New and Notable

Coupling between Neurotransmitter Translocation and Protonation State of a Titratable Residue during Na^+-Coupled Transport

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The functional significance of excitatory amino-acid transporters (EAATs) in clearing excess glutamates from central nervous system synapses is widely recognized. An archaeal aspartate transporter, Glt_{Ph}, from *Pyrococcus horikoshii* has broadly served as a model system for gaining structure-based insights into the mechanism of function of human EAATs, as the first structurally resolved member (1) of the family of glutamate transporters. Glt_{Ph} has since been resolved in multiple states, which helped us gain insights into the structural basis of the interactions involved in transport activity. Yet, translation of structural and dynamic data obtained by structural and computational studies for the archaeal transporter to human EAATs (subtypes hEAAT1-5) has been a challenge, partly due to the moderate sequence similarity (30–40%) between these orthologs, and the differences in their functional mechanisms. Recently, another ortholog, aspartate transporter, Glt_{Tk} from *Thermococcus kodakarensis* has been resolved in substrate-free form, providing new insights into the mechanism of reconfiguration back to an outward-facing state after the release of substrate and Na^+ ions (2).

Glutamate transport is a typical example of secondary transport process, fueled by the cotransport of sodium ions down their electrochemical gradient. EAATs (hEAAT1-3) also cotransport a proton, in addition to three Na^+ ions. Structure-based computations helped in recent years in elucidating the membrane-mediated mechanisms that permit the alternating access of the transporter between outward- and inward-facing states (3), the sequence of events during ion-coupled binding of substrate (4), and the position of sodium-binding sites (5,6). Yet, the mechanism of coupling between substrate release and proton cotransport is yet to be established. EAATs further function as chloride channels, and the transport cycle is completed by the countertransport of a K^+ ion, providing additional layers of complexity in neurotransmitter transport process.

Coupling between the protonation state of a titratable residue near substrate/ion-binding site and substrate binding/release has been recently noted in a number of sodium/proton-coupled transporters (7–9). The protonation of an acidic ion may (partly) shield the unfavorable electrostatic interactions with a negatively charged substrate near bound substrate, and substrate release may be succeeded by the deprotonation of the titratable residue upon exposure to aqueous environment. Similar phenomena have been observed by Heinzelmann and Kuyucak (10). Using homology models created for the outward- and inward-facing conformations of EAAT3, they confirmed with the help of free energy perturbation and thermodynamic integration methods that a glutamate, E373, is the most probable candidate for carrying the cotransported proton. The study further sheds light to a sequence of events and potential involvement of K^+ in enabling proton cotransport and resuming the transport cycle. E373 is protonated in the presence of substrate and one of the sodium ions (Na2). Its protonation favors neurotransmitter uptake and binding in the outward-facing state. In the inward-facing state, on the other hand, the dislocation of neurotransmitter and Na2, and the opening of the HP2 gate, prompt the release of neurotransmitter and the exposure of E373 to intracellular water, thus reducing the pKa near the binding pocket in favor of the deprotonation of E373. Proton release is completed upon binding of a K^+ ion to the same site. Binding of K^+ to the same site is consistent with newly resolved Glt_{Tk} structure, which showed that the vicinity of Glt_{Tk} Q321 (counterpart of E373 in EAAT3) could form a K^+ binding site (2).

The possible involvement of EAAT3 E373 as a proton acceptor has been proposed almost a decade ago (11), and the equivalent residue (E404) in the rat homolog of EAAT2 has been proposed to bind K^+ in 1997 (12). These observations confirm these original studies, consistent with the conservation of glutamate at this particular sequence position across hEAAT subtypes. The originality of the study lies, however, in the quantitative demonstration of the following:

1. The high affinity of E373 for being protonated in the outward-facing state,
2. Its effect on stabilizing the bound substrate,
3. The coupling of its protonation state to neurotransmitter release, and
4. The effect of bound cations (Na^+ or K^+) in modulating its protonation state.

The pKa values reported in Table 2 in Heinzelmann and Kuyucak (10) for a series of titratable residues near the binding pocket highlight the distinctive protonation propensities of E374.

Notably, in Glt_{Ph} as well as Glt_{Tk}, the same position is occupied by a glutamine, Q318 and Q321, respectively. The archaeal transporter does not require cotransporting of a proton, nor the countertransport of a K^+ ion (13,14). Notably, the terminal -OH group of glutamate (protonated E373)

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in hEAATs is replaced by an NH_2 group (Q318) in Glt_{Ph}. The involvement of Q318 in interactions that control the gating motions of the HP2 loop has been noted earlier (15), and microseconds simulations revealed the key role of HP2 in both extracellular and intracellular gating (16), in support of the involvement of this particular site in mediating substrate uptake and release.

The integrated molecular dynamics-free energy perturbation/thermodynamic integration approach adopted by Heinzelmann and Kuyucak (10) provides a comprehensive framework for assessing the protonation/deprotonation propensities of titratable residues that may (directly or indirectly) affect substrate-binding or -release. Compared to continuum calculations of pKa, the method presents the advantage of providing a full-atomic description of the coupled dynamics of the transporter and environment, and may be applicable to proton-coupled translocation events in general.

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