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Biochimica et Biophysica Acta

BBA
Bioenergetics

Does acetogenesis really require especially low reduction potential?

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A B S T R A C T

Acetogenesis is one of the oldest metabolic processes on Earth, and still has a major global significance. In this process, acetate is produced via the reduction and condensation of two carbon dioxide molecules. It has long been assumed that acetogenesis requires ferredoxin with an exceptionally low reduction potential of ≈ -500 mV in order to drive CO_2 reduction to CO and the reductive carboxylation of acetyl-CoA to pyruvate. However, no other metabolic pathway requires electron donors with such low reduction potential. Is acetogenesis a special case, necessitating unique cellular conditions? In this paper, I suggest that it is not. Rather, by keeping CO as a bound metabolite, the CO-dehydrogenase-acetyl-CoA-synthase complex can couple the unfavorable CO_2 reduction to CO with the favorable acetyl-CoA synthesis, thus enabling the former process to proceed using ferredoxin of moderate reduction potential of -400 mV. I further show that pyruvate synthesis can also take place using the same ferredoxins. In fact, the synthesis of pyruvate from CO_2, methylated-protein-carrier and -400 mV ferredoxins is an energy-neutral process. These findings suggest that acetogenesis can take place at normal cellular redox state. Mechanistic coupling of reactions as suggested here can flatten energetic landscapes and diminish thermodynamic barriers and can be another role for enzymatic complexes common in nature and a useful tool for metabolic engineering.

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1. Introduction

Acetogenic microbes, reducing CO_2 into acetate, have been isolated from diverse anaerobic habitats, including sediments, soils, acidic coal mine ponds, fecal material, psychrotrophic as well as thermophilic environments [1]. More than a billion tons of acetate is produced each year by these organisms, converting about a quarter of the total carbon within anaerobic soils into acetate [2].

The physiology and biochemistry of acetogens has been studied for 80 years, since Fischer et al. noticed H_2- and CO_2-dependent formation of acetate in sewage sludge [3]. However, only in the late 1980s and early 1990s were Wood and Ljungdahl able to elucidate the structure of the reductive acetyl-CoA pathway (Fig. 1) [1,2,4,5], considered today to be the most ancient carbon fixation route [6]. Still, many biochemical aspects of the pathway remain unknown and new discoveries are made every year (e.g. [7–11]). One of the most fascinating phenomena discovered recently is electron bifurcation, i.e. the concomitant coupling of favorable electron transfer from an electron donor (e.g. molecular

hydrogen, E' in the range of -400 to -350 mV) to an electron acceptor of higher reduction potential (e.g. NAD, $E' \geq -300$ mV) and an unfavorable electron transfer from the same donor to an acceptor of lower reduction potential (e.g. ferredoxin, $E' \leq -400$ mV) [12,13].
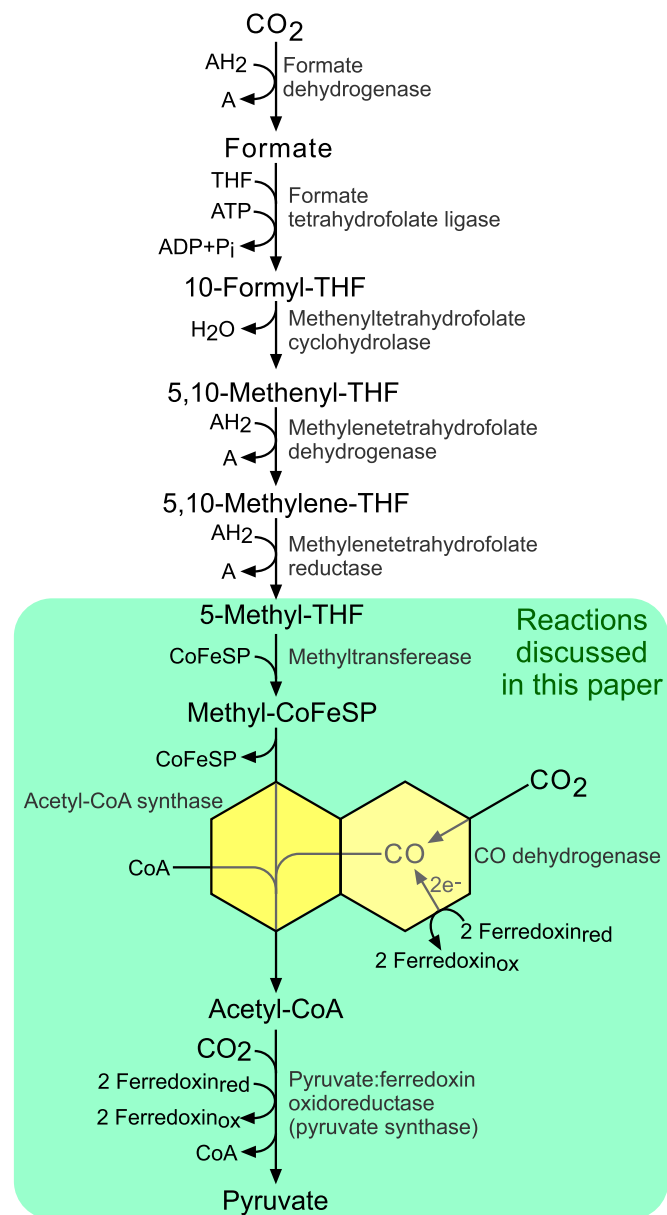
It has been suggested, and often repeated, that the ferredoxin that participates in acetogenesis should have an extremely low reduction potential ($E' \leq -500$ mV) in order to support the required reduction of CO_2 to CO by the CO-dehydrogenase-acetyl-CoA-synthase (CODH/ACS) complex ($E^{\circ\prime}$ (CO_2/CO) < -500 mV) (e.g. [5,9,14–18]). These studies proposed that the same low reduction potential ferredoxin is mandatory also for the reductive carboxylation of acetyl-CoA to pyruvate, acetyl-CoA + CO_2 + $2e^- >$ pyruvate + CoA, which is characterized by $E^{\circ\prime} \approx -500$ mV. However, as there are no other pathways strictly requiring electron donors with such a low reduction potential, it is difficult to accept this reasoning.

Usually, electron donors with moderate reduction potential are used in reactions which require especially low reduction potential, where ATP hydrolysis, coupled directly or indirectly to the reaction, provides the thermodynamic driving force [19]. Examples of unfavorable redox reactions that are activated via direct coupling to ATP-hydrolysis include the nitrogenese reaction [20], the ATP-dependent reduction of benzoyl-CoA to 1,5-cyclohexadiene-1-carboxyl-CoA [21], the activation of 2-hydroxyacyl-CoA dehydratases [21] and ATP-dependent carboxylic acid reductase [22–24]. Activation via indirect coupling to ATP hydrolysis is nicely exemplified in the ubiquitous reduction of a carboxy to a carbonyl via ATP-dependent activation of the carboxyl with a phosphoryl or a CoA moiety [19].

Fig. 1. The reductive acetyl-CoA pathway. The CO-dehydrogenase-acetyl-CoA-synthase complex is shown as two hexagons. The green area presents the reactions analyzed in the paper. 'AH₂' corresponds to a reduced electron donor and 'A' represents an oxidized electron donor. 'THF' corresponds to tetrahydrofolate and 'CoFeSP' represents corrinoid iron–sulfur protein.

Hence, assuming that acetogenesis can operate only with extremely low reduction potential electron donors suggests that there is something very 'special' about the energetics of this metabolic process. As acetogenesis is rooted at the origin of cellular life (e.g. [6]) and is still so widespread, it seems unlikely that it requires such specialized conditions.

In this paper, I demonstrate that acetogenesis and pyruvate synthesis can take place under a moderate reduction potential of −400 mV. I argue that one of the reasons that CO dehydrogenase and acetyl-CoA synthase work in a complex is to diminish internal energetic barriers [19], i.e. enabling CO_2 reduction without especially low reduction potential electron carriers. Also, I show that pyruvate synthesis can take place at reduction potential of −400 mV, if the concentrations of the reactants are modulated within a reasonable physiological range. In fact, the overall synthesis of pyruvate from methylated-protein-carrier,

two CO_2 molecules and four −400 mV ferredoxins is a completely reversible, energy-neutral process.

Importantly, numerous organisms are known to employ ferredoxins with reduction potentials of −500 mV or even lower (e.g. [25–29]). The analysis presented here does not suggest that these ferredoxins are not participating in acetogenesis. Rather, it is claimed that the usage of such ferredoxins is not a strict requirement of acetogenesis and that 'normal' ferredoxins, having moderate reduction potential, can suffice for pathway operation.

2. Methods

When possible, the thermodynamics of biochemical reactions were calculated using eQuilibrator, the biochemical thermodynamics calculator (http://equilibrator.weizmann.ac.il/) [30], according to the Gibbs energy of formations and mathematical framework given in [31]. pH and ionic strength were assumed to be 7 and 0.2 M, respectively. If available, values corresponding to 298.13 K were taken. CO_2 and CO were assumed to be in aqueous phase rather than gaseous phase. Hence, standard conditions for CO_2 and CO were taken as $[CO_2^{aq}] = [CO^{aq}] = 1$ M and not 1 atm. CO_2^{aq} refers to dissolved CO_2 and not to any of the hydrated forms of the compound (H_2CO_3, HCO_3^- and CO_3^{2-}), which were assumed to be in equilibrium with CO_2^{aq}. Protons should not be included in reaction equations since, according to the theorem developed in [31], they are not conversed under constant pH. However, for clarity, they are given in parentheses in each of the reactions. During all analyses the reduction potential of ferredoxin was assumed to be −400 mV.

To give a realistic picture of the energetic constraints imposed on reactions, I used $\Delta_r G'^m$ [19], the change in Gibbs energy under reactant concentrations of 1 mM [32–35]. A reaction with a positive $\Delta_r G'^m$ can still carry flux in the forward direction if the concentrations of the substrates are kept sufficiently above the concentrations of the products such that the actual $\Delta_r G'$ is negative. However, the concentrations of intracellular metabolites are limited: they are rarely above 10 mM or below 1 μM [32,35]. Thus, if $\Delta_r G'^m$ is sufficiently large, the reactants must acquire non-physiological concentrations to make the reaction favorable [36].

3. Results

3.1. Enzymatic complexes enable the diminishing of internal thermodynamic barriers

All reactions are formally reversible. So, regardless of the value of $\Delta_r G'^m$ associated with a reaction (change in Gibbs energy under reactant concentrations of 1 mM and constant pH), it is always possible to find reactant concentrations such that the actual $\Delta_r G'$ will be negative (Methods) [19]. However, if $\Delta_r G'^m$ is especially high, the concentrations of the substrates should be especially high or the products must have especially low concentrations to enable forward flux. Both alternatives may not be practical from a biological point of view. Especially high metabolite concentrations are deleterious since they create a large pool of material which is not assimilated into cellular macromolecules and further imposes an increased osmotic pressure. On the other hand, especially low metabolite concentrations will slow metabolic flux: when the concentration of a metabolite becomes lower than the affinity of the enzyme accepting it, it begins to limit the catalytic rate. Hence, metabolite concentrations within cells usually lie within a specific physiological range of 1 μM to 10 mM [32,35]. If metabolite concentrations are constrained to this range, reactions or pathways having high enough $\Delta_r G'^m$ can be considered infeasible [19].

This thermodynamic and biochemical analysis is based on the assumption that all metabolic intermediates are free and soluble within the cellular media. However, if one of the reactants is kept bound, the energetics of the reactions producing and consuming it can change

dramatically. Reactions operating within the same enzymatic complex, such that their intermediates are bound within the complex, cannot be treated as if they consume or produce free metabolites. Such reactions require a different thermodynamic analysis. Specifically, complexes can serve to diminish internal energetic barriers [19].

For example, consider two reactions; reaction R1 converts metabolite X to Y and has $\Delta_r G'^m$ of $+40$ kJ/mol, while reaction R2 converts metabolite Y to Z and has $\Delta_r G'^m$ of -50 kJ/mol. Although the overall conversion of X to Z is exergonic, catalyzing these reactions through independent enzymes will probably be infeasible, since in order for reaction R1 to proceed the concentration of X will have to be too high or the concentration of Y will have to be too low. However, if the two reactions are operated by a single enzymatic complex such that Y remains bound to the complex, Y_{bound} could be stabilized considerably as compared to Y_{free}. In such a case, the conversion of X to Y_{bound} can have, for example, $\Delta_r G'^m \approx 0$ kJ/mol, while the conversion of Y_{bound} to Z having $\Delta_r G'^m \approx -10$ kJ/mol. The overall conversion of X to Z still dissipates 10 kJ/mol but now there is no energetic barrier to constrain the process.

3.2. Coupling between endergonic CO₂ reduction to CO and exergonic acetyl-CoA synthesis

Does the CODH/ACS complex diminish internal thermodynamic barriers and thereby enable the reduction of CO_2 to CO using ferredoxin with moderate reduction potential of -400 mV? The reduction of CO_2 to CO is characterized by $E°' \approx -560$ mV (note that the compounds are assumed to be in aqueous phase and not in gaseous phase in which $E°' \approx -520$ mV). Hence, if -400 mV ferredoxins serve as the electron donors for this reduction, the overall reaction becomes highly endergonic, $\Delta_r G'^m > +30$ kJ/mol [30,37], too high to be feasible within the physiological range of metabolite concentrations [19]. However, the following reaction, which takes place within the same complex, acetyl-CoA synthase, is very exergonic ($\Delta_r G'^m < -30$ kJ/mol) [19]. If these two reactions are coupled via the CODH/ACS complex, the energetic barrier associated with CO_2 reduction might be diminished, enabling the net reaction to proceed unconstrained by thermodynamic barriers.

To analyze this option systematically, I propose three criteria that, if met, strongly indicate the coupling of the two reactions: (i) CO is a bound intermediate, produced by one reaction and consumed by the other; (ii) The internal energetic landscape of the reactions taking place within the CODH/ACS complex is flattened as compared to that of the free metabolites. (iii) The overall reaction of the CODH/ACS complex is characterized by a small $\Delta_r G'^m$.
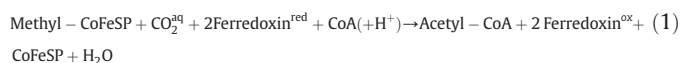
There is strong evidence that the first criterion indeed holds; specifically, CO appears to be a bound intermediate, which is shuttled ≈ 70 Å within an internal tunnel from the CO-dehydrogenase cluster to that of acetyl-CoA synthase [38–40]. On the other hand, the second criterion is difficult to assess. While we do have some information regarding the internal energetic landscape of isolated parts of the CODH/ACS complex (e.g. [7]), we simply do not have enough data about the entire complex. Importantly, since it was shown that different protein conformations considerably modulate the energetics of isolated parts of the complex [7], the energetic landscapes deciphered so far likely represent only a portion of the actual landscape of the full complex.

However, another thing that was found from studying isolated parts of the CODH/ACS complex, was that the reduction potential of the CO-dehydrogenase center is usually considerably higher than that of the pair CO_2/CO ($E' \gg -400$ mV) [41]. This suggests that reducing CO_2 to a complex-bound CO might not represent as high an energetic barrier as for the reduction of CO_2 to a free CO. In addition, the affinity of the CODH/ACS complex to exogenous CO was found to be high (~10 μM), indicating that bound CO is stabilized as compared to free CO [42]. Indeed, while acetogenic organisms generate significant quantities of CO (as an intermediate), they release only low levels of CO during growth [43,44].
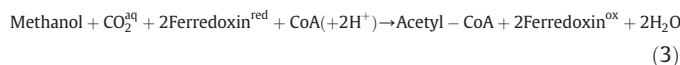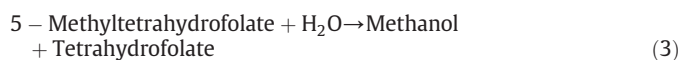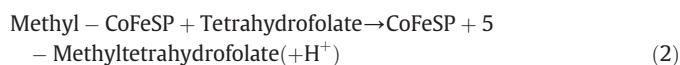
3.3. The energetics of the overall reaction of the CODH/ACS complex

The next important question is whether the overall reaction of the CODH/ACS complex (having only free and soluble reactants) is indeed characterized by a small $\Delta_r G'^m$. In a sense, this is the most important question. Complexes can be evolved to bind intermediates at different energy levels, thus decreasing internal thermodynamic barriers. However, the energetics of the overall reaction is an external constraint. In the analysis below I assume that reduction potential of ferredoxin is -400 mV. Also, by using $\Delta_r G'^m$ it is assumed that the concentration of dissolved CO_2 (CO_2^{aq}) is 1 mM (corresponding to $\approx 4\%$ atmospheric CO_2 at 30 °C–40 °C). This value is considerably higher than in ambient aerobic conditions, but lower than the concentration of CO_2^{aq} which is used to cultivate acetogens (in the range of 20–100% atmospheric CO_2) and is expected to prevail in these organisms' anaerobic habitats [2].

The overall reaction of the CODH/ACS complex is given by Eq. (1):

$$Methyl - CoFeSP + CO_2^{aq} + 2Ferredoxin^{red} + CoA(+H^+) \rightarrow Acetyl - CoA + 2Ferredoxin^{ox} + \quad (1)$$
$$CoFeSP + H_2O$$

where CoFeSP is the corrinoid iron–sulfur protein, which accepts a methyl group from 5-methyltetrahydrofolate [8,45–47]. This reaction can be decomposed into three reactions:

$$Methyl - CoFeSP + Tetrahydrofolate \rightarrow CoFeSP + 5$$
$$- Methyltetrahydrofolate(+H^+) \qquad (2)$$

$$5 - Methyltetrahydrofolate + H_2O \rightarrow Methanol$$
$$+ Tetrahydrofolate \qquad (3)$$

$$Methanol + CO_2^{aq} + 2Ferredoxin^{red} + CoA(+2H^+) \rightarrow Acetyl - CoA + 2Ferredoxin^{ox} + 2H_2O$$
$$(3)$$

$\Delta_r G'^m$ for reaction (2) is $\approx +6.5$ kJ/mol, as measured in ref. [48] at pH 7.6. Correcting to pH 7 results in $\Delta_r G'^m \approx +10$ kJ/mol. $\Delta_r G'^m$ for reaction (4) was calculated directly from the known Gibbs energies of formation of the reactants given in refs. [31,49] and was found to be -8.5 kJ/mol [30]. $\Delta_r G'^m$ for reaction (3) is more difficult to find as this reaction does not take place directly. As described in detail in the Appendix, I estimate this $\Delta_r G'^m$ to be ≈ -8 kJ/mol. Summing over reactions (2), (3) and (4) the $\Delta_r G'^m$ for reaction (1) is ≈ -6.5 kJ/mol.

A $\Delta_r G'^m$ of ≈ -6.5 kJ/mol represents a completely reversible reaction (especially considering that there are many substrates and products whose concentrations can be modulated [36]). The pathway can proceed in the forward direction given 1 mM reactant concentrations, but if the ratios [CoFeSP]/[Methyl-CoFeSP] and [acetyl-CoA]/[CoA] are kept at ≈ 10 the overall reaction changes direction.

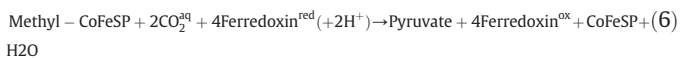### 3.4. The pyruvate synthase reaction

The CODH/ACS complex is fully reversible but what about the subsequent reaction — synthesis of pyruvate from acetyl-CoA — which is expected to impose a further energetic barrier? The reaction catalyzed by pyruvate synthase

$$Acetyl - CoA + 2Ferredoxin^{red} + CO_2^{aq}(+H^+) \rightarrow Pyruvate + 2Ferredoxin^{ox} + CoA$$
$$(5)$$
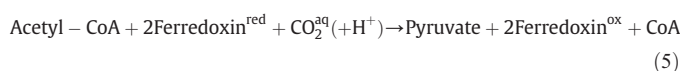
has $\Delta_r G'^m$ of $+7.5$ kJ/mol assuming that the reduction potential of ferredoxin is -400 mV [30]. This again represents a fully reversible reaction that can proceed in the forward direction if the ratios [acetyl-CoA]/[CoA] and [CO_2^{aq}]/[Pyruvate] are kept above 10. (The CODH/ACS complex can still produce acetate at [acetyl-CoA]/[CoA] > 10.) In fact, there are several reactions in central metabolism

that carry significant flux in the forward direction although having $\Delta_r G'^m >> +10$ kJ/mol (e.g. malate dehydrogenase, reducing malate to oxaloacetate using NAD(P)H as an electron donor; in order for this reaction to proceed the ratio [malate]/[oxaloacetate] should be kept very high).

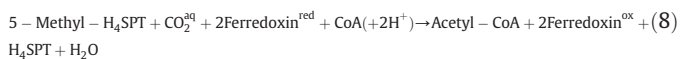When considering the overall reaction of the CODH/ACS complex and pyruvate synthase

$$\text{Methyl} - \text{CoFeSP} + 2\text{CO}_2^{aq} + 4\text{Ferredoxin}^{red} (+2\text{H}^+) \rightarrow \text{Pyruvate} + 4\text{Ferredoxin}^{ox} + \text{CoFeSP} + \quad (6)$$
$$\text{H2O}$$

one gets $\Delta_r G'^m \approx +1$ kJ/mol, indicating that the entire process is completely reversible. Conditions of [CoFeSP]/[Methyl-CoFeSP]$\leq$1, [acetyl-CoA]/[CoA]$\approx$10 and [CO_2^{aq}]/[Pyruvate]$\geq$10 supports acetate and pyruvate formation, while conditions of [CoFeSP]/[Methyl-CoFeSP]$\geq$10, [acetyl-CoA]/[CoA]$\approx$10 and [CO_2^{aq}]/[Pyruvate]$\leq$1 support pyruvate and acetate oxidation.

3.5. Energetics of the CODH/ACS complex in methanogenes

Instead of tetrahydrofolate, methanogenic archaea employ the C1 carrier tetrahydromethanopterin (H_4MPT) or its glutaminated variant tetrahydrosarcinapterin (H_4SPT) [50]. The molecular properties of these carriers are quite different from that of tetrahydrofolate and the energetics of the CODH/ACS reaction in methanogenes might differ from that in acetogenic bacteria [50,51].

In methanogenes reaction (1) can be decomposed into two reactions (H_4SPT as the C1 carrier):

$$\text{Methyl} - \text{CoFeSP} + \text{H}_4\text{SPT} \rightarrow \text{CoFeSP} + 5 - \text{Methyl} - \text{H}_4\text{SPT}(+\text{H}^+) \quad (7)$$

$$5 - \text{Methyl} - \text{H}_4\text{SPT} + \text{CO}_2^{aq} + 2\text{Ferredoxin}^{red} + \text{CoA}(+2\text{H}^+) \rightarrow \text{Acetyl} - \text{CoA} + 2\text{Ferredoxin}^{ox} + \quad (8)$$
$$\text{H}_4\text{SPT} + \text{H}_2\text{O}$$

$\Delta_r G'^m$ for reaction (7) is $\approx +7$ kJ/mol, as measured in ref. [52] (corrected to pH 7). $\Delta_r G'^m$ for reaction (8) is ≈ -14 kJ/mol as calculated in the Appendix. Hence, in methanogenes, $\Delta_r G'^m$ for reaction (1) is ≈ -7 kJ/mol, virtually identical to the value found for acetogenes and completely within the reversible range. Also, the energetics of reaction (8) suggest that it is reversible by itself; for example, keeping [Acetyl-CoA]/[CoA]>20 and [H_4SPT]/[5-Methyl-H_4SPT]>20 will support cleavage of acetyl-CoA and formation of 5-Methyl-SPT. This is essential to enable acetoclastic methanogenesis.

Importantly, it was found that pyruvate is not degraded to acetyl-CoA by methanogenes [53]. Considering the energetics of the pyruvate synthase enzyme there is no clear thermodynamic reason of why this is the case. It is possible that in these organisms the ratio [Acetyl-CoA]/[CoA] is kept high or that [pyruvate] is kept low, thus preventing a significant reverse flux. It is also possible that, at least in some methanogenic organisms, the reduction potential of the ferredoxins is kept very low (≈ -500 mV), preventing pyruvate degradation via the pyruvate synthase enzyme.

4. Discussion

The thermodynamic analysis presented here suggests that acetogenesis and pyruvate synthesis are fully reversible when operated both independently and consecutively even if supported by ferredoxins of moderate reduction potential of -400 mV. The analysis further indicates that the tunneling of CO from the CO-dehydrogenase C cluster to the acetyl-CoA synthase A cluster is more than just an elegant way to prevent this rich carbon and energy source escaping (e.g. [40]). Instead, operating the two clusters within a single complex, while keeping CO as a bound metabolite, plays a vital role in decreasing the energetic barrier for CO_2 reduction and thus allowing the entire process to operate at moderate cellular reduction potential.

Indeed, considering the recently found hydrogen threshold for the growth of an acetogenic bacterium, *Acetobacterium woodii* [9], a ferredoxin with a reduction potential of -400 mV makes more sense than one of -500 mV. *A. woodii* is able to oxidize hydrogen at 2500 ppm, corresponding to a redox potential of -340 mV. There is simply not enough energy to reduce a ferredoxin of -500 mV via electron bifurcation even if E' (NAD) $= -250$ mV, i.e. the energy gain from the favorable electron transfer from hydrogen to NAD is lower than the energy required for the unfavorable electron transfer from hydrogen to a -500 mV ferredoxin ($|(-500) - (-340)| > |(-250) - (-340)|$). However, the reduction of ferredoxin with a reduction potential of -400 mV via electron bifurcation can be possible even if E' (NAD) is in the region of -300 mV, so that E' (NAD) $= -280$ mV ($|(-400) - (-340)| = |(-280) - (-340)|$).

In addition, keeping the reduction potential of ferredoxin at ≈ -400 mV and that of NAD at ≈ -300 mV fits nicely to the proposed electron bifurcation scheme in which the favorable NADH-dependent reduction of methylene-THF to methyl-THF ($E' \approx -200$ mV, [9,50]) is coupled to the unfavorable NADH-dependent reduction of ferredoxin [9,54]. Finally, these reduction potentials can support NADP reduction via electron bifurcation in which ferredoxin and NADH serve as electron donors [16], and the reduction potential of NADP is ≈ -350 mV, as previously suggested [16,18].

The analysis presented here also accounts for the difference between CO dehydrogenases operating in acetogenic prokaryotes and that employed by microbes that use CO only as an electron source. While in the former organisms CO dehydrogenase is almost always coupled directly to acetyl-CoA synthase, the later organisms usually operate CO dehydrogenase as an independent enzyme, as no coupling is required to transfer the electrons from the highly energetic free CO to ferredoxin or another electron carrier [55,56].

The CODH/ACS complex formally couples three reactions that could have taken place separately (Fig. 2): (i) CO_2 reduction to CO ($\Delta_r G'^m > +30$ kJ/mol); (ii) condensation of CO with a methyl moiety bound to CoFeSP, to form an acetate moiety ($\Delta_r G'^m \approx -100$ kJ/mol) and (iii) formation of a thioester between acetate and CoA, producing acetyl-CoA ($\Delta_r G'^m > +50$ kJ/mol). The coupling of CO_2 reduction to the subsequent exergonic reaction was discussed in details in this manuscript, but the coupling of the endergonic thioester formation to the exergonic formation of acetate is of no less importance. This is achieved by creating a high energy acetyl-enzyme intermediate as the product of the condensation reaction (as opposed to a low energy acetate, Fig. 2). This enables an almost energy neutral attack of CoA, releasing acetyl-CoA. Indeed, the K_{eq} for the reaction E + Acetyl-CoA $\rightarrow$ E-Ac + CoA (E being the enzyme and E-Ac being the acetyl-enzyme intermediate) was found to be very close to 1 (0.2, [57]), indicating that the acetyl-enzyme intermediate is kept at high energy, similar to that of acetyl-CoA. In a sense, this is the opposite situation of CO-dehydrogenase, where the CO was kept at lower energy as compared to free CO (Fig. 2).

The CODH/ACS complex exemplifies the extraordinary ability of living organisms to cope with thermodynamic barriers. Similar solutions can be encountered in other pathways — for example, glycine synthesis/cleavage via the glycine cleavage system — and might represent a common biological solution to thermodynamic constraints. It will be interesting to investigate whether other abundant complexes are used as natural tools for flattening internal energetic landscapes. Complexes might also be used in metabolic engineering in order to ameliorate thermodynamic barriers by the coupling of otherwise independent reactions.
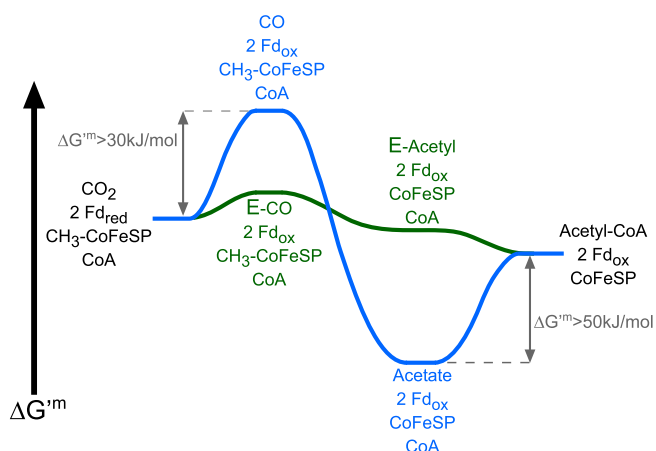
Fig. 2. A schematic representation of the energetics of acetyl-CoA formation. Blue represents reactions taking place by separate enzymes (not within a complex) and green corresponds to the reactions taking place within a single complex such that all intermediates are either bound or channeled. The reactions order is: (1) CO_2 reduction to CO; (2) CO condensation with the C1 moiety carried by CoDeSP and (3) formation of a thioester bond. 'Fd' corresponds to ferredoxin, 'CoFeSP' to corrinoid iron–sulfur protein, 'CH_3-CoFeSP' to methylated corrinoid iron–sulfur protein, 'E-CO' to carbon monoxide attached to the complex and 'E-Acetyl' to acetate attached to the complex.

Appendix

The thermodynamics of the 5-methyltetrahydrofolate cleavage reaction (reaction 3)

I used two methods to calculate the energetics of the 5-Methyltetrahydrofolate cleavage reaction: 5-Methyltetrahydrofolate + H2O → Methanol + Tetrahydrofolate

Method 1

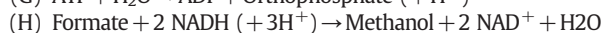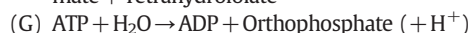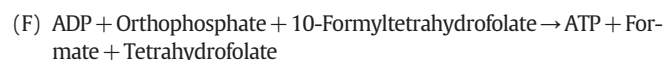The 5-Methyltetrahydrofolate cleavage reaction can be decomposed into the following reactions:

(A) NAD^+ + 5-Methyltetrahydrofolate → 5,10-Methylenetetrahydrofolate + NADH $(+H^+)$

(B) 5,10-Methylenetetrahydrofolate + H2O → Formaldehyde + Tetrahydrofolate

(C) Formaldehyde + NADH $(+H^+)$ → Methanol + NAD^+

$\Delta_r G'^m$ for reaction (A) is $+23$ kJ/mol (reduction potential of methylene-THF/methyl-THF is -200 mV) as given in [9,50]. $\Delta_r G'^m$ for reaction (B) is $+8.5$ kJ/mol as calculated in ref. [58]. $\Delta_r G'^m$ for reaction (C) is -37.5 kJ/mol as calculated directly from the known Gibbs energies of formation of the reactants [31,49], correcting the Gibbs energy of formation of formaldehyde as described in ref. [50]. Summing over reactions (A)–(C) the $\Delta_r G'^m$ for the 5-Methyltetrahydrofolate cleavage reaction is ≈ -6 kJ/mol.

Method 2

The 5-Methyltetrahydrofolate cleavage reaction can be decomposed into the following reactions:

(D) NAD^+ + 5-Methyltetrahydrofolate → 5,10-Methylenetetrahydrofolate + NADH $(+H^+)$

(E) 5,10-Methenyltetrahydrofolate + NAD^+ + H2O → 10-Formyltetrahydrofolate + NADH $(+H^+)$

(F) ADP + Orthophosphate + 10-Formyltetrahydrofolate → ATP + Formate + Tetrahydrofolate

(G) ATP + H2O → ADP + Orthophosphate $(+H^+)$

(H) Formate + 2 NADH $(+3H^+)$ → Methanol + 2 NAD^+ + H2O

$\Delta_r G'^m$ for reaction (D) is $+23$ kJ/mol as given in [9,50]. $\Delta_r G'^m$ for reaction (E) is -1 kJ/mol as calculated in ref. [50]. $\Delta_r G'^m$ for reaction (F) is $+10$ kJ/mol as also calculated in ref. [50]. $\Delta_r G'^m$ for reaction (G) is -55 kJ/mol as calculated directly from the known Gibbs energies of formation of the reactants [31,49]. $\Delta_r G'^m$ for reaction (H) is $+13$ kJ/mol as calculated directly from the known Gibbs energies of formation of the reactants [31,49]. Summing over reaction (D)-(H) the $\Delta_r G'^m$ for the 5-Methyltetrahydrofolate cleavage reaction is ≈ -10 kJ/mol.

Since both calculation methods are based on different reaction sets (except for the first reaction which is shared by both), the fact they result in such similar $\Delta_r G'^m$ values is quite convincing. For further calculations I used the average of the values produced by the two methods, i.e. $\Delta_r G'^m \approx -8$ kJ/mol.

The thermodynamics of reaction (8)

The equilibrium constant of the reaction:

(I) 5-Methyl-H4SPT + CO_2^{aq} + H2 + CoA → Acetyl-CoA + H4SPT + H2O
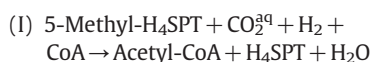
is $2.09 \cdot 10^6$ 1/M/atm, with $[CO_2]$ in molar units and $[H_2]$ in atm, at 37 °C [59]. This value corresponds to $\Delta_r G'^{\circ}$ of -36 kJ/mol. Note that this $\Delta_r G'^{\circ}$ is independent of the pH since protons are not produced or consumed during the reaction. At pH 7 and 37 °C, $E^{\circ'}$ (2 $H^+ + 2e^- \to H_2$) ≈ -430 mV (where standard conditions refer to 1 atmosphere of H_2 and not 1 M of dissolved H_2). Hence, the reduction potential of the half reaction:

(J) 5-Methyl-H4SPT + CO_2^{aq} + CoA + $2e^-$ → Acetyl-CoA + H4SPT + H2O

equals $E^{\circ'}$ (J) $= (-\Delta_r G'^{\circ}/2/F) - E^{\circ'}$ (2 $H^+ + 2e^- \to H_2$) ≈ -240 mV (F being the Faraday constant). Coupling this reaction to the oxidation of a ferredoxin gives reaction (8): 5-Methyl-H4SPT + CO_2^{aq} + 2 Ferredoxin^{red} + CoA → Acetyl-CoA + 2 Ferredoxin^{ox} + H4SPT + H2O.

The energetics of reaction (8) can therefore be calculated by the difference in reduction potentials, assuming -400 mV ferredoxins: $\Delta_r G'^{\circ} = 2*F*(0.24-0.4) \approx -31$ kJ/mol, or $\Delta_r G'^m \approx -14$ kJ/mol.

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