Hydrogen Metabolism and the Evolution of Biological Respiration

Two separate families of enzymes that oxidize hydrogen and also produce it arose through convergent evolution

Eric S. Boyd, Gerrit J. Schut, Michael W.W. Adams, and John W. Peters

Electron transport phosphorylation (ETP), one of three known biochemical mechanisms for conserving energy, couples oxidation-reduction reactions to the translocation of ions across cell membranes (Table 1). Although we tend to associate ETP with high potential acceptors such as oxygen, it also comes into play using low potential acceptors. In such cases, energy can accumulate through sequential translocation of ions across cellular membranes through low-energy-yielding reactions.

Like ETP, substrate-level phosphorylation (SLP) occurs widely in both oxic and anoxic settings, where it plays an important role in metabolic pathways such as glycolysis and the tricarboxylic acid cycle. SLP, which can combine with electron bifurcation (EB) in anaerobes such as hydrogen-oxidizing methanogenic archaea and acetogenic bacteria, is crucial for conserving energy in anoxic environments where electron acceptors, that is, oxidants, are limited. Presumably, early anaerobic organisms linked the oxidation of hydrogen with the reduction of carbon dioxide, much as we observe among modern methanogens and acetogens. Understanding how their hydrogenase enzymes evolved to harness energy thus may provide insights into some of the earliest forms of life on Earth.

Origins of Hydrogen Metabolism

Water interacting with the peridotitic minerals olivine and pyroxene that contain high levels of ferrous iron can form hydrogen, a process known as serpentinization (Fig. 1). This process was likely widespread on the early Earth and may have sustained early forms of hydrogen-dependent life. The ability to metabolize hydrogen is distributed across the three domains of life and is particularly prevalent in Archaea and Bacteria, where it plays a central role in aerobic and anaerobic physiologies and autotrophic and heterotrophic metabolisms. This wide distribution of hydrogen metabolism among diverse organisms is consistent with its ancient origin.

Two families of hydrogenase enzymes, one containing iron ([FeFe]-hydrogenase) and the other nickel and iron ([NiFe]-hydrogenase) metalloclusters, function in the metabolism of hydrogen. Members of both these enzyme families can oxidize hydrogen as well as produce it. Despite their structural similarities, however, these two types of hydrogenases are not related but appear to be the result of convergent evolution.

SUMMARY

➤ The ability of Archaea and Bacteria to metabolize hydrogen plays a central role in aerobic and anaerobic physiologies, and in autotrophic and heterotrophic metabolisms.
➤ Two unrelated families of hydrogenase enzyme, one containing iron ([FeFe]) and the other nickel and iron ([NiFe]) metalloclusters, arose through convergent evolution to catalyze the reversible oxidation of hydrogen.
➤ Ancient duplications of genes encoding hydrogen-oxidizing [NiFe]-hydrogenase likely occurred in methanogenic Archaea, suggesting this enzyme class first evolved to oxidize hydrogen.
➤ Much like modern methanogenic archaea and acetogenic bacteria, early anaerobic organisms linked hydrogen oxidation with carbon dioxide reduction.
➤ In strict anaerobes, electron bifurcating mechanisms play a central role in balancing the reduced and oxidized forms of cofactors such as ferredoxin (Fd), NADH, and flavins.
➤ Membrane-bound hydrogenases form multisubunit complexes with ion-translocating proteins, coupling the oxidation or reduction of Fd to hydrogen metabolism and the translocation of ions across cellular membranes. These complexes are the progenitors of modern-day respiratory complexes.
[FeFe]-hydrogenases are found in a limited number of strict anaerobic bacteria and a few unicellular eukaryotes but not in archaea. In contrast, [NiFe]-hydrogenase are widely distributed in both the archaeal and bacterial domains but not in eukaryotes. This pattern suggests that [FeFe]-hydrogenase evolved after the divergence of Archaea and Bacteria from the Last Universal Common Ancestor, while [NiFe]-hydrogenase likely arose prior to the divergence of Archaea and Bacteria, and may have played a role in primordial metabolism.

**TABLE 1.**

Modes of energy conservation in modern biology.

<table>
<thead>
<tr>
<th>Mode of Energy Conservation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron Transport Phosphorylation (ETP)</td>
<td>Coupling of oxidation-reduction reactions to the translocation of ions across cellular or organelle membranes. The retranslocation of ions to the cytoplasm is used to drive the synthesis of ATP. This process is common in Bacteria and Archaea and in the mitochondria of Eukarya and is more efficient than fermentative SLP.</td>
</tr>
<tr>
<td>Substrate Level Phosphorylation (SLP)</td>
<td>The transfer of a phosphoryl group from a donor compound to ADP to form ATP. This process is common in glycolytic and the tricarboxylic acid cycle and functions in all metabolisms.</td>
</tr>
<tr>
<td>Electron Bifurcation (EB)</td>
<td>The coupling of endergonic and exergonic reactions such that thermodynamically unfavorable reactions can be driven by thermodynamically favorable reactions. This process involves flavins and is common in anaerobic Bacteria and Archaea.</td>
</tr>
</tbody>
</table>
The [NiFe]-hydrogenases are prominent among hydrogenotrophic methanogens, which typically contain three phylogenetically and physiologically distinct hydrogen-oxidizing [NiFe]-hydrogenase enzymes (Fig. 1B and Fig. 2A). Among the [NiFe]-hydrogenases encoded by hydrogenotrophic methanogens are two representatives of the group 3 subclass of this enzyme, both of which are involved in reducing flavin cofactors. Group 3a enzymes [8-hydroxy-5-deazaflavin (F420) reducing [NiFe]-hydrogenases] couple the oxidation of hydrogen with reduction of F420, which serves as an electron carrier during methanogenesis (Fig. 3).

Group 3c [NiFe]-hydrogenase enzymes (Mvh) function in a complex with a flavin-containing heterodisulfide reductase, coupling hydrogen oxidation to exergonic reduction of the disulfide bond (S-S) linking coenzyme M (CoM) and coenzyme B (CoB) (Fig. 3). The energy released during this reaction drives the thermodynamically unfavorable reduction of ferredoxin (Fd), in a process termed electron bifurcation. Methanogens also contain group 4 membrane-bound [NiFe]-hydrogenase enzymes (Eha/Ehb) that couple oxidation of hydrogen with reduction of Fd, which is coupled with sodium ion translocation into the cell (Fig. 2B and Fig. 3). Reduced Fd then drives the initial reduction of carbon dioxide during methanogenesis.

The [NiFe]-hydrogenases are prominent among hydrogenotrophic methanogens, which typically contain three phylogenetically and physiologically distinct hydrogen-oxidizing [NiFe]-hydrogenase enzymes (Fig. 1B and Fig. 2A). Among the [NiFe]-hydrogenases encoded by hydrogenotrophic methanogens are two representatives of the group 3 subclass of this enzyme, both of which are involved in reducing flavin cofactors. Group 3a enzymes [8-hydroxy-5-deazaflavin (F420) reducing [NiFe]-hydrogenases] couple the oxidation of hydrogen with reduction of F420, which serves as an electron carrier during methanogenesis (Fig. 3).

Group 3c [NiFe]-hydrogenase enzymes (Mvh) function in a complex with a flavin-containing heterodisulfide reductase, coupling hydrogen oxidation to exergonic reduction of the disulfide bond (S-S) linking coenzyme M (CoM) and coenzyme B (CoB) (Fig. 3). The energy released during this reaction drives the thermodynamically unfavorable reduction of ferredoxin (Fd), in a process termed electron bifurcation. Methanogens also contain group 4 membrane-bound [NiFe]-hydrogenase enzymes (Eha/Ehb) that couple oxidation of hydrogen with reduction of Fd, which is coupled with sodium ion translocation into the cell (Fig. 2B and Fig. 3). Reduced Fd then drives the initial reduction of carbon dioxide during methanogenesis.
Likely Origins of the Hydrogenases

The occurrence of multiple phylogenetically related and hydrogen-oxidizing group 3a/3c and 4 [NiFe]-hydrogenase enzymes in methanogens suggests that they arose through a series of gene duplications. Because methanogen [NiFe]-hydrogenase enzymes in group 3 (Fig. 1B) and group 4 (Fig. 1B and Fig. 2A) branch lower (basal) in these lineages, we speculate that the triple branch point in the unrooted phylogenetic reconstruction (asterisk, Fig. 1) is the ancestral enzyme complex and may have been associated with a methanogen.

This scenario requires two gene duplications to generate these three phylogenetically related enzymes (Fig. 3). Because both group 3 enzymes are coupled to a bound flavin as a redox partner, the group 3 enzymes possibly emerged through duplication of the gene encoding an ancestral enzyme that also partnered with a flavin cofactor (Fig. 3).

Irrespective of coupling, the observation that the basal branching methanogen enzymes in subgroups 3 and 4 are involved in hydrogen oxidation suggests that the ancestral [NiFe]-hydrogenase enzyme also was involved in hydrogen oxidation, a hypothesis that is bolstered by the group 1 and most of the identified group 2 enzymes also being involved in hydrogen oxidation (Fig. 1). The group 1 enzymes, which indirectly
couple with oxidants such as oxygen, nitrate, and sulfate, likely evolved in response to selective pressure to harvest energy associated with these oxidants after they became more available about 2.4 billion years ago.

Group 2a enzymes are involved in harvesting hydrogen generated as a byproduct of nitrogen fixation, which is not considered a property of the Last Universal Common Ancestor and thus is not ancestral. Because methanogens harbor early branching hydrogenase subfamilies in two of these three enzyme subgroups, we further suggest that this ancestral [NiFe]-hydrogenase enzyme functioned as a hydrogen oxidizing enzyme that coupled with a variety of other redox partners such as flavin and Fd (Fig. 3). Such an enzyme may have functioned in the acetyl-CoA pathway in methanogens much like modern flavin-based EB [NiFe]-hydrogenase enzyme complexes that also involve Fd.

Hydrogen-Based Electron Bifurcation in Early Life

Generating cofactors with reduction potentials suitable for driving vital biosynthetic reactions is a paramount challenge in environments where energetic gradients are minimal. Methanogens and other anaerobic organisms, however, have mechanisms to meet this challenge, enabling them to persist in such environments.

For example, although reducing protons (Eo' - 420 mV, pH 7.0) via NADH (Eo' - 320 mV) is thermodynamically unfavorable, many anaerobes generate more hydrogen than expected solely from Fd-dependent reactions. Thus, some hydrogen is being derived from NADH, even though it is not thermodynamically favorable.

The coupling of thermodynamically favorable reactions with thermodynamically unfavorable reactions is made possible through a process called electron bifurcation (EB). Several [FeFe]- and [NiFe]-hydrogenase enzymes are part of flavin-containing EB complexes, most notably in microorganisms conducting fermentative, acetogenic, and methanogenic metabolisms. For instance, the [NiFe]-hydrogenase (group 3c)/heterodisulfide reductase complex of hydrogenotrophic methanogenesis—producing reduced Fd from hydrogen—is a critical energy-conserving step in methanogenesis. Thus, bifurcating mechanisms now play a central role in balancing the reduced and oxidized forms of cofactors such as Fd, NADH, and flavins in strict anaerobes, and they may well have done so on early Earth.

**[NiFe]-Hydrogenases as Progenitors to Complex Respiratory Systems**

What were the oxidants that supported life on early Earth before oxygen became widely available? Oxidant-limited anaerobes that lack well-defined respiratory systems may provide a clue. These organisms can recycle reduced Fd by forming hydrogen. The anaerobic oxidation of glucose is coupled with reduction of Fd and NAD to produce ATP. However, Fd has a redox potential similar to or lower than that of the H+/H2 couple (Eo' - 420 mV, pH 7.0), while NADH (Eo' - 320 mV) cannot be recycled through the reduction of protons to H2.

Several members of the euryarchaeal order *Thermococcales* circumvented this problem by using Fd as the sole redox carrier during sugar metabolism. The electrons that Fd gains are then disposed of by producing hydrogen by means of a group 4 ion-translocating, membrane-bound [NiFe]-hydrogenase (Mbh; Fig. 2B and Fig. 3).

Our phylogenetic analyses indicate that these group 4 [NiFe]-hydrogenases likely functioned early in evolution as oxidizing enzymes coupling hydrogen indirectly with carbon dioxide reduction in methanogenesis. Later, this type of enzyme diversified in function, eventually coupling numerous types of metabolism involving Fd and hydrogen (Fig. 2A and 2B).

Membrane-bound hydrogenases (Mbh), including Eha/Ehb, form multisubunit complexes with ion-translocating proteins, termed Mrp. Together, these complexes couple the oxidation or reduction of Fd to the oxidation or production of hydrogen. The outcome of this reaction determines the direction of ion translocation across the cellular membrane (Fig. 2B and Fig. 3). Ions enter the cell when Fd reduction is coupled to hydrogen oxidation (Eha/Ehb), whereas ions leave the cell when Fd is oxidized and protons are reduced.

Some organisms harbor additional Mrp-Mbh complexes that contain carbon monoxide dehydrogenase (CODH), formate dehydrogenase (FDH), or NADPH modules that couple the oxidation of these substrates to hydrogen production while translocating ions outside the cell for use in ATP synthesis (Fig. 2). Organisms that generate ATP through the coupling of Fd, carbon...
monoxide, or formate oxidation while generating hydrogen (group 4 enzymes) operate near the thermodynamic limits of life (-8 to -20 kJ/mol). They are considered to be among the most primitive respiratory complexes in extant life.

A more detailed phylogenetic analysis of group 4 [NiFe]-enzymes (Fig. 2A) indicates a complex history between the archaeal and bacterial lineages. Following the early branching of Eha/Ehb, two main lineages emerged. One of these lineages includes Mrp-Mbh from Crenarchaeaota in the order Desulfurococcales and other enzymes that do not activate hydrogen, including Fpo, Mrp-Mbx, and Nuo. The other lineage includes archaeal and bacterial enzymes that produce hydrogen, including an energy conserving hydrogenase (Ech) and an archaeal Mrp-Mbh.

The diversification of these lineages was likely driven in part by recruitment of accessory FDH, CODH, or NADPH modules that enable coupling with formate, carbon monoxide, and NADPH, respectively, for both Ech and Mrp-Mbh (Fig. 2A). The incorporation of FDH and CODH modules into these enzyme complexes would allow cells to oxidize these substrates, generating hydrogen and a Na\(^+\)-based PMF capable of driving ATP synthesis.

The large and small subunits of group 4 [NiFe]-hydrogenases show substantial homology with the subunits NuoD and NuoB of the NADH dehydrogenase (complex I), respectively, in Archaea, Bacteria, and Eukarya. Phylogenetic analysis indicates that Nuo proteins derive from Mbh or Ech (Fig. 1B and Fig. 2A). Additional subunits in the multimeric [NiFe]-hydrogenases (group 4) show sequence similarity to other subunits of respiratory complex I (NuoK, L, C, H, and I).

In addition to NuoK, the small and large subunits of group 4 [NiFe]-hydrogenases show substantial homology with the subunits NuoD and NuoB of the NADH dehydrogenase (complex I), respectively, in Archaea, Bacteria, and Eukarya. Phylogenetic analysis indicates that Nuo proteins derive from Mbh or Ech (Fig. 1B and Fig. 2A). Additional subunits in the multimeric [NiFe]-hydrogenases (group 4) show sequence similarity to other subunits of respiratory complex I (NuoK, L, C, H, and I).

Boyd: from Baseball to the Rockies, and from Rocks to Hydrogenases and Energy

Eric Boyd’s 7th-grade language arts teacher told him that he could earn an “A” if he would stop worrying about his grade and also complete the coursework. “Ironically, it was not until I was older before I realized what she was really trying to say to me—focus on working hard and doing a good job,” he says. “I have taken this approach to much of my life.”

Boyd, 35, is assistant professor of microbiology and immunology at Montana State University in Bozeman, where he studies microbial physiology and ecology as it relates to energy metabolism. “In particular, our work is focused on the biochemical processes that are likely to have supported the earliest forms of life—that is, life that is supported not by light-driven photosynthetic energy but rather chemical energy supplied by water-rock interactions,” he says. “Much of our work focuses on molecular hydrogen.”

Boyd was born in Des Moines, Iowa, and lived there as well as in Wisconsin and South Dakota while growing up. “Wherever we lived, however, my parents made certain that we were involved in sports and that we had access to the outdoors,” he says. His father recently retired after 40 years working at John Deere. His mother, a homemaker who cared for Boyd, an older brother, and a younger sister until Boyd was in third grade, worked in the insurance industry and, later, as a librarian. His parents live in Des Moines.

The family took many vacations in the West, including in the Rockies “where I developed a fascination with rocks, minerals, and the geological processes that are involved in their formation,” Boyd says. “Some of my earliest memories are trips to the Badlands, Rocky Mountain National Park, as well as Yellowstone. Who would have known that several decades later I would be studying hot-springs and the role of minerals in sustaining life in these systems?”

In his youth, Boyd spent much of his summers playing pickup baseball. “I couldn’t get enough of it—and as a result ended up wearing out my throwing arm and needing surgery to repair it.” The orthopedist predicted Boyd would never again throw a baseball or football. Ultimately, however, Boyd played pitcher, third baseman, and quarterback on his high school baseball and football teams. He earned a B.S. in biology in 2002 from Iowa State University and a Ph.D. in microbiology in 2007 from Montana State University (MSU). He was a NASA Astrobiology Institute postdoctoral fellow and is currently a NASA Early Career fellow at MSU.

He and his partner, Marisa, a self-employed gardener, live in Livingston, Mont., with their two dogs and a cat. “We used to have a flock of backyard chickens, but after nine years … they joined a friend’s flock,” he says. In addition to hiking and camping, Boyd also enjoys “the history of places. It’s a lot of fun reading about mining booms and busts, and then getting in the car and driving to the old mines or towns, if they still exist, and trying to imagine what it was like living during those times.”

Marlene Cimons

Marlene Cimons lives and writes in Bethesda, Md.
subunits of [NiFe]-hydrogenase share homology with FpoBD, which catalyzes the reduction of methanophenazine with F$_{430}$H$_2$ in methanogens, and with Mrp-MbxJL, which may be involved in respiring elemental sulfur. While we continue to make progress understanding the evolution of microbial respiratory systems, much remains to be learned.

Eric S. Boyd is Assistant Professor in the Department of Microbiology and Immunology, Montana State University, Bozeman, and the Wisconsin Astrobiology Research Consortium, University of Wisconsin, Madison; Gerrit Schut is a Research Scientist and Michael W. W. Adams is Professor in the Department of Biochemistry & Molecular Biology, University of Georgia, Athens; and John W. Peters is Professor in the Department of Chemistry and Biochemistry, Montana State University, Bozeman, and is director of the Biological Electron Transfer and Catalysis Energy Frontiers Research Center.

ACKNOWLEDGMENTS

This work was supported by NASA (NNX13AI11G and NNA13AA94A), the Division of Chemical Sciences, Geosciences and Biosciences, Office of Basic Energy Sciences of the Department of Energy (DE-FG05-95ER20175), and the Biological Electron Transfer and Catalysis Energy Frontiers Research Center funded by the Department of Energy, Office of Science.

SUGGESTED READING:


