Computational Characterization of N-acetylaspartylglutamate Synthetase: From the Protein Primary Sequence to Plausible Catalytic Mechanism

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N-acetyl-aspartyl-glutamate - the most abundant peptide neurotransmitter

Targets:

1. mGluR3 +, EC50 = 11-100 uM
2. NMDA -/+, EC50 = 666 uM

mGluR3 -> AC -> L-Ca^{2+}

A number of studies stipulate its implication in:

1. Schizophrenia
2. Ischaemia
3. Pain
4. Epilepsy
5. Traumatic Brain Injury

INN: spaglumic acid
Antiallergic - mast cell stabilizer

Metabolic pathway for NAAG biosynthesis in brain

\[
\begin{align*}
&\text{Acetyl-CoA} & \rightarrow & \text{NAT8L} & \rightarrow & \text{L-Glu, ATP} \\
&\quad & \text{NAT8L} & \quad & \text{RimklA} & \quad & \text{in vitro:} \\
&\quad & & & & \\
&\quad & & & & \\
\end{align*}
\]

\[
K_{M}^{(\text{Asp})} = 0.16 \pm 0.05 \text{ mM} \\
k_{\text{cat}} = 0.071 \pm 0.006 \text{ U/mg}
\]

\[
\text{in vitro:} \\
K_{M}^{(\text{Glu})} = 0.88 \text{ mM} \\
K_{M}^{(\text{mgATP})} = 0.065 \text{ mM} \\
k_{\text{cat}} = 2.6 \text{ s}^{-1}
\]


Coevolutionary-based protein structure prediction with AlphaFold2

Comparison of the homology model (orange) of RIMKLA structure to AlphaFold2 (green).

AlphaFold2* have been widely recognized as a new state-of-the art protein prediction method after its success in 14th Critical Assessment of protein Structure Prediction (CASP14)

Classical MD of RIMKLA

MD SETUP:
Software: GROMACS, NAMD
Number of atoms: ~ 40 000
Force field: CHARMM27
Periodic boundary conditions & Ewald summation scheme
T = 300 K, p = 1 atm
MD results in refinement of active site

But only with multiple trajectories, we can expect to see actual active site’s side chain packing improvement. Trajectories may also terminate in the actual collapse of the active site and the exit of ligands, despite the retention of the domain structure as a whole!
Proposed reaction mechanism:

1. Transfer of phosphate to N-Ac-Asp leading to acyl phosphate intermediate.
2. Nucleophilic attack N-C leading to NAAGS.

Another example of substrate-assisted catalysis !?
Reactionary configurations - QM/MM MD of RIMKLA-Intermediate complex

NAMD-TeraChem interface*

The QM part contained 135 atoms described by the density function theory with the PBE0 hybrid functional, D3 dispersion corrections and 6-31G** basis set with 1395 basis functions in total. The QM system included NAA-PO$_3$, ADP (cut on the C4’-C5’ bond), two magnesium atoms; Arg160, Arg201, Arg215, Glu273, Asp260, Asn275 side chains and water molecules.

Distance C-N across trajectories vary from 3Å to 5Å, but consistently low (3Å) distance signals that under the condition of attacking amino group deprotonation, the reaction is plausible.

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Conclusion

1. Starting from primary sequence, we obtained a model of RIMKLA in the holo-form and studied its dynamical properties using classical MD.
2. Notably, we were able to reconstruct the structure of the active site including reaction intermediate, coordinated with two Mg2+ ions, ADP and glutamate, that turned out to be stable in some of the trajectories we obtained, including those featuring QM/MM potential.
3. Obtained model suggests that catalysis is likely to proceed through substrate-assisted mechanism with acetyl phosphate or glutamate carboxyl groups as proton acceptors in the second step of the reaction.
Thank you for your attention!