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Significance and redox state of SH groups in pyruvate carrier isolated from bovine heart mitochondria

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The role and properties of -SH groups of purified pyruvate (monocarboxylate) carrier were investigated. After isolation, this protein has all -SH groups in the oxidized state. Upon reduction, the carrier can be labelled with eosin-5-maleimide. The shift in apparent M_r after the labelling points to the presence of at least two cysteine residues. Pyruvate uptake in the reconstituted system is inhibited by both permeable (eosin-5-maleimide at 1 mM concentration) and impermeable (mersalyl, *p*-chloromercuribenzoate) -SH group reagents. Phenylarsine oxide inhibits pyruvate transport only slightly (20%), but the inhibition is enhanced after preincubation with the substrate.

Introduction

Uptake of many metabolic substrates by mitochondria as well as their exchange between the cytosol and the matrix can occur due to the presence of several specialized transporting systems in the inner mitochondrial membrane [1]. Many of them have been extracted from the membrane and obtained in the reconstitutively active form [2], several, as adenine nucleotide translocase [3], phosphate-transporting protein [4], 2-oxoglutarate [5,6] and dicarboxylate [6,7] carriers have been already obtained in a pure form.

Recently, applying an affinity resin with immobilized 2-cyano-4-hydroxycinnamate as a ligand, we have succeeded in isolating a pyruvate transporting protein (monocarboxylate carrier) from bovine heart mitochondria [8,9]. The isolated monocarboxylate carrier (M_r 34000) had sensitivity towards inhibitors and specificity towards different substrates [8,9] which resembled those of the pyruvate-transporting system in intact mitochondria [10,11].

The presence of free -SH groups seems to be essential for the activity of all carriers from the inner mitochondrial membrane characterized till now. The first of these which was shown to be inhibited by organic mercurials was the phosphate transporter [12,13]. The use of -SH group reagents having different membrane permeabilities allowed two separate transporting systems to be distinguished: one, NEM-sensitive, catalyzing a net uptake of phosphate, and another, responsible for the exchange of divalent anions [14–16], which was insensitive to NEM. A similar insensitivity towards NEM was also exhibited by the oxoglutarate carrier [6]. Inhibition by -SH group reagents was confirmed for purified protiens in reconstituted system, as was reported for phosphate [17], dicarboxylate [18], oxoglutarate [6,19], citrate [20] and aspartate/glutamate [21] transporters.

Labelling of mitochondria and inside-out submitochondrial particles with either radioactive NEM or the fluorescent maleimide derivative, EMA, and subsequent purification of various carrier proteins enabled the polarity of the sulphydryl group environment to be determined. Based on these results, it was concluded that -SH groups essential for the activity of phosphate-transporting protein are located at the external side [22,23], whilst those in ADP/ATP translocator [23,24] and oxoglutarate carrier [19] at the inner side of the

Abbreviations: DTE, dithioerythritol; EMA, eosin-5-maleimide; Mops, 4-morpholinepropanesulphonic acid; NEM, *N*-ethylmaleimide; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulphonyl fluoride; pCMB, *p*-hydroxymercuribenzoate (*p*-chloromercuribenzoic acid); SDS, sodium dodecyl sulphate.

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inner mitochondrial membrane. The location of -SH groups of the adenine nucleotide translocator as well as the polarity of their environment were also investigated with spin-labelled maleimide derivatives [25]. For this protein [26], as in case of the phosphate carrier [27], the position in the amino-acid sequence of the cysteine responsible for binding of NEM was also determined.

Pyruvate translocator in intact mitochondria [11,28–30] and in a partially purified extract of mitochondrial membrane [31] was inhibited by -SH group reagents. Based on the results obtained with intact mitochondria, Halestrap [29] proposed a scheme of the active centre in which oxoacids are transported as thiol-addition compounds. The present study was carried out to clarify a possible role of -SH groups of the pyruvate carrier obtained recently in pure reconstitutively active form [8,9].

Materials and Methods

Materials

Bovine heart mitochondria were prepared according to Yu et al. [32] and stored frozen at concentration of 35 mg/ml.

[1-^{14}C]Pyruvic acid, sodium salt, was purchased from Amersham International. EMA was delivered by Molecular Probes, Junction City. Triton X-114, Amberlite XAD-2, diaminohexane, succinic anhydride, *N*-ethyl-*N*-3-(dimethylaminonpropyl)carbodiimide hydrochloride and phenylarsine oxide were from Fluka. Sodium deoxycholate, Mops, pyruvate, 2-cyano-4-hydroxycinnemate, mersalyl, pCMB and *p*-nitrobenzoylazide were obtained from Merck; Sephadex G-25 medium and CNBr-activated Sepharose 4B were from Pharmacia. Dowex 1-X8, Cl-form, 100–200 mesh and hydroxyapatite (Bio-Gel HTP) were provided by Bio-Rad. Rotenone and oligomycin were purchased from Sigma. All other chemicals were of analytical grade.

Affinity resin with immobilized 2-cyano-4-hydroxycinnamate was prepared from CNBr-activated Sepharose 4B [8,9]. Synthesis of the affinity resin was started by coupling a six-carbon spacer to Sepharose according to the procedure given by Szewczyk et al. [7], what was followed by an elongation of the spacer performed by addition of succinic anhydride (2 mmol/ml gel), followed by coupling the next diaminohexane molecule in the presence of *N*-ethyl-*N*-3-(dimethylaminopropyl)carbodiimide hydrochloride at pH 4.8. Nitrobenzoylazide attachment, reduction and diazotation were performed as described by Cuatrecasas and Anfinsen [33], except that coupling of 2-cyano-4-hydroxycinnamate as a ligand was done at pH 8.1.

Scintillation cocktail was prepared as described by Nałęcz et al. [34].

Isolation of the monocarboxylate carrier

Mitochondria were solubilized in 50 mM NaCl, 3% Triton X-114, 1 mM EDTA, 20 mM Mops (pH 7.2) for 20 min; the final protein concentration was 10 mg/ml. Material not solubilized was spun down at 120 000 × *g* for 45 min. 0.6 ml of the extract was loaded on 0.6 g of dry hydroxyapatite and eluted with 1.8 ml of the solubilization buffer. The pass-through fraction collected from hydroxyapatite was supplemented with 0.7% deoxycholate, its pH was adjusted to 6.2 and it was subjected to affinity chromatography. 0.7 ml of hydroxyapatite elutate was bound to 1 ml of the affinity resin equilibrated with 2% Triton X-114, 0.7% deoxycholate, 10 mM Mops (pH 6.2). The resin was subsequently washed with 2 ml of the equilibration buffer followed by 2.5 ml of the same medium containing 0.2 M NaCl, as described previously [8].

Reconstitution of the monocarboxylate carrier

Fractions eluted from the affinity column with 0.2 M NaCl, containing monocarboxylate carrier, were treated for 10 min with 50 mM DTE and passed in 0.2 ml portions through small (0.7 × 6 cm) Sephadex G-25 (medium) columns equilibrated with 2% Triton X-114, 50 mM KCl, 20 mM Mops (pH 8.0). Samples corresponding to void volumes were diluted 1 : 1 with the equilibration medium and subjected to reconstitution using Amberlite XAD-2 beads (batch procedure). In this technique preformed egg-yolk phosphatidylcholine vesicles (45 mg lipid/ml in 50 mM KCl, 20 mM Mops (pH 8.0)) were used. Such a suspension of liposomes was diluted 5 times with a sample containing protein, 2% Triton X-114, 50 mM KCl, 20 mM Mops and subsequently added to moist Amberlite XAD-2 beads, previously equilibrated with the lipids. The ratio of the detergent was 28 mg and 27 mg per mg of lipids and mg of moist beads of Amberlite, respectively. The reconstitution process lasted, under conditions of gentle mixing at 4 °C, for 5 h. The proteoliposomes were separated from the Amberlite beads and, in order to remove external buffer, were passed through small Dowex 1-X8 columns (0.4 × 5 cm) equilibrated with 170 mM sucrose. The pH value of the effluent was adjusted to 8.

Pyruvate uptake

ΔpH-driven pyruvate uptake of the reconstituted carrier protein was measured at 15 °C in a proportion of 2 μg protein per ml of incubation medium. After 3 min of preincubation in the absence or presence of inhibitors the reaction was started by a simultaneous addition of HCl (to decrease the external pH from 8 to 6.5) and 0.5 mM [1-^{14}C]pyruvate (about 1600 dpm/nmol). After the indicated time, the reaction was stopped by passing through small Dowex columns (0.4 × 3 cm) and trapped radioactivity was counted in a scintillation counter (Packard, Beckman).

Uptake of pyruvate into freshly isolated mitochondria was measured at 0 °C with the use of Millipore filtration technique (0.45 μm filters) [31]. The incubation medium contained 112 mM KCl, 20 mM succinate, 1 mM $MgCl_2$, 0.5 mM EDTA, 32.5 mM Mops (pH 6.9) and was supplemented with 2.5 μg/ml of oligomycin and 12 μM rotenone. Filters were equilibrated with this medium supplemented with 1 mM 2-cyano-4-hydroxycinnamate and 1 mM pyruvate. The reaction was started by addition of HCl and radioactive pyruvate after 3 min of preincubation either with or without inhibitor and was stopped by filtration of portions containing 0.5 mg protein and washed with 4 ml of the equilibration buffer per filter.

Miscellaneous techniques

For SDS-PAGE protein was precipitated by adding 5 vol. of precooled (−20 °C) acetone [31], stored for at least 1 h at −20 °C and dissolved in one of the following sample buffers:
buffer A: 0.1 M Tris-HCl (pH 6.8), 2% SDS, 2% 2-mercaptoethanol, 20% glycerol;
buffer B: 10 mM Tris-HCl (pH 7.2), 2% SDS;
buffer C: 10 mM Tris-HCl (pH 8.0), 2% SDS.
Gel electrophoresis was performed according to Laemmli [35] using a 17.5% acrylamide gel, as described by de Pinto et al. [36]. The polypeptides were stained with silver nitrate according to the Bio-Rad procedure.

For labelling of proteins with EMA, this compound was dissolved in 100 mM sodium phosphate (pH 7.2), subsequently diluted 10-times with water [37] and stored frozen in small portions. Samples containing 2–10 μg protein were dissolved in buffer B (if not otherwise stated) and 5 μl of the EMA-diluted solution was added. The reaction was carried out in darkness and was stopped by addition of 2-mercaptoethanol. Before loading on the polyacrylamide gel, the sample was supplemented with glycerol to diminish convection after loading. Portions of 40–200 μg of protein were loaded on the gel in order to visualize EMA-labelled band(s).

Reduction of disulphides was performed with tri-*n*-butylphosphine [38]. Protein after acetone precipitation was dissolved in buffer C. 1 μl of 1% tri-*n*-butylphosphine dissolved in 1-propanol was added to 1 μg of protein. A stream of nitrogen was blown over the surface of the sample. After 30 min EMA was added and further labelling occurred in darkness, as described above.

Protein was determined by the procedure of Lowry et al. [39] modified by adding 5% SDS, as described in Ref. 31 in order to avoid disturbances due to the high concentration of Triton.

Results

Pyruvate equilibration across the mitochondrial membrane occurs via an OH^- antiport or H^+ symport and thus depends on the transmembrane pH difference [10,11,28]. Isolated mitochondria are also capable of catalyzing the exchange of pyruvate with other 2-oxomonocarboxylic acids [10,11,28]. The uptake of pyruvate by mitochondria, like its efflux, is inhibited by mersalyl, NEM, *N*-phenylmaleimide and iodoacetate [28,30], the inhibition with both mersalyl and NEM being noncompetitive [28]. The exchange reaction, although inhibited by mersalyl, is, however, unaffected by NEM [28], an -SH group reagent penetrating through the membrane. These observations on exchange reaction have been subsequently confirmed in the reconstituted system [31]. In the case of bovine heart mitochondria the pyruvate uptake was significantly inhibited even by micromolar concentrations of pCMB (Fig. 1).

The activity of the pure monocarboxylate carrier reconstituted into liposomes was completely inhibited by mersalyl. However, pCMB at a concentration one order of magnitude larger than effective in mitochondria did not have the same effect (Fig. 2A). The effect of pCMB was much more pronounced after 2 and 4 min of incubation than at shorter times. The effect of pCMB on pyruvate uptake was also measured as a function of inhibitor concentration (Fig. 2B). The complete inhibition, i.e., the uptake level equal to that obtained in the presence of 2-cyano-4-hydroxycinnamate, was observed at concentrations of 100 μM and higher.

Table I shows the effect of EMA, an -SH group reagent capable of adequately penetrating the membrane at the concentration used (1.13 mM) [23,37,40], on the activity of the reconstituted pyruvate carrier. Apart from the fact that in the control vesicles for EMA treatment the activity was decreased due to a long incubation in the presence of phosphate as well as the dilution and loss of some material on the Sephadex column, EMA appeared to be a strong inhibitor of pyruvate transport. However, the purified carrier could

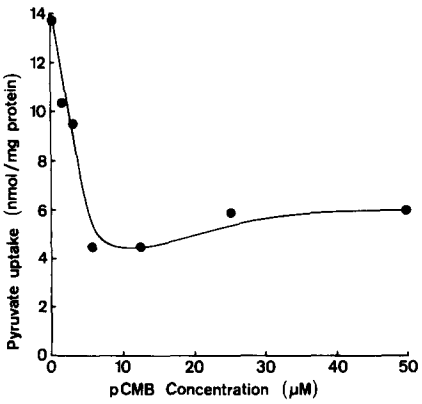

Fig. 1. Effect of pCMB on pyruvate uptake into freshly obtained bovine heart mitochondria. Pyruvate uptake was measured at 0 °C after 3 min of preincubation without or with various concentrations of pCMB. The transport reaction was stopped after 30 s, the time corresponding to the initial rate of the pyruvate uptake.

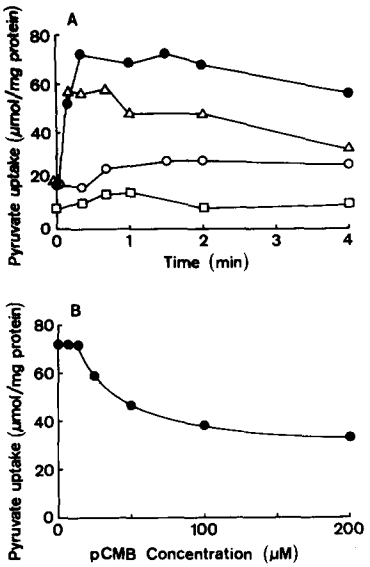

Fig. 2. Effect of SH group reagents on the pyruvate uptake by reconstituted monocarboxylate carrier. The pyruvate transporting protein was purified and incorporated into liposomes, transport measurements were performed at 15 °C.

(A) Time-course of pyruvate uptake after 30 min preincubation in the absence (●) or presence of the following compounds: 1 mM 2-cyano-4-hydroxycinnamate (○), 0.1 mM pCMB (△), 0.1 mM mersalyl (□).

(B) Effect of pCMB concentration on pyruvate uptake. Proteoliposomes were preincubated with the indicated concentration of pCMB for 5 min and the transport reaction was stopped after 20 s.

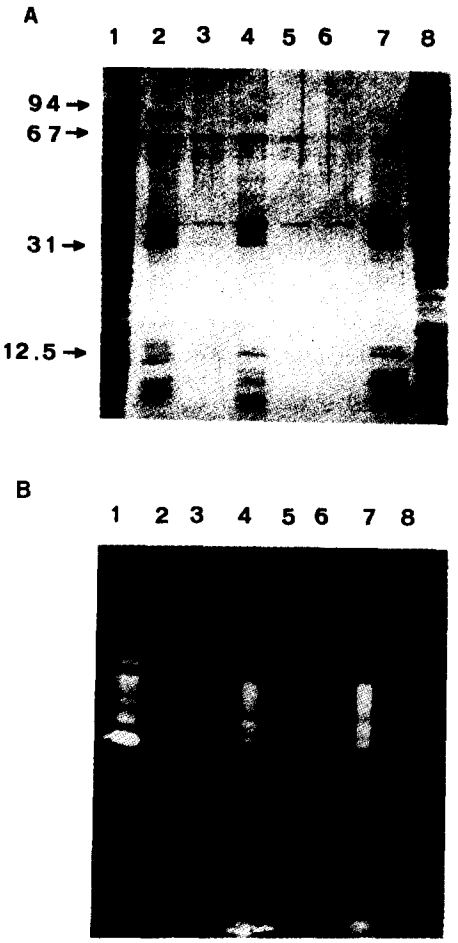

Fig. 3. SDS-PAGE pattern representing purification of pyruvate carrier under different conditions. Mitochondrial extract (lanes 1, 8) was subjected to chromatography on hydroxyapatite and the pass-through (lanes 2, 4, 7) was further purified on the affinity resin, as described in Materials and Methods. The purified monocarboxylate carrier is visualized in lanes 3, 5, 6. Lanes 6–8 represent the control purification procedure, lanes 4, 5 the same procedure performed in the presence of 1 mM PMSF. Lane 1 shows the mitochondrial extract labelled with EMA, lanes 2 and 3 – an HTP eluate and pyruvate carrier from an extract so treated, respectively. (A) The gel stained with silver nitrate; (B) the fluorograph. The following standard proteins were used as molecular weight markers: phosphorylase *b* (94000), bovine serum albumin (67000), carbonic anhydrase (31000) and cytochrome *c* (12500).

not be covalently labelled when treated with EMA either in solutions containing Triton X-114 and deoxycholate or precipitated with acetone and dissolved in sample buffer B (Fig. 3, lane 6). It should be noted that in the presence of 2% SDS all free -SH groups should have been made accessible to the reagent. SDS-protein complexes, when analysed by optical rotatory dispersion, could be described as either a fully extended α-helix folded back upon itself near its middle or a rod half the length of a fully extended α-helix [41,42], therefore all the functional groups should be accessible. No labelling with EMA was observed, either, when the monocarboxylate carrier was purified in the presence of the proteinase inhibitor, PMSF (lane 5), which was added on the assumption that proteolytic degradation could be responsible for the loss of cysteine(s) residue(s).

The lack of labelling of the monocarboxylate carrier with EMA could be the consequence of oxidation of thiol groups to disulphide bridges. Therefore, an attempt was undertaken to reduce the protein. An excess of tri-*n*-butylphosphine was chosen as the reducing agent [38] due to its specificity for disulphides and the rapid-

TABLE I

Effect of eosin-maleimide (EMA) on the activity of pyruvate uptake in reconstituted system

300 μl portions of proteoliposomes were incubated with 25 μl of EMA (10 mg/ml) in 10 mM phosphate buffer (pH 7.2). The incubation was performed in darkness at 4 °C for 45 min and was stopped by addition of 10 μl of 2-mercaptoethanol. Vesicles were passed through Sephadex G-25 (medium) columns (0.7×6 cm) equilibrated with 170 mM sucrose. Fractions corresponding to void volume were collected. Pyruvate uptake was measure for 20 s.

Treatment of vesicles	Pyruvate uptake (μmol/mg protein)
None	65
Preincubation with P_i, Sephadex filtration	36
Preincubation with P_i + EMA, Sephadex filtration	5.6

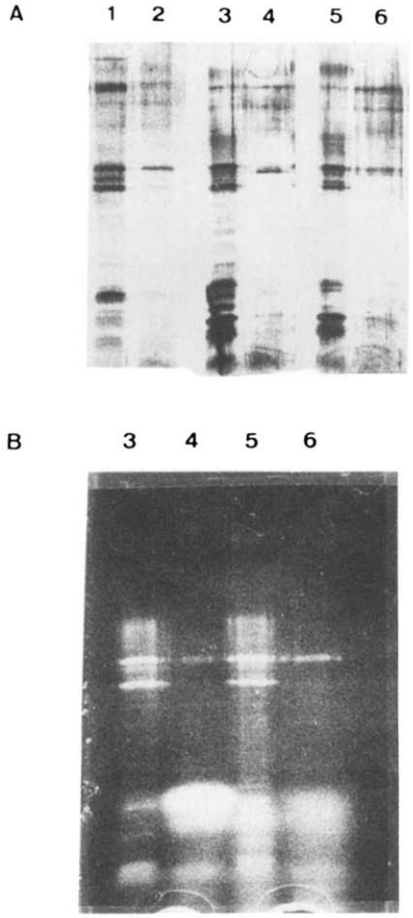

Fig. 4. Effect of reduction of disulphides with tributylphosphine on SH-group labelling with EMA. The eluate from hydroxyapatite columns (lanes with uneven numbers) and monocarboxylate carrier (lanes with even numbers) were dissolved after acetone precipitation either in sample buffer B (lanes 1, 2) or C (lanes 3–6). Lanes 5, 6 represent samples treated with tri-*n*-butylphosphine. All samples were subsequently treated with EMA, as described in Materials and Methods. (A) Gel stained with silver nitrate; (B) fluorograph.

ity of the reaction. As shown in Fig. 4 (lane 6), the monocarboxylate carrier became labelled with EMA after reduction with tri-*n*-butylphosphine. This was demonstrated by appearance of a fluorescent band (Fig. 4B, lane 6) and a shift of the molecular weight (Fig. 4A, lane 6), due to the addition of EMA. Fig. 4A presents the same gel as Fig. 4B (fluorograph) after staining with silver nitrate. Only the upper band is fluorescent and, due to a small increase in molecular weight, we conclude that the fluorescent band is a 34 kDa protein after binding of 2 molecules of EMA per molecule of protein. Therefore, on the fluorograph only one band is visible (lanes 4 and 6 of Fig. 4B) and the intensity of fluorescence (weaker for lane 4) corresponds to the amount of higher-molecular-weight band, as revealed after staining with silver nitrate (less in lane 4 than lane 6 of Fig. 4A). Incubation of the protein at high pH, necessary for the reduction reaction, already produced a certain reduction of -SH groups, as shown in Fig. 4 (lanes 4). From the silver-stained gel one could see that, after exposure of the protein to EMA at pH 8, most of the monocarboxylate carrier maintained its electrophoretic mobility and only a faint band of higher molecular weight was visible, interpreted as the addition product with EMA. The amount of labelled protein was higher when the reduction of disulphides was obtained by tri-*n*-butylphosphine, indicating that most of the protein was in the higher molecular weight form. These results indicate that the monocarboxylate carrier, when obtained from the affinity column, is in the oxidized state. This disulphide is formed between cysteines of the same polypeptide chain, because reduction does not change the molecular weight of the protein (a small observed shift of M_r is due only to the binding of EMA).

Before reconstitution, the monocarboxylate carrier was treated with DTE and one could thus expect that the protein was incorporated into liposomes in its reduced state. Fig. 5 demonstrates that DTE treatment for 10 min was sufficient to reduce disulphide(s) of the monocarboxylate carrier and enabled the protein to react with EMA.

From the fact that a disulphide bond can be formed in the monocarboxylate carrier one can conclude that this protein contains at least two cysteine residues. If they were in close vicinity they should have reacted with phenylarsine oxide [43]. The effect of this compound on pyruvate uptake in reconstituted system is presented in Table II. In contrast to pCMB, which inhibits the activity completely (the values obtained in the presence of pCMB are equal to those measured with liposomes without incorporated protein), phenylarsine oxide decreases the net uptake by 16–20% only. Preincubation

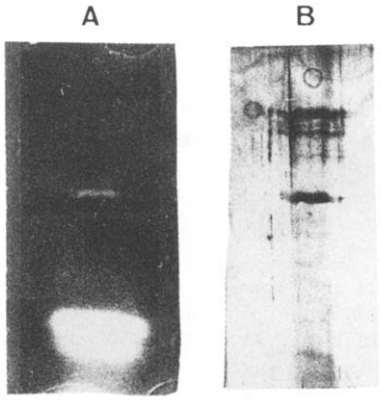

Fig. 5. SDS-PAGE of monocarboxylate carrier treated with EMA after reduction with DTE. Monocarboxylate carrier was isolated by affinity chromatography as described in Materials and Methods. It was next incubated with 10 mM DTE and passed through a Sephadex G-25 medium column (1×25 cm) equilibrated with 50 mM NaCl, 2% Triton X-114, 20 mM Mops (pH 8). Fractions corresponding to the void volume were collected, protein was precipitated with acetone and spun down for 1 h. Protein was dissolved in sample buffer B and labelled with EMA, as described in Materials and Methods. Lane A presents fluorograph, lane B the same gel stained with silver nitrate.

TABLE II

Effect of phenylarsine oxide on pyruvate uptake catalysed by purified monocarboxylate carrier reconstituted into phospholipid vesicles

Purification and reconstitution of monocarboxylate carrier were performed as described in Materials and Methods. Preincubation and transport were carried out at 15 °C. [1-^{14}C]Pyruvate (final 0.5 mM) and inhibitors (160 μM phenylarsine oxide or 100 μM pCMB) were added in indicated order, the reaction was started by pulse addition of HCl, decreasing the external pH to 6.6, and the reaction was terminated after indicated time by passing through Dowex colums, as specified in Materials and Methods.

Preincubation	Pyruvate uptake			
	Expt. 1 (1 min)		Expt. 2 (4 min)	
	(μmol/mg protein)	(% inhibition)	(μmol/mg protein)	(% inhibition)
Pyruvate 10 min	58.3	0	30.6	0
Pyruvate 2 min, then phenylarsine oxide 10 min	43.3	29.1	16.7	62.1
Phenylarsine oxide 10 min then pyruvate 2 min	50.0	16.1	25.8	21.4
pCMB 10 min, then pyruvate 2 min	6.7	100	8.2	100

with pyruvate before addition of phenylarsine oxide does not protect against this inhibition but, on the contrary, it seems to have an amplifying effect (30–60% inhibition) on the reaction with phenylarsine oxide. This would imply that the amount of -SH groups located in a lipid environment and being close to each other changes upon addition of the substrate.

Discussion

The monocarboxylate carrier as isolated from bovine heart mitochondria contains oxidized sulphydryl groups. It is not clear whether the disulphide bridge(s) is (are) formed during isolation procedure or are a characteristic of the native protein. The pyruvate carrier purified from mitochondria labelled with EMA was in the oxidized form, suggesting the presence of disulphide bond in the carrier already after extraction. One cannot exclude, however, the possibility that the protein fraction which contains reduced -SH groups and would react with EMA did not bind to the affinity column.

The disulphide bond is present in a single polypeptide and is not the result of crosslinking of two polypeptides of a dimer: in fact, no drastic change in molecular weight was found by electrophoretic analysis after reduction of the protein with tri-*n*-butylphosphine. From the fact that no reaction with EMA occurs upon formation of disulphide bridges, one can conclude that there is an even number of cysteine residues in the 34 kDa protein. After reduction of this protein with an excess of tri-*n*-butylphosphine, a process which should reduce completely all -S–S- bridges [38], and after the subsequent reaction with EMA, M_r 740, one can observe a shift of the band on SDS-PAGE, corresponding to an increase of the molecular weight close to 1500 and never exceeding 3000, indicating that the reaction occurred with two or at most four cysteines.

The question arises as to whether these cysteines are essential for the activity of the pyruvate carrier. The activation energy of pyruvate exchange in reconstituted system is 31 kJ/mol (7.4 kcal/mol) [31] and that of pyruvate uptake by intact mitochondria equalled 113 kJ/mol (27 kcal/mol) [10]. It has been impossible to estimate the latter value for the reconstituted pure monocarboxylate carrier due to the fact that at higher temperatures the activity of pyruvate uptake collapsed. Most probably, a difference of pH values was kept for a very short time after the pulse addition of HCl and/or the proteoliposomes were more leaky. The values of activation energy of both activities of the monocarboxylate carrier are significantly lower than the bond energies of typical -S–S- linkages, which are in the range of 53–89 kcal/mol [44]. Therefore, it seems more likely that the sulphydryl groups of the carrier remain in the reduced state and that the catalytic process does not involve their oxidation to -S–S- bridges, as was proposed for the phosphate-transporting protein [17].

Pyruvate uptake catalyzed by the purified monocarboxylate carrier is inhibited by permeable (EMA) and impermeable (pCMB, mersalyl) -SH group reagents. These results could indicate either that the carrier is randomly incorporated into proteoliposomes, or, if this is not the case, that essential -SH groups are exposed on both sides of the protein. A similar effect of permeable and impermeable -SH group reagents on the pyruvate uptake was observed in case of intact mitochondria [28]. Phenylarsine oxide does not inhibit totally pyruvate uptake, which could imply that either only a certain percentage of -SH groups are located in close vicinity one to the other, or that the mutual position of sulphydryl groups changes as a reflection of the conformation change of the carrier protein. Inhibition by phenylarsine oxide is much lower than 50%; moreover, the amount of cysteines is most probably not higher than four. Therefore, we would rather expect that sulphydryl groups change their position in relation to each other and that the distance between two -SH groups is regulated by the presence of substrate. Halestrap [29] proposed a model of the active centre of pyruvate carrier in which the interaction between the

substrate (the inhibitor) and the transporting protein involved one -SH group and two other positively charged groups (presumably lysine, arginine or histidine) [45]. Differences in inhibition of pyruvate uptake by phenylarsine oxide in the absence and presence of pyruvate suggest that conformational changes of the pyruvate carrier occur. Upon addition of the substrate two -SH groups exposed onto opposite sides of the membrane could move deeper into phospholipid bilayer, which could result in their much closer location relative to each other. This would explain a more pronounced inhibition by phenylarsine oxide in the presence of pyruvate. The lack of inhibition by NEM of the exchange process, in which the carrier protein is exposed to the substrate inside mitochondria or proteoliposomes, could be interpreted as well as the result of a deeper location of -SH group exposed on the inner side of the membrane.

These conformational changes of the pyruvate carrier could be regulated by addition of substrate and pH value on both sides of the membrane. A similar effect of pH on the reactivity of -SH groups has been recently reported for phosphate-transporting protein [46]. In this case, alterations of the reactivity of -SH groups were also influenced by matrix pH, in spite of the fact that -SH groups of the phosphate-transporting protein are located at or close to the outer surface of the inner mitochondrial membrane. As was suggested above for pyruvate transport, in case of the phosphate carrier it is proposed that changes in the reactivity of -SH groups are parallelled by conformational changes of the protein which can modify the function of the transport [22,47]. These similarities between both carriers are especially striking if one considers the fact that both of them catalyze the transport of anions driven by a pH gradient across the membrane.

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