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The Mitochondrial Adenine Nucleotide Translocator

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Adenine nucleotides in the cell are distributed in two main pools located inside and outside mitochondria, respectively. The ADP and ATP belonging to these pools are exchanged through a specific transport system embedded in the inner mitochondrial membrane. This exchange proceeds with a stoichiometry of 1 : 1, and under conditions of oxidative phosphorylation the uptake of one ADP by mitochondria is accompanied by the release of one ATP into the extramitochondrial cytoplasm. The role of the mitochondrial ADP/ATP exchange is therefore to replenish the cytoplasm in ATP.

The aim of this paper is not to review in detail the data which have accumulated on the adenine nucleotide transport in the last 10 years [cf. 1, 2], but to discuss some of its aspects which are more salient or are still controversial. For the sake of clarity and to place the discussion in perspective, the main properties of this transport are summarized in Table I. Some of them deserve brief comments.

1. Most of these properties are indicative of the protein nature of the adenine nucleotide transport system and of its catalytic function.

2. The oxidative phosphorylation of external ADP is controlled by the ADP transport at two levels, namely the rate and the substrate specificity.

3. Atractyloside, carboxyatractyloside, and bongkrekic acid used at low concentrations are specific inhibitors. Yet they differ in the nature of their inhibition and in their penetrability (cf. Table I). As shown by experiments carried out with labeled inhibitors, atractyloside and

TABLE I. General properties of adenine nucleotide translocation [cf. 1 and 2]

1 Nature of the transport: Exchange–diffusion process with a 1 : 1 stoichiometry [3–5].
2 Localization: Inner mitochondrial membrane [3].
3 Specificity: An intact adenine ring and at least two phosphate groups are required (ADP and ATP). AMP is not transported, neither are other natural nucleotides [3–8].
4 Kinetics: Saturation kinetics of Michaelis–Menten type; V_{max} for ADP, 3–8 nmol/min/mg protein at 2° in rat liver or rat heart; K_m for ADP, 1–7 μM [5, 9, 10]; K_m for ATP, 2–180 μM (depends on the energy state of mitochondria) [10]. Asymmetry of the adenine nucleotide translocation in well-coupled mitochondria: ADP is taken up more rapidly than ATP, which leads to a higher ATP/ADP ratio outside than inside mitochondria [2, 11, 12].
5 Temperature dependence: Transition in the Arrhenius plot of the transport rate of ADP at 8°. Activation energy 50 kcal/mol below 8°; 12 kcal/mol above 8° [5].
6 pH dependence: No effect on the rate of transport between pH 5 and 8.
7 Specific inhibitors:
  Atractyloside: competitive, does not penetrate the inner mitochondrial membrane [13].
  Carboxyatractyloside: noncompetitive, nonpenetrant [13].
  Bongkrekic acid: noncompetitive [14], or noncompetitive [15] depending on conditions of preincubation, inhibitory only below pH 7 [16] probably because it is a penetrant inhibitor at acidic pH.
  Binding parameters for atractyloside, carboxyatractyloside, and bongkrekic acid [cf. 2]: K_d = 5–20 nM; number of high affinity sites = 1–2 mol/mol cytochrome *a* in rat liver.
8 Inhibitors having possibly a regulatory function in cell metabolism: long-chain acyl-CoAs: competitive, nonpenetrant [16, 17]. They may be involved in the regulation of the ADP transport [18].
9 Specific ADP binding sites (atractyloside-removable ADP binding sites): 1–2 mol/mol cytochrome *a* in rat liver [2, 19, 20].
10 Turnover Number: 55–60 min^{-1} at 2°, 1600–2000 min^{-1} at 20° [1, 2].
11 Chemical and morphological changes induced by ADP transport: unmasking of SH groups [21, 22]; shrinkage of mitochondria [23, 24].
12 Genetic determination: nuclear mutants with altered kinetic properties have been isolated [25–27].

carboxyatractyloside remain bound to the outer surface of this membrane, whereas bongkrekic acid, when partially protonated, enters the inner mitochondrial membrane [unpublished]. Bongkrekic acid enhances the affinity of the adenine nucleotide transport system for ADP or ATP [28].

4. The ability to penetrate (bongkrekic acid) or not penetrate (atractyloside and carboxyatractyloside) into the inner mitochondrial membrane may explain the inner or outer localization of these inhibitors. However, it is equally plausible that the inhibitor binding sites are asymmetrically distributed, as suggested by the following observations. The number of atractyloside and carboxyatractyloside binding

sites does not increase when mitochondria are disrupted by sonication in the presence of excess inhibitor [13]. Furthermore, ADP transport assayed in submitochondrial particles, obtained by sonication of mitochondria in the presence of ADP, is inhibited by atractyloside only when this inhibitor has been added before sonication [29]; in these inside-out vesicles, atractyloside is inhibitory provided it has bound to the cytoplasmic face of the mitochondrial membrane beforehand. On the other hand, to be inhibitory, bongkrekic acid must be protonated, and its effect is likely to be related to its ability to reach the inner side of the mitochondrial membrane.

5. Experiments performed with yeasts have afforded evidence that the protein(s) belonging to the adenine nucleotide transport system are coded by nuclear genes [25–27]. Changes in the mitochondrial membrane equipment obtained either by special conditions of growth, for example anaerobiosis [30] or by cytoplasmic mutations [31, 32] do not modify the turnover rate of the adenine nucleotide transport, or the membrane affinity for ADP or ATP.

6. Membrane protein(s) able to bind specifically ADP [33] or atractyloside (or carboxyatractyloside) [34,35] have been recently isolated. They probably belong to the adenine nucleotide transport system. However, their identification as transport protein(s) requires the demonstration of their activity as catalysts in the ADP transport, after incorporation in phospholipid vesicles.

Some aspects of the mechanism of the transport itself and its regulation will be discussed now. They concern the asymmetry of the adenine nucleotide transport, the morphological and chemical changes in the mitochondrial membrane induced by the ADP transport, and the mobility of the ADP carrier.

Asymmetry of the Adenine Nucleotide Transport

In respiring, well-coupled mitochondria, external ADP is taken up preferentially to external ATP, and ADP uptake is accompanied by a release of internal ATP [7].

As the predominant forms of ADP and ATP, at physiological pH (7.2–7.5), are ADP^{3-} and ATP^{4-}, the transmembrane exchange of ADP_{ex} against ATP_{in} leads to an asymmetrical distribution of charges inside and outside the mitochondrial membrane. This electrogenic ADP_{ex}/ATP_{in} exchange cannot proceed extensively unless it is balanced by an inward movement of negative charges. The balancing charge may be carried by phosphate ion, based on the following observations: (1) external phosphate markedly increases the rate of ADP transport in respiring mitochondria [5], and (2) inhibition of phosphate transport by

N-ethylmaleimide (NEM) decreases the rate of ADP_{ex}/ATP_{in} exchange in mitochondria supplemented with phosphate [36].

The other facet of the asymmetry of the adenine nucleotide transport is the preferential uptake of ADP_{ex}. It has been interpreted in two different ways by Klingenberg et al. [37] and Kemp et al. [10]. Klingenberg et al. [37] did not find any significant difference in the K_m values for ADP and ATP, or in the K_d values relative to the dissociation of the ADP- and ATP-carrier complexes. On this basis, they eliminated the possibility that the asymmetry in the adenine nucleotide exchange is an intrinsic property of the transport system itself. They also found that the preferential uptake of ADP is abolished by uncouplers or by valinomycin plus K^+, i.e., by an increase of the membrane conductance to H^+ or K^+. This led them to propose an ADP carrier driven by a membrane potential which is negative inside. Under these conditions the ADP^{3-}-loaded carrier moves preferentially to the inner side of the membrane, whereas the ATP^{4-}-loaded carrier moves to the outer side. This results in an asymmetrical distribution of loaded carriers across the mitochondrial membrane.

In view of more recent experiments by Souverijn et al. [10], the membrane potential cannot be considered as the only factor governing the asymmetry of adenine nucleotide transport. The main conclusions of these authors are the following: uncouplers increase the $ADP_{ex}^{3-}/ADP_{in}^{3-}$ exchange despite the fact that this exchange is electroneutral; there is a preference for the ATP exchange to occur as an electroneutral ATP^{4-}/ATP^{4-} exchange; and most important, the energy state of mitochondria directly influences the kinetics of ATP uptake. In fact the K_m for ATP_{ex} is similar to the K_m for ADP_{ex} in conditions of low energy state, but it is more than 100 times higher in energy-rich conditions. Whereas the energy level of mitochondria controls the affinity for ATP, it has no effect on the maximal rate of transport which is the same for ATP and ADP. It is clear that the preferential uptake of external ADP in energy-rich conditions is due essentially to a higher affinity of the transport system for ADP than for ATP. The effect of the mitochondrial energy state on the binding affinity was explained by a change of conformation of the protein(s) involved in the transport itself. Such a conclusion is supported by observations of discrete chemical changes in the mitochondrial membrane as the result of the functioning of the ADP transport [cf. 2].

In brief, the two different interpretations which have been put forward to explain the preferential ADP_{ex}/ATP_{in} exchange are: (1) a difference in the mobilities of ADP- and ATP-loaded carriers as a consequence of an extrinsic parameter, the membrane potential [37], and (2) a difference in affinities for external ATP and ADP, especially when mitochondria are maintained in a high-energy state [10]; this could be an intrinsic property of the transport system possibly related to a conformational change of the carrier protein.

Changes in the Mitochondrial Membrane Induced by ADP Binding

When micromolar amounts of ADP are added to mitochondria, morphological and chemical changes in the inner mitochondrial membrane can be detected. A small number of SH groups are unmasked (one to two SH per cytochrome *a*), and furthermore a shrinkage occurs, characterized by a decrease of the space between the cristae (cf. Table I). The unmasked SH groups are probably located on the inner side or in the core of the inner mitochondrial membrane, as they are readily titrated by permeant, lipophilic, SH reagents such as NEM and fuscin [38]. The SH unmasking is maximal in energized mitochondria; this is of interest in view of the functional relationship between the ADP transport and the F_1-ATPase (see below) and also of the effect, discussed above, of the mitochondrial energy state on the affinity for external ATP [10].

The shrinkage of mitochondria is not modified by oligomycin and is therefore not related to the oxidative phosphorylation process. In contrast, it is abolished by atractyloside, and, for this reason, it was ascribed to a binding of ADP to the ADP translocator [24]. In support of this interpretation, bongkrekic acid, which increases the affinity of the ADP transport system for ADP [28], markedly enhances the ADP-dependent contraction of mitochondria [39].

The bilayer couple theory, recently put forward by Sheetz and Singer [40], may explain the opposite changes undergone by the mitochondrial membrane in contact with penetrant or nonpenetrant ligands. The bilayer couple theory states that the two halves of the bilayer may differentially expand or contract, depending on whether perturbing molecules are intercalated in one half or the other of the membrane bilayer. In erythrocytes for example, anionic amphipathic and nonpenetrant molecules induce a crenation of the membrane (crenators), whereas cationic amphipathic molecules, which are penetrant, induce a cup formation (cup formers). This is probably due to the preferential distribution of the anions in the outer half of the bilayer and of the cations in the inner half of the bilayer. It is tempting to speculate that such a mechanism also applies to the inner mitochondrial membrane. Bongkrekic acid, which is a penetrant inhibitor in the undissociated form, would remain intercalated in the inner layer, and its action would be antagonized by atractyloside and carboxyatractyloside, which are nonpenetrant inhibitors.

Does the Adenine Nucleotide Transport Involve a Mobile Carrier or a Channel?

In the fluid mosaic model of biological membrane postulated by Singer and Nicholson [41], the membrane proteins, dispersed in a matrix of

fluid lipids, are able to move in the plane of the membrane. This model may apply to the inner mitochondrial membrane. The following observations indicate that the adenine nucleotide transport system is in a lipid environment and that its functioning is dependent on the fluidity of the surrounding phospholipids. (1) The Arrhenius plot of the rate of ADP transport is characterized by a break at 8° C (cf. Table I) which can be related to a phase transition of lipid surrounding the transport protein(s). (2) A higher degree of saturation of membrane phospholipids in *Candida utilis* mitochondria is accompanied by a decrease of the maximal turnover rate of the ADP transport, the binding affinity, and capacity for ADP being unaltered [42]. (3) Atractyloside, carboxyatractyloside, and long-chain acyl-CoA esters, which are amphiphilic, nonpenetrant inhibitors, plunge their apolar moiety into the outer half of the phospholipid bilayer, their polar moiety remaining attached on the outer surface of the membrane. Atractyligenin and free fatty acids, which are the apolar moieties of atractyloside and acyl-CoAs, respectively, inhibit by themselves the ADP transport [43, 44]. (4) Spin-labeled long-chain acyl-CoAs give immobilized spectra when bound to the ADP translocator. The line shapes of the spectra change upon addition of ADP or of carboxyatractyloside, and they become identical to that of the corresponding spin-labeled free fatty acid, i.e., mobile in a lipid phase [45]. This indicates that acyl-CoAs, when displaced from the ADP translocator, diffuse directly into the phospholipid bilayer.

The lateral diffusion of membrane proteins in the phospholipid bilayer may be constrained by protein–protein interactions occurring in the core of the membrane which result in large protein complexes that are not easily moved [41, 46]. Protein–protein interactions between the ADP transport system and the F_1-ATPase are suggested by the following observations: (1) oxidative phosphorylation of external ADP can be accomplished without an obligatory flux of adenine nucleotides into and out of the mitochondrial matrix [47, 48], and (2) external ADP is phosphorylated by the ATP-synthetase without prior dilution in the matrix pool of nucleotides [49].

The mobile-carrier hypothesis fits with the fact that the adenine nucleotide transport depends on the nature of the lipid environment; it also fits with the carrier-distribution model [1], according to which carrier molecules loaded with ADP or ATP are distributed on the inner or outer side of the inner mitochondrial membrane, depending on the charge which is carried and on the value of the membrane potential. Finally it is easy to conceive that a mobile carrier catalyses a 1 : 1 stoichiometric exchange between internal and external adenine nucleotides. On the other hand, the interaction of the F_1-ATPase with the adenine nucleotide transport system is in better agreement with a fixed-pore model of transport than with a mobile-carrier model. Besides, it has been argued [50], by analogy with the rather slow flip-flop of

phospholipids in biological membranes, that the transverse mobility of transport proteins is probably not the correct explanation for transport mechanisms.

One is therefore faced with the dilemma that both mobility and fixity are attributes of the adenine nucleotide transport system. As a tentative explanation, it can be proposed that this transport system is made of two units, one mobile and one fixed, each one located in one half of the phospholipid bilayer. The fixed unit would be a channel located in the inner half of the bilayer in close contact with the F_1 ATPase located on the same side. The mobile unit could be a rotating carrier located in the outer half of the inner membrane; by rotation it would be able to transport external ADP to the immediate proximity of the channel and conversely to transport to the outside the ATP delivered by the channel. The channel unit may be made of an aggregate of proteins interacting with each other and defining a space whose dimensions depend on the strength of interaction between the aggregated proteins. A coordination in the contraction–expansion movements of the channel and in the rotation of the mobile carrier would be required for an adequate functioning of the transport system.

Concluding Remarks

Despite its complexity, adenine nucleotide transport represents a system of choice for studying membrane functions at the molecular and cellular level. In fact, thanks to the very high affinity of specific inhibitors such as atractyloside, it has been possible to undertake the isolation, from the mitochondrial membrane, of an atractyloside-binding protein which is probably related to the ADP transport system [35]. This research will probably develop with reconstitution experiments dealing with incorporation of purified transport protein(s) into phospholipid vesicles. On the other hand, the functioning of the adenine nucleotide transport in the cell and its regulation by inhibitory metabolites such as long-chain acyl-CoAs may be topics of interest along with current research on mitochondrial–cytosolic interrelationships in cell metabolism [cf. 51]. In this line of thinking, one may wonder whether there is a coordination between the transport of ADP, phosphate, and oxidizable anions of the tricarboxylic cycle. As discussed above, coordination between ADP and phosphate transport must occur in phosphorylating mitochondria to maintain a charge balance during the ADP_{ex}/ATP_{in} exchange. It would be interesting to know whether long-chain acyl-CoAs, which inhibit not only the ADP transport, but also the malate and citrate transport [17, 52] have a coordinating function in the overall anion transport activity of mitochondria.

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