Lactate racemization, a story of so much more than just a nickel

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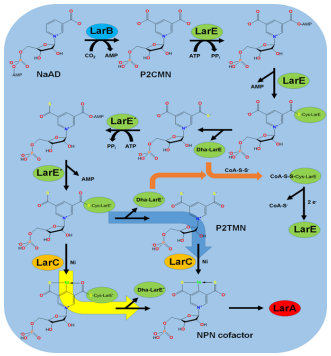
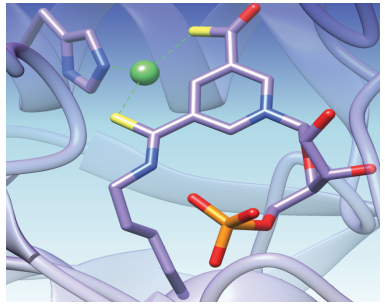
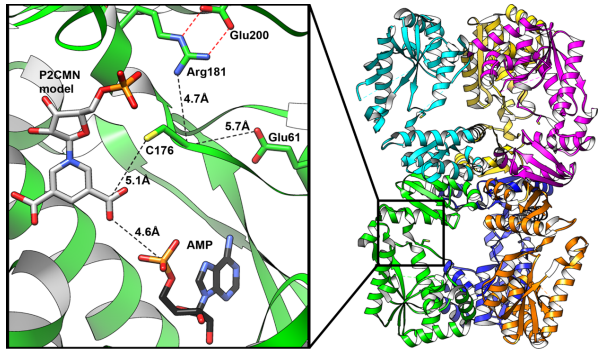


Lactate racemase **LarA**, the ninth discovered nickel-dependent enzyme, was shown to contain a newly identified vitamin B3 derived Ni cofactor (P2TMN-Ni = NPN cofactor). The cofactor is bound via Lys184 with the Ni being coordinated by His200.
Synthesis of the cofactor involves three proteins **LarB**, **LarC**, and **LarE**:
Cofactor biosynthesis begins with **LarB**, a carboxylase/hydrolase of NaD.
LarE, a new member of the PP-loop ATP Pyrophosphatase family, then inserts two sulfur atoms. Structural analysis, combined with structure-guided mutagenesis establishes **LarE** as a paradigm for sulfur transfer through sacrificing its catalytic cysteine residue, only the second sacrificial sulfur transferase to be described[1,2].
Finally, **LarC** inserts a nickel atom to form a five-membered nickellacycle structure in which a stable nickel-carbon bond, as well as a nickel-sulfur bond is created (a metallacycle). **LarC** is therefore the first cyclometallase identified in nature.
Structure-function characterization discovered that **LarC** requires cytidine triphosphate (CTP) hydrolysis[3].
LarA then utilizes the cofactor for lactate racemization. Again using functional and structural methods we provided compelling evidence, that this is accomplished via a proton-coupled hydride transfer mechanism[4].
Lactate racemization is involved in lactate metabolism and cell wall assembly but the cofactor may also be used for a wide range of other yet to be discovered reactions.



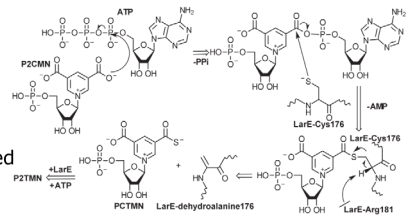
P2TMN-Ni cofactor in LarA

LarE a sacrificial sulfur transferase[1,2]

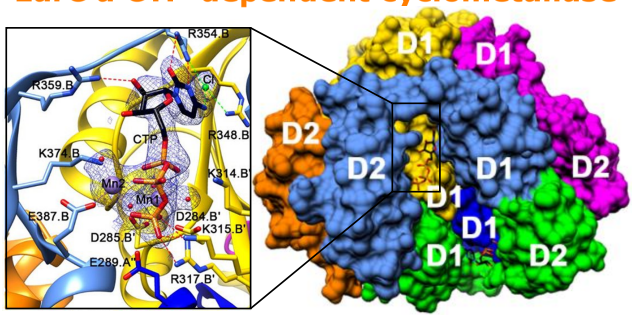


LarE forms a hexamer (6 monomers). It binds ATP-Mg to activate its substrate P2CMN via AMPylation (model based on nicotinamide mononucleotid bound structure - PDB 5UDR). The activated P2CMN is attacked by Cys176 and the sulfur is transfered from the protein to the cofactor.

This makes LarE a sacrificial sulfur transferase and two LarE have to be sacrificed to form P2TMN. LarE could possibly be regenerated via a CoA-persulfide.[4]

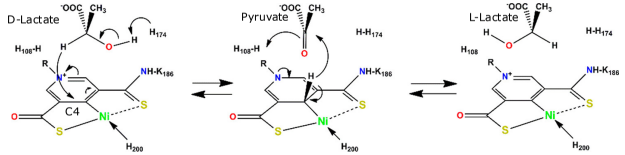


LarC a CTP-dependent cyclometallase[3]



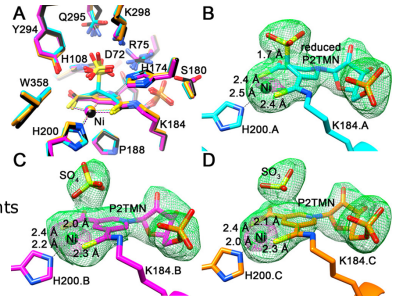
LarC contains a N-terminal C1 domain, responsible for P2TMN and Ni binding. The above structure (PDB 6BWQ) shows the C-terminal C2 domain with a novel CTP binding pocket.
The C2 domain forms a hexamer (6 monomers) and each C2 monomer contains two domains D1 and D2. CTP is bound by the D1 and D2 domains of two separate monomers.
No structure of C1 is available, therefore the mechanism of LarC to create the cofactor metal cycle (Ni-S and Ni-C bonds) using the C2 bound CTP remains unclear.

LarA Operates by a Proton-Coupled Hydride Transfer (PCHT) Mechanism[4]



Functional studies established a PCHT mechanism instead of a proton-coupled electron transfer (PCET):
- The intermediate pyruvate was detected
- No Ni EPR signal was detected (PCHT proposes inactive Ni^{2+} instead of PCET involves EPR active Ni^{3+})
-A normal substrate Substrate Kinetic Isotope Effect of kH/kD of 3.1 is consistent with direct cleavage of the C–H bond (PCHT) instead of C-C bond cleavage in (PCET).

- A structure of LarA in complex with the inhibitor sulfide was solved (PDB 6C1W), showing ring C4 modification as proposed in PCHT is possible.



A) LarA active site alignments
B) Sulfide bound
C) Sulfate bound
D) Sulfide intermediate

Future directions, beyond lactate racemase

10% bacteria have LarA, many might have different substrate
Additional 15% have only LarBCE and no LarA
-> undiscovered protein uses cofactor for unknown activiy
Lactate and other racemase reaction utilization for industry
Role of these proteins in pathogenic bacteria - drug targets

1. Fellner, M.,/Desguin, B. et al. (2017) Structural insights into the catalytic mechanism of a sacrificial sulfur insertase of the N-type ATP pyrophosphatase family, LarE, Proc Natl Acad Sci U S A 114, 9074-9079.
2. Fellner, M. et al. (2018) Analysis of the Active Site Cysteine Residue of the Sacrificial Sulfur Insertase LarE from Lactobacillus plantarum, Biochemistry, in press
3. Desguin, B.,/Fellner, M. et al. (2018) Biosynthesis of the nickel-pincer nucleotide cofactor of lactate racemase requires a CTP-dependent cyclometallase, J Biol Chem., 293, 12303-12317.
4. Rankin, J. A. et al. (2018) Lactate Racemase Nickel-Pincer Cofactor Operates by a Proton-Coupled Hydride Transfer Mechanism, Biochemistry 57, 3244-3251.

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