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Does acetogenesis really require especially low reduction potential?

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ABSTRACT

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].

Abbreviations: CODH/ACS, CO-dehydrogenase-acetyl-CoA-synthase; CoFeSP, Corrinoid iron–sulfur protein; E', Reduction potential at a constant pH (7); $E^{\circ\prime}$, Reduction potential under standard conditions (1 M concentration of reactants) and at a constant pH (7); $\Delta_r G'$, Gibbs energy of a reaction at a constant pH (7); $\Delta_r G^{\circ\prime}$, Gibbs energy of a reaction under standard conditions (1 M concentration of reactants) and at a constant pH (7); $\Delta_r G'^{m}$, Gibbs energy of a reaction under reactant concentrations of 1 mM and at a constant pH (7)

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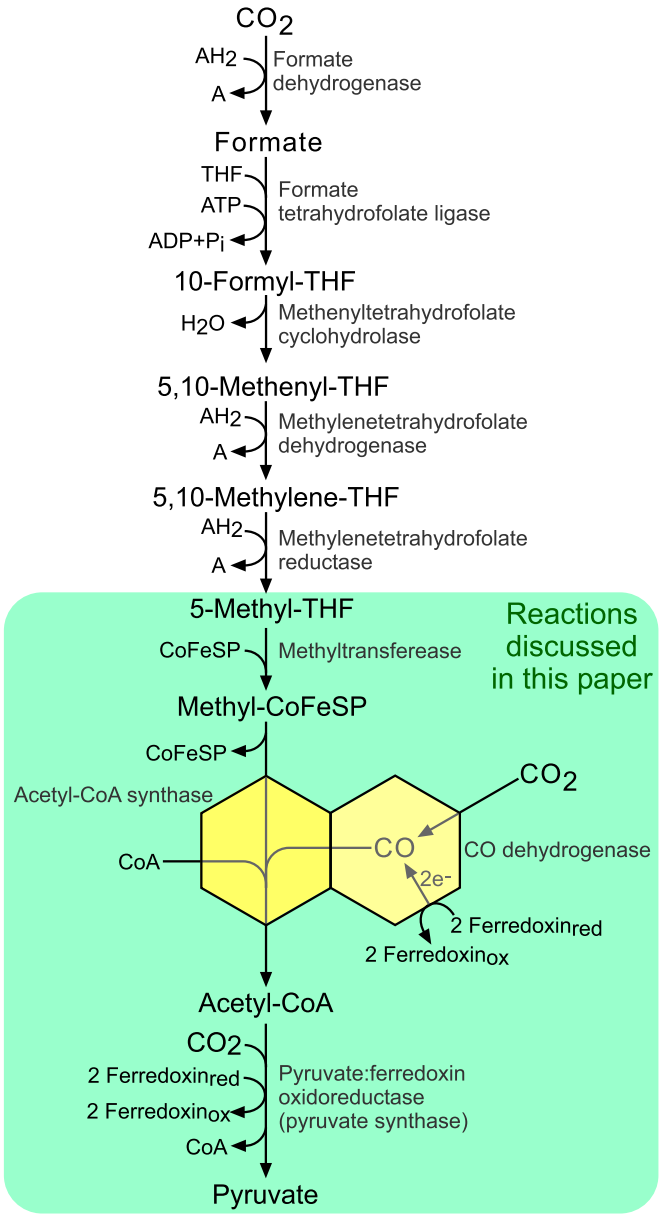

Fig. 1. The reductive acetyl-CoA pathway. The CO-dehydrogenase-acetyl-CoA-synthase complex is shown as two hexagons. The green area presents the reactions analyzed in the paper. 'AH_2' corresponds to a reduced electron donor and 'A' represents an oxidized electron donor. 'THF' corresponds to tetrahydrofolate 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 $\Delta_rG'^m$ [19], the change in Gibbs energy under reactant concentrations of 1 mM [32–35]. A reaction with a positive $\Delta_rG'^m$ 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 Δ_rG' is negative. However, the concentrations of intracellular metabolites are limited: they are rarely above 10 mM or below 1 μM [32,35]. Thus, if $\Delta_rG'^m$ 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 $\Delta_rG'^m$ 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 Δ_rG' will be negative (Methods) [19]. However, if $\Delta_rG'^m$ 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 $\Delta_rG'^m$ 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

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