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Kinetic characterization of the reconstituted tricarboxylate carrier from rat liver mitochondria

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The tricarboxylate carrier from rat liver mitochondria was purified by chromatography on hydroxyapatite/celite and reconstituted in phospholipid vesicles by removing the detergent using hydrophobic chromatography on Amberlite. Optimal transport activity was obtained by using a Triton X-114/phospholipid ratio of 0.8, 6% cardiolipin and 24 passages through a single Amberlite column. In the reconstituted system the incorporated tricarboxylate carrier catalyzed a first-order reaction of citrate/citrate or citrate/malate exchange. The activation energy of the exchange reaction was 70.1 kJ/mol. The rate of the exchange had a pH optimum between 7 and 8. The half-saturation constant was 0.13 mM for citrate and 0.76 mM for malate. All these properties were similar to those described for the tricarboxylate transport system in intact mitochondria. In proteoliposomes the maximum exchange rate at 25 °C reached 2000 μmol/min per g protein. This value was independent of the type of substrate present at the external or internal space of the liposomes (citrate or malate).

Introduction

The transport of citrate through the inner mitochondrial membrane is catalyzed by a specific transport system, known as the tricarboxylate carrier (for a review see Refs. 1 and 2). This transport system plays an important role in fatty acid synthesis, gluconeogenesis and the transfer of reducing equivalents across the membrane [3]. Besides tricarboxylates, also phospho*enol*pyruvate, L-malate and other dicarboxylates are transported by a strict antiport mechanism. The carrier is inhibited by some impermeable tricarboxylate analogues like 1,2,3-benzenetricarboxylate as well as by sulphydryl reagents, but not by *N*-ethylmaleimide. The kinetic properties of the tricarboxylate carrier have also been characterized in intact liver mitochondria [4,5]. Interestingly, the activity of the tricarboxylate carrier is high in liver and virtually absent in heart and brain [6,7].

The tricarboxylate carrier has recently been isolated from rat liver mitochondria by chromatography on hydroxyapatite and celite [8] and from bovine liver mitochondria by chromatography on hydroxyapatite and Silica Gel 60 [9]. In SDS-containing gels, the purified fraction from rat liver consists of a single band with M_r 30000 [8], whereas that from bovine liver of a single band with M_r 37000–38000 [9]. In both cases, after incorporation into liposomes the purified protein has been functionally identified as the tricarboxylate carrier [8,9] by its requirement for a counteranion as well as its substrate specificity and inhibitor sensitivity.

In this paper the conditions for optimal reconstitution of the mitochondrial tricarboxylate carrier are described. The basic kinetic parameters of the citrate/citrate and malate/citrate exchange in reconstituted liposomes are reported.

Materials and Methods

Materials

Hydroxyapatite (Bio-Gel HTP) and Dowex AGl-X8 were purchased from Bio-Rad; Celite 535 from Serva; Amberlite XAD-2 from Fluka; [1,5-^{14}C]citrate and L-[U-^{14}C]malate from Amersham International (Amersham, U.K.); egg yolk phospholipids (L-α-phosphatidyl-

Abbreviations: EYPL, egg yolk phospholipids; Pipes, 1,4-piperazinediethanesulphonic acid; SDS, sodium dodecyl sulphate.

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choline from fresh turkey egg yolk), phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, dioleylphosphatidylcholine, dipalmitoylphosphatidylcholine, distearylphosphatidylcholine, cardiolipin, Pipes, Triton X-100 and Triton X-114 from Sigma; Sephadex G-75 from Pharmacia. Other reagents were obtained as reported [5,8].

Isolation and reconstitution of tricarboxylate carrier

The tricarboxylate carrier of rat liver mitochondria was purified in Triton X-100 as described previously [8]. Reconstitution of the tricarboxylate carrier into liposomes was performed by removing the detergent with a hydrophobic ion-exchange column [10,11]. In this procedure, the mixed micelles containing detergent, protein and phospholipids were repeatedly passed through Amberlite XAD columns. The composition of the mixture used for reconstitution was: 200 μl of the purified tricarboxylate carrier (about 0.1 μg protein) containing 0.8 mg cardiolipin, 90 μl of 10% Triton X-114, 100 μl of 10% egg yolk phospholipids in the form of sonicated liposomes prepared as described in Ref. 12, 10 mM citrate or 20 mM malate, 10 mM Pipes (pH 7.0) in a final volume of 700 μl. Unless otherwise specified in the legends to tables and figures, the initial Triton X-114/phospholipid ratio (w/w) was 0.8. After vortexing, this mixture was passed 24 times through the same Amberlite column (0.5 × 3.6 cm) preequilibrated with a buffer containing 10 mM Pipes and the substrate present in the starting mixture at the same concentration. All the operations were performed at 4°C except the passage through Amberlite, which was carried out at room temperature.

Transport measurements

The external substrate was removed by passing the proteoliposomes through a Sephadex G-75 column preequilibrated with 50 mM NaCl and 10 mM Pipes (pH 7.0). The first 600 μl of the turbid eluate from the Sephadex column were collected, transferred to reaction vessels (150 μl each), incubated at 25°C (for 4 min), and then used for transport measurements by the inhibitor stop method [13]. Transport was initiated by adding the labelled substrate ([^{14}C]citrate or [^{14}C]-malate) at the concentrations indicated in the legends to tables and figures, and stopped, after the desired time interval, by the addition of 20 mM 1,2,3-benzenetricarboxylate, a known inhibitor of the tricarboxylate carrier. In control samples, the inhibitor was added together with the labeled substrate at time zero. The external radioactivity was removed by passing the samples through a Dowex AG1-X8 column, 100–200 mesh, chloride form (0.5 × 4 cm equilibrated with 50 mM NaCl). The liposomes eluted with 1 ml of 50 mM NaCl were collected in 4 ml of scintillation mixture, vortexed and counted. The transport activity was calculated by subtracting the control from the experimental values. The 1,2,3-benzenetricarboxylate-insensitive radioactivity associated to the control samples was always less than 10% with respect to the 1,2,3-benzenetricarboxylate-sensitive citrate or malate uptake. K_m and V values were determined by a computer-fitting program based on linear regression analysis. Control experiments showed that the rate of both citrate and malate uptake was maximal when the proteoliposomes were loaded with 10 mM citrate or 20 mM malate.

Other methods

Protein was determined by the Lowry method modified for the presence of Triton [14]. The samples used for protein determination were subjected to acetone precipitation and redissolved in 1% SDS. The internal volume of the total liposomes (i.e., liposomes with and without incorporated carrier protein) was determined as described in Ref. 10.

Results

Optimal conditions of reconstitution

For a detailed characterization of the kinetic properties of a purified carrier protein, the conditions leading to optimal reconstitution have to be thoroughly investigated. We have chosen three appropriate criteria for this analysis describing the state of the reconstituted protein and/or the liposomes: (a) the activity of the citrate/citrate exchange derived from the kinetics within the first minute, (b) the total exchange, i.e., the amount of labeled citrate taken up after reaching equilibrium (120 min after addition of label), and (c) the intraliposomal volume.

Fig. 1 illustrates the dependence of these three parameters on the detergent/phospholipid ratio during reconstitution. It should be noted that on the one hand the value given for the intraliposomal volume means the volume of all the liposomes produced (see Methods), on the other hand the number (or size) of the liposomes which contain active carrier can be inferred from the total exchange. At an initial ratio detergent/phospholipid lower than 0.8, all three parameters, i.e., the exchange activity, the total exchange and the intraliposomal volume, increased in a similar way on increasing the concentration of Triton X-114. This means that, in this range of detergent/phospholipid ratio, both the amount of active carrier incorporated and the number (and/or size) of total liposomes increased on increasing the detergent concentration. Above the optimum detergent/phospholipid ratio of 0.8, all three parameters decreased. The exchange activity and the total activity, however, were definitely more affected than the intraliposomal volume. This indicates that again both the number (and/or size) of the liposomes and, to a greater extent, the amount of incorporated active carrier pro-

tein are affected. A similar influence of Triton X-114 on the efficiency of reconstitution of other mitochondrial carriers has been observed using the same method of reconstitution [10,15].

As shown in Fig. 2, the rate of citrate/citrate exchange progressively increased with the number of passages through Amberlite, reaching a maximum after 21–24 passages. This behaviour is similar to that reported for other mitochondrial carriers [10,15], although in our case a higher number of passages through Amberlite was required for optimal activity. The intraliposomal volume markedly increased on increasing the number of amberlite passages up to 15; after 15 passages it remained practically constant. Correspondingly, the total exchange was more or less independent of the number of column passages from 15 to 30.

It is known that lipids definitely influence the activity of reconstituted mitochondrial anion carriers [16–19]. In particular it has been reported that the tricarboxylate carrier is activated specifically by cardiolipin [20]. Since cardiolipin is needed for the elution of the tricarboxylate carrier from celite and is therefore always present in the purified preparation of this carrier protein [8], the effect of lipids has been investigated using the enriched carrier preparation after the previous step of purification, i.e., the hydroxyapatite chromatography. Fig. 3 illustrates the effect of cardiolipin on the activity of the tricarboxylate carrier when added to EYPL (egg yolk

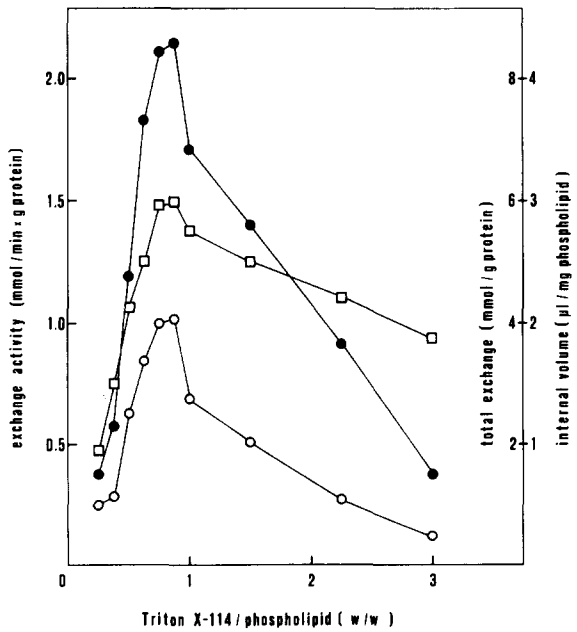

Fig. 1. Dependence of the efficiency of reconstitution of the tricarboxylate carrier on the detergent/phospholipid ratio. The proteoliposomes were prepared as described in Materials and Methods except that increasing concentrations of Triton X-114 were used. 0.1 mM [^{14}C]citrate was added to proteoliposomes which contained 10 mM citrate. The exchange activity measured after 1 min (○), the total exchange calculated from the exchange equilibrium after 120 min (●) and the internal volume (□) were determined.

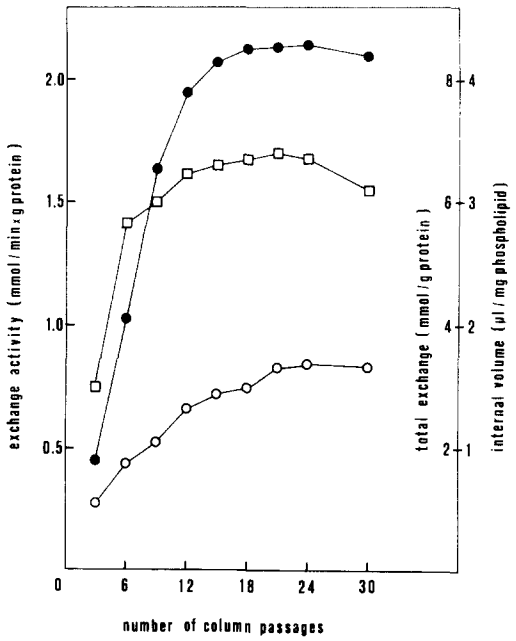

Fig. 2. Dependence of the efficiency of reconstitution of the tricarboxylate carrier on the number of the Amberlite column passages. The proteoliposomes were prepared as described in Materials and Methods except that the number of passages through the same Amberlite column was varied as indicated. 0.1 mM [^{14}C]citrate was added to proteoliposomes which contained 10 mM citrate. The exchange activity measured after 1 min (○), the total exchange calculated from the exchange equilibrium after 120 min (●) and the internal volume (□) were determined.

phospholipids) during reconstitution. The rate of citrate/citrate exchange increased on increasing the concentration of cardiolipin up to 6% (w/w). Above this concentration the activity decreased, being still 33% higher than the control value (without cardiolipin) at 20% added cardiolipin. The internal volume was increased by cardiolipin reaching a maximum at 10%, then remaining constant up to 20%. As shown in Fig. 3, cardiolipin also affected the total exchange which exhibited a definite plateau between 6 and 10%. The specificity of this activating effect was tested by adding various phospholipids at different concentrations to EYPL in the reconstitution mixture (Table I). At a concentration of 6%, besides cardiolipin also the two other acidic phospholipids, i.e., phosphatidylinositol and phosphatidylserine, increased the rate of citrate/citrate exchange. Addition of phosphatidylethanolamine had no effect on the transport activity. The rate of the exchange was, on the other hand, inhibited by dioleylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearylphosphatidylcholine. The fact that the total exchange was decreased by added phosphatidylcholines, without a significant change in the intraliposomal volume, suggests a negative influence of these phospholipids on the incorporation of the tricarboxylate carrier into the liposomes. Vice versa the increase in the total exchange by phosphatidylinositol and phospha-

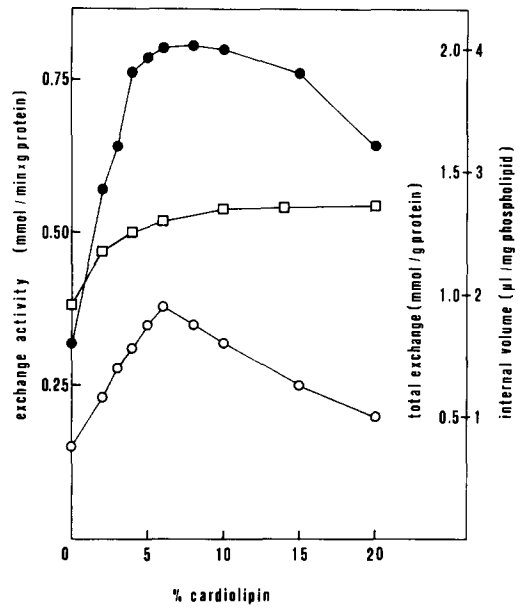

Fig. 3. Dependence of the efficiency of reconstitution of the tricarboxylate carrier on the concentration of cardiolipin. The proteoliposomes were prepared as described in Materials and Methods except that the hydroxylapatite eluate was used instead of the purified tricarboxylate carrier and the concentration of cardiolipin with respect to the EYPL was varied as indicated in the figure. 1 mM [^{14}C]citrate was added to proteoliposomes which contained 10 mM citrate. The exchange activity measured after 1 min (○), the total exchange calculated from the exchange equilibrium after 120 min (●) and the internal volume (□) were determined.

tidylserine and, to a greater extent, by cardiolipin has to be attributed at least in part to an increased incorporation of the carrier molecules into the liposomes, because it cannot sufficiently be accounted for by the observed small increase in the internal volume. The possible alternative explanation that the carrier incorporated is activated by acidic phospholipids is unlikely since the citrate/citrate exchange activity, which should be the most sensitive parameter, is increased less than the total exchange. In the presence of a higher concentration (20%) of phospholipids added to EYPL, the rate of the citrate/citrate exchange was slightly increased only by cardiolipin; addition of all the other phospolipids inhibited the transport activity. The total exchange was not affected by phosphatidylinositol and phosphatidylserine, indicating that under these conditions the amount of carrier incorporated is the same, but the carrier is less active, which is also supported by the reduced specific activity. In the light of the results shown above, in all subsequent experiments we used a Triton X-114/phospholipid ratio of 0.8, 6% cardiolipin and 24 passages through a single Amberlite column.

Reaction order, temperature- and pH-dependence of [^{14}C]citrate uptake

In Fig. 4, the time course of the [^{14}C]citrate uptake in proteoliposomes loaded either with citrate or with malate is reported. Citrate uptake increased linearly with time for about 2 min in citrate-loaded liposomes and for about 1 min in malate-loaded liposomes. These results were obtained in experiments where several kinetic measurements were carried out within the first minutes (not shown). In both cases the rate of citrate uptake evaluated from the initial, approximately linear range was 0.79 mmol/min per g protein at 25 °C (at 0.1 mM citrate). The total amount of citrate/g protein taken up into the proteoliposomes, on the other hand, was different in the two types of vesicle, being 35% lower in the case of malate-loaded liposomes. The observed difference in the equilibrium exchange value can easily be rationalized taking into account the difference in the affinities of malate and citrate to the carrier (see below). The reac-

TABLE I

Influence of different phospholipid composition of liposomes on the reconstituted citrate/citrate exchange activity

Reconstitution was performed with hydroxyapatite eluate, instead of purified tricarboxylate carrier, and with liposomes prepared from EYPL or a mixture of EYPL and the indicated phospholipids present at a concentration of 6% or 20%. Abbreviations: EYPL, egg yolk phospholipids (L-α-phosphatidylcholine from turkey eggs, Sigma); DPG, cardiolipin; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; DOPC, dioleylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearylphosphatidylcholine. 1 mM [^{14}C]citrate was added to proteoliposomes loaded with 10 mM citrate.

Phospholipid composition	Expt. 1 (with 6% phospholipids added to EYPL)			Expt. 2 (with 20% phospholipids added to EYPL)		
	specific activity (μmol/min per g protein)	total exchange (μmol/120 min per g protein)	internal volume (μl/mg phospholipid)	specific activity (μmol/min per g protein)	total exchange (μmol/120 min per g protein)	internal volume (μl/mg phospholipid)
EYPL	220	809	2.0	180	709	2.0
+ DPG	417	2544	2.6	244	1057	2.7
+ PE	215	886	2.0	108	410	2.6
+ PI	337	1763	2.2	68	700	1.9
+ PS	359	1752	2.3	117	730	2.0
+ DOPC	140	558	1.9	70	332	2.9
+ DPPC	155	700	2.1	96	431	2.5
+ DSPC	135	530	1.9	60	320	2.6

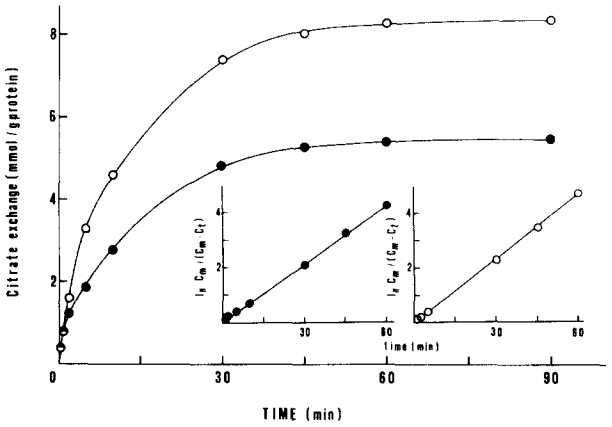

Fig. 4. Time-course of the citrate/citrate and citrate/malate exchange in reconstituted liposomes. 0.1 mM [^{14}C]citrate was added to proteoliposomes which contained 10 mM citrate (○) or 20 mM malate (●). The insets are logarithmic plots of ln $C_m/(C_m-C_t)$ where C_m is the maximum citrate exchange/g 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 8.35 and 5.48 mmol/g protein for the citrate/citrate exchange and the citrate/malate exchange, respectively.

tion order of the citrate/citrate and citrate/malate exchanges was investigated by plotting the natural logarithm of the fraction of equilibrium $citrate_{max}/(citrate_{max}-citrate_t)$ against time [21]. As shown in the insets of Fig. 4, straight lines were obtained, demonstrating that the two exchange reactions follow a first-order kinetics similar to the results obtained for the citrate/malate exchange in intact mitochondria [5]. The first-order rate constant, k, was 0.07 min^{-1} for the citrate/malate exchange and 0.08 min^{-1} for the citrate/citrate exchange.

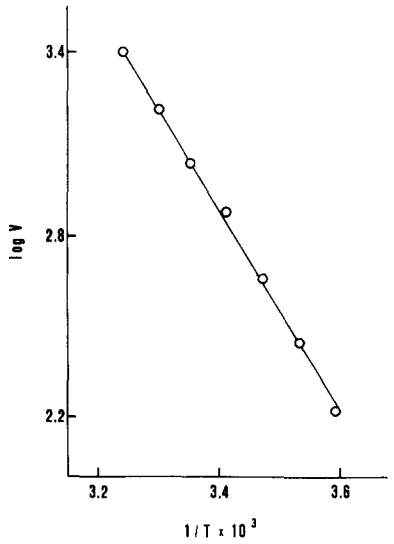

Fig. 5. Temperature dependence of the rate of the reconstituted citrate/citrate exchange. 0.1 mM [^{14}C]citrate was added to proteoliposomes which contained 10 mM citrate and were incubated at the indicated temperatures. The exchange activity, V, is expressed in μmol/min per g protein.

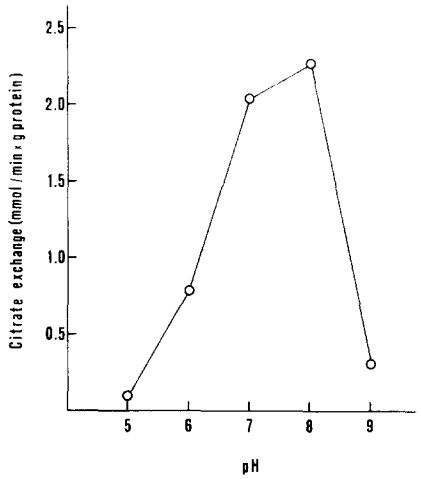

Fig. 6. pH dependence of the rate of reconstituted citrate/citrate exchange. 1 mM [^{14}C]citrate was added to proteoliposomes which contained 10 mM citrate and were incubated at the indicated pH values. The data represent the mean of the values obtained in six different experiments. The standard deviations are 0.024 and 0.030 at pH 7 and pH 8, respectively.

The temperature dependence of the rate of citrate/citrate exchange is shown in Fig. 5. The rate of citrate exchange increased about 15-times with increasing the temperature from 5 to 35 °C. A straight line was obtained in an Arrhenius plot over this range with an activation energy of 70.1 kJ/mol.

Fig. 6 illustrates the influence of the external pH on the rate of citrate/citrate exchange. In the presence of a nearly saturating concentration of external citrate (1 mM), high transport rates were obtained in the range between pH 7 and 8. The pH optimum was reproducibly found to be pH 8. At pH values lower than 7 and higher than 8 the rate of citrate exchange markedly decreased.

K_m and V values of citrate and malate transport

The dependence of the exchange rate on substrate concentration was studied by changing the concentration of externally added [^{14}C]citrate or [^{14}C]malate at constant internal concentration of 10 mM citrate and 20

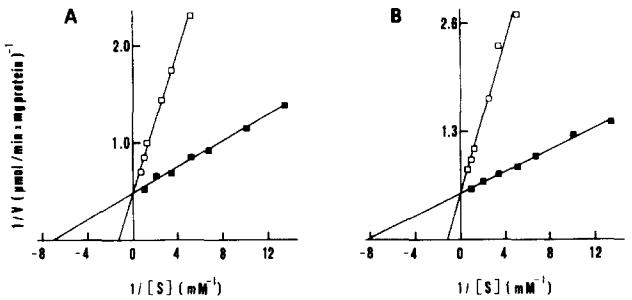

Fig. 7. The dependence of the rate of citrate and malate uptake in proteoliposomes on substrate concentration. [^{14}C]Citrate (■) or [^{14}C]malate (□) was added at the concentrations indicated to proteoliposomes which contained 10 mM citrate (A) or 20 mM malate (B).

TABLE II

K_m and V values for the uptake of citrate and malate in citrate-loaded or malate-loaded proteoliposomes

Experimental conditions as in Fig. 7. The values given in the table are the means ± S.E. of 4–20 experiments.

Substrate	Internal substrate	K_m (mM)	V(μmol/min per g protein)	No. expts.
Citrate	citrate	0.13 ± 0.01	2049 ± 94	20
Malate	citrate	0.76 ± 0.04	1868 ± 106	6
Citrate	malate	0.14 ± 0.03	2059 ± 129	4
Malate	malate	0.80 ± 0.04	1983 ± 155	4

mM malate, respectively. The data from typical experiments are shown in Fig. 7 as Lineweaver-Burk plots. In both citrate-loaded (Fig. 7A) and malate-loaded (Fig. 7B) liposomes, linear functions were obtained showing common intersections at the ordinate. This indicates that V (approx. 2100 μmol/min per g protein for the citrate/citrate and malate/citrate exchanges and approx. 1800 μmol/min per g protein for the citrate/malate and malate/malate exchanges) is independent of the type of substrate present on the two sides of the membrane. The different slopes, however, indicate that the apparent transport affinity constants are different. In these experiments, the apparent K_m for citrate (0.13 mM) was about 6-fold lower than that for malate (0.8 mM). Table II reports mean values and standard errors of K_m and V for the uptake of citrate and malate both in citrate-loaded and malate-loaded proteoliposomes. The statistical analysis of these data shows that the differences in K_m values are highly significant when different external substrates are present, while the V values are not significantly different within the experimental error.

TABLE III

K_i values for substrates and substrate analogues competing with citrate for the exchange reaction

The K_i values were calculated from double reciprocal plots of the rate of citrate/citrate exchange versus substrate concentrations. The experimental conditions were the same as in Fig. 7. The competing anions were added simultaneously with [^{14}C]citrate at the appropriate concentrations.

	K_i (mM)	No. expts.
cis-Aconitate	0.04 ± 0.02	3
threo-D$_s$-Isocitrate	0.08 ± 0.02	3
1,2,3-Benzenetricarboxylate	0.08 ± 0.02	4
Phospho*enol*pyruvate	0.18 ± 0.03	3
Succinate	2.5	2
Malonate	3.1	2
Benzylmalonate	2.8	2
p-Iodobenzylmalonate	1.0	2

Inhibition by substrates and substrate analogues

The inhibition of the reconstituted citrate/citrate exchange by various compounds was analyzed in the presence of different substrate concentrations. Isocitrate and other tricarboxylates, malate and other dicarboxylates as well as phosphoenolpyruvate were all identified as competitive inhibitors, since they were found to increase the apparent K_m without changing the V of the citrate exchange (not shown). The inhibition constants, K_i, are summarized in Table III.

Discussion

In this study we have applied a method of reconstitution based on detergent removal by chromatography on Amberlite [10,11]. This method resulted in several-fold higher transport activities than those observed using the freeze-thaw-sonication procedure (data not shown) and led to the formation of larger proteoliposomes, which are more appropriate for kinetic studies. Among the parameters which may influence the efficiency of reconstitution of the purified tricarboxylate carrier, particularly the ratio of detergent to phospholipids, the number of passages through the Amberlite column and the lipid composition of the liposomes were found to be important for obtaining high transport activities.

In the reconstituted system we have determined the basic kinetic data of the tricarboxylate carrier from rat liver mitochondria. The reconstituted tricarboxylate carrier catalyzes a first-order reaction. The temperature dependence of the transport reaction from 5 to 35 °C is linear in an Arrhenius plot. When comparing the temperature dependence of several reconstituted mitochondrial carrier proteins it became obvious that most of them do not show break points (Refs. 15, 22, 23 and this paper), whereas two (namely the ADP/ATP carrier and the phosphate carrier) exhibit a definite break between 20 and 25 °C [24,25]. The activation energy of the tricarboxylate carrier was determined to be 70.1 kJ/mol, which is close to the values obtained for the other reconstituted mitochondrial carriers and for the ADP/ATP carrier in the higher temperature range [15,22–25]. As observed for citrate transport in mitochondria [5], the reconstituted tricarboxylate carrier shows a marked pH optimum, being strongly inactivated at pH values below 7 and above 8. A detailed study with respect to the dependence of the reconstituted tricarboxylate carrier activity on pH in relation to the species ($citrate^{2-}$ or $citrate^{3-} + H^+$) transported by the carrier is currently in progress in our laboratory.

Under optimal conditions, a V value of 2000 μmol/min per g protein was measured at 25 °C. This value was found to be very similar for two different external substrates and did not at all depend on the nature of the internal substrate. Thus, similar to the situation found for the dicarboxylate carrier [15], the

translocation step within the transport cycle of the tricarboxylate carrier seems not to be influenced very much by the kind of substrate transported. The specific activity estimated in this study is several-fold higher than that reported by Claeys and Azzi (376 nmol/min per mg protein at 30 °C) for the tricarboxylate carrier purified from bovine liver [9], which, however, may also be due to different reconstitution procedures. The turnover number, which was calculated assuming that the isolated protein is pure and consists of a monomer of 30 kDa, corresponds to 60.1 min^{-1}. This turnover number is in the same order of magnitude as found for the reconstituted ADP/ATP carrier and the reconstituted dicarboxylate carrier [15,26] and is lower than that reported for the reconstituted phosphate carrier [25,27,28]. The half-saturation constants and K_i values reported in this paper for the known substrates and inhibitors of the reconstituted tricarboxylate carrier are very similar to those found in intact mitochondria [5]. This held true in particular for the much higher affinity of the reconstituted carrier for citrate in comparison to malate. It is important that identical apparent K_m values were found for external citrate and malate in both citrate-loaded and malate-loaded proteoliposomes. This indicates that the half-saturation constant of the carrier for a particular substrate does not depend on the counteranion under saturating internal concentrations.

Cardiolipin was already known to enhance the activity of the reconstituted tricarboxylate carrier [19,20], although it has not been investigated whether this effect is due to increased incorporation of carrier protein or to the activation of the incorporated carrier. Also the influence of cardiolipin on the size and number of liposomes has not been tested. In this paper we present evidence that the activity of the reconstituted tricarboxylate carrier was increased not only by cardiolipin but also by two other acidic phospholipids, phosphatidylinositol and phosphatidylserine. This effect can to a larger extent be attributed to an increased incorporation of the carrier into the liposomes and only to a lower degree to an activation of the incorporated carrier. In comparison to this, the effect of a slightly increased volume of the proteoliposomes seems not to be as important. This conclusion is based on the observation that optimal concentrations of acidic phospholipids increase the total exchange considerably more than the specific exchange activity (Table I). Similar to the tricarboxylate carrier, the reconstituted ADP/ATP carrier and the reconstituted phosphate carrier are known to be activated by cardiolipin or acidic phospholipids [16–18]. In contrast, the reconstituted dicarboxylate carrier and the reconstituted oxoglutarate carrier are inhibited by cardiolipin and phosphatidylinositol [15,22].

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