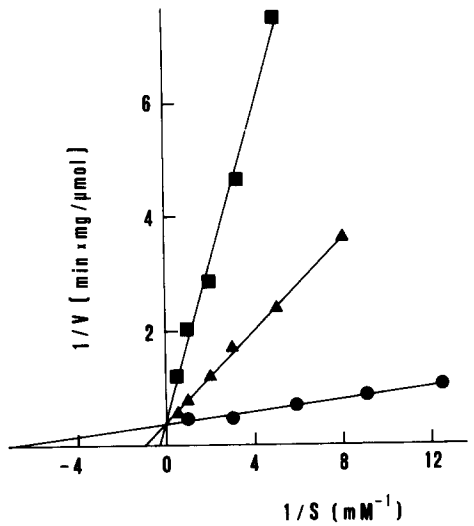

Fig. 5. The dependence on substrate concentration of the rate of ornithine, citrulline or lysine uptake in proteoliposomes. [^{14}C]ornithine (●), [^{14}C]citrulline (■) or [^{14}C]lysine (▲) was added at the concentrations indicated to proteoliposomes loaded with 30 mM ornithine. The incubation medium contained 60 mM sucrose and 10 mM Hepes-NaOH (pH 8).

The inhibition of the rate of [^{14}C]ornithine uptake by various compounds was analyzed in the presence of different substrate concentrations. The data of Fig. 6 demonstrate that lysine, citrulline and arginine inhibit the rate of ornithine exchange in a competitive manner. The K_i values of lysine, citrulline and arginine for [^{14}C]ornithine uptake, evaluated from four experiments, were 1.1 ± 0.3, 3.0 ± 0.5 and 0.9 ± 0.2 mM respectively, and were, therefore, very close to the K_m values of lysine and citrulline reported in Table 2.

3.4. Influence of pH

In the previous paper on the purification of the ornithine carrier from rat liver mitochondria [4], optimal transport activity was obtained by solubilizing the mitoplasts at pH 6 and by performing the reconstitution at pH 8. Here the influence of the pH of the proteoliposomal incubation medium on the reconstituted ornithine/ornithine exchange activity was inves-

Table 2
K_m and V_{max} values for the uptake of ornithine, citrulline an lysine in ornithine-loaded proteoliposomes

Substrate	K_m (mM)	V (mmol/min g prot.)	No. expts.
Ornithine	0.16 ± 0.02	3.2 ± 0.7	15
Citrulline	3.6 ± 0.7	2.9 ± 1.2	5
Lysine	1.2 ± 0.3	3.0 ± 1.5	3

Experimental conditions as in Fig. 5. The values given in the table are the means ± S.E. of 3–15 experiments.

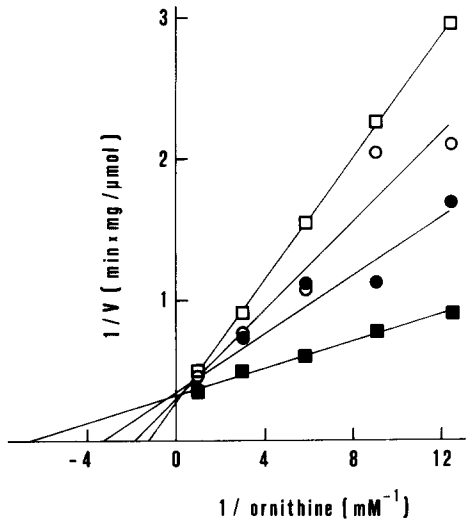

Fig. 6. Inhibition of the reconstituted ornithine/ornithine exchange by citrulline, lysine and arginine. [^{14}C]ornithine was added to proteoliposomes under the experimental conditions reported in the legend to Fig. 5 (■). Where present, 2.5 mM citrulline (●), 2.5 mM lysine (□) or 2.5 mM arginine (○) was added simultaneously with [^{14}C]ornithine.

tigated (Fig. 7). In the presence of a nearly saturating concentration of external ornithine (1 mM), the pH optimum was reproducibly found to be pH 8 (Fig. 7A). Double-reciprocal plots of the exchange rate versus ornithine concentration show that decreasing the pH from 8 to 7 increased the K_m of ornithine and decreased the V_{max} (Fig. 7B), indicating that the inhibition of ornithine uptake by protons is of mixed type [20]. It should be noted that the inhibition of ornithine exchange by acidification from pH 8 to 6.5 was completely reversible (data not shown). Only below pH 6.5 the ornithine carrier became irreversibly inactivated. On the basis of these results, all the other experiments were carried out at pH 8.0.

3.5. Influence of cations

The dependence of the rate of ornithine/ornithine exchange on cations is shown in Table 3. The results demonstrate that all the cations tested inhibited the activity of the ornithine carrier significantly. Within the same group of cations, the extent of inhibition depended on the type of cation, since it was more or less constant in the presence of different Na^+ salts and varied on changing the cation in the presence of the same anion. The divalent cations Ca^{2+} and Mg^{2+} were more effective inhibitors of the ornithine/ornithine exchange than the monovalent cations. The order of effectiveness of the latter was $NH_4^+ > K^+ > Li^+ > Na^+$.The trivalent La^{3+} and Pr^{3+} were the most effective cations; however, it must be considered that their inhibition may be rather unspecific due to their strong binding to lipid headgroups. The inhibition of the ornithine carrier activity by cations was analyzed by changing the concentration of substrate and inhibitors. In the experiment illustrated in Fig. 8A the external substrate was varied from 0.08 to 1 mM and the concentration of NaCl from 10 mM (present as Hepes-NaOH buffer) to 40 mM. The straight lines of Fig. 8A, obtained by plotting the results according to Lineweaver–Burk, intersected in a common point between the abscissa and the ordinate, indicating a mixed type of inhibition [20]. From this and similar experiments the K_i value for Na^+ with respect to the ornithine/ornithine exchange was calculated to be 21 ± 2.7 mM in five experiments. In order to evaluate the 'true' K_m of ornithine in the absence of cations, the apparent K_m values of ornithine, measured in the presence of 10–40 mM NaCl (Fig. 8A), were replotted as a function of NaCl concentration (Fig. 8B). From the secondary plot the K_m of ornithine at 0 mM NaCl (cation-independent K_m) was extrapolated to be 0.1

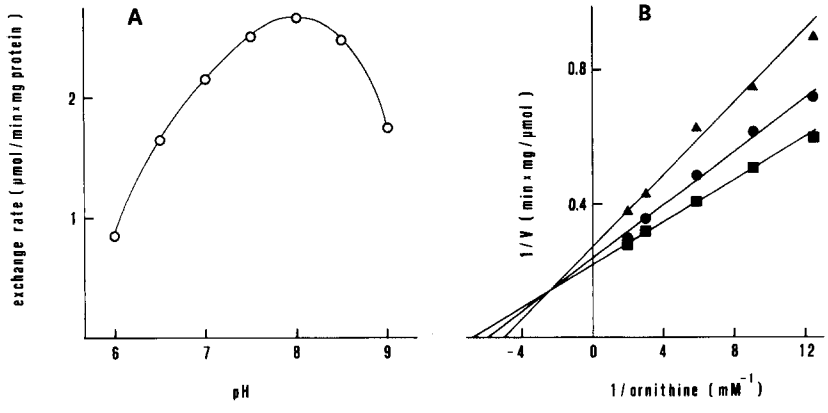

Fig. 7. Effect of pH on the reconstituted ornithine/ornithine exchange. (A) 1 mM [^{14}C]ornithine was added to proteoliposomes which were previously loaded with 30 mM ornithine and incubated in 60 mM sucrose and 10 mM Hepes-Tris at the indicated pH values. (B) [^{14}C]ornithine was added to proteoliposomes under the experimental conditions reported in the legend to Fig. 5 at pH 8.0 (■), pH 7.5 (●) and pH 7.0 (▲).

Table 3
Effect of cations on the rate of the reconstituted ornithine/ornithine exchange

Addition	mM	% inhibition	No. expts.
NaCl	10	27± 5	4
Na_2SO_4	5	27± 5	4
$NaOOC_2H_3$	10	23± 3	3
NaF	10	21	1
KCl	10	40± 6	3
LiCl	10	32± 7	4
NH_4Cl	10	54±10	4
Tris-Cl	10	35	2
$CaCl_2$	5	68± 8	3
$CaCl_2$	10	78± 6	4
$MgCl_2$	10	72	2
$LaCl_3$	5	57	2
$LaCl_3$	10	73	2
$PrCl_3$	5	78	2
$PrCl_3$	10	95	2

The proteoliposomes were loaded with 30 mM ornithine and the exchange was initiated by adding 0.1 mM [^{14}C]ornithine. The indicated salts were added together with the labelled substrate. The values given in the table are the means ± S.E. of % inhibition. The control value of the uninhibited ornithine exchange rate varied between 530 and 620 μmol/min per g protein.

mM (0.09 ± 0.02 mM in five experiments). This value is considerably lower than the half-saturation constant (K_m) for ornithine measured in the presence of a finite concentration of Na^+. In further experiments, similar to those illustrated in Fig. 8A, K^+, Ca^{2+} and Mg^{2+} were also found to inhibit the ornithine/ornithine exchange with a mixed type of inhibition. The K_i of K^+, Ca^{2+} and Mg^{2+} for ornithine exchange were 13 ± 3.5, 1.5 ± 0.3 and 1.4 ± 0.4 mM, respectively (from three determinations). Taking into account these results, in the experiments reported in this paper we avoided the presence of cations in the incubation mixture except the Na^+ ions present in the Hepes buffer.

4. Discussion

The experiments reported here on optimization of the reconstitution of the ornithine carrier were performed in order to obtain a reliable basis for the determination of the kinetic data of this carrier protein. In this study we applied a method of reconstitution based on detergent removal by chromatography on Amberlite XAD-2 [5,6]. This method not only resulted in several-fold higher transport activities than those observed using the freeze-thaw-sonication procedure (data not shown) but also led to the formation of larger proteoliposomes, which are more suitable for kinetic studies. Among the parameters which may influence the efficiency of reconstitution, it was particularly the amount of Amberlite, the ratio of detergent and phospholipids and the number of passages through the Amberlite column that were found to be important for obtaining high transport activities. Optimal activity of the ornithine carrier was obtained at an Amberlite/detergent ratio considerably lower than that needed for the reconstitution of other mitochondrial carriers [5,16–18,21]. The optimum detergent/phospholipid ratio for the reconstitution of the ornithine carrier was the same as that found for the carnitine and the phosphate carriers [18,22] and considerably higher than that determined for the reconstitution of other mitochondrial carriers [16,17,21]. Optimal activity of the ornithine carrier was obtained after a number of passages through Amberlite higher than that observed for the dicarboxylate and oxoglutarate carriers [16,21] and lower than that observed for other carriers [5,17,18,22]. The absolute concentration of phospholipid, which had to be present initially in the reconstitution mixture for optimal activity of the ornithine carrier, was similar (9–14 mg/ml) for all mitochondrial carriers reconstituted with the Amberlite procedure ([5,16–18,21,22]

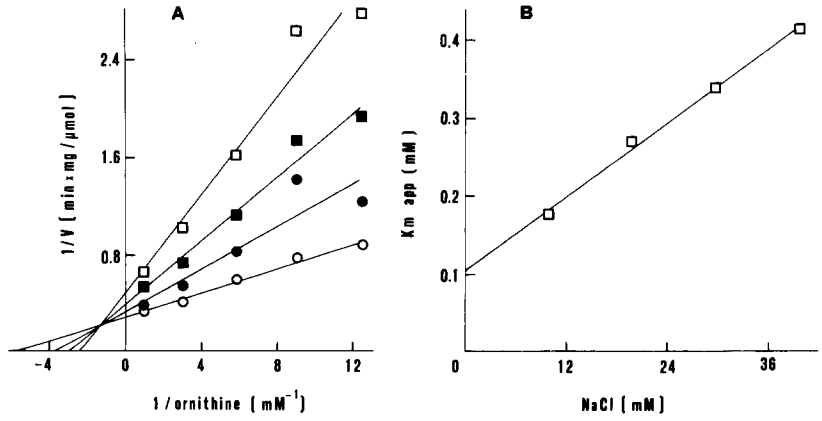

Fig. 8. (A) Inhibition of the reconstituted ornithine/ornithine exchange by NaCl. [^{14}C]ornithine was added at the concentrations indicated to proteoliposomes which were previously loaded with 30 mM ornithine and incubated in 60 mM sucrose and 10 mM Hepes-NaOH pH 8 (○). Additional 10 mM (●), 20 mM (■) or 30 mM (□) NaCl was added simultaneously with the labelled substrate. (B) Replot of apparent K_m values for ornithine extrapolated from (A) versus NaCl concentration.

and this paper). The presence of particular lipids, on the other hand, influenced the activity of the reconstituted ornithine carrier. Interestingly, as observed for other reconstituted mitochondrial carriers [11–13,17,18,23], the ornithine carrier is activated by acidic phospholipids.

We have determined the basic kinetic data of the ornithine carrier in proteoliposomes under optimal conditions. A V_{max} value of 3 mmol/min per g protein was measured at 25°C. This value was found to be very similar for three different externally added substrates and did not depend on the nature of the internal substrate. Thus, similar to the situation found for other carriers [16,17,21], the translocation step catalyzed by the ornithine carrier seems not to be influenced very much by the kind of substrate transported. The turnover number, which was calculated assuming a pure isolated protein monomer of 33.5 kDa, corresponds to 107 min^{-1}. This turnover number is in the same order of magnitude as found for other reconstituted carriers [15–18,24,25] and is lower than that reported for the reconstituted phosphate carrier and pyruvate carrier [24–27]. The half-saturation constant of ornithine for the reconstituted ornithine carrier is much lower than that of lysine and especially that of citrulline. The affinity of the carrier for arginine is about the same as for lysine as deduced from competition experiments. It is important that identical apparent K_m values were found for external ornithine in ornithine-loaded and citrulline-loaded proteoliposomes. This indicates that the half-saturation constant of the carrier for ornithine does not depend on the type of countersubstrate under saturating internal concentrations. The ornithine/ornithine and ornithine/citrulline exchanges catalyzed by the reconstituted ornithine carrier can be described as a first-order reaction. When comparing the temperature dependence of several reconstituted mitochondrial carriers, it appears that most of them, including the ornithine carrier, show no break points between 10 and 35°C ([15–18,28] and this paper), whereas two (namely the ADP/ATP carrier and the phosphate carrier) exhibit a definite break between 20 and 25°C [29,30]. The activation energy of the reconstituted ornithine carrier was determined to be 89 kJ/mol, which is close to the values obtained for the other reconstituted mitochondrial carriers [25].

A distinct feature of the reconstituted ornithine carrier, not observed for the other mitochondrial metabolite carriers, is its inhibition by protons and cations. The results reported in this paper agree with the observations made in intact mitochondria by Aronson and Diwan that ornithine uptake is greater at pH 8 than at pH 7 and is inhibited by choline, Mg^{2+} and Tris buffer [31]. As regards the protons, these authors hypothesized a competition with ornithine for acidic carrier groups. In the present paper the effects of external pH and cations on the reconstituted ornithine carrier activity were extended and analyzed kinetically. Our results support the idea that protons and cations compete with the substrate for negatively-charged residues. However, the effects of protons and cations are definitely more complex since they also cause a decrease in V_{max} of ornithine transport. One possible explanation for the mixed type of inhibition observed in this investigation might be an interaction of protons and cations not only with the active center, but also with a separate binding site of the ornithine carrier.

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