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Nmar
207 & 1308
Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO$_2$ fixation

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Archaea of the Phylum Thaumarchaeota

Most abundant prokaryotes on Earth

Widely distributed in marine, terrestrial, and geothermal environments

Contribute the most to the nitrogen and carbon cycles across the globe
Nitrosopumilus Maritimus
(N. Maritimus)

• Used N. maritimus because it is a representative of the phylum
• Has an extremely high affinity for ammonia → allows them to grow at really low ammonia concentrations (in the low nanomolar range)
• Autotrophic carbon dioxide fixation is the central anabolic process used
The authors provide biochemical evidence that the thaumarchaeal ammonia oxidizers assimilate inorganic carbon via a modified version of the autotrophic hydroxypropionate/ hydroxybutyrate (HP/HB) cycle of Crenarchaeota that is far more energy efficient than any other aerobic autotrophic pathway.
Further, the authors conclude that the "high efficiency of anabolism exemplified by this autotrophic cycle perfectly suits the lifestyle of ammonia-oxidizing archaea, which thrive at a constantly low energy supply, thus offering a biochemical explanation for their ecological success in nutrient-limited environments".
Hydroxypropionate/Hydroxybutyrate (HP/HB) Cycle

• In general…
• Step 1:
  • 1 acetyl Co-A and 2 bicarbonate molecules are converted via 3-hydroxypropionate to succinyl Co-A
• Step 2:
  • Succinyl Co-A is converted via 4-hydroxybutyrate to 2 molecules of acetyl Co-A and one of those molecules serves as a carbon precursor
Hydroxypropionate/ Hydroxybutyrate (HP/HB) Cycle

More specifically, ...

Thaumarchaeota possess a modified version of the HP/HB cycle that is distinct from the cycle operating in Crenarchaeota. This shows that the cycle apparently evolved 2x in archaea.

Additionally, this is the most efficient carbon dioxide fixation mechanism in the presence of oxygen.
4-Hydroxybutryl-CoA dehydratase
Catalyzes reaction from the 4-hydroxybutryl-CoA → crotonyl-CoA

Crotonyl-CoA hydratase
Catalyzes the reaction from crotonyl-CoA → 3-hydroxybutryl-CoA

3-Hydroxypropionyl-CoA dehydratase
Catalyzes the reaction from 3-hydroxypropionyl CoA → acryloyl Co-A + H₂O
• 1308 has substantially higher specific activity in comparison to the other enzyme present in the HP/HB cycle.
Methods:

• Screen the protein (Nmar 207 & Nmar 1308) with various conditions, including pH’s and concentrations, to determine optimal crystalizing conditions

• Plate the optimal conditions with the protein and substrate and allow the crystals to form

• Harvest the crystals using a loop and microscope and freeze them in liquid nitrogen

• Use X-ray crystallography to collect data to determine the structure of the protein

• Use Pymol and COOT for structural analysis
The Nmar_207 structure was solved at 1.55 Å, showing overall structure to be that of a homotetramer.

The tetramer contained four active sites, each of which contained one $[4\text{Fe}-4\text{S}]^{2+}$ cluster and one FAD molecule.

The larger interfaces come together by flipping one of the two monomers from the mirror-image position 180 degrees. In this conformation, the monomer-monomer interface is composed mainly of interactions between corresponding regions of alpha-helices and beta sheets.
Nmar 207 with substrate binding site

- In this position, the substrate has its CoA-thioester pointing toward the flavin moiety, leaving the 2’ and 3’ carbon positions directly above FAD. These results largely confirm prior characterizations of the C. amino active site, which featured these three residues interacting with the substrate.
The active site is accessed through a narrow binding channel near the surface of a monomer-monomer interface. The walls of this channel are formed by both interacting monomers. FAD rests in this channel, with the isoalloxazine ring most proximal to the channel’s entry.

The other substrate in this key dehydration reaction, 4-hydroxybutyryl-CoA, was also captured in our crystallized active site.
Discussion on Nmar 207

- 4-hydroxyburyl-CoA dehydratase originally found in strict anaerobes
- These strictly anaerobes constitute a distinct anaerobe family in the 4HBD phylogenetic tree
- Two independent clusters are formed by hyperthermophilic Crenarchaea that are unable to ferment
- The number of 4HBD genes identified is comparable with the RuBisCO large subunit
- Extra 4Fe-4S layer in proximity of the reaction (3 Å apart) shows the influence of the iron on the FAD
- Adding an additional iron to the structure allowed for a clear pathway of the water molecule being dehydrated to be visualized
Pymol Structure Nmar 1308

- The Nmar 1308 structure was solved at 2.1 Å
- Hexameric
Nmar 1308 with 2DUB

(ENOYL-COA HYDRATASE COMPLEXED WITH OCTANOYL-COA)
Nmar 1308 B factor map
2DUB
Nmar 1308
Catalytic Residues

Nmar 1308 aligned with 2DUB
Nmar 1308 and $B$-factor loop of 2DUB
Discussion on Nmar 1308

• Further, with this information we look to hypothesize about its promiscuous behavior such as:
  • Why the protein can accommodate 3-4 carbon long structure but nothing bigger
  • How it can catalyze a forward and reverse reaction
  • Why the hexameric structure is an advantage
Achieved

• We aimed to determine the structures of both Nmar 207 and Nmar 1308 in order to better understand the role they play in the modified version of the HP/HB cycle

Future Endeavors

• Determine the structures of other enzymes in the cycle and therefore ascertain how the HP/HB cycle is the most energy efficient cycle
• Make the HP/HB cycle still more efficient long-term
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