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Purification of reconstitutively active α-oxoglutarate carrier from pig heart mitochondria *

F. Bisaccia, C. Indiveri and F. Palmieri **

Department of Pharmaco-Biology, Laboratory of Biochemistry, University of Bari and CNR Unit for the Study of Mitochondria and Bioenergetics, Via Amendola 165/A, 70126 Bari (Italy)

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The α-oxoglutarate carrier from pig heart mitochondria has been solubilized with Triton X-114 and purified by chromatography on hydroxyapatite and celite in the presence of cardiolipin. When applied to SDS gel electrophoresis, the purified protein consists of only a single protein band with an apparent M_r of 31.5 kDa. It corresponds to band 4 of the five protein bands previously identified in the hydroxyapatite pass-through of Triton X-114 solubilized heart mitochondria (Bisaccia, F. and Palmieri, F. (1984) Biochim. Biophys. Acta 766, 386–394). When reconstituted into liposomes the α-oxoglutarate transport protein catalyzes a phthalonate-sensitive α-oxoglutarate/α-oxoglutarate exchange. It is purified 250-fold with a recovery of 62% and a protein yield of 0.1% with respect to the mitochondrial extract. The properties of the reconstituted carrier, i.e., the requirements for a counteranion, the substrate specificity and the inhibitor sensitivity, are similar to those described for α-oxoglutarate transport in mitochondria.

Introduction

The inner mitochondrial membrane contains a specific carrier system for the transport of α-oxoglutarate (for a review, see Refs. 1 and 2). Under physiological conditions this system catalyzes a strict counterexchange of α-oxoglutarate and malate, which is important for several metabolic processes like gluconeogenesis from lactate, the malate-aspartate shuttle, the isocitrate-α-oxoglutarate shuttle and nitrogen metabolism [3,4].

Since 1967, when the existence of a specific carrier for α-oxoglutarate has been proposed [5,6], the properties of the α-oxoglutarate carrier have been extensively investigated in intact mitochondria. Besides α-oxoglutarate and malate, this carrier accepts also other dicarboxylates, i.e. malonate, succinate and oxaloacetate, although with lower affinity [7–10]. It is inhibited by several SH reagents [11] and some impermeable dicarboxylate analogues like phenylsuccinate, butylmalonate and *p*-iodobenzylmalonate [9,12], which also inhibit other transport systems in mitochondria [5,11–16]. A specific inhibitor of the α-oxoglutarate carrier is phthalonate [17]. In contrast to the dicarboxylate and the tricarboxylate carriers, which have low activity in heart [18,19], the α-oxoglutarate carrier is very active both in heart and in liver [8,9,20]. Although the existence of this carrier is well documented and elucidated in mitochondria in some detail, this protein has not yet been isolated.

* This paper is dedicated to the memory of Prof. Giuseppe Porcellati.

** To whom correspondence should be sent at the Instituto di Biochimica, Università di Bari, Via Amendola, 165/A, 70126 Bari, Italy.

Abbreviations: $C_{12}E_8$, octa-ethyleneglycolmono-*n*-dodecyl ether; Pipes, 1,4-piperazinediethanesulphonic acid.

In previous studies we have demonstrated that, upon application of high-resolution gradient SDS-gel electrophoresis, the hydroxyapatite pass-through of Triton-solubilized heart mitochondria contains five proteins bands of very similar molecular weight [21,22]. Out of these five distinct bands, band 5 corresponds to the ADP/ATP carrier, band 3 was identified to be the phosphate carrier [21] and band 2 was shown to be porin from the outer mitochondrial membrane [22]. The function of the remaining two bands, however, and in particular of band 4 which is more abundant than band 1, remained to be established.

In this paper we describe the purification of the α-oxoglutarate carrier from pig heart mitochondria using functional reconstitution as a monitor of the carrier activity during isolation. The purified protein when incorporated into liposomes closely resembles the properties of the α-oxoglutarate transport system as characterized in mitochondria. Upon high resolution SDS-gel electrophoresis the purified α-oxoglutarate transport protein appears to be homogeneous and has an apparent molecular weight of 31.5 kDa. It corresponds to band 4 of the five protein bands present in the hydroxyapatite pass-through of Triton X-114 solubilized heart mitochondria.

Materials and Methods

Materials. Hydroxyapatite (Bio-Gel HTP) and Dowex AG1-X8 were purchased from Bio-Rad, Celite 535 from Roth, α-[1-^{14}C]oxoglutarate, L-[U-^{14}C]malate, [2-^{14}C]malonate, [1,5-^{14}C]citrate, [^{32}P]phosphate, [2-^{3}H]ADP and L-[U-^{14}C]aspartate from Radiochemical Centre (Amersham, U.K.), egg yolk phospholipids from Fluka, cardiolipin from Avanti-Polar Lipids, Triton X-114, Triton X-100, cholic acid, Lubrol WX, Tween 20, Pipes and D-malic acid from Sigma, Brij 58 from Atlas, octylglucoside from Calbiochem, Genapol X-80 from Hoechst, $C_{12}E_8$ from Nikko Chemicals and α-cyanocinnamate from R. Emmanuel Wembley. *n*-Octylpolydisperseoligooxyethylene was kindly supplied by Dr. J.P. Rosenbusch and glisoxepide by Bayer. Phthalonic acid was a gift of Drs. G. Randazzo and A. Evidente. Other reagents were obtained as reported [9].

Isolation of the α-oxoglutarate transport protein. Pig heart mitochondria prepared as described in Ref. 23, were solubilized in 3% Triton X-114 (w/v)/50 mM NaCl/10 mM Pipes (pH 7) at a final concentration of 16 mg protein/ml. After 10 min at 0°C the mixture was centrifuged at 138 000 $\times g$ for 40 min to obtain a clear supernatant referred to as extract.

500 μl of the extract (3.5–4.5 mg protein) supplemented with cardiolipin (2 mg/ml) were applied on cold hydroxyapatite columns (pasteur pipettes containing 600 mg of dry material) and eluted with the solubilization buffer. The first 500 μl of the eluate from two hydroxyapatite columns were pooled and applied on cold celite columns (pasteur pipettes containing 250 mg of dry material). Elution was performed with the solubilization buffer. The first ml was collected from each celite column.

Incorporation of the α-oxoglutarate transport protein into liposomes. Liposomes (10 ml) were prepared by sonication of 1 g of egg yolk phospholipids in a buffer containing 20 mM NaCl/10 mM Pipes/1 mM EDTA (pH 7), with a Branson-Sonifier B-15 under a stream of N_2 for 60 min at 0°C (20 s sonication, 20 s intermission). Where indicated, α-oxoglutarate or other substrates were included in the sonication buffer at the concentrations specified in the legends to tables and figures.

The α-oxoglutarate transport protein was incorporated into liposomes by the freeze-thaw-sonication procedure (24,25). 0.6 ml liposomes were mixed with sonication buffer and 30 μl of mitochondrial extract or 50 μl of celite or hydroxyapatite eluates in a final volume of 1 ml. After 5 min at 0°C the mixture was frozen in liquid N_2, thawed in a water bath at 10°C and then pulse-sonicated (0.3 s sonication, 0.7 s intermission) for 6 s at 0°C.

Assay of α-oxoglutarate exchange in proteoliposomes. In order to measure α-oxoglutarate exchange using externally added α-[^{14}C]oxoglutarate, the substrate outside the proteoliposomes was removed by passing the proteoliposomes through an anion exchange column (Dowex AG1-X8, 50–100 mesh) in acetate form preequilibrated with 1.2 ml of 50 mM NaCl and eluted with the same solution. The eluted proteoliposomes were tempered at 25°C for 4 min and used for exchange

measurements by the inhibitor stop method [26]. Transport was initiated by adding 1 mM α-[^{14}C] oxoglutarate (50000–70000 cpm) and stopped, after the desired time interval, by the addition of 20 mM phthalonic acid. In control samples phthalonic acid was added together with the labelled substrate at time zero. To remove the external radioactivity each sample was applied on a column of Dowex AG1-X8, 100–200 mesh, acetate form (0.5 × 4 cm equilibrated with 1.2 ml of 50 mM NaCl). The liposomes eluted with 1 ml of 50 mM NaCl were collected, vortexed with 4 ml of scintillation mixture (Maxifluor Baker, The Netherlands) and counted. The exchange activity was calculated by subtracting the control values from the experimental samples. In some experiments other labelled substrates were used instead of α-[^{14}C]oxoglutarate.

SDS-gel electrophoresis. Polyacrylamide slab gel electrophoresis of acetone-precipitated samples was performed in the presence of 0.1% SDS according to Laemmli [27]. The separation gel contained 17.5% acrylamide and an acrylamide-to-bisacrylamide ratio of 150 to give a high resolution of polypeptides of an M_r value close to 30000 [21]. In some experiments, the electrophoretic systems generally applied for the resolution of the cytochrome b-c_1 complex [28] and of the cytochrome c oxidase complex [29] were also used. Staining was performed with the silver nitrate method [30]. The molecular weights were determined with the help of Pharmacia low-molecular weight markers.

Other methods. Protein was determined by the Lowry method modified for the presence of Triton [31]. The activity of other transport systems was assayed as described above for the reconstituted α-oxoglutarate carrier using the following stop inhibitors: *N*-ethylmaleimide (phosphate carrier), butylmalonate (dicarboxylate carrier), 1,2,3-benzenetricarboxylate (tricarboxylate carrier), carboxyatractyloside (ADP/ATP carrier) and pyridoxalphosphate (aspartate/glutamate carrier).

Results and Discussion

Solubilization and purification of the α-oxoglutarate carrier

The conditions for solubilization of the α-oxoglutarate carrier in undenatured state were optimized by measuring the reconstituted α-oxoglutarate/α-oxoglutarate exchange activity in mitochondrial extracts of pig heart. In Table I a number of detergents used for solubilization of the active carrier protein are compared. Among these detergents, Triton X-100 and Triton X-114 are most effective in solubilizing the active α-oxoglutarate carrier. The non-ionic detergent Genapol X-80, the polyoxyethyleneglycol homologues of Triton Brij 58, Lubrol WX and Tween 20, and the ionic detergent cholate also solubilize the α-oxoglutarate carrier in an active form, but they are considerably less effective. In the extracts obtained by using other non-ionic detergents like octylglucoside, $C_{12}E_8$ and *n*-octylpolydisperseoligooxyethylene there is virtually no α-oxoglutarate exchange activity.

Since hydroxyapatite has successfully been used to purify several transport proteins [21,32–36], the same method was applied to the mitochondrial extracts of pig heart which possess α-oxoglutarate exchange activity. Table I shows that a considerable portion of the transport activity is recovered in the pass-through of hydroxyapatite columns only if Triton X-114 or Triton X-100 were used as solubilizing detergents.

In view of the striking effect of cardiolipin on the elution of the phosphate carrier from hydroxyapatite [21], the influence of this phospholipid on the isolation procedure of the α-oxoglutarate carrier was investigated. The data reported in Table II show that the amount of carrier protein, i.e., the total α-oxoglutarate exchange activity measured after reconstitution, is only slightly increased by the addition of cardiolipin to the mitochondrial extract which was applied to hydroxyapatite. The specific activity, however, is substantially increased suggesting a retention of other proteins. This is also indicated by the decreased amount of protein eluted from the column.

For further purification, the hydroxyapatite pass-through was subjected to chromatography on celite (cf. Table II). By this purification step the specific activity of the reconstituted α-oxoglutarate transport is increased 1.4-fold without added cardiolipin and 1.9-fold in the presence of this phospholipid. With cardiolipin added the entire procedure increases the specific activity 247-

TABLE I

EFFECT OF VARIOUS DETERGENTS ON THE SOLUBILIZATION OF THE ACTIVE α-OXOGLUTARATE CARRIER PROTEIN AND ITS ELUTION FROM HYDROXYAPATITE

Pig heart mitochondria were extracted by the indicated detergents at a 3% concentration (w/v). The extracts which possess reconstituted α-oxoglutarate transport activity were chromatographed on hydroxyapatite. The activity was measured in proteoliposomes containing 1 mM internal α-oxoglutarate and 1 mM external α-[^{14}C]oxoglutarate. The values of α-oxoglutarate transport represent the total activity present in 1 ml of mitochondrial extract and 1 ml of the corresponding hydroxyapatite pass-through obtained as described in Materials and Methods. n.d., not determined, Octyl-POE, *n*-octylpolydisperseoligooxyethylene.

Detergents	Solubilized protein (%)	α-Oxoglutarate transport (μmol per 6 min)	
		Extract	Hydroxyapatite eluate
Triton X-114	47	331	274
Triton X-100	37	296	207
Genapol X-80	33	158	54
Brij 58	22	91	0
Lubrol WX	21	104	9
Tween 20	12	94	0
Cholate	28	103	0
Octylglucoside	37	5	n.d.
Octyl-POE	29	9	n.d.
$C_{12}E_8$	28	0	n.d.

fold with respect to that of the mitochondrial extract. Approx. 62% of the total transport activity is recovered with a protein yield of 0.1%.

Fig. 1 shows a SDS-polyacrylamide gel electrophoresis of the hydroxyapatite and celite pass-through obtained, in the presence or in the absence of cardiolipin, from mitochondria solubilized with Triton X-114. Without added cardiolipin, the first 500 μl of the hydroxyapatite pass-through contain four protein bands, called bands 2–5 (Fig. 1, lane C), since band 1 appears only in later fractions (see Ref. 21). Chromatography of the hydroxyapatite fraction on celite results in elution of the same four protein bands, although a relative increase in band 2 can be seen (Fig. 1, lane D). Clearly in the absence of cardiolipin both band 3 and band 4 are not absorbed by celite. With added cardiolipin, on the other hand, the hydroxyapatite fraction contains only two bands namely band 3 and band 4 (Fig. 1, lane G). This is in agreement with previous results, showing that band 2 is retained and band 5 is retarded by

TABLE II

PURIFICATION OF THE α-OXOGLUTARATE CARRIER

Conditions as described in Materials and Methods, except that, where indicated, cardiolipin was not added to the Triton X-114 extract. The activity of the reconstituted α-oxoglutarate exchange, measured as in Table I, is expressed as μmol/6 min per g protein (specific activity) and μmol/6 min (total activity).

	Without added cardiolipin				With added cardiolipin			
	Protein (mg)	α-oxoglutarate transport		purification (fold)	protein (mg)	α-oxoglutarate transport		purification (fold)
		specific activity	total activity			specific activity	total activity	
Mitochondria	16				16			
Triton X-114 extract	6.4	40	256	1	6.4	42	269	1
Hydroxyapatite	0.08	2850	228	71	0.04	5580	223	133
Celite	0.06	3960	237	99	0.016	10390	166	247

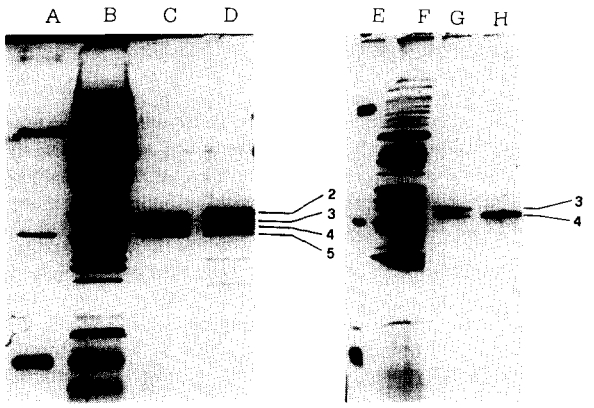

Fig. 1. SDS gel electrophoresis of fractions obtained during the purification of the α-oxoglutarate transport protein. A and E, marker proteins (bovine serum albumin, carbonic anhydrase and cytochrome c); B and F, mitochondrial extract; C and G, hydroxyapatite pass-through; D and H, celite pass-through. F, G, and H with added cardiolipin; B, C and D without added cardiolipin.

hydroxyapatite in the presence of cardiolipin [21]. Purification of the band 4 protein can be improved when only the first part of the hydroxyapatite pass-through – as described in the Methods section – is applied on a celite column in the presence of cardiolipin. Fig. 1, lane H shows that under these conditions band 4 with an apparent M_r of 31.5 kDa is eluted from celite in pure form, whereas band 3 is completely retained.

Since the isolated protein catalyzes a highly active α-oxoglutarate exchange (Table II) and since the purified fraction contains only band 4 (Fig. 1), this protein can be identified as the α-oxoglutarate carrier.

The protein purified by the procedure described above consists of a homogeneous band in SDS-gel electrophoresis not only in our gel system, but also in two other systems [28,29] generally applied to the resolution of the cytochrome b-c_1 complex (complex III) and the cytochrome c oxidase (complex IV), respectively (not shown).

In other experiments (not shown) it was found that Triton X-114 cannot be substituted by Triton X-100 for the isolation of band 4. This is mainly due to the fact that band 5, i.e. the ADP/ATP carrier, is present in large amount in the hydroxyapatite pass-through obtained by the use of Triton X-100 even in the presence of cardiolipin. Under these circumstances the celite column does not retain the ADP/ATP carrier completely. In agreement with these observations, the specific activity of the reconstituted α-oxoglutarate exchange is considerably lower in both hydroxyapatite and celite pass-through, if the protein was solubilized by Triton X-100 instead of Triton X-114.

Properties of the reconstituted α-oxoglutarate carrier

In all experiments described in the following the reconstituted system consists of purified band 4 incorporated into liposomes.

Accurate measurements of α-oxoglutarate uptake in proteoliposomes requires an effective stop inhibitor. Preliminary experiments have shown that phthalonate, a specific and potent inhibitor of the α-oxoglutarate carrier in mitochondria [17], is a suitable stopping reagent for measuring α-oxoglutarate transport in the reconstituted system, since

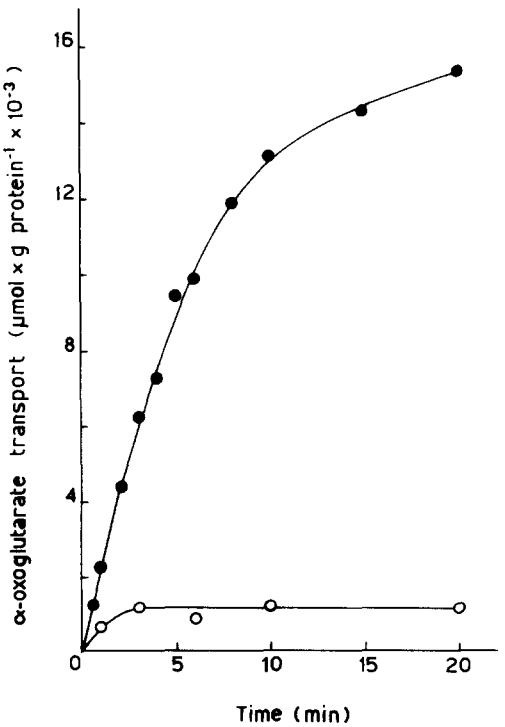

Fig. 2. Time-course of α-oxoglutarate uptake in reconstituted liposomes. 1 mM α-[^{14}C]oxoglutarate was added at time zero to reconstituted liposomes with 20 mM α-oxoglutarate as internal substrate (●——●), or to reconstituted liposomes without internal substrate (○——○).

it blocks the α-oxoglutarate uptake completely in less than 4 s.

Fig. 2 illustrates the time-course of α-oxoglutarate uptake by proteoliposomes which have been loaded before with the same substrate. The initial transport rate is about 2300 μmol/min per g protein at 25°C. The uptake of labelled α-oxoglutarate, on the other hand, is very small and time-independent after 3 min when the proteoliposomes were not loaded with α-oxoglutarate. No uptake at all occurs without incorporation of the carrier protein into the liposomes (not shown). In intact mitochondria the α-oxoglutarate carrier is known to catalyze an obligatory counterexchange of anions [1,2,37]. The dependence on internal α-oxoglutarate of the transport catalyzed by the reconstituted protein and the inhibition by the specific inhibitor phthalonate, proves that in fact the isolated α-oxoglutarate carrier has been reconstituted.

The dependence of the α-oxoglutarate carrier activity on intraliposomal counteranions has been further investigated in proteoliposomes loaded with a variety of substrates. The intraliposomal concentration of the anions used was 20 mM and the exchange time was 10 min. The data reported in Table III show that labelled α-oxoglutarate exchanges not only against α-oxoglutarate but also against internal L-malate, malonate, succinate, oxaloacetate and maleate which are known to be substrates of the α-oxoglutarate carrier in mitochondria [9–10]. In contrast, labelled α-oxoglutarate does not significantly exchange against fumarate, D-malate, oxomalonate and substrates of other mitochondrial carriers, like citrate, phos-

TABLE III

DEPENDENCE OF THE α-OXOGLUTARATE TRANSPORT IN RECONSTITUTED LIPOSOMES ON INTERNAL SUBSTRATE.

The proteoliposomes were loaded with the indicated substrates. Transport was initiated by the addition of 1 mM α-[^{14}C]oxoglutarate.

Internal substrate (20 mM)	α-oxoglutarate transport (μmol/g protein per 10 min)
– (Cl^- present)	510
α-Oxoglutarate	14660
L-Malate	10615
Malonate	6080
Succinate	3380
Oxaloacetate	3570
Maleate	3420
Fumarate	250
D-Malate	330
Oxomalonate	490
Citrate	345
Phosphate	500
Sulphate	445
Aspartate	695
ADP	230

TABLE IV

SENSITIVITY OF THE α-OXOGLUTARATE EXCHANGE IN RECONSTITUTED LIPOSOMES TO INHIBITORS AND EXTERNALLY ADDED SUBSTRATES

Proteoliposomes were loaded with 20 mM α-oxoglutarate and the exchange was started with 1 mM external α-[^{14}C]oxoglutarate. The SH reagents were added 2 min before the labelled substrate at 1 mM concentration. The other inhibitors and external anions were added together with α-[^{14}C]oxoglutarate at a concentration of 10 mM, except carboxyatractyloside (0.1 mM), α-cyanocynnamate (1 mM) and glisoxepide (5 mM). The control values of α-oxoglutarate exchange were 13.14 and 17.93 mmol/10 min per g protein in experiments 1 and 2.

Additions	% Inhibition
Experiment 1	
Phthalonate	100
Phenylsuccinate	50
Butylmalonate	48
p-I-benzylmalonate	85
Phthalate	46
p-Hydroxymercuribenzoate	83
Mersalyl	76
N-Ethylmaleimide	13
1,2,3-Benzenetricarboxylate	6
Carboxyatractyloside	8
α-Cyanocynnamate	0
Glisoxepide	5
Experiment 2	
D-malate	12
L-malate	85
Oxalate	0
Oxomalonate	9
Malonate	77
Oxaloacetate	53
Succinate	51
α-Oxoglutarate	88
Glutarate	14
Glutamate	4
Fumarate	0
Maleate	55

phate, sulphate, aspartate and ADP. The low activity in the presence of these anions is approximately the same as the obviously unspecific activity observed in the presence of Cl^-.

The sensitivity of the α-oxoglutarate/α-oxoglutarate exchange in reconstituted liposomes to inhibitors of various anion-transporting systems was also tested. Table IV, experiment 1, shows that the α-oxoglutarate exchange is inhibited completely by 10 mM phthalonate. *p*-Iodobenzylmalonate is less effective than phthalonate, but more than the other dicarboxylate analogues in agreement with their affinities to the carrier protein as determined in mitochondria [9,17]. The exchange is also inhibited by the SH-blocking reagents *p*-hydroxymercuribenzoate and mersalyl but not by *N*-ethylmaleimide, as found in mitochondria [11]. In contrast, 1,2,3-benzenetricarboxylate, carboxyatractyloside, α-cyanocynnamate and glisoxepide, which inhibit other mitochondrial transport systems [2], have no effect. In addition, the α-oxoglutarate exchange in reconstituted liposomes is inhibited by 10 mM externally added L-malate, malonate, succinate, oxaloacetate and maleate, whereas it is not significantly affected by D-malate, oxalate, oxomalonate, glutarate, glutamate and fumarate (Table IV, experiment 2). Also citrate, phosphate, sulphate, ADP and pyruvate have no effect (not shown). The same inhibition pattern was observed in mitochondria [9].

The specificity of the reconstituted α-oxoglutarate carrier was further characterized by variation of labelled external substrates. In some experiments malate was chosen as internal counteranion, since it is known to be a substrate not only for the α-oxoglutarate carrier, but also for the dicarboxylate and the tricarboxylate carriers [1,2], and it would thus reveal contamination by these transport proteins. The results of Table V show that, besides α-oxoglutarate, also labelled malate and malonate can exchange against internal malate in a phthalonate-sensitive reaction. In contrast, citrate (a typical substrate of the tricarboxylate carrier) and phosphate (a substrate of the dicarboxylate carrier) do not significantly exchange against internal malate. This holds true both when the activity is measured with the stop inhibitor phthalonate, to assay the α-oxoglutarate carrier,

TABLE V

UPTAKE OF LABELLED SUBSTRATES BY RECONSTITUTED LIPOSOMES LOADED WITH MALATE OR α-OXOGLUTARATE

Proteoliposomes were loaded with 20 mM malate or 20 mM α-oxoglutarate. 20 mM phthalonate was used as stop inhibitor. Where indicated, 1,2,3-benzenetricarboxylate (1,2,3-BTA) or butylmalonate at a concentration of 20 mM were used instead of phthalonate.

External substrate (1 mM)	Substrate uptake (μmol/g protein per 10 min)
Internal substrate: 20 mM malate	
α-[^{14}C]Oxoglutarate	11900
[^{14}C]Malate	4870
[^{14}C]Malonate	4140
[^{14}C]Citrate (inhibitor phthalonate)	100
[^{14}C]Citrate (inhibitor 1,2,3-BTA)	340
[^{32}P]Phosphate (inhibitor phtalonate)	760
[^{32}P]Phosphate (inhibitor butylmalonate)	190
Internal substrate: 20 mM α-oxoglutarate	
α-[^{14}C]Oxoglutarate	16270
[^{14}C]Malate	7790
[^{14}C]Malonate	6530
[^{14}C]Citrate	290
[^{32}P]Phosphate	530
No internal substrate, though Cl^- present	
α-[^{14}C]Oxoglutarate	400

and when it is measured with the stop inhibitor used for the tricarboxylate carrier, i.e., 1,2,3-benzenetricarboxylate, and that used for the dicarboxylate carrier, i.e., butylmalonate. Similar results were obtained with α-oxoglutarate-loaded proteoliposomes (Table V). Further experiments (not shown) demonstrated that liposomes reconstituted with the band 4 protein do not catalyze the exchange reactions citrate/citrate (tricarboxylate carrier), ADP/ADP (adenine nucleotide carrier), aspartate/aspartate (aspartate-glutamate carrier) and phosphate/phosphate (phosphate carrier and dicarboxylate carrier) when measured with the appropriate stop inhibitors reported in Materials and Methods. Thus, in agreement with the purity shown by the SDS-gel electrophoresis, the isolated protein is not contaminated by other transport systems including those which are not adsorbed by hydroxyapatite like the ADP/ATP carrier and the phosphate carrier.

Conclusion

The results presented demonstrate that the protein isolated by the procedure described in this paper is the α-oxoglutarate carrier of the inner mitochondrial membrane. A strong argument in favour of this conclusion is the fact that the isolated protein reconstituted in liposomes catalyzes transport of α-oxoglutarate by a strict counterexchange. As in mitochondria, besides α-oxoglutarate, also malate, malonate, succinate, oxaloacetate and maleate can be used as counteranions. The reconstituted α-oxoglutarate carrier exhibits the same high specificity of the transport system in mitochondria, as indicated by the observation that externally added D-malate, fumarate, oxomalonate, oxalate, glutarate and glutamate do not inhibit the exchange of α-[^{14}C]oxoglutarate. Furthermore, the inhibitor sensitivity of the reconstituted protein is identical to that of the mitochondrial α-oxoglutarate carrier.

The isolation of the α-oxoglutarate carrier as a homogeneous protein without contamination by other transport proteins proves that it is a separate molecular entity although it shares some properties with the dicarboxylate and the tricarboxylate carrier, e.g., the common substrate malate.

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