Hypothesis

A model for metalloprotein-catalysed ADP,ATP transport in mitochondria

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We propose a hypothetical model for the transmembrane exchange reaction catalysed by the mitochondrial adenine nucleotide carrier protein, which basically consists of an alternating reorientation of a transitory carrier–metal–nucleotide complex. The key features of the model are: the participation of an intrinsic divalent metal ion in the course of transport catalysis; the different stability constants of protonated and deprotonated nucleotide–metal complexes; the exposure and retraction of strategic arginyl residues; the alternating reorientation of the active center involving a change from the cytosolic conformation (C_c) to the matrix conformation (C_m).

ADP,ATP-transport *Mitochondria* *Model* *Metalloprotein* *Catalysis*

1. INTRODUCTION

The carrier-mediated transport of ADP and ATP across the inner mitochondrial membrane represents a key process for the energy supply of eukaryotic aerobic cells (review [1,2]). The use of chemically modified adenine nucleotides proved to be of exceptional utility in delineating the basic steric, contact and structural elements which are prerequisites for substrate binding and additionally for subsequent transport catalysis. This approach substantiated the remarkable specificity of this anion-transport system and led to a direct insight into the molecular mechanism of ADP and ATP translocation [3–5].

We have found a new class of active site-directed probes for this transport system [6]. A structure–activity study with these anionic mono-azo dyes conclusively showed that their metal-chelating properties are responsible for the specific inhibition of carrier-mediated ADP uptake in rat liver mitochondria. It was postulated that a metal ion, possibly Mg^{2+}, which is tightly bound to the carrier protein, plays an essential role for transport catalysis. Based primarily on these as well as on the findings with substrate analogs we propose a new model for carrier-mediated mitochondrial adenine nucleotide transport. A preliminary report of this model has been made [7].

2. RESULTS AND DISCUSSION

The key features of the hypothetical model in fig.1 are:

(i) The participation of an intrinsic divalent meti ion in the course of transport catalysis;

(ii) The different stability constants of protonate and deprotonated nucleotide–metal complexes;

(iii) The exposure and retraction of strategic argin residues;

(iv) The alternating reorientation of the active cente

The authors wish to congratulate Professor Friedrich Cramer on the occasion of his sixtieth birthday

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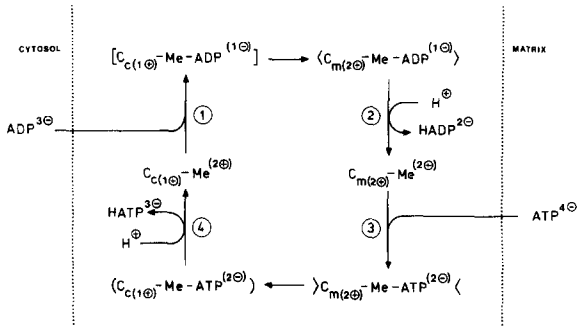

Fig.1. Transmembrane exchange of ADP *vs* ATP via ternary carrier–metal–nucleotide complexes.

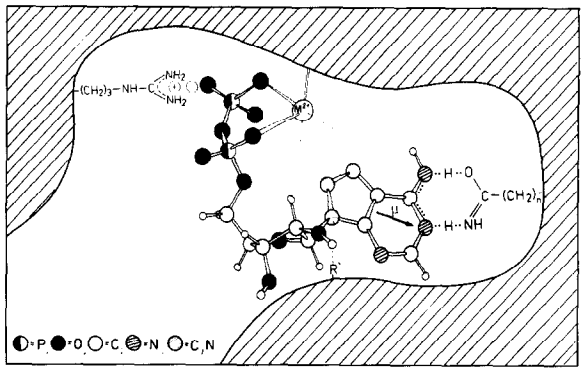

Fig.2. Schematic representation of the cytosol facing active center with the basic steric, contact and structural elements which are prerequisite for ADP binding (recognition) and transport [3–5]. Essential for transport catalysis is an overall ADP conformation with an anti-positioned heterocycle, a C(2′)-endo–C(3′)-exo ribose pucker and a *gauche–gauche* orientated exocyclic group C(5′)–O(5′). The invariant nucleobase binding site is proposed to be an asparagine (n = 1) or glutamine (n = 2) residue. For specific binding, the N(1)-amide attraction is sufficient whereby μ denotes the permanent dipole moment whose direction towards the N(1) atom (indicated by an arrow) correlates with the π-electron charge distribution in the amidine region. The transfer step requires the participation of the N(1)–C(6)(-NH_2) amidine region, thereby inducing an amino⇌imino tautomerism (indicated by dotted lines). R represents a hydrogen-accepting amino acid residue. Broken lines indicate the H-bonding interaction; i.e., the trigger-function of the *trans*-positioned C(2′)-hydroxyl group. The protein-bound bivalent metal ion is shown in an octahedral configuration forming a bidentate chelate with the α- and β-oxygen functions of the phosphate moiety. Cross-hatched areas represent carrier protein regions of the active center.

involving a conformational change from the cytosolic conformation (C_c) to the matrix conformation (C_m).

In general, our model consists of four steps:

Step 1: ADP^{3-} ($ADP^{3-} + H^+ \rightleftharpoons HADP^{2-}$, pK_a = 6.88 [8] binds to the cytosol facing active center of the carrier protein (C_c). The two negative charges at the α- and β-phosphate group are neutralized by chelation with the protein-bound metal ion (cf. fig.1,2). With Mg^{2+} as the metal ion, the stability constant K for such a bidentate nucleotide-metal complex ($ADP^{3-} + Mg^{2+} \rightleftharpoons$ Mg-ADP^{1-}) is 1023 M^{-1} [8]. The values given for K and pK_a are valid for aqueous solutions but should not differ in principle for hydrophilic protein matrices. The non-chelated oxygen at the terminal phosphate group is fully ionized at neutral pH, because complex formation lowers the pK_a from 6.8–5.3 [9]. The remaining negative charge at the β-phosphate is supposed to be neutralized by the guanidino group of an arginyl residue (cf. fig.2). Several independent lines of evidence indicate that this amino acid plays an essential role for cytosolic substrate as well as inhibitor binding. These are:

(i) The selective chemical modification of strategic arginine residues at the nucleotide and the inhibitor (atractyloside, bongkrecic acid) binding sites [10];
(ii) The findings with substrate analogs modified at the phosphate moiety (review [3]) and with the dianionic phosphonomethyl derivative ADPβCH_3 which shows only a weak interaction with the carrier protein (unpublished);
(iii) The structure–inhibitor activity study with metallochromic pseudosubstrates [6]. Besides the charge-neutralization, the metal-complex formation exerts an additional and functionally important effect, i.e., the metal ion profoundly alters the solvation of the phosphate moiety thus accounting for a large and favorable entropy change [11].

The partially desolvated and apparently electroneutral ternary protein–metal–ADP complex

reorients across the membrane due to a conformational change of the active center from the C_c- to the C_m-state. This transition [2] presumably is triggered by the interaction of the *trans*-positioned C (2′) hydroxyl group of the substrate with a specifically spaced hydrogen-accepting group at the active center of the carrier protein (cf. fig.2) [4,12].

Step 2: In the course of the conformational change from the C_c- to the C_m-state a second arginyl residue, denoted as (2+), is exposed at the active center. This is in line with [10] where two strategic arginyl residues were found at the active center when it faces the matrix side. To avoid charge isolation during the C_c-state of the protein a solvent anion or the polar head of a phospholipid is assumed to act as a transitory counterion for this guanidino group. After transition to the C_m-state the counterion is supposed to be 'neutralized' by the surface potential. As a consequence the active center (i.e., the ternary carrier–metal–nucleotide complex) becomes positively charged $\langle C_m\text{–Me–ADP}\rangle^{1+}$.

Finally, protonation of the terminal phosphate group of ADP in the ternary complex liberates $HADP^{2-}$ at the matrix side. This event is based on the following findings:

(i) Protonation of the non-chelated oxygen at the terminal phosphate group in an α,β-bidentate $MgADP^{1-}$ complex decreases the complex stability by about 30-fold ($MgHADP \rightleftharpoons Mg^{2+} + HADP^{2-}$, $K = 30\ M^{-1}$) [8];

(ii) Metal-complexation of ADP converts the β-phosphate from a secondary into a primary phosphate group. The pK_a value of the latter one, however, is 5.3 and not as expected around 1 [11];

(iii) The pK_a value of the terminal phosphate in a ternary enzyme–metal–nucleotide complex can be shifted to the more alkaline region. This was shown for arginine kinase where the ternary complex has a pK_a about 1.5 pH units higher than the free $MgADP^{1-}$ complex [13]. The above conformational change of the active center could change the environment of the β- and γ-phosphate group, respectively (step 4) thereby inducing such a pK_a shift;

(iv) The thiophosphate analog ADPβS shows the same carrier-specific binding properties as ADP. Its transport activity, however, is drastically reduced [14]. Furthermore, it was shown that the binary complex of $ADP\beta S^{3-}$ and Mg^{2+} has a pK_a (terminal phosphate group) at least one unit lower than $MgADP^{1-}$ [9] as well as a stability constant close to $MgADP^{1-}$ (H. Strotmann, personal communication). These findings support our model in as much as liberation of $HADP\beta S^{2-}$ at the matrix side is rendered more difficult due to the very low pK_a (<4) which hardly can be overcome in this case by the proposed conformationally induced pK_a shift.

Step 3: ATP^{4-} binds to the carrier protein in its $C_{m(2+)}$–Me^{2+} state thereby forming an α,β- or more likely a β,γ-bidentate complex ($MgATP^{2-} \rightleftharpoons Mg^{2+} + ATP^{4-}$, $K = 11500\ M^{-1}$) [8]. The two remaining negative charges of the phosphate moiety are compensated by the above two strategic arginyl residues. A comparison of the stability constants of $MgATP^{2-}$, $MgADP^{1-}$ and MgHADP shows that ATP^{4-} preferentially should bind to the C_m-state. The overall apparently electroneutral ternary carrier–metal–nucleotide complex (i.e., $\rangle C_{m(2+)}\text{–Me–}ATP^{2-}\langle$), then reorients according to the above conformational change to the cytosol whereby one arginyl residue remains masked at the matrix side. Due to the fact that the transported substrate itself was a transitory counterion for the masked arginyl residue the ternary complex carries one negative charge when facing the cytosolic side $(C_c\text{–Me–ATP})^{1-}$.

Step 4: Protonation of the γ-phosphate group of ATP in the ternary complex liberates $HATP^{3-}$ at the cytosolic side ($MgHATP^{1-} \rightleftharpoons Mg^{2+} + HATP^{3-}$, $K = 30\ M^{-1}$ [8]). Again, a conformationally induced pK_a shift of the terminal phosphate group has to be postulated, because the pK_a of this group is lowered to 5.3 upon complexation with Mg^{2+} [9].

The overall exchange reaction catalysed by the adenine nucleotide carrier protein thus basically consists of an alternating reorientation of a transitory ternary carrier–metal–nucleotide complex. As far as the topographical asymmetry of this

transport protein is concerned the model depicted does not contradict:

(i) Klingenberg's gated pore model with a common reorienting binding center for substrate and both inhibitor types (atractyloside, bongkrecic acid) attaining its asymmetry by two different conformations (C_c, C_m [15]; or
(ii) Vignais's double inhibitor site gated carrier model; i.e., a model with two separate preexisting binding sites for the inhibitors. These sites belong to the same active center which can reorient. The asymmetry is given by the different inhibitor binding sites [16].

The distribution of the two conformational states of the carrier protein (C_c,C_m) (i.e., the transport rate or transition rate of the ternary carrier–metal–nucleotide complexes) can be modulated extrinsically by the membrane potential due to the inversely charged ternary complexes ($(C_c\text{–Me–ATP})^{1-}$; $\langle C_m\text{–Me–ADP}\rangle^{1+}$). Thus, with a membrane potential positive outside the heteroexchange mode external ADP *vs* internal ATP is highly preferred. An analogous effect of the membrane potential on the influx and efflux rate (electrophoretic effect) is discussed in [17,18]. The functional asymmetry in our model cannot only be modulated by the membrane potential but might also be an intrinsic property of the active center. The preferential uptake of ADP as well as the preferential extrusion of ATP in energized

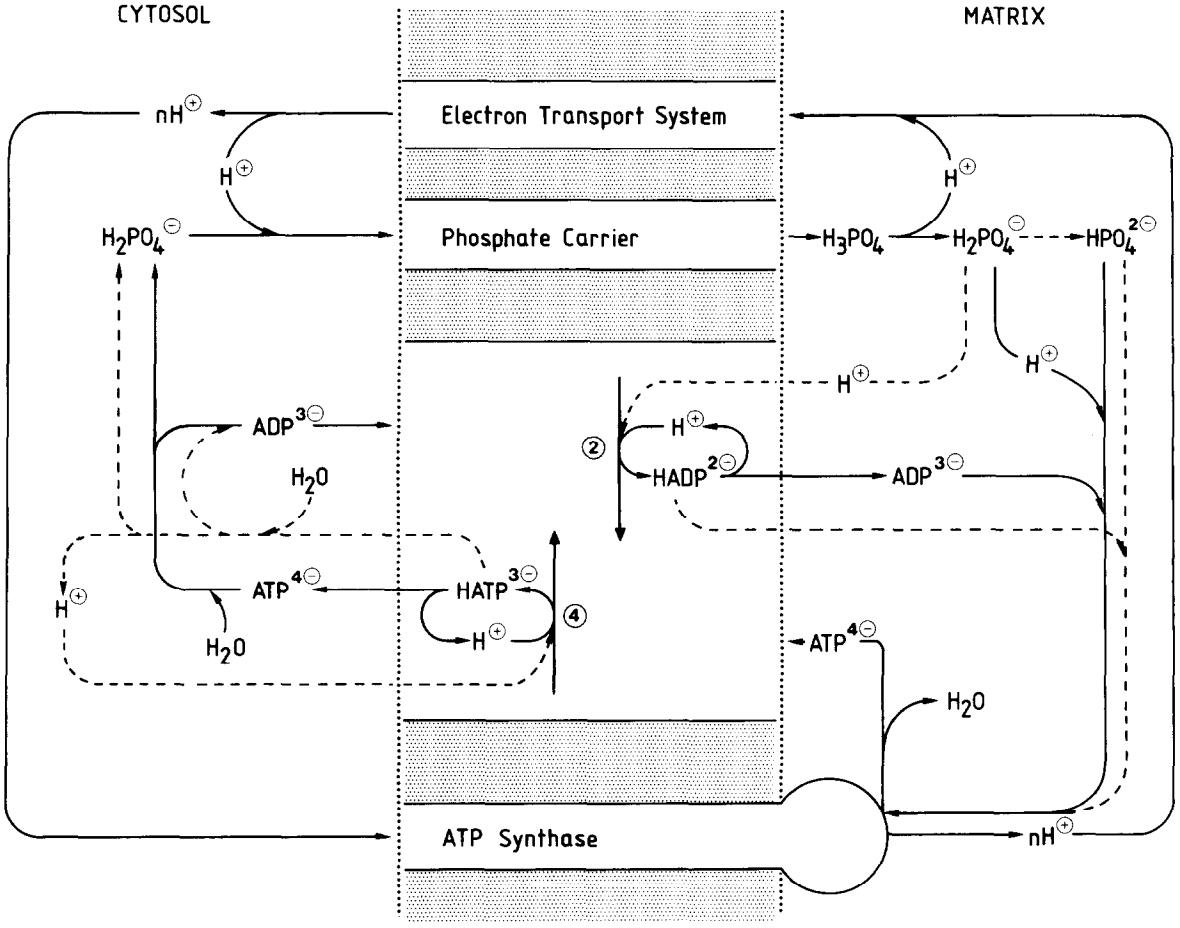

Fig.3. Protonation of the ternary carrier–metal–nucleotide complex (cf. fig.1, step 2 and 4) by membrane intrinsic cyclic protons (indicated by full lines) or bulk phase protons (dashed lines).

mitochondria could be due to the different stability constants of the corresponding nucleotide–metal complexes.

Metalloproteins exhibit particular, configurational or conformational features which account for a remarkable substrate specificity. Furthermore, the metal ion which probably forms an octahedral complex with the protein and the substrate (cf. fig.2) might itself function as an intrinsic center of asymmetry. As far as the protonation during step (2) and (4) in fig.2 is concerned we suggest two possible modes (fig.3):

(i) Protonation of the β-phosphate and γ-phosphate, respectively, in the ternary complexes by membrane intrinsic cyclic protons (indicated by full lines);

(ii) Protonation by bulk phase protons (indicated by dashed lines) which, e.g., can result from the dissociation of dihydrogenphosphate and cytosolic orthophosphate, respectively.

Cytosolic phosphoryl transfer reactions involving ATP^{4-} or $HATP^{3-}$ are reduced to hydrolysis for clearness. In its physiological heteroexchange mode the transport system is electrically imbalanced; i.e., for the cytosolic delivery of ATP^{4-} one negative charge moves outside. This negative charge is compensated by a parallel electrogenic proton extrusion; i.e., $(n + 1)$proton via the respiratory chain linked proton pumps. Thus, the model readily can be integrated into the metabolic relationships with the ATP-synthase, the phosphate carrier and the proton fluxes linked to the respiratory chain [19].

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