Volume 327, number 1, 54–56 FEBS 12722 July 1993

Probing the active center of the mitochondrial dicarboxylate transporter

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Received 24 May 1993

2-*n*-Alkylmalonates with various length of the alkyl residue have been used to study the topography of the active center of the dicarboxylate transporter in intact rat liver mitochondria. Measurements of the K_i values of these competitive inhibitors suggest that in the transporter there is a large hydrophobic region at least 1.7 nm in size, containing a polar domain (ca 0.5 nm) and situated close to a substrate-binding site. These zones are assumed to be involved in the mechanism of dicarboxylate transport.

Dicarboxylate transporter; 2-*n*-Alkylmalonates; Active center

1. INTRODUCTION

The dicarboxylate transporter fulfils an important function of dicarboxylic acid transport through the mitochondrial inner membrane [1,2]. The carrier transports dianions (malate, malonate, succinate, and HPO_4^{2-}) by the electroneutral exchange. It is known that the substrate-binding site of the active center of the carrier has two positive charges located at a short distance (0.24–0.28 nm) [3]. The binding places of dicarboxylates and phosphate are not identical [4] and overlap one another [5]. There is a hydrophobic region in the vicinity of the substrate-binding site [3].

Analysis of possible molecular mechanisms of the substrate transport through biological membranes (e.g. [6–10]) and of suggested models of the transporter structure (e.g. [11–13]) allow us to conclude that the transporter active center should contain: (i) at least one site for the substrate binding, which is accessible alternatively from the inner and outer sides of the membrane; (ii) hydrophobic domains isolating the binding site from the outer or the inner medium of mitochondria; (iii) polar domains controlling the approaches to the substratebinding site(s).

From this it follows that some evidence for the topography of the active center of transporters may be obtained with the aid of hydrophobic derivatives of their substrates. The objective of this work was to investigate the active center of the dicarboxylate transporter of the mitochondria, using its competitive inhibitors, 2-*n*-alkyl derivatives of malonate. Varying the length of the aliphatic chain of the inhibitor we probed the active center and determined the size and polarity of its regions.

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2. EXPERIMENTAL

The activity of the dicarboxylate transporter in intact mitochondria was determined by measuring the rate of the succinate oxidase under conditions where the transporter controlled the rate of the process and the alkylmalonates had no noticeable effect on succinate dehydrogenase [14] The synthesized alkylmalonates contained 97–99.8% of the basic compound. Oxygen uptake was measured polarographically at 25°C in a medium containing sucrose (125 mM), tris-(hydroxymethyl)aminomethane (20 mM, pH 7.2), EDTA (2 mM), potassium chloride (20 mM), magnesium chloride (2.5 mM), phosphate (10 mM), rotenone (1 μM), 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalonitrile (0.2 μM), and rat liver mitochondria (0.5 mg/ml).

The average values of the observed inhibition constants (K_i^{obs}) were determined using 3–8 preparations of mitochondria. The actual values of K_i were corrected according to the equation [15]:

$$pK_i = pK_i^{obs} + \lg(R\lambda + 1),$$

where R is the partition coefficient of inhibitor between the mitochondrial membranes and the medium, λ is the concentration of the lipid component of mitochondrial membranes in the medium. The R value was determined from plots of the K_i versus the concentration of mitochondria [14,15] λ was calculated from lipid contents in mitochondria (0.133 mg/mg of protein [16]).

3. RESULTS AND DISCUSSION

Fig. 1 shows that the plot of the observed K_i as a function of the length of the alkyl substituent of the inhibitor has two plateaus in the regions of C_4–C_7 and $\geq C_{12}$ malonates. Since an increase in the length of the alkyl residue of the inhibitor leads to an increase in its partition coefficient between the mitochondrial membrane and the medium [14], the second plateau is primarily due to the decrease in the equilibrium concentration of the inhibitor in the incubation medium (see Fig. 1, dotted line). The actual values of K_i for inhibitors (see section 2) were used to calculate of the increments of free energy of the methylene group binding ($\Delta\Delta G$) in the transporter active center.

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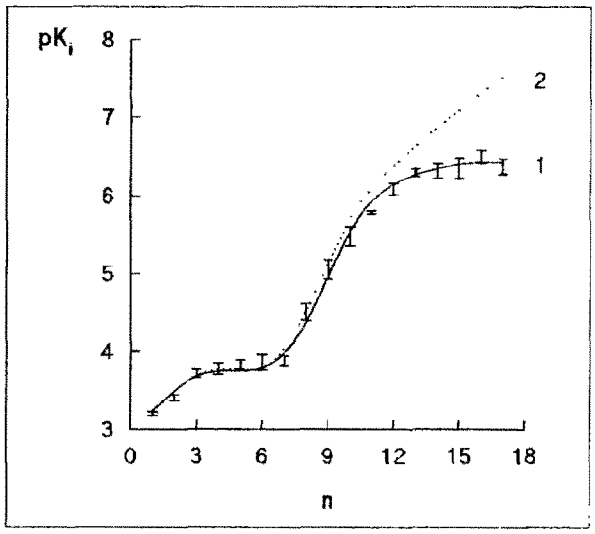

Fig. 1. Plots of the observed (1) and corrected (2) inhibition constant of the dicarboxylate transporter of rat liver mitochondria versus the number of carbon atoms (n) in the alkyl residue of 2-n-alkylmalonates.

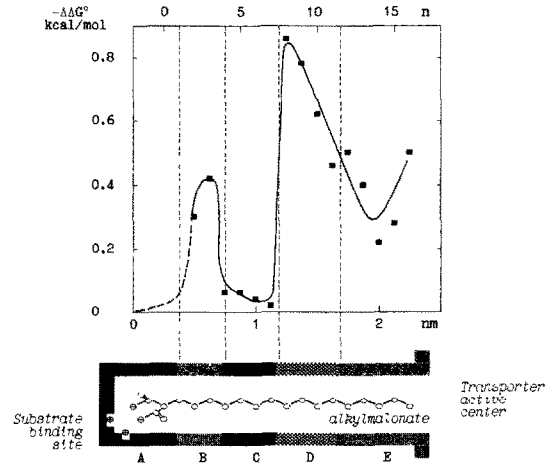

Fig. 2. Energy profile of the active center of the dicarboxylate transporter of rat liver mitochondria.

As follows from Fig. 2, five zones may be recognized in the transporter active center. Thus, in the vicinity of the substrate-binding site (zone A) the lipophilic areas are situated (zones B and D) which differ in hydrophobicity, and are separated by a polar domain (zone C). Whereas the hydrophobicity of zone D corresponds to that of octanol [17], the hydrophobicity of the most distant region (zone E) is close to that of the mitochondrial membrane for which increment of the free energy of transfer of the methylene group is equal to 0.33 kcal/mol [14]. In this connection it could be assumed that the external part of the active center (zone E) is formed by phospholipid, e.g. cardiolipin, which is known to be essential for the reconstitution of the functionally active dicarboxylate transporter [18].

The data obtained allow for the structural peculiarities of the transporter active center, as discussed in the Introduction, to be specified. The supposed localization of above-mentioned zones is illustrated in Fig. 3 by a one-center transporter model. From the results obtained it also follows that the size of the transporter active center (see Fig. 2) is commensurable with the thickness of the membrane lipid monolayer, since the competitive effect [14] and the decrease in the K_i of alkylmalonates are retained even though the length of the aliphatic chain of the inhibitors increases up to 2 nm.

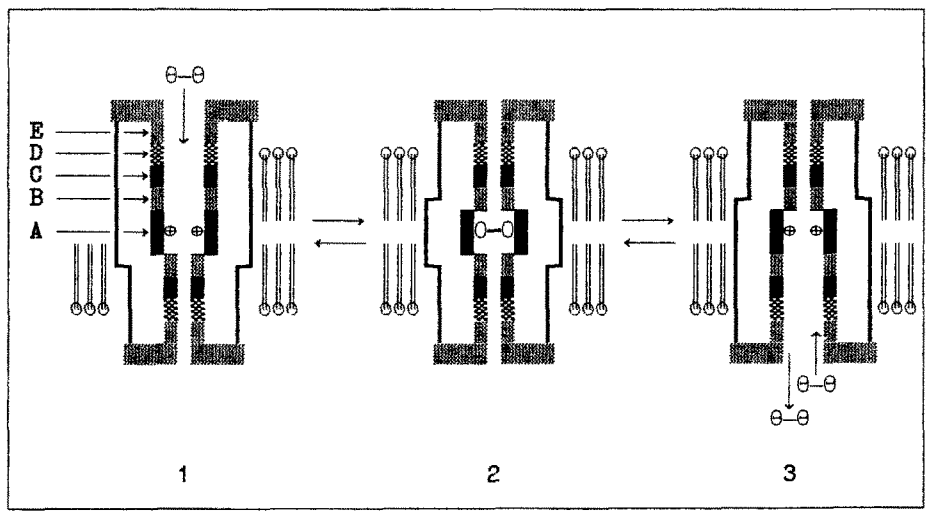

Fig. 3. Supposed one-center mechanism of the dicarboxylate transporter. In state 1, the open active center binds the substrate dianion in zone A. In state 2, the entry into the active center closes due to polarity change in zones A and C, zones B, D and E ensuring the isolation of the substrate-binding site. In state 3, the active center may open on both sides of the membrane with equal probability; at the same time the exchange with another substrate dianion takes place.

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