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Kinetic Singularities of Transport ATPases

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ABSTRACT. The molecular mechanisms of Tr-ATPases (Na, K-ATPase, Ca-ATPase, Zn-ATPase, Cu-ATPase, Ni-ATPase, Mg-ATPase, Mn-ATPase (cationic ATPases) and HCO_3-ATPase (anionic ATPase) and principal kinetic schemes of their work have been studied. As a result, two general singularities have been established: 1. bell-like geometric shape of kinetic $V=f(x)$ curve is certainly specific to all Tr-ATPases and is a necessary but insufficient condition for the identification of Tr-ATPase system; 2. another feature of ionic Tr-ATPases is that the MgAPT complex is their substrate. This is also a necessary but insufficient condition for the identification of Tr-ATPase system. The best manifestation of general kinetic singularities of Tr-ATPases is the kinetic scheme (molecular mechanism) of Na,K-ATPase system and conditions of regulation of their transport, which are localized in synaptic membranes and microsome of rat brain. *© 2007 Bull. Georg. Natl. Acad. Sci.*

Key words: *P-type ATPase, Tr-ATPase, molecular mechanisms.*

Transport of substances, their transmembrane transport, is an indispensable link not only in the substance exchange but also for the viability of the whole living organism, beginning with bacteria including the animal cell. Active transport of both anions and cations is mediated via the special ion pumps, which, in turn, represent the molecular vehicles localized within the membrane, providing ion transport against the concentration gradient, chiefly at the expense of ATP chemical energy. Proceeding from this, ion pumps are characterized by ATP-hydrolase activity and, therefore, instead of ion pumps the term "transport ATPases" (Tr-ATPase) is often used. Their study has a great theoretical and practical significance from the functional point of view.

Discovered so far have been many Tr-ATPases, such as Na,K-ATPase, Ca-ATPase, Zn-ATPase, Cu-ATPase, Ni-ATPase, Mg-ATPase, Mn-ATPase (cationic ATPases); HCO_3-ATPase, Cl-ATPase (anionic ATPases) and K,H-ATPase (proton ATPase). Existence of Tr-ATPase was shown both in animal and bacterial membranes [6;8;10;13;14]; the Laboratory of Membranology is engaged in the study of the molecular mechanism of the ATPases in question (the principal kinetic schemes of their work are most approximated to the genuine mechanism of a relevant enzyme). Deciphering of the molecular mechanism and establishing the principles of their regulation enables to specify kinetic singularity of Tr-ATPases, particularly, of P-type ATPases.

A characteristic feature of P-type ATPases, to which all the Tr-ATPases mentioned above belong, is that they have a phosphorylated intermediate (EP) being, of crucial importance in the mediation of transport. In terms of general principles of Tr-ATPases work, an enzyme should by all means possess minimum two conformational states stipulated by EP availability which determines the two diverse (low and high) affinities for the transportable ion. For the transport to be effected, at the very beginning the enzyme with high affinity should bind an ion, whereas after the transport has been effected, due to decay of ion affinity, the enzyme should get released from that ion. Graphically, the kinetic curve of transport enzyme velocity $V=f(x)$ (x - transported ion concentration) has a complex geometric shape. The reaction rate curves are of bell-like shape (Fig. 1) whose origin, the ascending phase corresponds to ion binding with a high affinity, and the descending – its release with a low affinity. Occasionally,

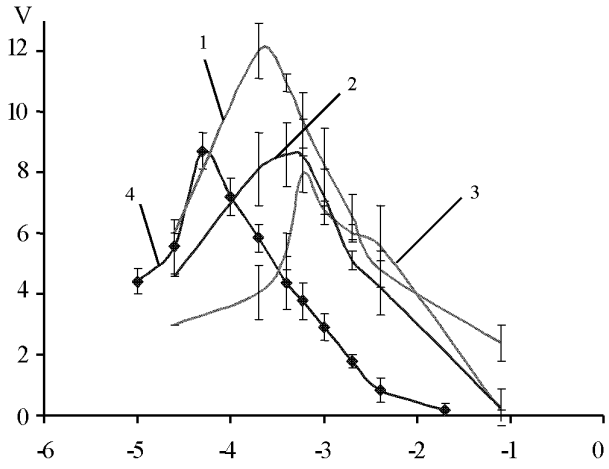

Fig. 1. Tr-ATPase activity dependence on transported cation concentration, V = f(lg$M$).
1 – Ni-ATPase (Ni^{2+}); 2 – Zn-ATPase (Zn^{2+});
3 – Mn-ATPase (Mn^{2+}); 4 –Mg-ATPase (Mg^{2+}).

in a definite concentration range of a transport ion after the ascending phase the curve passes into a clear-cut plateau, i.e. attains an imaginary constrained velocity followed by the descending phase.

The above-said kinetic curve shape has been common to all Tr-ATPases which mediate one type of ion transport. In the case of a cotransport, when two type ions are simultaneously transported (e. g. Na,K-ATPase), we obtain a graph of a similar shape (Fig. 2), where the enzyme velocity is plotted on the ordinate , while on the abscissa, changes of $[Na^+]/[K^+]$ ratio, provided their sum is constant of ($[Na^+]+[K^+]$=const); i. e. depending on the concentration of these ions, affinity for them changes respectively (see the Fig.2). If the concentration of one of them is constant, the bell-like V=f(x) curve remains unaltered.

Such geometric shape of kinetic V=f(x) curve is certainly specific to all Tr-ATPases and is a necessary but insufficient condition for the identification of Tr-ATPase system.

Mg^{2+}-ion has to play a special role in the work of all ionic Tr-ATPases. It is, in principle, an inhibitor for ATPases, since it binds to a substrate site and represents a competitive inhibitor for bivalent cations. But at the same time, Mg^{2+} is an essential activator as well, for the MgATP complex and not free ATP is a real substrate for all P-type, ionic Tr-ATPases [1;8;9]. The complex can be built on the enzyme itself: first ATP free binds to the substrate site and only then Mg^{2+} binds to ATP. We think that MgATP complex, being the substrate of ionic Tr-ATPases, accounts for the formation of the phosphorylated intermediate.

Thus, another feature of ionic Tr-ATPases is that the MgAPT complex is their substrate. This is also a necessary but insufficient condition for the identification of the Tr-ATPase system.

The best manifestation of general kinetic singularities of P-type transport ATPases is their kinetic scheme. On the basis of kinetic analysis of the multi-sited enzyme systems [2] which yield curves of a complex geometric shape (with turning and inflexion points available) it became possible to decipher the molecular mechanism of the Na,K-ATPase activity and to devise an accurate principal scheme (minimal model), which relies on the evidence reported in the literature and on our own experiments. The scheme in question explains the experimental findings available on this issue and involves all possible models of Na,K-ATPase operation [3-5, 7].

The genuine substrate of Na,K-ATPase, as mentioned above, is the MgATP complex and not free ATP. According to this scheme, Na,K-ATPase has an oligomeric structure per one subunit (α) with a single nucleotide site.

Adhering to a minimal model principle (that implies selection of the minimal number of enzyme forms and interreaction steps which are bound in a definite way and at any ligand concentration provide coincidence of geometric shapes of theoretical and experimental curves), Na,K-ATPase represents a dimer with $\alpha\beta$ subunit per each monomer. The condition of monomer is linked with transitions of another monomer. It is remarkable that the monomer's conformation state in a dimer is determined on the one hand by a bound ligand and by the monomer's conformation state, on the other. Thus it may be stated that monomers in a dimer interact in a consistent pattern. In conformity with the principal kinetic scheme, Na,K-ATPase can operate both in consecutive and simultaneous transport regime: in the enzyme oligomeric structure each protomer interacts with ions in succession, whereas the work of the entire complex is synchronized in such a way that one protomer is bound with Na^+, while the other is thereat bound with K^+. In the consecutive regime only the catalytic site of high affinity participates, while in the simultaneous – the low affinity site.

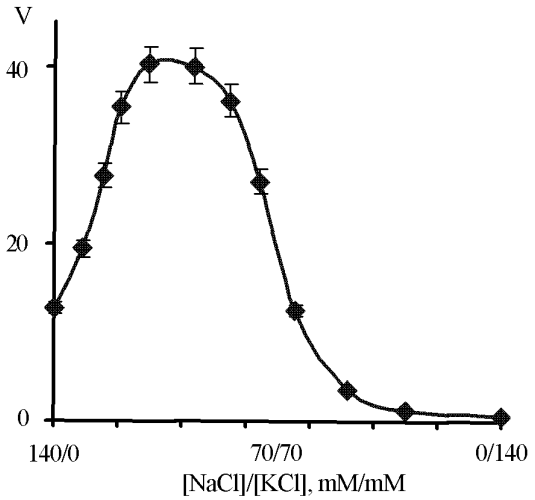

Fig. 2. Na,K-ATPase activity dependence on [Na+] / [K^+] ratio.

The Na,K-ATPase kinetic scheme envisages that there is one catalytic site per one subunit. To this site may bind ATP_f and Mg^{2+}. This scheme, as distinct from others, envisages the conformational changes induced by the action of these ligands.

An extremely important fact, revealed by our investigations, is remarkable: the Na,K-ATPase systems localized in the synaptic membranes and other fractions differ from each other by the mechanism of their molecular activity. We think that this must be due to Na,K-ATPase, as has been already noted, being an oligomeric system, and there are several isomers of its basic α and β subunits which are unevenly distributed in the subcellular fractions [11].

The molecular mechanism of Na,K-ATPase activity localized in the synaptic membrane fractions appears to be far more complex than the kinetic scheme of the protoplasmic membrane Na, K-ATPase [12]. The Na, K-ATPase localized in the synaptic membranes, depending on the concentration ratio of ligands: ATP_f, Mg^{2+}, and MgATP, may operate in different regimes: Mg^{2+}-dependent ($[Mg^{2+}]>>[ATP_f]$); ATP-dependent ($[ATP_f]>>[Mg^{2+}]$) and MgATP-dependent ($[MgATP]>>[ATP_f]=[Mg^{2+}]$). It is remarkable that in Mg^{2+}-dependent regime Na^+ and K^+ transport stoichiometry alters and instead of $3Na^+ : 2K^+$ becomes $3Na^+ : 1K^+$, or else $3Na^+ : 0K^+$ [4].

The molecular mechanism of protoplasmic membrane Na,K-ATPase activity, as has been already stated, is simpler and does not have Mg^{2+}-dependent and ATP-dependent regimes of work [12]. The enzyme system operates only in MgATP-dependent regime, wherein Na^+ and K^+ transport stoichiometry is $3Na^+ : 2K^+$. It is namely due to the lack of Mg^{2+}-dependent and ATP-dependent regimes that protoplasmic Na,K-ATPase is insensitive to neurotransmitters, while these latter are important regulators of the synaptic Na,K-ATPase and their activity is unequivocally associated with chemical synaptic transmission [3]. By the action of neurotransmitters the Na,K-ATPase localized in the synaptic membranes is inhibited and from MgATP-dependent regime it turns into Mg^{2+}-dependent one that is followed by Na^+ and K^+ transport stoichiometry change which, instead of $3Na^+ : 2K^+$, renders $3Na^+ : 1K^+$ or $3Na^+ : 0K^+$. Respectively, the electrogeneity coefficient increases and contribution of synaptic membrane Na,K-ATPase to the generation of transmembrane potential enhances.

Thus, on the basis of P-type transport ATPases studies and their comparative analysis, some kinetic singularities of P-type ATPases have been established.

ბიოქიმია

P-ტიპის ტრანსპორტული ATPაზების ზოგიერთი კინეტიკური თავისებურება

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ნივთიერებათა ტრანსპორტი, მათი ტრანსმემბრანული გადაადგილება აუცილებელი რგოლია არა მარტო ნივთიერებათა ცვლაში, არამედ მთლიანად ცოცხალი ორგანიზმის ცხოველმოქმედებისათვის. კათიონებისა და ანიონების აქტიური ტრანსპორტი ხორციელდება მემბრანაში ლოკალიზებული მოლეკულური მანქანების — ტრანსპორტული ATPაზების საშუალებით, რომლებიც მიეკუთვნებიან P-ტიპის ATPაზებს. დღეისათვის აღმოჩენილია მრავალი P-ტიპის ATPაზა, რომელთაგან მხოლოდ Na,K-ATPაზაა კინეტიკურად სრულად შესწავლილი. მემბრანოლოგიის ლაბორატორიაში მიმდინარეობს P-ტიპის ATPაზების (როგორიცაა Zn-ATPაზა, Ni-ATPაზა, Mn-ATPაზა, Mg-ATPაზა, HCO_3-ATPაზა, Cl-ATPაზა) მოქმედების მოლეკულური მექანიზმისა და მათი რეგულაციის გზების დადგენა. ჩატარებული კვლევების საფუძველზე, ჩამოყალიბდა P-

ტიპის ტრანსპორტული ATPაზების ზოგიერთი კინეტიკური თავისებურება: 1) ყველა ტრანსპორტული P-ტიპის ATPაზასათვის ტრანსპორტირებადი იონის კონცენტრაციიდან ფერმენტული რეაქციის სიჩქარის დამოკიდებულების ამსახველ მრუდს აქვს ზარისებური ფორმა. 2) P-ტიპის ყველა ტრანსპორტული ATPაზას სუბსტრატს წარმოადგენს MgATP კომპლექსი.

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