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Probability analysis of mammalian glutamate transporter activity

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Abstract. Mammalian glutamate transporters are essential for the most aspects of normal brain functioning including cognition, memory and learning. Its structure and functional properties are vigorously investigated now. However, there is no compliance amongst researchers with the elementary events sequence of glutamate transporter cycle. The sequence of elementary events was analysed using stochastic simulation of the processes. It was shown that in the case of invariant equilibrium constants of reactions of substrate and co-transported ions associations transposition in binding events sequence leads to alteration of protein affinity to substrate, but has insufficient impact on maximal transport velocity. Moreover, it was indicated that glutamate should be bound to the protonated form of transporter after the second sodium ion association.

1. Introduction

Excitatory amino acid transporters (EAATs 1-5) perform the precise control of synaptic transmission of major mammalian excitatory amino acid L-glutamate operating as secondary active transporters and ion channels at the same time [1]. However, excessive glutamate stimulation can result in excitotoxicity (glutamate-related cell injury or death). This type of damage may contribute to a large number of severe neurodegenerative disorders [2]. Therefore, understanding of the glutamate transporter functional mechanism and its kinetic properties is of the great importance.

One of unsettled question in EAATs functioning is the sequence of elementary events of substrate and co-transported ions associations on the extracellular part of the membrane and consequently its dissociation on the intracellular part. The conformational changings related to association events leads to additional conformational changings possessing anion channel activity [3]. This fact evidences about meaningful biological importance as glutamate transporter carries the net of three positive charges inside the neuron cell in each transport cycle and its anion channel neutralizes this effect to avoid membrane depolarization.

So far the overall structure of EAATs is not fully described and the existing data on bacterial homologues are usually used. Thus mammalian transporters are considered also to be a bowl-shaped trimeric proteins with aqueous basin contacting extracellular solution and extending halfway across the membrane bilayer and a pointed base facing the cytoplasm. Each protomer consists of eight transmembrane alpha-helical segments (TM1-8) and two helical hairpins (HP1-2) which are divided in two functional domains: a scaffold domain (TM1-6) and a transport domain (TM 7-8 and HP1-2). Each protomer of glutamate transporter is an independently and stochastically functioning subunit. The transport cycle of individual subunits is described as a co-transport of 1 glutamate with 3 sodium

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ions and 1 proton ion into the cytosol, and counter-transport of 1 potassium ion into the external solution [1].

The protein undergoes several conformational changes during its whole transport cycle. Some of them are related with substrate binding and its translocation inside the cell across the membrane, another comes along with substrate dissociation and relocation of the transporter to the outside position.

In addition, EAATs possess thermodynamically-uncoupled anion conductance, which is a chloride current under physiological conditions. It is a common understanding now, that anion channel opening is achieved via additional branching conformational change and is not a part of the transport cycle [4]. Existence of such a current has been proposed to preserve excessive cell excitation and prevent additional glutamate release.

Molecular dynamic (MD) simulations of an association process on the external part of a membrane clarify the experimentally observed cooperativity in sodium ions binding and also in substrate binding. Association of a Na^+ in the first sodium binding site facilitates conformational changes in transport domain of the protein therefore increasing affinity for another sodium ion. Association of Na^+ in the third sodium binding site stabilizes such a conformational changes and also promotes another conformational changes that favor for an association of a substrate [5].

2. The sequence of elementary events in glutamate transporter cycle

Binding of the first sodium ion to the empty transporter was previously determined as initial translocation step in the whole glutamate transport cycle. However, it still remains unclear whether the second sodium ion associates to the transporter before substrate binding or after. It is also known that the glutamate transporter has to be protonated to permit sodium and substrate binding [6]. Finally, at least one sodium ion determined to associate with glutamate bound form of transporter [7].

The precise sequence of dissociation events in the intracellular part of the membrane during glutamate transporter cycle is the most controversial question. The reason is that there is little known about conformational changes through this process and bacterial homologues crystallographic data with MD simulations cannot fully describe it.

An existing assumption builds upon proximity of Na^+ in the second sodium binding site to intracellular solution [5]. So the dissociation of sodium off this binding site could be an initial step. Further, an observed movement of HP1 generates a small opening to another ions and substrate binding sites, just as it is for HP2 [3]. There is also a consumption based on a “first-in-first-out” mechanism, so that deprotonation of the glutamate transporter should be a further dissociation step [8].

3. Virtual simulator for probability analysis of mammalian glutamate transporter functioning

Virtual computer simulator was proposed to examine the kinetic properties of a single neuronal glutamate transporter. At the core of it is stochastic modelling algorithm based on probability coefficients of each elementary step of protein transport cycle, such as substrate binding and dissociation or translocation across the membrane. The structural properties of the protein are implicit in a probability values, which are generally derived from equilibrium constants of each elementary reaction (step) or differences of free energies of the substrates binding. The timescale parameters of each step could be settled independently.

Such an approach provides an opportunity to change the sequence of elementary events and so to take into account different schemes of protein transport cycle. Besides, virtual experiments could be performed at different ambient conditions, such as substrate and ions concentrations. Furthermore, a significant advantage of this approach is ability to obtain detailed information about the functional properties of the transporter at a rather short time.

3.1. Scheme of protein transport cycle

The most feasible scheme of protein cycle is shown on figure 1. However, along with permutation with repetitions there are 400 variants of scheme for sequence of substrate association and dissociation

elementary events in glutamate transporters which describe its turnaround kinetic properties. All of them were examined.

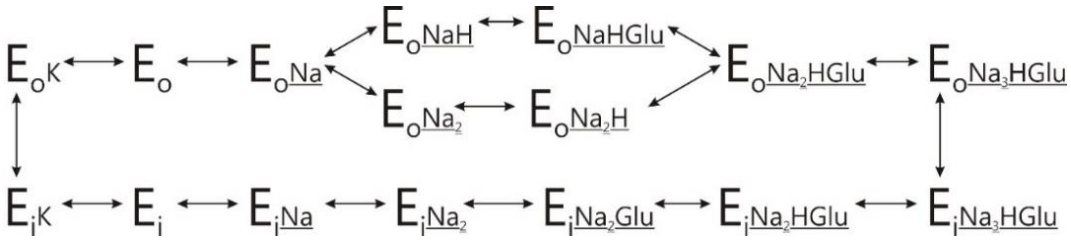

Figure 1. The scheme of elementary events of glutamate transporter cycle.

3.2. Results

In case of glutamate amino acid and protons, transport kinetic dependences approximation was performed by Michaelis–Menten equation, and Hill equation was applied to sodium ions transport kinetic dependence for the same purpose.

Under physiological ambient conditions transport kinetics of EAATs doesn't depend on the dissociation steps order (data not shown). In fact, the association steps order also has insufficient impact on maximum rate achieved by the system.

Difference between possible variants of scheme for sequence of elementary events in glutamate transporters reflects on $K_{1/2}$ values introduced in figure 2.

Substrate	Order	$K_{1/2}$
Glu	1st	2.2 ± 0.1 mM
	2nd	1.88 ± 0.01 mM
	3rd	1.89 ± 0.03 mM
	4th	2.20 ± 0.02 mM
	5th	3.56 ± 0.03 mM

Substrate	Order	$K_{1/2}$
H^+	1st	8.60 ± 0.04 nM
	2nd	7.8 ± 0.6 nM
	3rd	7.56 ± 0.04 nM
	4th	7.56 ± 0.04 nM
	5th	14.3 ± 0.1 nM

Substrate	Order	$K_{1/2}$
Na^+	1st, 2nd, 3rd	52.8 ± 1.7 mM
	2nd, 3rd, 4th	53.1 ± 0.4 mM
	3rd, 4th, 5th	56.9 ± 0.4 mM
	1st, 2nd, 4th	48.5 ± 0.2 mM
	1st, 2nd, 5th	48.9 ± 0.1 mM
	1st, 3rd, 4th	48.5 ± 0.4 mM
	1st, 4th, 5th	51.3 ± 0.2 mM
	2nd, 4th, 5th	52.5 ± 1.1 mM
	2nd, 3rd, 5th	50.5 ± 0.3 mM
	1st, 3rd, 5th	46.6 ± 0.3 mM

Figure 2. $K_{1/2}$ values of transport kinetic dependences approximation.

Kinetic dependences for the optimal sequence of elementary events in glutamate transporter cycle represented the highest affinity for substrates consistent with literature data are performed at figure 3. These dependences exhibit qualitative characterization, and the time course of elementary steps is to be validated.

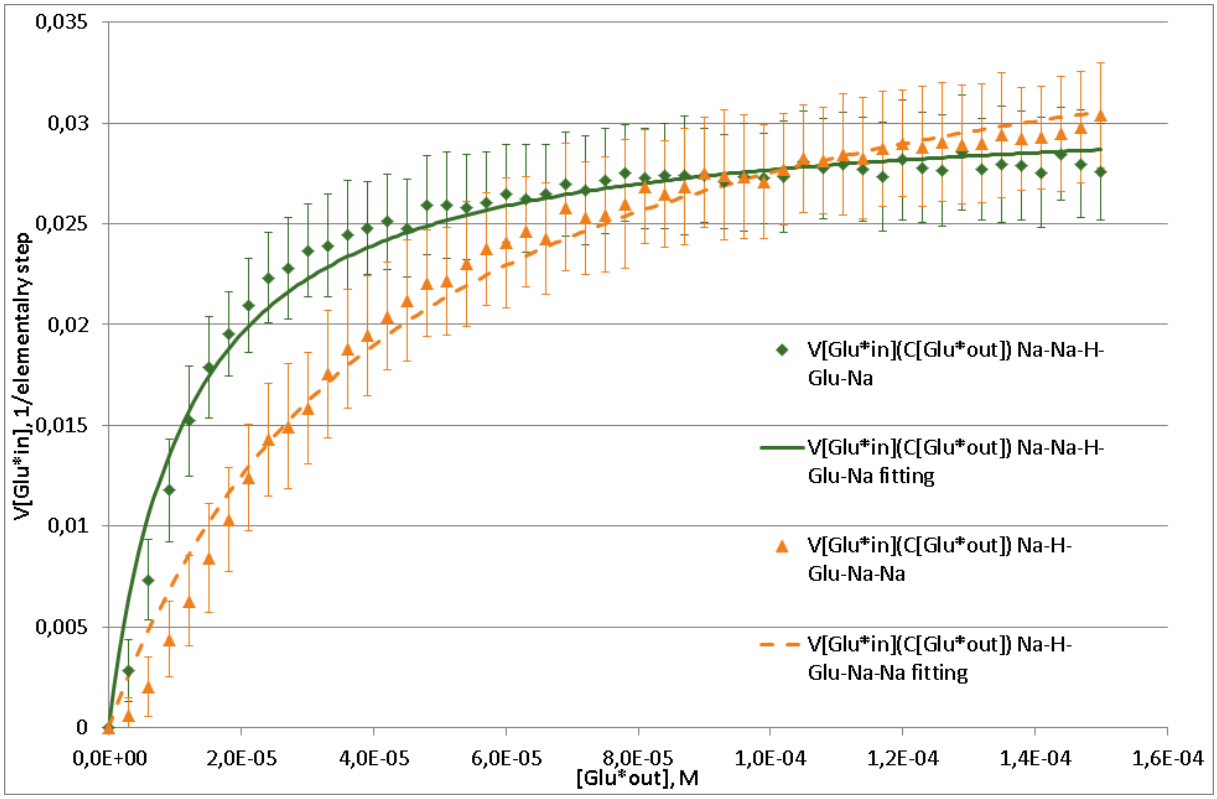

Figure 3. Kinetic dependences of elementary events in glutamate transporter cycle.

4. Discussion

The developed virtual simulator can be used for stochastic modelling of EAATs transport cycle and allows to predict some protein features which should be experimentally proved.

It is shown that under physiological concentration of substrates the kinetic properties of EAATs are independent of glutamate, sodium ions and protons dissociation steps order in the cytosol.

On the terms of invariance of equilibrium ratios for substrates association alteration of elemental steps order in the extracellular space leads to changing in enzyme affinity for transported amino acid, though doesn't affect the maximum transport rate.

On the base of EAATs activity computer modeling it was shown that the most optimal sequence of elemental events is implemented in the scheme wherein glutamate molecule associates to protonated form of transporter after the second sodium ion binding.

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