Lanthanide-dependent Methanotroph Thrives on Radioactive Promethium

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Abstract

Promethium is the only Lanthanide (Ln) that exists exclusively as unstable isotopes, which consequently prevents its occurrence in appreciable amounts in nature. Lns have shown to be biologically relevant for methanotrophic and methylotrophic bacteria. This paper presents growth studies on the methanotrophic bacterium *Methylacidiphilum fumariolicum* SolV with Pm-147 along with enzymatic assays of its Ln-dependent methanol dehydrogenase. The results indicate that Pm is positioned precisely as assumed within the Ln series in a biological context, despite its radioactivity.

Introduction

Lanthanides are a group of elements that generally exhibit similar chemical properties, but with promethium (Pm) standing out due to its radioactivity and rarity. Pm-147 is typically produced via neutron bombardment of Nd-146, which forms Nd-147 through neutron capture. Nd-147 then undergoes β^--decay (neutron is converted to proton under emission of an electron) to form Pm-147.[1] This isotope itself decays via β^--emission to Sm-147 with a half-life of 2.6234 years (Fig. 1). These distinct characteristics set Pm apart from the otherwise stable Ln-series. Its β^--emitting properties make it valuable for applications such as radiotherapy, nuclear batteries for pacemakers and spacecraft, and thin-film measurement technologies.[2–4] Chemically, it occurs in the +3 oxidation state and its coordination chemistry follows the expected lanthanide contraction. Despite recent insights, Pm's coordination chemistry remains underexplored due to its scarcity and instability.[2] While Lns are known to play essential roles in certain biological processes, the role of Pm remains entirely unexplored due to the aforementioned reasons.

The field of Ln-using bacteria is thriving since the discovery that Ln are essential cofactors for methanol dehydrogenase enzymes.[5–13] Ln-dependent bacteria, such as the thermoacidophilic methanotroph *Methylacidiphilum fumariolicum* SolV (SolV),[14] have evolved to utilize specific Lns for key C_1-metabolic processes, particularly in methanol dehydrogenase (MDH, XoxF-MDH) enzymes.[10] MDH catalyzes the conversion of methanol to formaldehyde, a vital step in the bacterial energy metabolism, and requires Lns as Lewis acids in the active site in addition to pyrroloquinoline quinone (PQQ) as redox cofactor (Fig. 1). These bacteria show a preference for larger Lns like lanthanum and cerium. The smaller Lns, such as dysprosium and holmium, are less efficiently taken up and used, reflecting the importance of ionic radius and coordination chemistry in the selective uptake and function of these elements.[15] Remarkably, strain SolV is also able to grow on trivalent actinides such as curium (Cm) and americium (Am). Bacteria such as SolV tolerate these α-emitting actinides, and even preferentially use and thrive on these, in the presence of other Lns.[16]

Although Cm and Am are α-emitters, their radiation thus highly damaging but less penetrating due to the distinct properties of the emitted α-particles the impact of the two actinides on bacterial growth seems minimal. This suggests that radiation damage may be mitigated or even irrelevant to these specialized strains, although this has not yet been studied in depth on a molecular level. Further, how these Ln-using bacteria interact with β^--emitters such as Pm-147, which is characterized by an exceptionally high specific activity of around 34.4 TBq/g, is entirely unknown.[17]

This could alter the structure and function of critical proteins and enzymes[18] and impair bacterial growth. To investigate this, we evaluated the capability of β^--emitting Pm-147 to

support the growth of strain SolV in comparison to non-radioactive Lns (Fig. 1). This approach enabled us to determine whether Pm's radioactivity uniquely influences bacterial metabolism or enzyme activity. Using strain SolV, the now most comprehensively characterized Ln-using methanotroph in terms of Ln and An interactions, we further assessed whether Pm aligns with its expected position within the Ln series both in terms of growth and MDH activity.

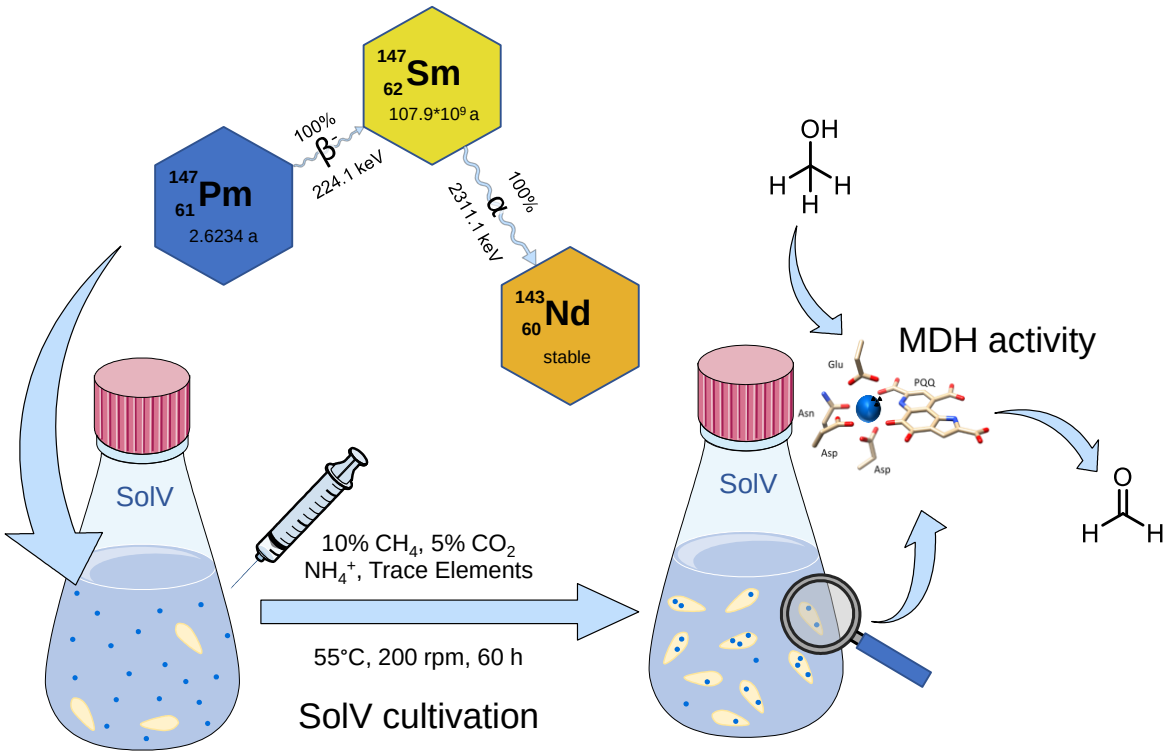

Figure 1. Excerpt from the nuclide chart illustrating the β^--decay of Pm-147 into the isotope Sm-147. Schematic overview of the cultivation process and the incorporation of Pm-147 into the active site of MDH.

Results and Discussion

To systematically compare the chemical behaviour of Pm-147 with other Ln, we conducted *in vitro* measurements of Ln-MDH enzymatic activity using metal-free apo-protein (apo-XoxF-MDH). For this, the *xoxF* gene from strain SolV was expressed in *Escherichia coli* (*E. coli*) as reported previously.[16] After purification, the metal-free apo-protein was incubated with equimolar concentrations of the redox cofactor pyrroloquinoline quinone (PQQ) and the respective Ln. To ensure accurate comparisons and effectively evaluate the place of Pm within the Ln-series, we conducted separate assays with Lns ranging from lanthanum (La) to gadolinium (Gd).

Subsequently, the enzymatic activities of different Ln-MDH towards its native substrate methanol were assessed using a dye-coupled assay.[19] Consistent with previously published data, enzymatic activity increased from La to Nd, followed by a distinct decrease.[10,16] This decrease in activity has been previously observed and attributed, among other things, to less effective cofactor activation and reduced binding affinity of smaller Ln within the active site of the enzyme.[20–22] As depicted in Figure 2a, Pm-147 exhibits enzymatic activity precisely within the gap between Nd and Sm, corroborating its expected position in the Ln-series. Importantly, there was no observable influence from Pm-147 inherent radioactivity on the enzymatic activity.

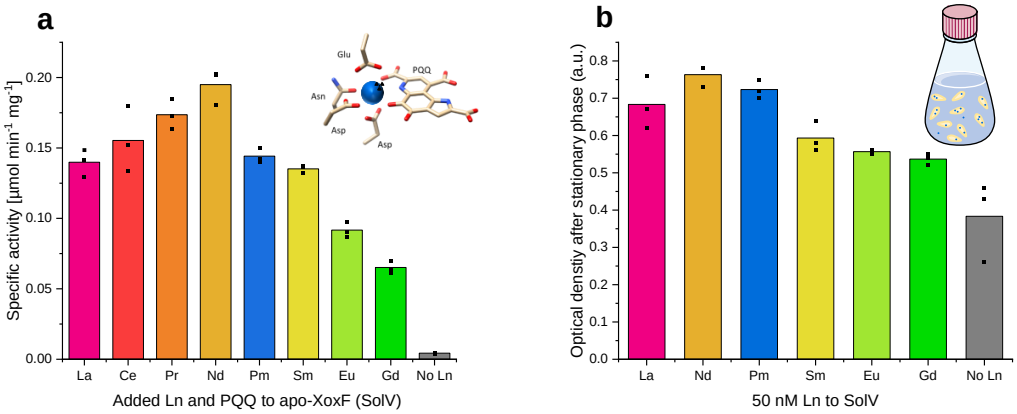

Figure 2 a. Enzymatic activity of reconstituted Ln-MDH. Each bar shows the average of three replicates, each marked with a black dot. **b.** Optical densities at 600 nm after stationary phase of strain SolV, grown with approximately 50 nM of indicated Lns over 60 h. Each bar shows the average of three replicates, each marked with a black dot. In cases where multiple measurements yielded the same value, resulting in overlapping dots, only a single dot is displayed.

Following the enzymatic *in vitro* studies, strain SolV emerged as an ideal candidate for subsequent *in vivo* investigations with Pm-147. This bacterial strain is known for its ability to grow in presence of the trivalent radioactive actinides Am and Cm.[16] Given that Pm-147 is a completely different type of emitter, we were particularly interested whether SolV could tolerate even higher levels of radioactivity and whether other radiation types would impact its growth.

For cultivation experiments with various Ln-sources, the extremophilic strain SolV requires specific conditions: a pH of 2-3, a cultivation temperature of 55 °C, and an atmosphere supplemented with methane (CH_4) and carbon dioxide (CO_2). Given that sealed glass bottles can leach Lns in non-negligible amounts under these conditions, we employed polyethylene (PE) injection bottles typically used for medical applications to mitigate this issue.

Figure 2b presents the final optical density (OD) values, measured at 600 nm, after 60 hours of cultivation of SolV with 50 nM of different Lns including Pm-147. The results clearly demonstrate that Pm-147 fits well within the Ln-series aligning with existing literature about this element.[7] This study suggests that, despite the high radioactivity of Pm-147, SolV exhibits comparable growth patterns to those observed with other Lns. Within the course of 60 hours the radioactivity of Pm-147 does not have an immediate negative impact on growth.

To verify that Pm-147 was assimilated and utilized as a Ln-source, liquid scintillation counting (LSC) measurements were conducted with the supernatant following the growth studies. The initial concentration of Pm-147 in the medium was determined to be 53.5 ± 0,1 nM. Post-cultivation measurements showed a significant reduction of 98 %, resulting in a concentration of 0.9 ± 0.2 nM of Pm-147 in solution. This marked decrease, concomitant with increased optical density/bacterial growth indicates that SolV effectively took up Pm-147 from the medium. The substantial reduction in external Pm-147 concentration suggests that the radioactivity is predominantly linked with the bacterial cells rather than remaining in the external environment.

Outlook

Growth studies with the acidophilic methanotroph SolV and assays with its methanol dehydrogenase in the presence of Pm, places this element exactly as one would expect within the lanthanide series. Remarkably, despite its high radioactivity, the β^--emissions of this isotope did not indicate impairment of bacterial growth and allowed strain SolV to use this Ln in its metabolism. This is the first time that it has been shown that Pm-147 is of biological relevance. Given strain SolV's established ability to accumulate Ln, successful cultivation with Pm-147 could extend this capability, facilitating advanced bioremediation strategies by leveraging the bacterium's resilience in metal-rich environments to detoxify contaminated sites.[15]

Conflicts of interest

There are no conflicts to declare.

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References

1. Broderick, K. *et al.* Reactor production of promethium-147. *Appl. Radiat. Isot.* **144**, 54–63 (2019).

2. Driscoll, D. M. *et al.* Observation of a promethium complex in solution. *Nature* **629**, 819–823 (2024).

3. Hu, F. *et al.* Pm-149 DOTA bombesin analogs for potential radiotherapy. *Nucl. Med. Biol.* **29**, 423–430 (2002).

4. White, F. *et al.* Probing the Subtle Differences between Promethium and Curium. Preprint at https://doi.org/10.21203/rs.3.rs-4788524/v1 (2024).

5. Mattocks, J. A. & Cotruvo, J. A. Biological, biomolecular, and bio-inspired strategies for detection, extraction, and separations of lanthanides and actinides. *Chem. Soc. Rev.* **49**, 8315–8334 (2020).

6. Hu, P. *et al.* Genome-Resolved Metagenomic Analysis Reveals Roles for Candidate Phyla and Other Microbial Community Members in Biogeochemical Transformations in Oil Reservoirs. *mBio* **7**, e01669-15 (2016).

7. Skovran, E. & Martinez-Gomez, N. C. Just add lanthanides. *Science* **348**, 862–863 (2015).

8. Fitriyanto, N. A. *et al.* Molecular structure and gene analysis of Ce3+-induced methanol dehydrogenase of Bradyrhizobium sp. MAFF211645. *J. Biosci. Bioeng.* **111**, 613–617 (2011).

9. Peplow, M. Unlocking the Lanthanome. *ACS Cent. Sci.* **7**, 1776–1779 (2021).

10. Pol, A. *et al.* Rare earth metals are essential for methanotrophic life in volcanic mudpots. *Environ. Microbiol.* **16**, 255–264 (2014).

11. Wegner, C.-E. *et al.* Extracellular and Intracellular Lanthanide Accumulation in the Methylotrophic *Beijerinckiaceae* Bacterium RH AL1. *Appl. Environ. Microbiol.* **87**, e03144-20 (2021).

12. Chistoserdova, L. Lanthanides: New life metals? *World J. Microbiol. Biotechnol.* **32**, 138 (2016).

13. Wehrmann, M., Billard, P., Martin-Meriadec, A., Zegeye, A. & Klebensberger, J. Functional Role of Lanthanides in Enzymatic Activity and Transcriptional Regulation of Pyrroloquinoline Quinone-Dependent Alcohol Dehydrogenases in *Pseudomonas putida* KT2440. *mBio* **8**, e00570-17 (2017).

14. Pol, A. *et al.* Methanotrophy below pH 1 by a new Verrucomicrobia species. *Nature* **450**, 874–878 (2007).

15. Singer, H. *et al.* Learning from nature: recovery of rare earth elements by the extremophilic bacterium *Methylacidiphilum fumariolicum*. *Chem. Commun.* **59**, 9066–9069 (2023).

16. Singer, H. *et al.* Minor Actinides Can Replace Essential Lanthanides in Bacterial Life**. *Angew. Chem., Int. Ed.* **62**, e202303669 (2023).

17. Valentin, J. Relative biological effectiveness (RBE), quality factor (*Q*), and radiation weighting factor (w_R): ICRP Publication 92: Approved by the Commission in January 2003. *Ann. ICRP* **33**, 1–121 (2003).

18. Hellweg, C. E. *et al.* The space experiment CERASP: Definition of a space-suited radiation source and growth conditions for human cells. *Acta Astronaut.* **63**, 906–914 (2008).

19. Phi, M. T. *et al.* Assessing Lanthanide-Dependent Methanol Dehydrogenase Activity: The Assay Matters. *ChemBioChem* **25**, e202300811 (2024).

20. Featherston, E. R. *et al.* Biochemical and Structural Characterization of XoxG and XoxJ and Their Roles in Lanthanide-Dependent Methanol Dehydrogenase Activity. *ChemBioChem* **20**, 2360–2372 (2019).

21. Lumpe, H., Pol, A., Op Den Camp, H. J. M. & Daumann, L. J. Impact of the lanthanide contraction on the activity of a lanthanide-dependent methanol dehydrogenase – a kinetic and DFT study. *Dalton Trans.* **47**, 10463–10472 (2018).

22. Jahn, B. *et al.* Similar but Not the Same: First Kinetic and Structural Analyses of a Methanol Dehydrogenase Containing a Europium Ion in the Active Site. *ChemBioChem* **19**, 1147–1153 (2018).