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Role of rare earth elements in methanol oxidation

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For decades rare earth elements (or lanthanides) were considered not to be involved in biological processes, until their discovery in the active site of the XoxF-type methanol dehydrogenase of the methanotrophic bacterium *Methylacidiphilum fumariolicum* SolV. Follow-up studies revealed the presence of lanthanides in other pyrroloquinoline quinone-containing enzymes involved in alcohol metabolism. This review discusses the biochemistry of the lanthanide-dependent enzymes and the ability of these metals of influencing the gene expression and the type of methanol dehydrogenase used by microorganisms. Furthermore, it highlights novel insights on the uptake mechanism of rare earth elements into bacterial cells.

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Introduction

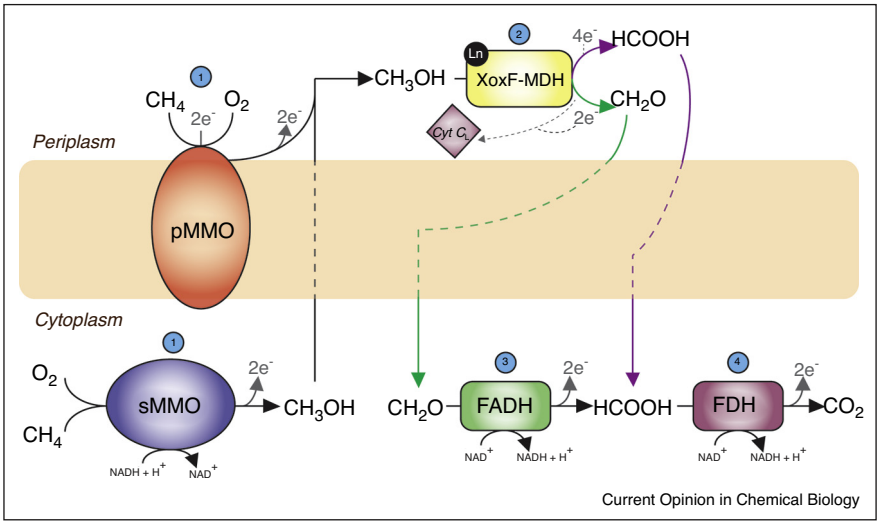
Rare earth elements (REEs) are a group of elements that includes lanthanides (Ln) from lanthanum to lutetium in the periodic table, plus yttrium and scandium. These metals are often divided in 'light' lanthanides (LREEs) which comprise elements with atomic number (Z) from 57 to 63 (La-Eu), and 'heavy' lanthanides (HREEs) indicating elements with Z from 64 to 71 (Gb-Lu). Despite their name, REEs are very abundant in the environment, with a concentration that, on average, represents 0.015% of the Earth crust [1]. Lanthanides are usually present in a trivalent form, except for cerium (Ce^{3+}, Ce^{4+}) and europium (Eu^{2+}, Eu^{3+}) and their ionic radius decreases with increase of atomic number, a feature known as 'lanthanide contraction' [1,2]. Besides their chemical properties, REEs were considered not to be involved in biological processes, but this dogma was challenged by the discovery of a lanthanide-dependent enzyme involved in methane metabolism (Figure 1) of the extremophilic bacterium *Methylacidiphilum fumariolicum* SolV [3••]. This microorganism was completely dependent on REEs for growth and the crystal structure of its methanol dehydrogenase (MDH) revealed a lanthanide ion in the active site. The enzyme was encoded by the gene *xoxF*, a homologue of the calcium-dependent MDH *mxaFI*, so far considered to be the only enzyme capable of methanol oxidation in methanotrophic and methylotrophic bacteria. This study initiated a completely new field of research that explored the role of lanthanides in biological systems and expanded it to enzymes outside methane metabolism. Furthermore, the addition of REEs to cultivation methods permitted the isolation of novel and uncharacterized bacteria from a variety of different habitats [4–8,9••], allowing researchers to study microorganisms so far considered uncultivable. Currently, this area of study is growing with exciting new discoveries, which will be highlighted in this review.

Rare earth elements in methane/methanol oxidation

Biochemical characteristics of lanthanide-dependent enzymes

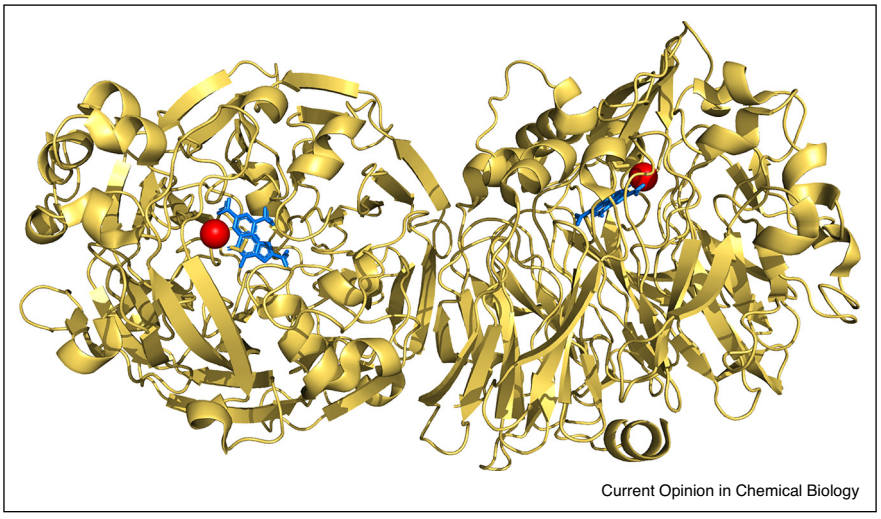
The lanthanide-dependent methanol dehydrogenase (XoxF-MDH) was purified from different microorganisms and it was shown to be a α_2 homodimer with periplasmic localization and a pyrroloquinoline quinone (PQQ) cofactor (Figure 2) [3••,10–12]. When compared to the calcium dependent MDH MxaF, XoxF showed a substitution of an alanine with an aspartate residue at the active site (Asp^{301} — numbering based on *M. fumariolicum* SolV), to coordinate the REE ion. Lanthanides are stronger Lewis acids than calcium and, as shown by Density Function Theory (DFT) calculations, they represent an advantage in the redox cycling of the PQQ [13] and in the formation of the nucleophilic agent [14] compared to Ca^{2+}. Furthermore, XoxF showed optimal activity at pH 7 and no ammonium activation was needed, while MxaFI performed best at pH 9 and had to be activated [15,16]. In addition, both enzymes can oxidize a range of primary alcohols and formaldehyde [17•] but Ln-MDH had higher affinity for methanol and faster conversion rates [3••,13]. Another difference between the two MDHs involves the oxidation of methanol to formaldehyde by Ca-MDH and directly to formate by Ln-MDH in a 4 electrons process [3••,13]. However, it was recently demonstrated that XoxF purified from *Methylobacterium extorquens* AM1 produced formaldehyde as final product [18], challenging the assumption that all Ln-MDHs would generate formate.

Figure 1



Aerobic methane oxidation pathway in Ln-dependent bacteria. The oxidation of methane (CH_4) to methanol (CH_3OH) (1) is catalyzed by the methane monooxygenase (MMO). Bacteria can have two types of MMOs: a soluble, iron-binding protein, called sMMO and/or a particulate methane monooxygenase (pMMO) that is a membrane-bound enzyme and uses copper as cofactor. The methanol produced by MMO is oxidized by XoxF MDH (2), that shuttles electrons to a C_L cytochrome [41,42], encoded by the gene *xoxG* [31] (or the *xoxJG* fusion gene in thermophilic Verrucomicrobia), and releases formaldehyde (CH_2O) or formate (HCOOH) as product, which are finally converted to CO_2 by (3) formaldehyde dehydrogenase (FADH) and (4) formate dehydrogenase (FDH). In *M. fumariolicum* SolV only pMMO is present and methanol is converted directly to formate.

Figure 2



X-ray crystal structure of *M. fumariolicum* SolV XoxF-type methanol dehydrogenase illustrating the dimeric structure with the PPQ co-factor shown in blue and the lanthanide ion in red.

Experimental data showed that purified XoxF worked with different lanthanides, but the activity was higher with the light REEs compared to the heavy ones [3••,12,19,20•,21•] — (Table 1). This data supported *in vivo* observations, where growth rate and/or gene expression was influenced by the type of lanthanide used, with a clear preference for light REEs [12,22–24]. To test the effect of a heavy REE in the active site of the enzyme, the crystal structure of the XoxF enzyme from a culture of *M. fumariolicum* SolV grown with europium (Eu^{3+}) was analyzed [20•]. No significant structural change was observed when comparing it with the mixed-Ln XoxF from the same organism, but the Eu^{3+} culture had a slower growth rate and lower enzymatic activity. These