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# Kinetics of the Reconstituted Tricarboxylate Carrier from Eel Liver Mitochondria[1]

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The tricarboxylate carrier from eel liver mitochondria was purified by chromatography on hydroxyapatite and Matrix Gel Blue B and reconstituted into liposomes by removal of the detergent with Amberlite. Optimal transport activity was obtained by using a phospholipid concentration of 11.5 mg/ml, a Triton X-114/phospholipid ratio of 0.9, and ten passages through the same Amberlite column. The activity of the carrier was influenced by the phospholipid composition of the liposomes, being increased by cardiolipin and phosphatidylethanolamine and decreased by phosphatidylinositol. The reconstituted tricarboxylate carrier catalyzed a first-order reaction of citrate/citrate or citrate/malate exchange. The maximum transport rate of external [$^{14}$C]citrate was 9.0 mmol/min per g of tricarboxylate carrier protein at 25°C and this value was virtually independent of the type of substrate present in the external or internal space of the liposomes. The half-saturation constant ($K_m$) was 62 $\mu M$ for citrate and 541 $\mu M$ for malate. The activation energy of the citrate/citrate exchange reaction was 74 kJ/mol from 5 to 19°C and 31 kJ/mol from 19 to 35°C. The rate of the exchange had an external pH optimum of 8.

**KEY WORDS:** Tricarboxylate carrier; mitochondria; transport; liposomes; kinetics; reconstitution; eel.

## INTRODUCTION

The inner membrane of liver mitochondria contains a specific transport system, known as the tricarboxylate (or citrate) carrier, which catalyzes the efflux of citrate from the mitochondria in exchange for a tricarboxylate, a dicarboxylate (malate) or phosphoenolpyruvate (for reviews, see Refs. 1 and 2). Because of its metabolic importance,[3-6] the tricarboxylate carrier from higher eukaryotic cells has been extensively studied.[7-13] The transporter has been purified,[14-16] functionally characterized after reconstitution into liposomes,[14-18] cloned,[19] and overexpressed.[20] The amino acid sequence of the carrier from different mammals [19,21,22] demonstrates that it belongs to the carrier protein family of the mitochondrial inner membrane (for reviews, see Refs. 23 and 24). The tricarboxylate carrier is asymmetrically inserted in the membrane and its N- and C-termini are exposed at the cytoplasmic side of the membrane.[25] Only one gene for this transporter has been detected in man and cow,[22] of which the human one has been localized to chromosome 22q11 within a region implicated in DiGeorge syndrome, velo-cardio-facial syndrome, and schizophrenia.[26] Functionally, the tricarboxylate carrier catalyzes a strict 1 : 1 exchange of substrates and the substrate exchange follows Michaelis-Menten kinetics.[7] The citrate/malate exchange is electroneutral because the carrier accepts solely the single protonated form of citrate (H-citrate$^{2-}$) and the unprotonated form of malate (malate$^{2-}$).[18] The carrier is inhibited by impermeable substrate analog such as 1,2,3-benzenetricarboxylate, as well as by sulfydryl reagents, but not by

[1] Abbreviations: EYPL, egg yolk phospholipids; Pipes, 1,4-piperazinediethanesulphonic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCA, trichloroacetic acid.

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*N*-ethylmaleimide.[7,9] Similarly to the majority of the mitochondrial metabolite carriers (for reviews, see Refs. 23 and 27), the tricarboxylate carrier functions according to a simultaneous (sequential) reaction mechanism.[18] Furthermore, the activity of the tricarboxylate carrier, which is high in liver and virtually absent in heart and brain,[28,29] is affected by hormonal and nutritional factors, being reduced in diabetes mellitus [30] and in starved animals [31] and stimulated in hyperthyroidism.[32]

In contrast to the situation with higher eukaryotic cells, very little is known about the tricarboxylate carrier in fish, although lipogenesis is particularly important in these animals because of the role played by lipids in environmental adaptation.[33] Recently, the tricarboxylate carrier, which is the only mitochondrial carrier protein so far investigated in fish, has been purified to homogeneity from eel (*Anguilla anguilla*) liver mitochondria. [34] Upon SDS gel electrophoresis, the purified carrier from eel consisted of a single polypeptide with an apparent molecular mass of 30.4 kDa.[34] After incorporation into liposomes, the purified protein has been functionally identified as the tricarboxylate carrier for its requirement for a counteranion, as well as its substrate specificity and inhibitor sensitivity, which are both similar to those of the tricarboxylate carrier, as characterized in rat liver mitochondria. The kinetic properties of the eel tricarboxylate carrier have not yet been characterized either in intact mitochondria or in the reconstituted system with the purified protein. In this paper, we describe the conditions for optimal reconstitution of the tricarboxylate carrier purified from eel liver mitochondria. In addition, the values of transport rate and related kinetic parameters of uptake of citrate and malate by reconstituted liposomes loaded with citrate or malate have been determined.

## MATERIALS AND METHODS

### Materials

Hydroxyapatite (Bio-Gel HTP) and Bio-Rad Protein Assay were purchased from Bio-Rad; Matrex Gel Blue B was obtained from Amicon; Amberlite XAD-2, Dowex AG1-X8, Sephadex G-75, Pipes, Triton X-100, Triton X-114, bovine heart cardiolipin, bovine liver phosphatidylethanolamine, bovine liver phosphatidylinositol, bovine brain phosphatidylserine, synthetic dioleylphosphatidylcholine, synthetic dipalmitoylphosphatidylcholine, and synthetic distearylphosphatidylcholine from Sigma; [1,5-$^{14}$C]citrate and L-[U-$^{14}$C]malate from Amersham International (Amersham, U.K.); and egg yolk phospholipids (EYPL; phosphatidylcholine from eggs) from Fluka. All other reagents were of analytical grade.

### Isolation and Reconstitution of the Tricarboxylate Carrier

Silver eels (*Anguilla anguilla*) (200–300 g) were purchased from sea-water aquaria and liver mitochondria were prepared as previously described.[35] The tricarboxylate carrier was purified from eel liver mitochondria according to Zara *et al.*[34] The purified carrier protein was reconstituted into liposomes by cyclic removal of the detergent with a hydrophobic column.[36] The composition of the initial mixture used for reconstitution was: 40 μl of the purified tricarboxylate carrier (about 0.25 μg protein), 70 μl of 10% Triton X-114, 80 μl of 10% egg yolk phospholipids in the form of sonicated liposomes, 10 m*M* citrate or malate, and 10 m*M* Pipes (pH 7.0) in a final volume of 700 μl. After vortexing, this mixture was recycled ten times through an Amberlite column (0.5 × 5 cm) preequilibrated with a buffer containing 10 m*M* Pipes and the substrate present in the starting mixture at the same concentration.

### Transport Measurements

The external substrate was removed by chromatography on a Sephadex G-75 column (0.8 × 15 cm) preequilibrated with 50 m*M* NaCl and 10 m*M* Pipes (pH 7.0). The first 600 μl of the turbid eluate from the Sephadex column were collected and distributed in reaction vessels (180 μl each). Transport was initiated by the addition of [$^{14}$C]citrate or [$^{14}$C]malate at the indicated concentrations and stopped, after the desired time, by adding 20 m*M* 1,2,3-benzenetricarboxylate. In control samples, the inhibitor was added together with the labeled substrate. The assay temperature was 25°C. The external radioactivity was removed by passing each sample of proteoliposomes (180 μl) through a Dowex AG1-X8 column, 100–200 mesh, chloride form. The proteoliposomes eluted with 1 ml of 50 m*M* NaCl were collected in 4 ml of scintillation mixture, vortexed, and counted. The experimental values were

corrected by subtracting the respective control value. $K_m$ and $V_{max}$ values were determined by a computer-fitting program based on linear regression analysis.

### Other Methods

Since the purified tricarboxylate carrier is overestimated by the Lowry method modified for the presence of Triton [37] because of the presence of cardiolipin, protein was determined from Coomassie Blue-stained SDS-PAGE gels with an LKB 2202 Ultroscan laser densitometer, with bovine serum albumin as protein standard. The samples used for protein determination were subjected to TCA precipitation, washed with acetone, and redissolved in 1% SDS. The internal volume of total liposomes (i.e., liposomes with and without incorporated carrier protein) was determined as previously described.[38]

## RESULTS

### Optimal Conditions of Reconstitution

In order to accurately determine the kinetic parameters of the tricarboxylate carrier purified from eel liver mitochondria, we first optimized the reconstitution procedure by adjusting the parameters that influence the efficiency of carrier incorporation into liposomes. In these experiments three criteria describing the state of the reconstituted protein and/or the liposomes were analyzed: (a) the initial citrate/citrate exchange rate, which gives information on the specific activity of the carrier, (b) the total exchange, i.e., the amount of labeled substrate taken up after reaching equilibrium (90 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.

In Fig. 1, the influence of the lipid concentration and of the detergent/lipid ratio on the reconstituted citrate/citrate exchange is shown. Both the exchange rate and the total exchange increased on raising the lipid concentration and the detergent/phospholipid ratio, reaching their maximum at 11.5 mg phospholipid/ml and a detergent/phospholipid ratio of 0.9. Above these values the activity decreased, whereas the total liposomal volume remained virtually constant up to 20 mg phospholipid/ml and up to a detergent/

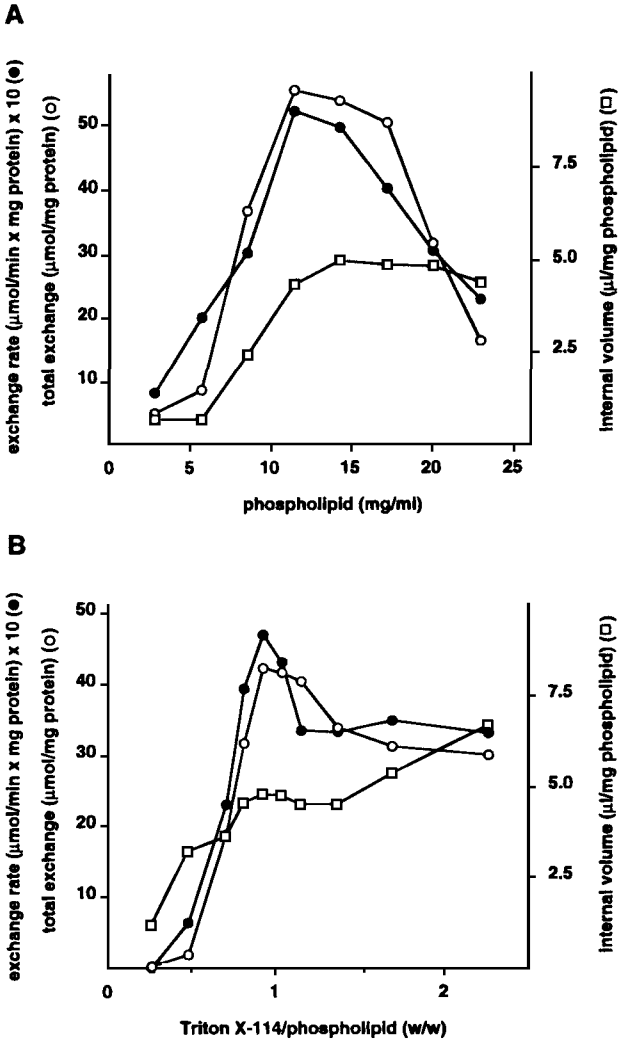


**Fig. 1.** Dependence of the efficiency of reconstitution of the eel tricarboxylate carrier on the phospholipid concentration (A) and on the detergent/phospholipid ratio (B). The proteoliposomes were prepared as described in Materials and Methods except that increasing concentrations of phospholipids (A) or increasing concentrations of Triton X-114 (B) were used. [$^{14}$C]Citrate (0.1 m*M*) was added to proteoliposomes which contained 10 m*M* citrate. The exchange rate measured after 1 min of incubation with the labeled substrate (●), the total exchange calculated from the exchange equilibrium after 90 min of incubation with the labeled substrate (○) and the internal volume (□) were determined as described in the Materials and Methods Section.

lipid ratio of 1.4. The citrate/citrate exchange rate and the total exchange were also strongly influenced by the number of passages through Amberlite showing an optimum after nine to ten passages (Fig. 2). The internal volume, however, remained approximately the same from four to thirteen passages. In other experiments (not shown), it was found that both the transport

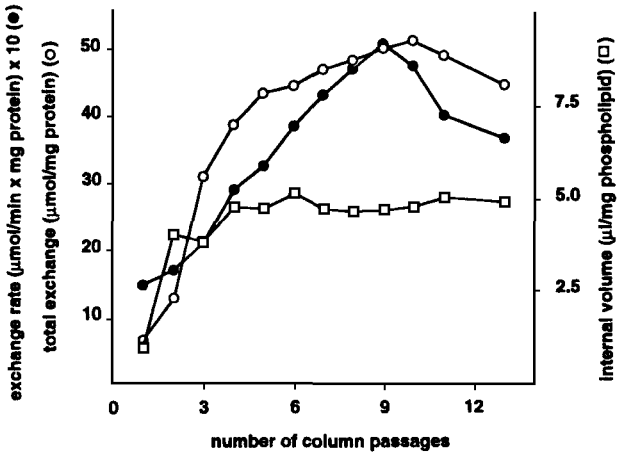


**Fig. 2.** Dependence of the efficiency of reconstitution of the eel tricarboxylate carrier on the number of the Amberlite column passages. The proteoliposomes were prepared as described in Materials and Methods except that the number of passages through the same Amberlite column was varied as indicated. [$^{14}$C]Citrate (0.1 m*M*) was added to proteoliposomes which contained 10 m*M* citrate. The exchange rate measured after 1 min (●), the total exchange calculated from the exchange equilibrium after 90 min (○), and the internal volume (□) were determined as described in the Materials and Methods Section.

rate and the total transport increased linearly with protein concentration up to 0.8 μg/ml of purified protein. On the basis of these findings, in the subsequent experiments a lipid concentration of 11.5 mg/ml, a detergent/phospholipid ratio of 0.9, a protein concentration below 0.8 μg/ml, and ten Amberlite column passages were used.

Lipids have been reported to modulate the activity of mammalian mitochondrial transporters.[39–42] The influence of various phospholipids on the activity of the eel tricarboxylate carrier when added to EYPL (egg yolk phospholipids) during reconstitution is shown in Fig. 3. In these experiments, the effect of lipids has been investigated using the enriched carrier preparation obtained after the hydroxyapatite chromatography step, since cardiolipin is needed for the elution of the carrier from Matrex Gel Blue B and is, therefore, always present in the purified preparation of the eel tricarboxylate carrier (see Ref. 34). As shown in Fig. 3A, both cardiolipin and phosphatidylethanolamine, at a concentration of 5%, markedly increased the rate of citrate exchange (+70 and +50%, respectively) in comparison to the exchange rate measured in the presence of EYPL alone. Interestingly, the total exchange was less stimulated by these phospholipids, i.e., +34 and +19% for cardiolipin and phosphatidylethanolamine, respectively. Moreover, the percentage increase

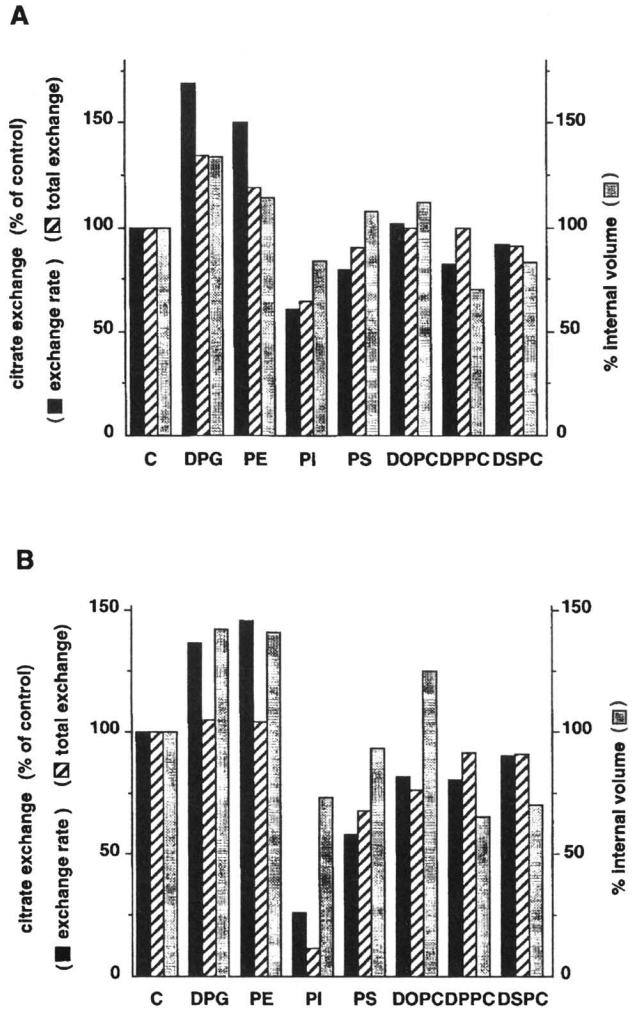


**Fig. 3.** Dependence of the reconstituted citrate/citrate exchange activity on the phospholipid composition of liposomes. Reconstitution was performed with the hydroxyapatite eluate, instead of purified tricarboxylate carrier, and with liposomes prepared from EYPL (phosphatidylcholine from egg yolk, Fluka) (control) or a mixture of EYPL and the indicated phospholipids present at a concentration of 5% (A) or 15% (B). Abbreviations: C, control; DPG, cardiolipin; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; DOPC, dioleylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearylphosphatidylcholine. [$^{14}$C]Citrate (0.5 m*M*) was added to proteoliposomes preloaded with 10 m*M* citrate. The exchange rate and the total exchange in proteoliposomes prepared with EYPL alone (C) were 127 nmol/min × mg protein and 2447 nmol/mg protein, respectively. The internal volume of these proteoliposomes was 2.5 μl/mg phospholipid. All these values were set to 100 in the control sample (C).

in the internal volume was comparable to that in the total exchange. In the presence of a higher concentration of cardiolipin and phosphatidylethanolamine (15%; Fig. 3, panel B), only the specific activity of

the tricarboxylate carrier was stimulated, while the total activity was virtually unaffected. Under these conditions, a marked increase in the intraliposomal volume was also found. The finding that 5% cardiolipin or 5% phosphatidylethanolamine increases the exchange rate more than the total exchange indicates that the stimulatory effect of these two phospholipids is due to a direct activation of the incorporated protein more than to an increased incorporation of carrier protein. This interpretation is supported by the fact that at higher concentrations (15%) of cardiolipin and phosphatidylethanolamine, the exchange rate was increased, but the total exchange was almost unaffected, whereas the internal volume was increased, which indicates not only an activation of the incorporated protein, but also a negative influence of cardiolipin and phosphatidylethanolamine on the incorporation of the eel tricarboxylate carrier into liposomes. Figure 3 also shows that phosphatidylinositol and phosphatidylserine, particularly at a concentration of 15%, decreased both the exchange rate and the total exchange of the eel liver tricarboxylate carrier. On the other hand, the phosphatidylcholines tested, i.e., dioleylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearylphosphatidylcholine, did not significantly influence the exchange rate and the total exchange of the eel liver tricarboxylate carrier even at 15% (Fig. 3B).

## Reaction Order, Temperature, and pH Dependence of Citrate Transport

Figure 4A shows the time course of 0.1 m*M* [$^{14}$C]citrate uptake by proteoliposomes containing either citrate or malate. The curves of Fig. 4A represent an exponential approach to isotopic equilibrium as demonstrated by the straight lines reported in Fig. 4B, where the natural logarithm of the fraction of equilibrium $citrate_{max}/(citrate_{max}\text{-}citrate_t)$ is plotted against time.[43] This means that both citrate/citrate and citrate/malate exchange reactions follow a first-order kinetics. The first-order rate constants, $k$, extrapolated from the slope of the logarithmic plots, were 0.063 $min^{-1}$ for the citrate/citrate exchange and 0.049 $min^{-1}$ for the citrate/malate exchange. The initial rate of citrate uptake, calculated as the product of $k$ and the intraliposomal citrate at equilibrium, was 3.75 mmol/min per g protein at 25°C in the case of the citrate/citrate exchange and 2.62 mmol/min per g protein in the case of the citrate/malate exchange. This difference can be explained by taking into account the different affinities of malate and citrate to the carrier (see below). In order to determine the initial rate accurately, further experiments were performed in which several kinetic measurements were carried out within the first minutes (not shown). From these experiments it was concluded that citrate uptake increased linearly with time for about 3 min in citrate-loaded liposomes and for only about 1 min in malate-loaded liposomes.

The temperature dependence of the reconstituted citrate/citrate exchange activity of the carrier is presented in Fig. 5 in the range from 5 to 35°C. The Arrhenius plot shows two linear ranges with different slopes. The break occurred at 19°C. The activation energies, as derived from the slopes, were 74.3 and 30.7 kJ/mol between 5 and 19°C and 19 and 35°C, respectively.

In Fig. 6, the effect of the external pH on the rate of citrate/citrate exchange is shown. In the presence of a nearly saturating concentration of external citrate (0.5 m*M*), high transport rates were obtained in the range between pH 6.5 and 8.5. The pH optimum was reproducibly found to be pH 8. At pH values lower than 6.5 and higher than 8.5, the rate of citrate transport decreased markedly.

## $K_m$ and $V_{max}$ Values of Citrate and Malate Transport

The dependence on substrate concentration of the rate of eel tricarboxylate carrier was studied by changing the concentration of external [$^{14}$C]citrate or [$^{14}$C]malate at a constant internal concentration of 10 m*M* citrate or malate. Table I reports mean values and standard errors of $K_m$ and $V_{max}$ for the uptake of citrate and malate in citrate-loaded or malate-loaded liposomes. 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 two substrates as compared in one experiment were not significantly different. The results of Table I show that, in the presence of external citrate or malate, the $V_{max}$ value is independent of the type of substrate, whereas the $K_m$ is about tenfold higher for malate than for citrate. Furthermore, virtually the same $K_m$ and $V_{max}$ values for external [$^{14}$C]citrate were found when the rate of citrate uptake was measured

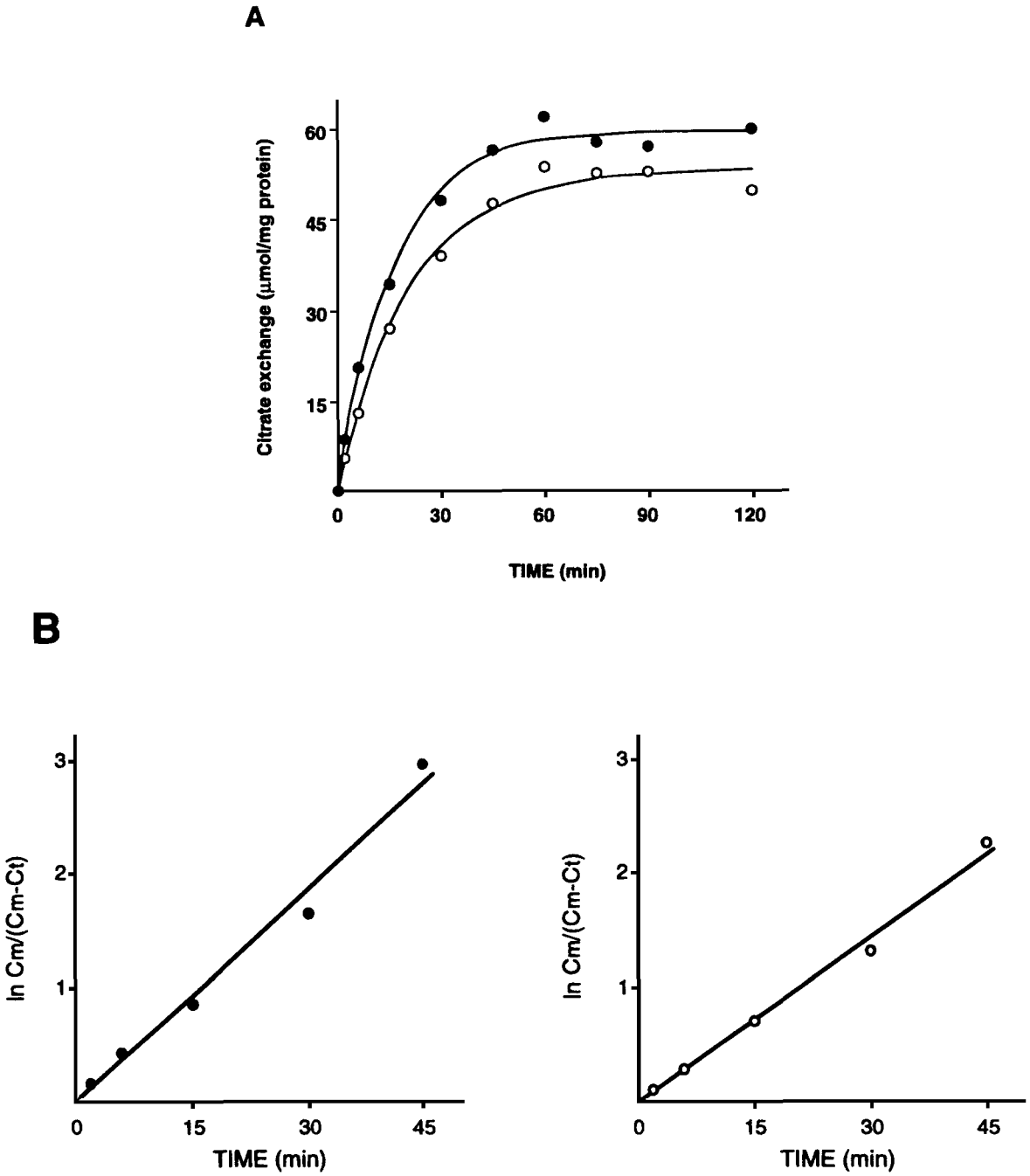


**Fig. 4.** Time course of the citrate/citrate exchange and citrate/malate exchange in reconstituted liposomes. (A) [$^{14}$C]Citrate (0.1 m*M*) was added to proteoliposomes which contained 10 m*M* citrate (●) or 10 m*M* malate (○). (B) Logarithmic plots of ln $C_m/(C_m - C_t)$ where $C_m$ is the maximum citrate exchange/g protein and $C_t$ is the citrate exchange at time $t$, according to the relation ln $C_m/(C_m - C_t) = kt$. The amount of citrate taken up after reaching equilibrium was measured after 120 min; it was 59.6 and 53.4 mmol/g protein for the citrate/citrate exchange and the citrate/malate exchange, respectively.

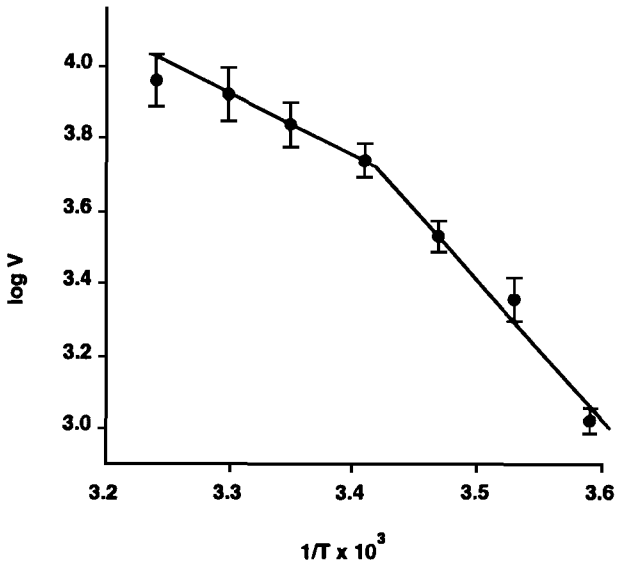


**Fig. 5.** Temperature dependence of the rate of the reconstituted citrate/citrate exchange. [$^{14}$C]Citrate (0.1 m*M*) was added to proteoliposomes, which contained 10 m*M* citrate and were incubated at the indicated temperatures. The exchange activity, *V*, is expressed in nmol/min × mg protein. The means ± S.D. of four experiments are reported.

in proteoliposomes containing a constant internal concentration (10 m*M*) of either citrate or malate.

The inhibition of the rate of [$^{14}$C]citrate uptake in citrate-loaded proteoliposomes by various compounds

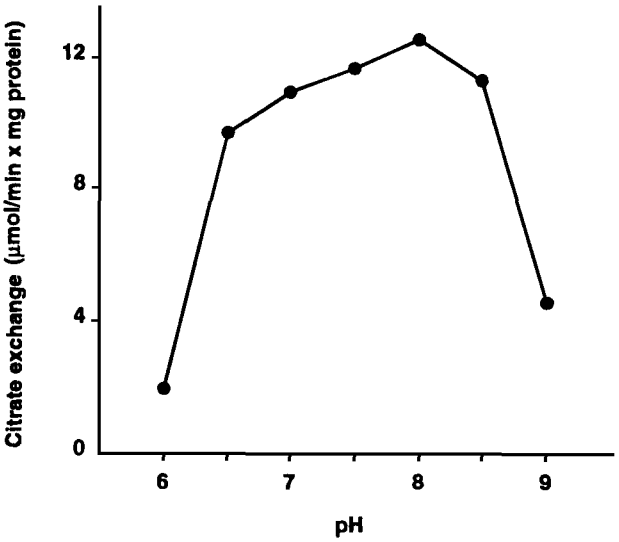


**Fig. 6.** pH dependence of the rate of reconstituted citrate/citrate exchange. [$^{14}$C]Citrate (0.5 m*M*) was added to proteoliposomes which contained 10 m*M* citrate and were incubated at the indicated pH values. The data represent the means of four different experiments.

**Table I.** $K_m$ and $V_{max}$ Values for the Uptake of Citrate and Malate in Reconstituted Liposomes[a]

| Substrate | Internal substrate | $K_m$ (µ*M*) | $V_{max}$ (mmol/min × g protein) | No. expt. |
|---|---|---|---|---|
| Citrate | Citrate | 62 ± 5 | 9.0 ± 0.7 | 10 |
| Citrate | Malate | 68 ± 4 | 7.3 ± 0.5 | 6 |
| Malate | Citrate | 541 ± 75 | 8.3 ± 0.8 | 7 |

[a] [$^{14}$C]Citrate, 0.0125–0.25 m*M*, or [$^{14}$C]malate, 0.1–1.5 m*M*, was added to proteoliposomes containing either 10 m*M* citrate or 10 m*M* malate. The values given in the table represent the means ± S.E. of six to ten experiments.

was analyzed in the presence of different substrate concentrations. 1,2,3-Benzenetricarboxylate and other tricarboxylates, succinate and malonate, as well as phosphoenolpyruvate were all identified as competitive inhibitors, since they were found to increase the apparent $K_m$ without changing the $V_{max}$ of the citrate/citrate exchange. The inhibition constants, $K_i$, are summarized in Table II.

## DISCUSSION

In a previous paper[34] we characterized the purified and reconstituted tricarboxylate carrier from eel liver with respect to substrate specificity and inhibitor sensitivity. In this study, we first optimized the reconstitution procedure of the eel tricarboxylate carrier in order to obtain a reliable basis for the determination of the kinetic data of this transport protein. To this

**Table II.** $K_i$ Values for Substrate Analogs Competing with Citrate for the Exchange Reaction[a]

| Inhibitor | $K_i$ (m*M*) |
|---|---|
| *cis*-Aconitate | 0.034 |
| *threo*-$D_s$-Isocitrate | 0.056 |
| 1,2,3-Benzenetricarboxylate | 0.015 |
| Phosphoenolpyruvate | 0.155 |
| Succinate | 3.2 |
| Malonate | 5.8 |

[a] The $K_i$ values were calculated from double reciprocal plots of the rate of the [$^{14}$C]citrate/citrate exchange versus substrate concentrations. Conditions as in Table I. The competing substrate analogs were added simultaneously with [$^{14}$C]citrate at the appropriate concentrations. The data represent the means of three different experiments.

end, we used a method of reconstitution based on a cyclic removal of the detergent by chromatography on Amberlite,[36] which results in higher transport activities and larger proteoliposomes than those obtained by the freeze–thaw sonication procedure. The results reported above show that the relative amount of the components of the reconstitution mixture, i.e., detergent, protein, and phospholipids, and the number of passages through the Amberlite column markedly influence the efficiency of reconstitution of the purified eel tricarboxylate carrier protein. Optimal activity of the eel tricarboxylate carrier was obtained with nine to ten passages through Amberlite, a number which is considerably lower than that found for the rat liver tricarboxylate carrier[17] and other mitochondrial carriers.[44-46] The detergent/phospholipid ratio, which had to be present initially in the reconstitution mixture for optimal conditions, was, however, about the same as that observed for the rat liver tricarboxylate carrier,[17] the oxoglutarate carrier,[47] and the dicarboxylate carrier,[48] and definitely lower than the values obtained with other mitochondrial transporters.[44,45]

Under optimal conditions, a $V_{max}$ value of 9 mmol/min per g protein was measured at 25°C. This value is four- to fivefold higher than that reported for the tricarboxylate carrier purified from rat liver[17] and even more than the value reported for the same carrier from bovine liver.[16] The high activity of the eel liver tricarboxylate carrier is in agreement with the higher rate of lipogenesis measured in the liver of the fish with respect to that determined in the liver of mammals.[49] The turnover number, calculated by assuming a pure isolated protein monomer of 30.4 kDa, corresponds to 304 $min^{-1}$. It is approximately the same as that found for the reconstituted dicarboxylate, oxoglutarate, and ADP/ATP carrier[48,50,51] but definitely lower than that of the phosphate carrier[52] and higher than that of the tricarboxylate, carnitine, and ornithine carrier from rat liver.[17,44,45] The half-saturation constants of citrate (62 $\mu M$) and malate (541 $\mu M$) for the eel tricarboxylate carrier are lower than those determined for the tricarboxylate carrier isolated from rat liver.[17] Similarly, the $K_i$ values evaluated from competition experiments (Table II) show that the affinities of the eel tricarboxylate carrier for *cis*-aconitate, isocitrate, 1,2,3-benzenetricarboxylate and phosphoenolpyruvate are higher than those of the rat liver tricarboxylate carrier. On the contrary, the affinities of the eel tricarboxylate carrier for succinate and malonate are remarkably lower than those of the rat liver tricarboxylate carrier. Another major kinetic difference between the eel and the rat tricarboxylate carrier is demonstrated by the finding that the temperature dependence of the former, but not of the latter,[17] shows a definite break point at 19°C. The activity of the purified eel liver tricarboxylate carrier is influenced by the lipid composition of the liposomes (Fig. 3). In particular, cardiolipin and phosphatidylethanolamine were found to increase the activity of the eel tricarboxylate carrier, whereas phosphatidylinositol and phosphatidylserine were found to decrease it. The stimulatory effect by phosphatidylethanolamine on the activity of the eel tricarboxylate carrier appears to be rather characteristic. First, phosphatidylethanolamine does not share its effect with any of the phosphatidylcholines that were tested. Second, phosphatidylethanolamine does not have any effect on the activity of the rat liver tricarboxylate carrier[17] or on that of any other mitochondrial carrier investigated so far.[44,45,48] The effect of cardiolipin on the eel tricarboxylate carrier also requires some comment. With the exception of the dicarboxylate and oxoglutarate carriers, that are inhibited by cardiolipin,[48,50] this phospholipid is known to enhance the activity of the mitochondrial carriers.[38,40,44,45] However, the activity of most carriers was enhanced not only by cardiolipin but also by two other acidic phospholipids, namely phosphatidylinositol and phosphatidylserine. This behavior, which has also been shown for the rat tricarboxylate carrier,[17] contrasts markedly with the stimulation of the eel tricarboxylate carrier by cardiolipin and its inhibition by phosphatidylinositol and phosphatidylserine. The observed inhibitory effects exerted *in vitro* by the latter two phospholipids are consistent with the fact that their content in the inner mitochondrial membrane of eel liver is considerably lower than in the inner membrane of rat liver mitochondria (V. Zara and V. Gnoni, personal communication). In summary, the influence of phospholipids on the activity of the eel tricarboxylate carrier is quite different from that previously found for the tricarboxylate carrier of rat liver mitochondria.[17] This striking behavior may be due to differences in the primary structure of the eel citrate carrier and to the different lipidic microenvironment that surrounds the carrier protein in the fish inner mitochondrial membrane.

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