Mechanism of chloride channeling by Excitatory Amino Acid Transporters

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GLUTAMATERGIC SIGNALING

References

EAAT’s Alternating Access Mechanism

Jensen et al., 2013
GltTk from Thermococcus kodakarensis

Verdon & Boudker, 2012

References

Cooperative Intersubunit Motions are Essential to Function

Jiang et al., PNAS 2011

Glutamate uptake blocked by disulfide cross-linking between ‘distant’ pairs
Transition of one subunit at a time: a mechanism revealed by our experiments & computations


Transition of one subunit at a time: a mechanism revealed by our experiments & computations

Outwards

Inwards

Two outwards

Two inwards

IFS

OFS

IFS

IFS

RMSD = 1.382 Å


Intermediates mediate permeation of polar solutes

MD setup for Glt$_{ph}$ (PDB:3V8G)

The putative anion pathway suggested by Verdon and Boudker shows a constriction zone near S65, Y195, M286 and P304
Intermediates mediate permeation of polar solutes

MD setup for Glt$_{\text{ph}}$ (PDB:3V8G)

Energy barrier $\sim 20$ kcal/mol

A intermediate water-channeling state iChS forms during the transition to inward-facing conformations.
A intermediate water-channeling state $i\text{ChS}$ forms during the transition to inward-facing conformations.
**iChS** favors anion permeation

**Pore radius of iChS**

![Graph showing pore radius (Å) vs. PMF (kcal/mol)]

- **PMF (kcal/mol)** vs. **Channel Z (Å)**
  - Energy barrier < 5 kcal/mol

**Hydrophobic gate**

**Selectivity filter**

**iChS**

**Channel Z (Å)**

- **R276**
- **R397**
- **F50**
- **G357**
- **V51**

**iChS**
iChS distinct from OF and IF states
Same channel confirmed in EAAT1

Chloride channel

EAAT1

closed-channel

open-channel

solvent accessibility

Glt_{Ph}

SASA (\text{Å}^2)

hEAAT1

EC IC

V51 L212 F50 V209 M202 M395 R276 Y195

0 80 160

M89 L296 L88

12±05 Å

M89 L88
Hypothesis:

Three residues are found to control the channel opening or closure, and they are solvent-exposed prior to transitioning to closed state.

Suppose we bind at those positions a bulky molecule at those positions.

Do we observe an effect on channel permeability?
Experiments confirm the key role of L296C in glutamate uptake.

**Electrophysiology**

MTS-reagents that are covalently bound to cysteines:
- **MTSES**: negatively-charged
- **MTSET**: positively-charged
- **MTSACE**: neutral
- **DTT**: reagent to reduce the disulfide bridge

**SCAM**

**Glu uptake**
M89 in EAAT1 also controls anion permeation
L88 controls both anion permeation and substrate transport

### Current-voltage

- **WT**
- **L88R**

### ion selectivity

- $P_x/P_{Cl}$
- WT
- L88R

### Glu uptake

- Uptake/Surface Expression (%)
- WT
- L88R

### Normalized $I_{max}$

- $I_{max}$/Surface expression (%)
- WT
- L88R
Conclusion

- Anion permeation takes place in the intermediate channeling state, $iChS$.
- Channel opening is enabled by
  - elevator-like downward movement of transport core in the substrate-loaded state
  - repacking of a cluster of hydrophobic residues (L88, M89 and L296 in EAAT1)
- Robustly shared by both the archaeal and mammalian transporters.