Biological Lanthanides: The Unexpected Elements of Life

Yiwei Liu Literature Seminar October 15, 2019

The lanthanide series (Ln) comprises 15 f-block elements from lanthanum to lutetium. Even though lanthanides are considerably abundant in the crust of Earth, they were not recognized as biologically essential elements for many reasons.[1] Their poor solubility in water ($K_{sp} \approx 10^{-21}$) hinders the uptake, transportation and storage in living systems.[1] Carboxylate ligands, such as aspartate and glutamate, may bind and stabilize lanthanide in living system. However, the high coordination number of lanthanide ions would result in a compact and highly negative binding site, which is difficult to achieve by biological ligands.[2]

The discovery of biological lanthanides started in 2007. A strain of methylotrophic bacteria (organisms using C1 carbon substrates, like methane or methanol, as energy source), named *Methylacidiphilum fumariolicum* (*M. fumariolicum*) SolV, was found in volcanic mud pot of Solfatara, Italy.[3] The extreme condition was of special interest to researchers. The soil was hot (55°C), strongly acidic (pH=1-2), and more importantly, rich in lanthanides (2 μM). Lanthanide-containing materials, either from the original volcanic mud or from added lanthanide salt, were essential to cell growth. The growth was negligible in the absence of lanthanide, even with calcium as a substitute (**Figure 1**).[4] More examples of Ln-dependent organisms were reported in recent years. The *Deepwater Horizon* oil spill in 2010 caused vast proliferation of methylotrophs, along with the significant depletion of local lanthanum, cerium and praseodymium.[5] Such strong lanthanide affinity was also demonstrated recently, where neodymium(III) ions in computer hard drives were effectively extracted by cultured *Methylobacterium extorquens* (*M. extorquens*) as lanthanide source.[6]

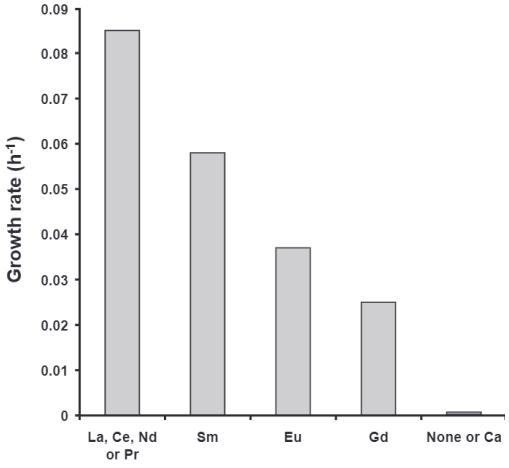

Figure 1. Growth rate of *Methylacidiphilum fumariolicum SolV* with and without Lanthanide.

Inspired by the above findings, investigators have been trying to elucidate molecular basis of lanthanide dependency. In 2011, Hibi et al discovered a protein expressed by *Bradyrhizobium* sp. MAFF211645 with La^{3+} or Ce^{3+} in the culture.[7] The protein has similar molecular mass to methanol dehydrogenase (MDH), a calcium-dependent enzyme. Remarkable progress was made in 2014, when the lanthanide-dependent protein was isolated, characterized, and identified as a new methanol dehydrogenase.[4] The X-ray crystal structure (**Figure 2**) revealed the lanthanide (in this case, cerium) binding site consisting of four carboxylate residues and a pyrroloquinoline quinone (PQQ) cofactor. Despite the discrepancy of the protein sequence between Ca-MDH and Ln-MDH, the metal binding site is highly similar, with one additional aspartate in Ln-MDH to achieve a coordination number of 10.

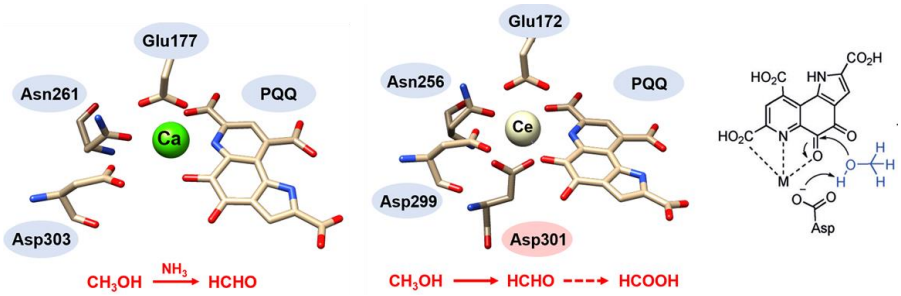

Figure 2. Active site and reaction of Ca-MDH (left) and Ln-MDH (middle), and proposed hemiketal mechanism by nucleophilic addition of methanol (right).

The property of Ln-MDH was different from Ca-MDH.[4] The methanol binding affinity (K_M) was determined to be 0.8 μM, which was higher than the calcium counterpart (20 μM). The catalytic efficiency (k_{cat}/K_M), according to Michaelis-Menten kinetics, was also considerably greater than Ca-MDH (11600 vs. 800 $s^{-1}mM^{-1}$). The stronger Lewis acidity of lanthanides was proposed to activate PQQ cofactor for nucleophilic addition by methanol (**Figure 2**), which is the first and essential step of the hemiketal mechanism.[1] Such an explanation received both computational and experimental support. Prejanó et al revealed the low-lying LUMO of Ln-MDH in the rest state, which facilitated the nucleophilic addition of methanol to PQQ. The HOMO in Ln-MDH-methanol complex, a proposed reaction intermediate, was stabilized by the lanthanide ion.[9] The computational result corroborated the experimental data from Lumpe et al. The UV-Vis spectrum revealed a greater change of PQQ electronic structure upon lanthanide binding compared to the calcium counterpart.[12]

Other proteins involved in the uptake and regulation of lanthanides where also discovered. In 2018, a lanthanide-binding protein named lanmodulin (LanM, **Figure 3**) was identified after co-purified with Ln-MDH.[10] Like calmodulin (CaM), this lanthanide protein contains multiple carboxylate-rich lanthanide binding loops joint by α-helices. Unlike Ln-MDH, where the active site includes one extra aspartate, the ligands in CaM and LanM were identical. Three aspartate, one glutamate and one threonine formed the binding site in both proteins. The ligands in LanM preferred bidentate form to provide extra coordination required for lanthanide. A picomolar binding affinity was observed for early lanthanides, which was 10^8-fold greater compared to the affinity for Ca^{2+} (**Figure 3**). Origin of such selectivity has not been well explained. Mutating the conserved proline residue into alanine increased the affinity of Ca^{2+} by 10^2-fold, yet the selectivity of lanthanide was still as high as 10^6.

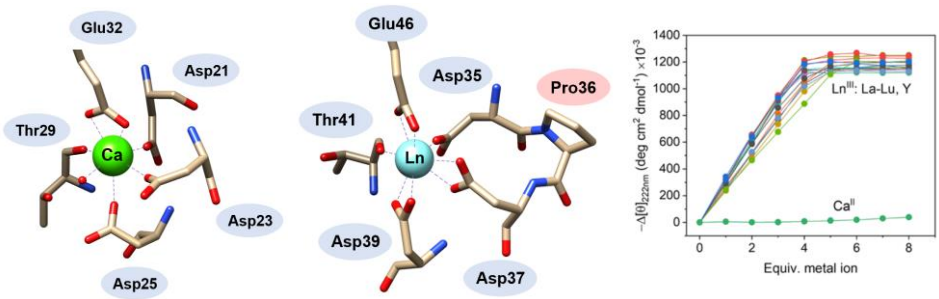

Figure 3. Calcium binding site of CaM (left); Lanthanide binding site of LanM (middle), and binding of lanthanides and calcium to LanM monitored by circular dichroism spectrum (right).

Lanmodulin and Ln-MDH, along with other transporters, were found to be relevant in *M. extorquens* PA1. (**Figure 4**) [11] A TonB-dependent ion transporter, and ABC, an inner-membrane transporter, were adjacent to Ln-MDH and lanmodulin genes. Knockout of these transporters caused decreased cell growth in Ln culture. Surprisingly, knockout of LanM gene showed no significant impact on cell growth, which indicated an indirect involvement of LanM in uptake and regulation. This system was proposed to be responsible to the regulation between Ca- and Ln-MDH pathways. Expression of Ln-MDH and suppression of Ca-MDH were achieved at nanomolar level.[11] However, no molecular evidence was presented to support the genomic study results.

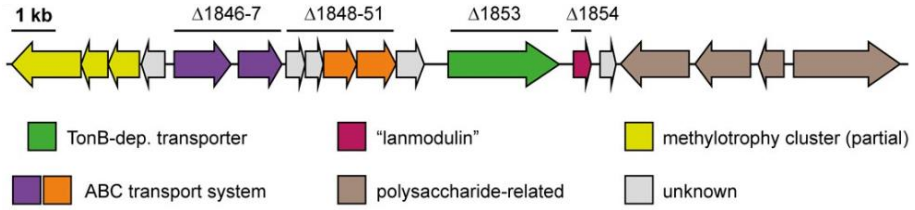

Figure 4. Genes relevant to lanthanide metabolism in *M. extorquens* PA1.

In summary, two types of lanthanide proteins have been reported in living system. Ln-MDH is indispensable for the oxidation of methanol to formaldehyde in certain methylotrophs. The structure and reactivity are different compared to Ca-MDH. Lanmodulin binds lanthanides with picomolar affinity and 10^8 selectivity over calcium. It is potentially involved in the uptake and regulation of cellular lanthanide. In future studies, new chemistry of biological lanthanides, like the redox properties, will potentially inspire new design in artificial systems. New lanthanide proteins, such as enzymes and transporters, may reveal greater biological significance of lanthanides.

References

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