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DISPATCH

Carbon fixation: “Let things flow naturally forward in whatever way they like”

Wolfgang Nitschke

Laboratoire de Bioénergétique et Ingénierie des Protéines (BIP) UMR 7281, IMM FR3479, CNRS/Aix-Marseille University, Marseille, France

Email: nitschke@imm.cnrs.fr

Summary:
Mixed-acid fermentation generates H_2 and CO_2 from formate. As shown in a recent study, the formate oxidation reaction can be driven backwards when sufficiently high partial pressures of the gases are applied, suggesting potentially interesting biotechnological applications.

Escherichia coli grown in the presence of glucose — but in the absence of electron acceptors — ferments the sugar to a mix of organic acids; specifically, lactic, succinic and formic acids. The formic acid produced in this ‘mixed-acid fermentation’ is further reacted with protons to yield two gases, CO_2 and H_2. In this redox process, the electrons derived from the oxidation of formate to CO_2 are used for the reduction of two protons to yield molecular hydrogen. Oxidising formate to CO_2 (which escapes into the gas phase) by using ubiquitously available protons as electron acceptors is a clever way to prevent the main fermentation reaction from stalling due to product inhibition. All this was elucidated almost a century ago [1,2], and the process has since become a standard entry in textbooks on microbial physiology. As a sorted-out mechanism, it was further studied only by a fringe community of microbiologists in more recent years.

That changed dramatically when molecular details of the enzyme carrying out the final step (oxidation of formate to CO_2) started to emerge [3,4]. The so-called ‘formate hydrogenlyase’ (FHL) turned out to be a textbook example for the construction-kit strategy that life almost exclusively applies during evolutionary innovation and diversification [5]. FHL revealed itself to be composed of protein subunits belonging to the vast superfamily of

Molybdo-di-pterin enzymes [6], to that of [NiFe]-hydrogenases [7,8] and to membrane-integral ion transporters (Figure 1B). Even more stunningly, FHL emerged from these comparisons [9] as an evolutionary precursor of Complex I (*alias* NADH:quinone oxidoreductase type I), the mastodon enzyme playing a pivotal role in mitochondrial oxidative phosphorylation and in numerous respiratory chains of prokaryotes [10]. Seven essential subunits in Complex I have clear-cut sequence homologs in FHL, although the catalytic ones among them have undergone severe shifts in substrate specificities. Further studies into the mutual phylogenetic relationships of these homologous subunits will almost certainly provide thrilling insights into the evolutionary history of the Complex I superfamily.

In contrast to its low appeal to fundamental research in microbiology, from the point of view of applied microbiology, the process of mixed-acid fermentation was intriguing all along due to its ability to produce hydrogen gas, a potential fuel. Still, the massive research efforts into biological generation of H_2 — driven by the growing awareness that we are heading full speed towards the fossil-fuel cliff edge (which will either take the form of energy shortage or catastrophic climate change or, most likely, both) — have yielded a plethora of biological systems [11] more efficient than the mixed-acid fermentation pathway and have thus diminished interest in the latter process. However, a recent article by Magali Roger and colleagues [12] now offers a new potential biotechnological application of this microbiological mechanism by proposing to use the 'reverse' rather than the 'forward' reaction of FHL. But in addition, their study also suggests some interesting implications for our understanding of non-biotech topics, such as evolution and enzymatics.

This is where the deeper meaning of the quote in the title comes in. The ancient Chinese philosopher Laozi's thermodynamic wisdom perfectly captured that there is no pre-destined forward or backward direction, but rather that (like all chemical reactions) enzymatic reactions flow naturally so that the Gibbs free energy ΔG will be negative; that is, the reaction is exergonic, and this is what 'forward' really means. The 'standard' redox midpoint potential, determined at 1 M for solutes and at 1 atm for gases, of the formate–CO_2 couple is -430 mV, whereas that of the H_2–$2H^+$ couple is -414 mV. At standard conditions, these two redox couples are therefore almost at equilibrium (Figure 1A). However, the actual environmental concentrations usually encountered by *E. coli* are a far shot from standard conditions. Present-day CO_2 levels are close to 400 ppm, while formate concentrations produced by fermentatively grown *E. coli* can reach the millimolar range. As a result, the operating potential of the couple will lie at around -500 mV, and even under pre-industrial CO_2 levels this operating potential was only a few millivolts more positive. On the other hand, H_2 concentrations are close to nil,

yielding effective potentials for the H_2–$2H^+$ couple that are likely more positive than -300 mV. Thermodynamics then tells us that 'forward' means production of CO_2 and H_2 with quite some excess of free energy to do work in energy conversion (Figure 1A).

Now, our planet has certainly been quite different in the distant geological past. CO_2 levels in the early Archaean (more than 3 billion years ago) are thought to have reached 10 bars and above. It was only biological carbon fixation and chemical weathering, together with subsequent geological burying and subduction of carbon compounds, that gradually, over eons, drew CO_2 levels down to present day values. CO_2 concentrations at or above 10 bars, by contrast, shift the effective electrochemical potential of the formate–CO_2 couple more positive than that of the H_2–$2H^+$ couple (Figure 1A), yielding CO_2 fixation rather than formate oxidation as the new (or more appropriately, *old*) 'forward'. High concentrations of H_2, as are likely in some of the locales proposed as habitats for early life, exacerbate this bias towards reducing CO_2 to formate. It has consequently been proposed that an FHL-related enzyme may have provided reduced carbon compounds derived from formate to life in the early Archaean and that the primordial function of FHL was indeed carbon fixation [13].

Magali Roger and colleagues [12] have devised an experimental time-machine transporting *E. coli* back to Earth's early days. Using an ingenious and technically challenging experimental setup, these authors succeeded to grow *E. coli* under a 10-bar atmospheric mixture of CO_2 and H_2, while strictly controlling all fundamental growth parameters — a technical tour-de-force. Key to the feasibility of this experiment was the prior construction of genetically engineered *E. coli* strains devoid of two further hydrogenases encoded by the genome, leaving FHL as the sole enzyme able to redox convert H_2 and protons. With this strain, they were able to attain formate yields exceeding 500 mM in their bioreactor volume. Further deletion of two additional formate-converting, and therefore potentially formate-metabolising, enzymes (respiratory formate dehydrogenase and pyruvate formatelyase) further increased this yield slightly. Finally, absence of formate release in a strain that also had FHL knocked out provided ironclad proof that the observed phenomenon was indeed due to thermodynamic constraints forcing FHL to go into reverse gear. Seeing reversal of FHL *in vivo* under appropriate conditions beautifully vindicates both thermodynamics and the proposed evolutionary scenario.

However, the ramifications of the results reported in this article go beyond fundamental research. As discussed by the authors, both formate production and CO_2 capture are interesting from a biotechnological point of view. Formate is a starting material for a plethora of chemical syntheses and is discussed as an essential feedstock for the microbiological production of value-added molecules [14,15]. The perspective of using a biological production mechanism for

formate relying on the direct reduction of CO_2 by H_2 appears extremely attractive. Roger *et al.* [12] furthermore point out that this mechanism, although yielding a valuable chemical and potential fuel, at the same time captures CO_2 and thus can be seen as a way to combat global warming by pulling down carbon directly from the atmosphere. However, as the authors fittingly also discuss, further process-oriented optimisation and up-scaling as well as long-term stabilisation of the process (for example, for use in flow reactors) are required. Although the interest of producing the valuable molecule formate is undeniable, the carbon-capture aspect certainly would need a continuous and large-scale process to become viable. In this context, it is instructive to compare the yield of carbon capture of 500 mmols into formate per liter of reactor fluid to the C-content of the *E. coli* cell paste used to produce this formate, which amounts to a little over 2 mols (assuming a C-content in biomass of about 50%). Growing the same amount of an autotrophic organism (likely even within a shorter time span) will therefore capture more carbon than obtained *via* the reversed FHL-reaction. Maybe we shouldn't quit watching our carbon footprints just yet…

References

1. Stephenson, M., and Stickland, L.H. (1932). Hydrogenlyases. Bacterial enzymes liberating molecular hydrogen. Biochem. J. *26,* 712–724.

2. Woods, D.D. (1936). Hydrogenlyases. The synthesis of formic acid by bacteria. Biochem. J. *30,* 515–527.

3. Bagramyan, K., and Trchounian, A. (2003). Structural and functional features of formate hydrogen lyase, an enzyme of mixed-acid fermentation from *Escherichia coli*. Biochemistry (Moscow) *68,* 1159–1170.

4. Andrews, S.C., Berks, B.C., McClay, J., Ambler, A., Quail, M.A., Golby, P., and Guest, J.R. (1997). A 12-cistron *Escherichia coli operon* (hyf) encoding a putative proton-translocating formate hydrogenlyase system. Microbiol. *143,* 3633–2647.

5. Baymann, F., Lebrun, E., Brugna, M., Schoepp-Cothenet, B., Giudici-Orticoni, M.-T., and Nitschke, W. (2003). The redox protein construction kit; Pre-LUCA evolution of energy conserving enzymes. Philos. Trans. R. Soc. Lond. B *358,* 267–274.

6. Grimaldi, S., Schoepp-Cothenet, B., Ceccaldi, P., Guigliarelli, B., and Magalon, A. (2013). The prokaryotic Mo/W-bisPGD enzymes family: a catalytic workhorse in bioenergetics. Biochim. Biophys. Acta *1827,* 1048–1085.

7. Vignais, P.M., and Billoud, B. (2007). Occurrence, classification and biological function of hydrogenases: an overview. Chem. Rev. *107,* 4206–4272.

8. Brugna-Guiral, M., Tron, P., Nitschke, W., Stetter, K.-O., Burlat, B., Guigliarelli, B., Bruschi, M., and Giudici-Orticoni, M.-T. (2003). [NiFe] hydrogenases from the hyperthermophilic bacterium *Aquifex aeolicus*; properties, function and phylogenetics. Extremophiles *7,* 145–157.

9. Sargent, F. (2016). The model [NiFe] hydrogenases of *Escherichia coli*. Adv. Microb. Physiol. *68,* 433–507.

10. Ohnishi, T. (1993). NADH-quinone oxidoreductase, the most complex complex. J. Bioenerg. Biomembr. *25,* 325–329.

11. van Niel, E.W. (2016). Biological processes for hydrogen production. Adv. Biochem. Eng. Biotechnol. *156,* 155–193.

12. Roger, M., Gabrielli, W., Brown, F., and Sargent, F. (2018). Efficient hydrogen-dependent carbon-dioxide reduction by *Escherichia coli*. Curr. Biol. *28,* xxxx-xxxx.

13. Nitschke, W., and Russell, M.J. (2009). Hydrothermal focusing of chemical and chemiosmotic energy, supported by delivery of catalytic Fe, Ni, Mo/W, Co, S and Se, forced life to emerge. J. Mol. Evol. *69,* 481–496.

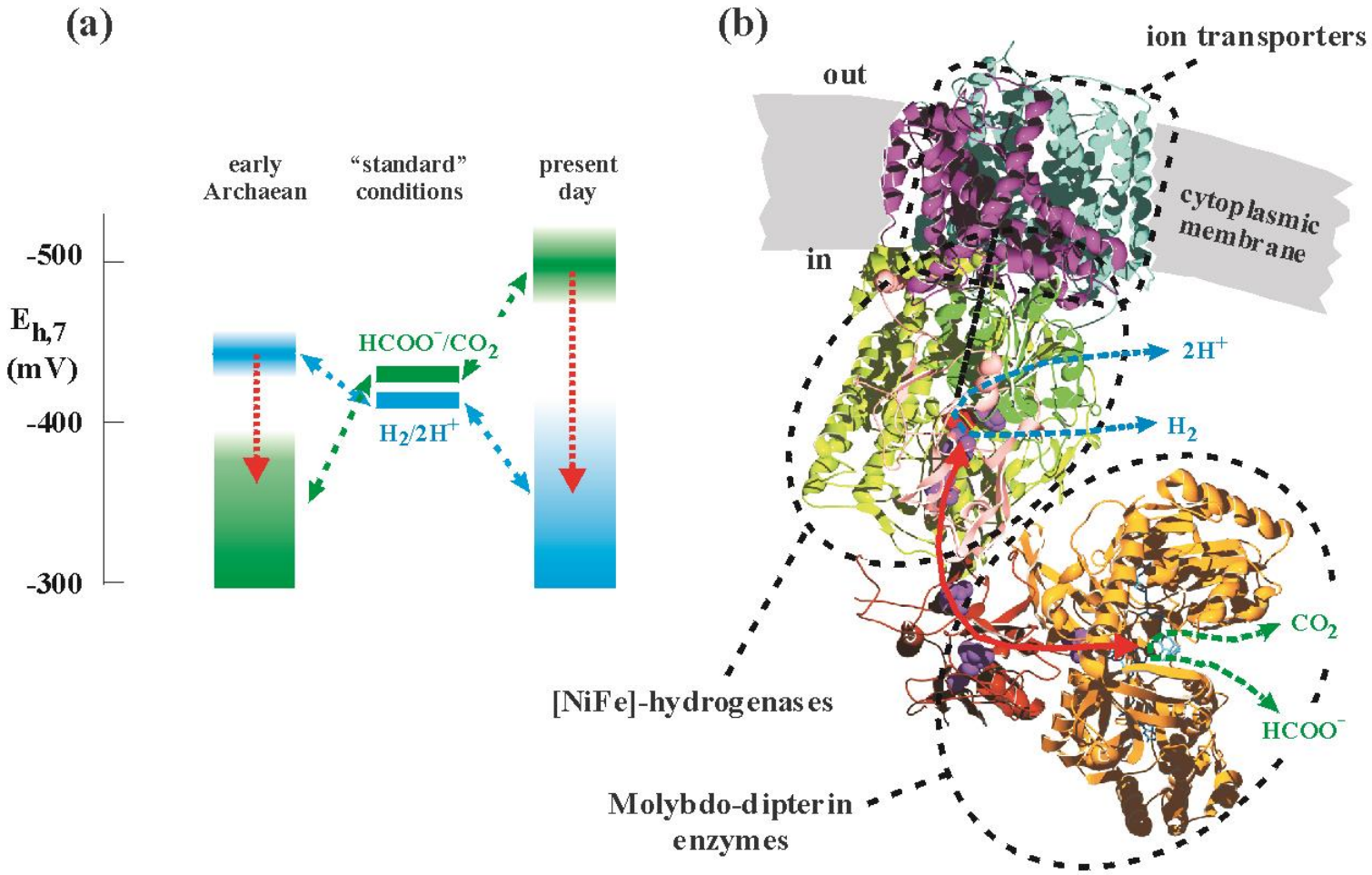
14. Windman, T., Zolotova, N., Schwandner, F., and Shock, E.L. (2007). Formate as energy source for microbial metabolism in chemosynthetic zones of hydrothermal ecosystems. Astrobiology *7,* 873–890.

15. Bar-Evan, A. (2016). Formate assimilation: The metabolic architecture of natural and synthetic pathways. Biochemistry *55*, 3851–3863.

Figure 1. The FHL-enzyme and the thermodynamics of its catalytic reactions.
(A) Best-guess representation of the FHL enzyme based on the homology of its constituent subunits to certain subunits of Complex I (Protein Database entry 4HEA, www.rcsb.org/pdb/home.do). FHL's mosaic make-up featuring units from various enzyme superfamilies is indicated. (B) Illustration of the effect of substrate concentrations on the effective redox potentials (with respect to the Standard Hydrogen Electrode) of the involved couples and on the corresponding driving forces for electron flow between couples.

In Brief

Mixed-acid fermentation generates H2 and CO2 from formate. As shown in a recent study, the formate oxidation reaction can be driven backwards when sufficiently high partial pressures of the gases are applied, suggesting potentially interesting biotechnological applications.


(a)
early Archaean
"standard" conditions
present day
-500
$E_{h,7}$ (mV)
-400
-300
$HCOO^-/CO_2$
$H_2/2H^+$
(b)
ion transporters
out
in
cytoplasmic membrane
$2H^+$
H_2
CO_2
$HCOO^-$
[NiFe]-hydrogenases
Molybdo-dipterin enzymes