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Kinetic characterization of the reconstituted carnitine carrier from rat liver mitochondria

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The carnitine carrier was purified from rat liver mitochondria and reconstituted into liposomes by removing the detergent from mixed micelles by Amberlite. Optimal transport activity was obtained with 1 μg/ml and 12.5 mg/ml of protein and phospholipid concentration, respectively, with a Triton X-100/phospholipid ratio of 1.8 and with 16 passages through the same Amberlite column. The activity of the carrier was influenced by the phospholipid composition of the liposomes, being increased in the presence of cardiolipin and decreased in the presence of phosphatidylinositol. In the reconstituted system the incorporated carnitine carrier catalyzed a carnitine/carnitine exchange which followed a first-order reaction. The maximum transport rate of external [^{3}H]carnitine was 1.7 mmol/min per g protein at 25°C and was independent of the type of countersubstrate. The half-saturation constant (K_m) for carnitine was 0.51 mM. The affinity of the carrier for acylcarnitines was in the μM range and depended on the carbon chain length. The activation energy of the carnitine/carnitine exchange was 133 kJ/mol. The carrier function was independent of the pH in the range between 6 and 8 and was inhibited at pH below 6.

Introduction

The inner mitochondrial membrane contains a specific system for the transport of carnitine and acylcarnitines, known as the carnitine carrier (for review, see Ref. 1). This transport system plays an important role in the translocation of fatty acids as acylcarnitines into the mitochondrial matrix, where the acyl groups are released to be used for fatty acid oxidation.

The properties of the carnitine carrier have been investigated in intact mitochondria since 1975 [2–8]. This carrier catalyzes an exchange between carnitine and acylcarnitines of various length [7]; it is inhibited by SH-reagents like mersalyl and *N*-ethylmaleimide and by substrate analogues like sulphobetaines [4,5]. Some kinetic properties of the carnitine carrier have also been characterized in intact mitochondria [5,8].

Attempts have been made to reconstitute the activity of the carnitine carrier from a total mitochondrial extract [9] or a partially purified preparation [10]. Recently we have isolated the carnitine transport protein from rat liver mitochondria by chromatography on hydroxyapatite and celite [11]. In SDS-containing gels, the purified fraction consists of a single band with an apparent M_r of 32500. After incorporation into liposomes the purified protein has been functionally identified as the carnitine carrier [11] by its substrate specificity and inhibitor sensitivity, which are both very similar to those described for the carnitine transport system in mitochondria.

In this paper, the conditions for optimal reconstitution of the purified mitochondrial carnitine carrier are described. In addition, the values of transport rate and related kinetic parameters of uptake of carnitine by reconstituted liposomes loaded with carnitine or acetylcarnitine have been measured.

Materials and Methods

Materials. Hydroxyapatite (Bio-Gel HTP) was purchased from Bio-Rad, Celite 535 from Roth, Amberlite XAD-2 from Fluka, Sephadex G-50 and G-75 from Pharmacia, L-[^{3}H]carnitine from Amersham. L-α-Phosphatidylcholine from fresh turkey egg yolk (EYPL),

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

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L-α-phosphatidylcholine-dipalmitoyl, -dioleoyl, -distearoyl, L-α-phosphatidylethanolamine, L-α-phosphatidylinositol, L-α-phosphatidyl-L-serine, cardiolipin, Pipes (1,4-piperazinediethanesulphonic acid), Triton X-100, L-carnitine, DL-octanoylcarnitine, DL-myristoylcarnitine and L-palmitoylcarnitine were obtained from Sigma, L-acetylcarnitine, DL-lauroylcarnitine, DL-palmitoylcarnitine and DL-stearoylcarnitine from Serva. L-α-Methylbutyrylcarnitine, D-carnitine and L-isobutyrylcarnitine were a gift of Prof. N. Siliprandi. All other reagents were of analytical grade.

Isolation and reconstitution of the carnitine carrier. The carnitine carrier from rat liver mitochondria was purified by chromatography on hydroxyapatite and celite as described previously [11]. Liposomes were prepared as described previously [12] by sonication of 100 mg egg yolk phospholipids in 1 ml water for 60 min at 0°C. The purified carnitine carrier was reconstituted by a method based on detergent removal by hydrophobic chromatography on Amberlite [13]. A mixture containing protein, lipids and detergent was applied several times on the same Amberlite column. Unless otherwise specified in the legends to tables and figures, the composition of the initial mixture was: 380 μl of the purified carnitine carrier (about 1 μg protein in 3% Triton X-100), 20 μl of 10% Triton X-100, 100 μl of liposomes (7.5 mg phospholipids), 13 mM carnitine, 30 mM sodium phosphate (pH 7) in a final volume of 680 μl. After vortexing, this mixture was passed 16 times through the same Amberlite column (0.5 × 3.0 cm) preequilibrated with a buffer containing 30 mM sodium phosphate (pH 7) and 13 mM carnitine. All the operations were performed at 4°C except the passages through Amberlite which were carried out at room temperature.

Transport measurements. In order to remove the external substrate, 550 μl of proteoliposomes were passed through a Sephadex G-75 column (0.7 × 15 cm), preequilibrated with 60 mM NaCl and 10 mM Pipes (pH 7). The eluted proteoliposomes (700 μl), distributed in reaction vessels (100 μl), were used for transport measurements by the inhibitor stop method [14]. Transport was started by adding 10 μl of [^{3}H]carnitine at the concentrations indicated in the legends to tables and figures, and stopped, after the desired time interval, by adding 5 μl of 30 mM *N*-ethylmaleimide, a known inhibitor of the carnitine carrier. In control samples the inhibitor was added together with the labeled substrate at time zero. The incubation temperature was 25°C. In order to remove the external radioactivity, each sample was passed through a Sephadex G-50 column (0.6 × 8 cm). The liposomes eluted with 1.3 ml 60 mM NaCl were collected in 4 ml of scintillation mixture, vortexed and counted. The transport activity was evaluated as the difference between the experimental and the control values, which were measured after 2 and 4 min, i.e., within the initial linear range of [^{3}H]carnitine uptake into the proteoliposomes. The *N*-ethylmaleimide-insensitive radioactivity associated to the proteoliposomes was always less than 5% of the *N*-ethylmaleimide-sensitive radioactivity taken up during the transport assay. K_m and V_{max} values were determined by a computer-fitting program based on linear regression analysis.

Measurements of intraliposomal volumes. The total internal volume of the liposomes (i.e., liposomes with and without incorporated carrier protein) was determined as described in Ref. 13. The 'active' intraliposomal volume (i.e., that of liposomes with incorporated active carrier molecules) can be calculated for a carrier operating by an exchange mechanism, by the equation: V_a (μl/ml) = $(dpm_{in}/dpm_{ex}) \times (S_{ex}/S_{in}) \times 1000$, where dpm_{in} and dpm_{ex} represent the radioactive label inside and outside the liposomes after equilibrium has been reached, and S_{ex} and S_{in} are the concentrations of substrate present outside and inside the liposomes. Since the amount of the purified protein added to the reconstitution mixture was very low with respect to phospholipids (protein/phospholipid ratio (w/w) = $1.3 \cdot 10^{-4}$), the active internal volume was only about 2.5/100 of the total intraliposomal volume.

Other methods. Protein was determined by the Lowry method modified for the presence of Triton [15]. All the samples used for protein determination were subjected to acetone precipitation and redissolved in 1% SDS [11]. The possible loss of carnitine from the active intraliposomal space was tested after labeling the internal active pool (by incubation of the proteoliposomes with 1 μM [^{3}H]carnitine for 20 min) and removing the external radioactivity.

Results

In previous papers the [^{3}H]carnitine/carnitine exchange was measured in reconstituted liposomes to monitor the presence of the carnitine carrier during the purification procedures [9–11]. In these studies, long transport times were used to obtain a sufficient amount of labelled substrate inside the liposomes. The aim of this work is to determine the kinetic parameters of the transport catalyzed by the purified carnitine carrier. For this purpose, an accurate measurement of the initial rate of transport was necessary. Therefore the reconstitution procedure, performed by removing the detergent from mixed micelles of detergent, lipid and protein, has been optimized by adjusting the parameters that influence the efficiency of carrier incorporation into the liposomes. In these experiments both the initial transport rate and the total transport calculated from the exchange equilibrium after 60 min were measured. The first parameter gives information on

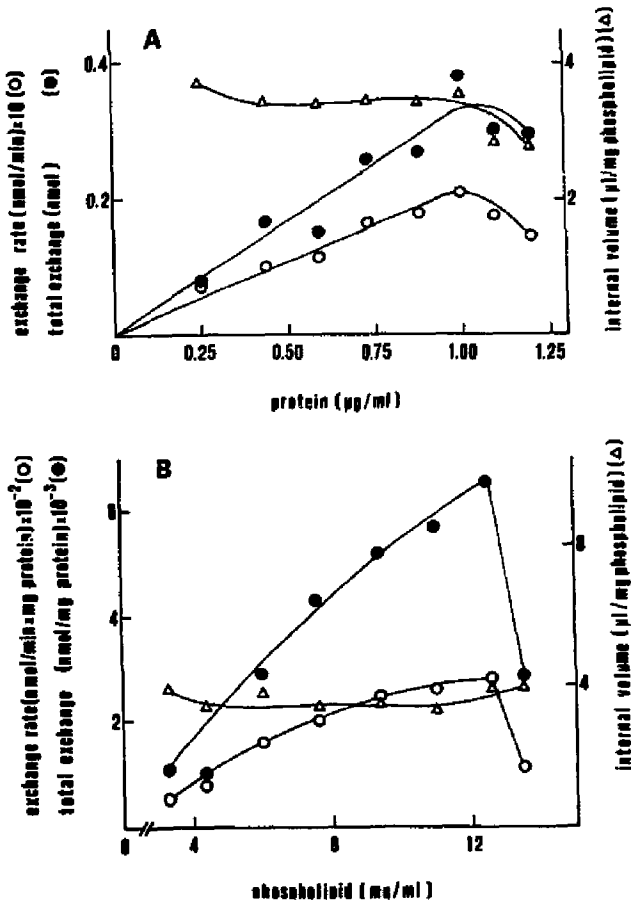

Fig. 1. Reconstitution of the carnitine carrier: variation of the protein (A) and phospholipid (B) concentration. The proteoliposomes were prepared as described in Materials and Methods except that increasing concentrations of protein (A) or phospholipids (B) were used. 0.1 mM [^{3}H]carnitine was added to proteoliposomes which contained 13 mM carnitine. The transport rate measured within the first 4 min (○), the total transport calculated from the exchange equilibrium after 60 min (●) and the total internal volume (△) were determined.

the specific activity of the carrier, whereas the uptake of radioactivity after 60 min is correlated to the number (and/or size) of the liposomes loaded with active carrier. Moreover the intraliposomal volume has been measured to verify the efficiency of reconstitution.

In Fig. 1 the influence of the protein and lipid concentration on the reconstituted carnitine/carnitine exchange is shown. Both the transport rate and the equilibrium transport (total transport) increased linearly with protein concentration (Fig. 1A) up to 1 μg/ml. Above this value a loss of activity corresponding to a reduction of the total liposomal volume was observed, indicating that higher concentrations of protein cause the formation of less or smaller liposomes and at the same time reduce the incorporation of active carrier into the liposomes. The maximal amount of labelled carnitine taken up by the liposomes (0.34 nmol) is in reasonably good agreement with the theoretical value of 0.8 nmol as calculated on the basis of the exchangeable internal carnitine present in the active intraliposomal volume (see Methods). The dependence of the exchange on the lipid concentration is shown in Fig. 1B. Maximum transport rate and total transport were found at 12 mg phospholipid/ml. Above this concentration we observed a marked decrease of activity, whereas the total liposomal volume remained constant. Probably in the presence of high lipid concentrations the incorporation of protein molecules is drastically decreased.

Other critical parameters for the method of reconstitution used in this work are the detergent/lipid ratio and the number of passages of the reconstitution mixture through the Amberlite column (see Methods). Both the exchange activity and the total exchange showed a maximum at a detergent/lipid ratio of 1.8 (Fig. 2A). Above this value these parameters decreased probably due to a decrease of the number and/or the size of liposomes, indicated by the reduction of the total internal volume, and perhaps also due to a reduction of the amount of carrier incorporated into the liposomes. A similar influence of Triton X-100 or Triton X-114 on the efficiency of reconstitution of other mitochondrial carriers was observed using the same method of reconstitution [13,16,17]. The number of column passages was not critical between 14 and 17 (Fig. 2B). In this range both the activity and the total internal volume reached their maximum. More than 20 passages led to a decrease of activity, whereas the internal volume remained constant. This may be accounted for by an increase in protein adsorption to the Amberlite material.

Lipids have been reported to modulate the activity of reconstituted mitochondrial carriers [16–21]. The influence of various phospholipids on the activity of the carnitine carrier when added to EYPL (egg yolk phospholipids) during reconstitution is shown in Table I. 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 carnitine carrier from celite and is therefore always present in the purified preparation of this carrier protein [11]. At a concentration of 5%, the carnitine/carnitine exchange activity was markedly increased by cardiolipin, decreased by phosphatidylinositol and not significantly influenced by all the other phospholipids tested. The fact that the exchange at equilibrium as well as the total volume of the liposomes were not influenced by cardiolipin suggests that the observed increase in the carrier activity can be explained by an activation of the carrier molecules incorporated rather than by an increased incorporation. The inhibition by phosphatidylinositol, on the other hand, is accompanied by a decrease both in the total exchange and in the total

liposomal volume. It is therefore difficult to decide whether this inhibition is due to inactivation or reduced incorporation of the carrier protein. When the concentration of the phospholipids added to EYPL in the reconstitution mixture was increased up to 20%, the rate of carnitine exchange was diminished by all the phospholipids tested with the only exception of dioleoylphosphatidylcholine. Since the exchange at equilibrium and the total volume of the liposomes were not affected by 20% cardiolipin, it is likely that at high concentrations this phospholipid inactivates the carrier.

The kinetics of [^{3}H]carnitine/carnitine exchange in proteoliposomes is shown in Fig. 3. The curve represents an exponential approach to isotopic equilibrium, which is demonstrated by the straight line obtained in the inset where the ln of maximum carnitine transport minus carnitine transport at a given time is plotted against time [22]. This means that the isotopic equilibration of carnitine catalyzed by the reconstituted carnitine carrier follows a first order kinetics. The first-order rate constant, k, extrapolated from the slope of the logarithmic plot, was 0.074 min^{-1}. We have performed control experiments under the conditions of Fig. 3 in order to test whether the carnitine content of the 'active' liposomes decreased during the long incubation times used (see Methods). It was found that the proteoliposomes lost 7–10% of the internal carnitine in 60 min (three experiments). When taking into account these results the reported constant rate could possibly be overestimated by about 10%.

In order to obtain the basic kinetic data of the carnitine carrier the dependence of the exchange rate on substrate concentration was studied by changing the concentration of externally-added [^{3}H]carnitine at a constant internal concentration of 13 mM carnitine or acetylcarnitine. The latter value was chosen on the basis of control experiments showing that the rate of carnitine exchange was maximal when the proteoliposomes were loaded with carnitine or acetylcarnitine from 10 mM to 30 mM. The data from a typical experiment are shown in Fig. 4 as a double-reciprocal plot. In both carnitine-loaded and acetylcarnitine-loaded liposomes, straight lines were obtained which were virtually undistinguishable. The K_m and V_{max} values for carnitine exchange at 25°C were approximately 0.51 mM and 2.1 mmol/min per g protein, respectively. Thus, the kinetic constants of the carnitine carrier are independent of the type of countersubstrate. In 20 experiments for the substrate carnitine an average value of 0.51 ± 0.14 mM for the K_m and 1.70 ± 1.3 mmol/min per g protein for the V_{max} was determined. The surprisingly high standard error of the V_{max} values when comparing different experiments, must be attributed to variations in the amount of active carrier molecules present in the different preparations of the purified carrier. Nevertheless, the V_{max} values for the two countersubstrates carnitine and acetylcarnitine, as compared in one experiment, were not significantly different (Fig. 4).

Fig. 4 also shows that by addition of 30 μM DL-myristoylcarnitine to the labeled substrate, the rate of [^{3}H]carnitine/carnitine exchange was inhibited in a purely competitive way, leading to a K_i of 4.3 μM. All the other acylcarnitines tested, as well as D-carnitine, (see Table II) were identified as competitive inhibitors, since they were found to increase the K_m without changing the V_{max} of the carnitine/carnitine exchange. The inhibition constants, K_i, are summarized in Table II.

Fig. 5 shows the temperature dependence of the rate of carnitine/carnitine exchange. In an Arrhenius

TABLE I

Dependence of the reconstituted carnitine/carnitine exchange activity on the phospholipid composition of liposomes

Reconstitution was performed with the hydroxyapatite eluate, instead of purified carnitine carrier, and with liposomes prepared from EYPL or a mixture of EYPL and the indicated phospholipids present at a concentration of 5%, 10% or 20%. 1 mM [^{3}H]carnitine was added to the proteoliposomes loaded with 13 mM carnitine. Abbreviations: EYPL, egg-yolk phospholipids (L-α-phosphatidylcholine from turkey eggs, Sigma); DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; DPG, cardiolipin; S.A., specific activity expressed in μmol/min per g protein; T.E., total exchange expressed in μmol/60 min per g protein; V_i, total internal volume expressed in μl/mg phospholipid.

	5%			10%			20%		
	S.A.	T.E	V_i	S.A.	T.E.	V_i	S.A.	T.E	V_i
EYPL	85	1310	1.9	78	1280	1.9	90	1410	1.8
+DOPC	78	1370	2.0	61	1310	2.1	71	1510	2.0
+DPPC	81	1250	2.1	50	1110	2.1	40	710	2.1
+DSPC	88	1330	1.9	48	970	2.0	37	780	2.0
+PI	54	970	1.2	47	815	1.1	30	510	0.7
+PS	71	1010	1.9	54	780	1.9	45	670	1.1
+PE	95	1400	2.0	77	1370	2.0	56	850	1.9
+DPG	190	1248	2.0	107	1110	1.9	57	1370	2.0

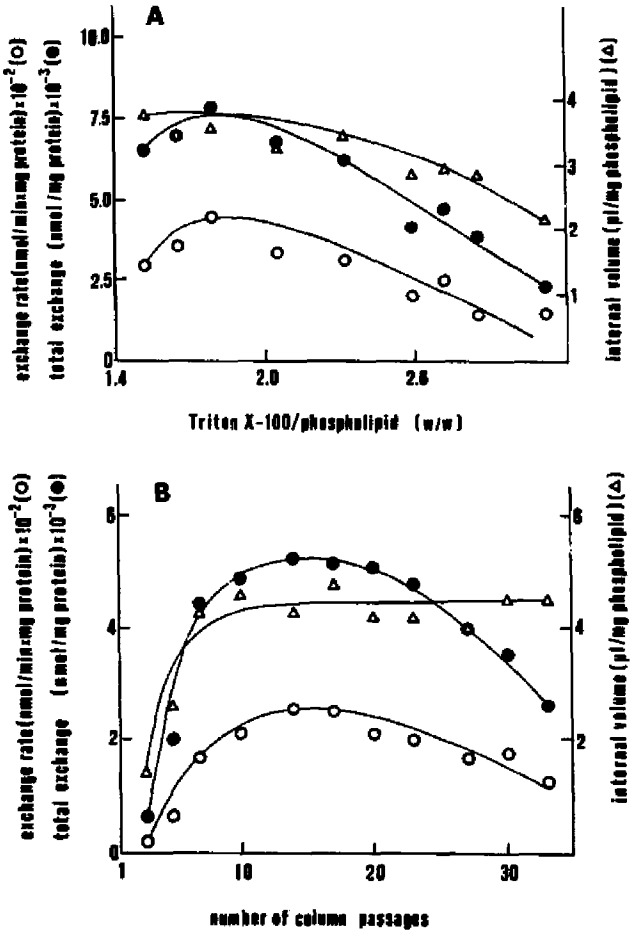

Fig. 2. Reconstitution of the carnitine carrier: variation of the detergent/phospholipid ratio (A) and the number of the Amberlite column passages (B). The proteoliposomes were prepared as described in Materials and Methods except that (A) increasing concentrations of Triton X-100 were used or (B) the number of passages through the same Amberlite column was varied. 0.1 mM [^{3}H]carnitine was added to proteoliposomes which contained 13 mM carnitine. The transport rate measured within the first 4 min (○), the total transport calculated from the exchange equilibrium after 60 min (●) and the total internal volume (△) were determined.

plot a straight line was obtained in the range from 8.5 °C to 27.5 °C (Fig. 5A). The activation energy as derived from the slope was 133 kJ/mol. Moreover, as shown in Fig. 5B, the K_m for carnitine was approximately the same at 25 °C and at 17 °C, i.e., 0.50 mM and 0.57 mM, respectively. The V_{max} of transport, on the other hand, was reduced 4.8-fold when the temperature was decreased from 25 to 17 °C.

The influence of external pH on the rate of carnitine exchange is illustrated in Fig. 6. In the presence of a substrate concentration of 0.1 mM, the rate of carnitine exchange showed an optimum at pH 6.5 (Fig. 6A). If, on the other hand, the substrate was present at high concentrations (2 mM), the rate of exchange was more or less independent of the pH from 6.5 to 8.0 (Fig. 6A).

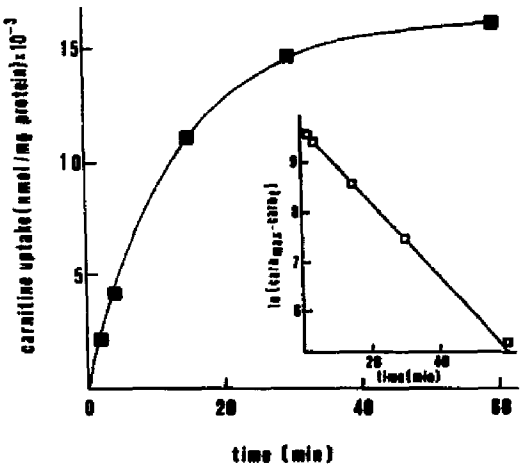

Fig. 3. Time-course of the carnitine/carnitine exchange in reconstituted liposome. Conditions as described in Materials and Methods. 1 mM [^{3}H]carnitine was added to proteoliposomes containing 13 mM carnitine. The inset represents the logarithmic plot of $\ln(carn_{max} - carn_t)$ according to the relation $\ln(carn_{max} - carn_t)/\ln carn_{max} - kt$. $Carn_{max}$ is the maximum carnitine exchange/g protein and $carn_t$ is the carnitine exchange at time t. The value of $carn_{max}$ was extrapolated at infinite time by a computer non linear regression analysis; it was 16.4 mmol/g protein.

The influence of pH on carnitine exchange was further analyzed in Fig. 6B, showing a double-reciprocal plot at three different external pH values. Alkalinization of the medium (pH 8.5) did not change the kinetic parameters significantly, whereas acidification (pH 5.5) led to a decrease of V_{max}.

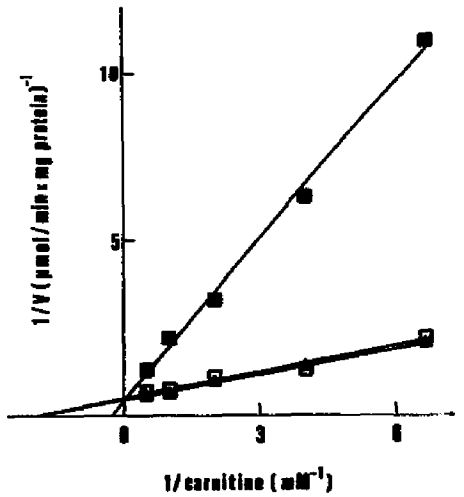

Fig. 4. Dependence of the rate of carnitine exchange in proteoliposomes on substrate concentration. Conditions as described in Materials and Methods. [^{3}H]Carnitine was added at the indicated concentrations to proteoliposomes loaded with 13 mM carnitine (▲) or 13 mM acetylcarnitine (□). In one experiment, additionally 30 μM myristoylcarnitine (■) was added simultaneously with the labeled substrate to proteoliposomes containing 13 mM carnitine.

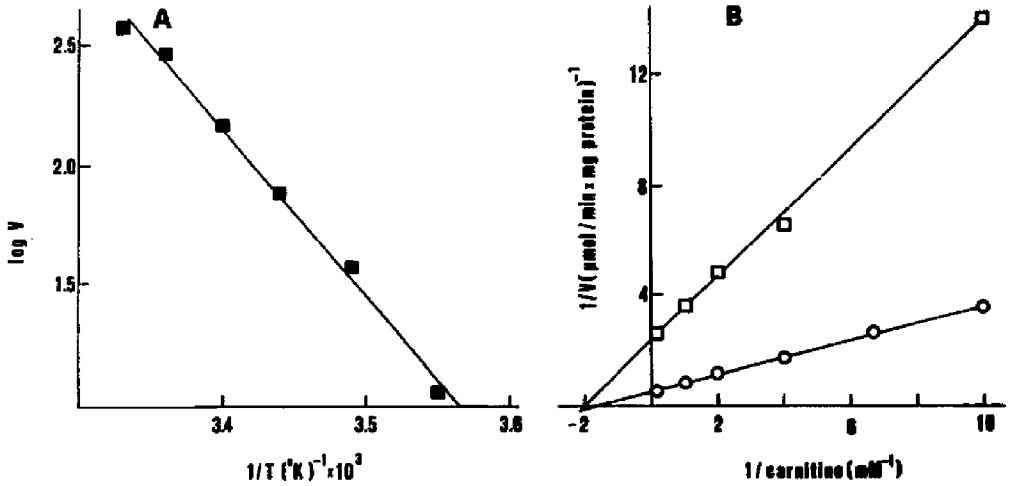

Fig. 5. Temperature dependence of the rate of the reconstituted carnitine/carnitine exchange. (A) Arrhenius plot: 0.1 mM [^{3}H]carnitine was added to proteoliposomes which contained 13 mM carnitine and were incubated at the indicated temperatures. (B) Lineweaver-Burk plots performed at 17°C (□) and 25°C (○): [^{3}H]carnitine was added at the indicated concentrations to proteoliposomes containing 13 mM carnitine and incubated at 17°C or 25°C.

Discussion

We analyzed the kinetics of carnitine transport catalyzed by the mitochondrial carnitine carrier in reconstituted liposomes. Thereby transport rates could be measured without interference of metabolites and/or metabolic pathways present in mitochondria. The optimization of the reconstitution procedure was performed in order to obtain a reliable basis for the determination of the kinetic data of this carrier protein. We have used a method of reconstitution based on detergent removal by chromatography on Amberlite [13], which results in higher transport activities than those obtained by the freeze-thaw-sonication procedure. It turned out that the relative amounts of the components of the reconstitution mixture, i.e., detergent, protein and phospholipids, as well as the number of passages through the Amberlite column significantly influence the efficiency of reconstitution of the purified carnitine carrier. Optimal activity of the carnitine/carnitine exchange was obtained at a detergent/phospholipid ratio considerably higher than that observed for the reconstitution of other mitochondrial carriers [13,16,17]. The optimum number of passages through Amberlite was the same as that found for the aspartate/glutamate carrier [13] and was between that

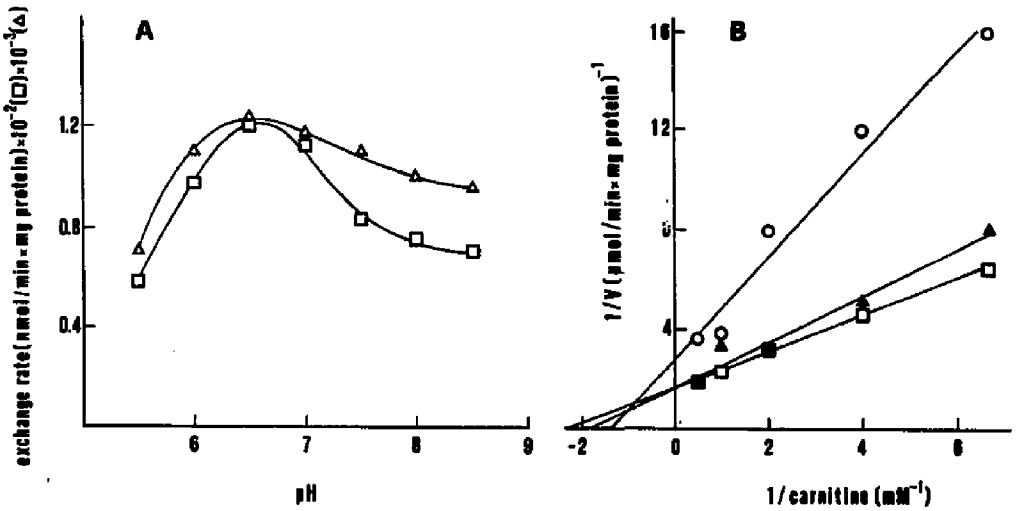

Fig. 6. Effect of pH on the reconstituted carnitine/carnitine exchange. (A): 0.1 mM (□) or 2 mM (△) [^{3}H]carnitine was added to proteoliposomes, which contained 13 mM carnitine and were reconstituted at the indicated pH values. (B) Experimental conditions as in Fig. 6A at pH 5.5 (○), 7 (□) and 8.5 (▲) except that [^{3}H]carnitine was added to the proteoliposomes at the indicated concentrations.

TABLE II

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

The K_i values were calculated from double reciprocal plots of the rate of [^{3}H]carnitine/carnitine exchange versus substrate concentrations. The experimental conditions were the same as in Fig. 4. The competing substrates were added simultaneously with [^{3}H]carnitine at the appropriate concentrations.

Inhibitor	Acyl carbons atoms	K_i (μM)	Number of expts.
D-Carnitine	0	510 ± 70	4
L-Acetylcarnitine	2	82 ± 19	5
DL-Octanoylcarnitine	8	15 ± 4	5
DL-Lauroylcarnitine	12	3.8 ± 1.2	4
DL-Myristoylcarnitine	14	5.1 ± 1.5	6
L-Palmitoylcarnitine	16	5.1 ± 1.7	5
DL-Palmitoylcarnitine	16	5.4 ± 1.5	7
DL-Stearoylcarnitine	18	30 ± 5	5
L-Isobutyrylcarnitine	4	630 ± 130	5
L-α-Methylbutyrylcarnitine	5	380 ± 110	6

required for the dicarboxylate carrier and for the tricarboxylate carrier [16,17], respectively. The absolute concentration of phospholipid, which had to be present initially in the reconstitution mixture for optimal conditions, was similar (10–14 mg/ml) for all mitochondrial carriers reconstituted with the Amberlite procedure [13,16,17].

The activity of the reconstituted carnitine carrier was influenced by the lipid composition of the liposomal membranes. In this regard the most interesting result is the increased activity caused by cardiolipin. This effect has been observed previously [9–11] but it was not investigated whether it is due to an increased incorporation of carrier protein or to the activation of the incorporated carrier. In this paper we present evidence that the stimulation by cardiolipin can be attributed to a direct activation of the incorporated carrier molecules. It is remarkable that the stimulatory effect of cardiolipin seemed to be specific since all the other phospholipids tested including the acidic ones did not activate. On the contrary, phosphatidylinositol inhibited the activity even when added only at 5% of total lipids.

We have determined the basic kinetic data of the carnitine carrier in proteoliposomes under optimal conditions. The carnitine/carnitine exchange catalyzed by the reconstituted carnitine carrier can be described as a first-order reaction. The activation energy of the reconstituted carnitine carrier was determined to be 133 kJ/mol, and thus closely resembles that measured in mitochondria [5]. This value is significantly higher than the activation energy reported for several other reconstituted mitochondrial carriers, but is similar to that found for the ADP/ATP carrier in the lower temperature range [16,17,23–25]. As observed for the uptake of carnitine in intact mitochondria [4], the reconstituted carnitine carrier is practically independent of the external pH in the range between 6 and 8. The V_{max} of carnitine uptake is found to be 1.7 mmol/min per g protein at 25°C in the presence of internal carnitine or acetylcarnitine, respectively, as countersubstrate. The turnover number, calculated by assuming that the isolated protein is pure and consists of a monomer of 32.5 kDa, corresponds to 55 min^{-1}. This value is similar to that found for the tricarboxylate carrier and is about one order of magnitude lower than those reported for other reconstituted mitochondrial carriers [16,17,23,24,26–28]. The half-saturation constant of carnitine for the reconstituted carnitine carrier (0.51 mM) is similar to the value found in intact mitochondria [8]. It is important to note that we measured identical K_m values for carnitine in both carnitine-loaded and acetylcarnitine-loaded proteoliposomes. This indicates that the K_m for carnitine does not depend on the type of countersubstrate. The competition experiments (Table II) clearly show that the affinity of the carrier for acylcarnitines is much higher as compared to that for carnitine. The half-saturation constant of the carrier is very low (5 μM) for carnitine esters containing fatty acids from 12 to 16 carbon atoms. In previous studies in intact mitochondria, only the affinity of acetylcarnitine and octanoylcarnitine has been investigated [8]. The K_i values reported in these studies are remarkably higher than those found by us in the reconstituted system.

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