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Nickel–pincer nucleotide cofactor

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A novel organometallic cofactor, nickel pyridinium-3,5-dithiocarboxylic acid mononucleotide, was recently discovered in lactate racemase (LarA) of *Lactobacillus plantarum*. This review summarizes the substantial progress made in uncovering the function of this cofactor as a transient hydride acceptor in the LarA mechanism. The latest developments related to cofactor biosynthesis reveal insights into a pathway in which LarB serves as a nicotinic acid adenine dinucleotide hydrolase/carboxylase, LarE acts as a sacrificial sulfur transferase, and LarC functions as a nickel insertase, forming the nickel–pincer nucleotide cofactor that becomes covalently tethered to LarA in some bacteria. Bioinformatic studies reveal a widespread occurrence of *larA*, *larB*, *larC*, and *larE* orthologs in microorganisms, and additional roles for the cofactor are considered.

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Introduction

A new organometallic cofactor (Figure 1a), nickel bound to pyridinium-3,5-dithiocarboxylic acid mononucleotide (P2TMN), was identified in lactate racemase from *Lactobacillus plantarum* by mass spectrometry and X-ray crystallography in 2015 [1••]. Inorganic chemists have characterized many similar-appearing planar organic ligands that tri-coordinate metals and termed these synthetic compounds pincer complexes [2,3]. The lactate racemase cofactor, herein called the nickel–pincer nucleotide (NPN), is the first biological example of such a species. This review summarizes insights gained during the past two years from biochemical, biomimetic, computational, spectroscopic, crystallographic, and bioinformatic studies related to the role of the NPN cofactor in lactate racemization, the biosynthesis of NPN by LarB, LarE, and LarC (Figure 1b), and the possible alternative roles for this coenzyme.

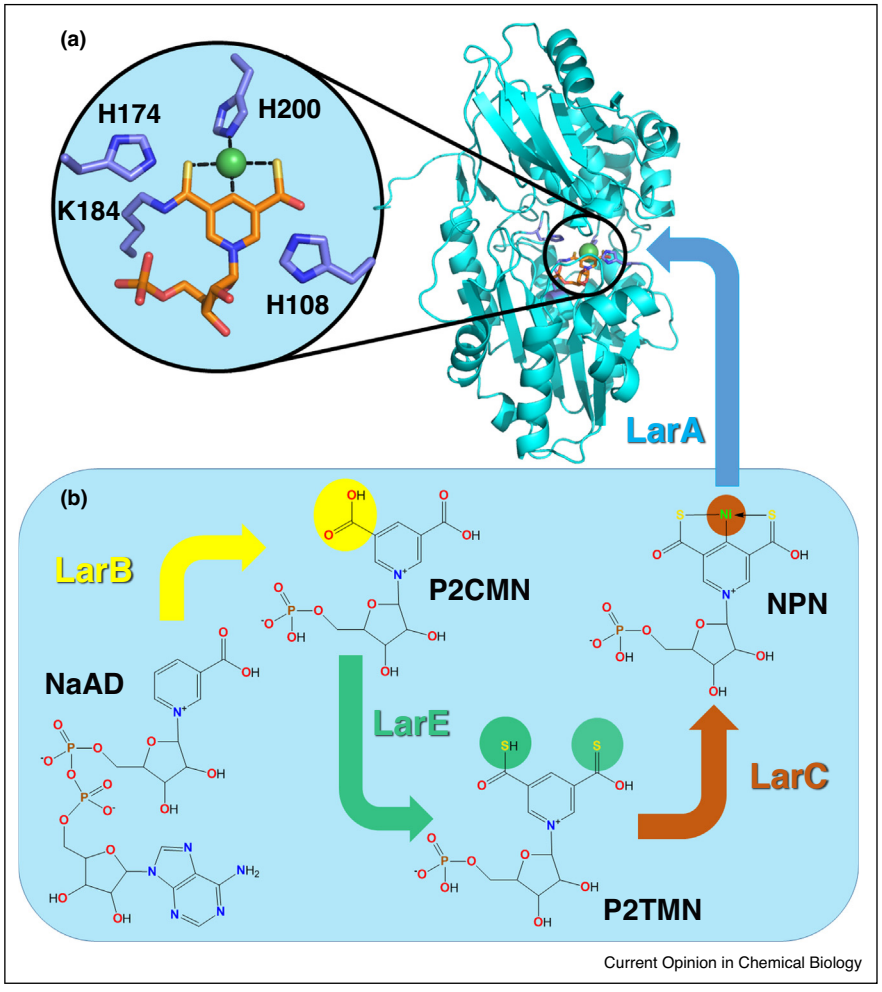
Role of the NPN cofactor in lactate racemase

When first identified, the LarA NPN cofactor was proposed to participate in a proton-coupled hydride transfer (PCHT) reaction in which (i) the D- or L-lactate hydroxyl groups are deprotonated by H108 or H174, (ii) pyruvate forms as hydride transfers to the cofactor, (iii) the same hydride returns to either face of pyruvate (known since 1965 [4]), and (iv) the D- or L-alkoxide products are reprotonated [1••]. Hydride was depicted as transferring to C4 of the pyridinium, akin to standard nicotinamide-type chemistry, but the alternative possibility of a nickel–hydride intermediate was mentioned.

Synthetic models and computational studies have since provided important insights into the LarA mechanism. For example, a synthetic nickel-dithioamidepyridinium species resembling the NPN cofactor was shown to catalyze the dehydrogenation of alcohols, and accompanying density functional theory (DFT) calculations indicated hydride transfer to C4 of the pyridinium [5•]. The authors did not observe racemization due to instability of the reduced pincer complex. Other groups carried out DFT calculations on 139-atom [6] or 200-atom [7] models of the LarA active site. In both cases, the authors favored PCHT to the pyridinium C4 atom and ruled out hydride transfer to nickel. Calculations by Zhang and Chung indicated the energy barriers for the NPN cofactor were greater than those for the Ni-free species or for NAD^+, and these authors suggested the tethered, nickel-bound cofactor destabilizes the intermediate thus promoting racemization [6]. By contrast, Yu and Chen indicated the NPN cofactor exhibits greater hydride addition reactivity than NAD^+ for environments with medium to high polarity [7]. Qiu and Wang computationally predicted the racemization activities of scorpion-like nickel–pincer compounds with an appended imidazole group and found similar free energy barriers for racemization [8].

In contrast to these DFT-based proposals suggesting a PCHT mechanism, Wang and Shaik used quantum mechanical/molecular mechanical calculations and

Figure 1



The lactate racemase cofactor. **(a)** The NPN cofactor forms an enzyme adduct with *L. plantarum* LarA by covalent attachment to K184. The square-planar nickel is tri-coordinated by the pincer ligand using two sulfurs and one carbon atom, with H200 also serving as a metal ligand. The well-positioned residues H108 and H174 likely facilitate catalysis. **(b)** The overall biosynthetic pathway for the NPN cofactor, starting with NaAD carboxylation/hydrolysis by LarB, sulfur insertion by LarE, and nickel insertion by LarC.

proposed a proton-coupled electron transfer (PCET) mechanism [9]. These authors posited a resting enzyme with a Ni(III) redox state that is reduced to Ni(II) during the reaction. Furthermore, they invoked the transient cleavage of the C1–C2 bond of lactate during the reaction, yielding a CO_2 anion radical and acetaldehyde. Rotation of the acetaldehyde and reversal of the preceding steps provided for lactate racemization.

Direct experimental results using LarA argue against a PCET mechanism and support PCHT [10•]. Electron paramagnetic resonance spectroscopy indicated a diamagnetic Ni(II) in LarA, quenching a sample of enzyme plus lactate revealed the presence of pyruvate, and kinetic isotope effect studies found $k_H/k_D = 3.11$ when using L-2-^{2}H-lactic acid, consistent with cleavage/reformation of the C–H bond during catalysis. In addition, optical absorption spectroscopy revealed a perturbation of the cofactor chromophore upon addition of lactic acid, consistent with hydride addition to the pyridinium ring. Notably, sulfite also altered the spectrum. The crystal structure of enzyme in the presence of sulfite (PDB access code 6C1W) revealed a sulfite adduct with the pyridinium C4, mimicking the proposed reactivity of hydride [10•]. Earlier DFT calculations indicating hydride addition to C4 were reproduced, and hydride transfer to the nickel was shown to be feasible if H200 dissociates. Significantly, planar nickel–hydride pincer complexes have been extensively characterized [11].

To summarize, LarA uses the NPN cofactor to facilitate a PCHT mechanism (Figure 2) via a P2TMN-hydride species (top) and may also access a nickel–hydride species (bottom). Indeed, the availability of two interconverting