Energetics of syntrophic fatty acid oxidation

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Abstract: Fatty acids are key intermediates in methanogenic degradation of organic matter in sediments as well as in anaerobic reactors. Conversion of butyrate or propionate to acetate, (CO_2), and hydrogen is endergonic under standard conditions, and becomes possible only at low hydrogen concentrations (10^{-4}–10^{-5} bar). A model of energy sharing between fermenting and methanogenic bacteria attributes a maximum amount of about 20 kJ per mol reaction to each partner in this syntrophic cooperation system. This amount corresponds to synthesis of only a fraction (one-third) of an ATP to be synthesized per reaction. Recent studies on the biochemistry of syntrophic fatty acid-oxidizing bacteria have revealed that hydrogen release from butyrate by these bacteria is inhibited by a protonophore or the ATPase inhibitor DCCD (*N,N'*-dicyclohexyl carbodiimide), indicating that a reversed electron transport step is involved in butyrate or propionate oxidation. Hydrogenase, butyryl-CoA dehydrogenase, and succinate dehydrogenase acitivities were found to be partially associated with the cytoplasmic membrane fraction. Also glycolic acid is degraded to methane and CO_2 by a defined syntrophic coculture. Here the most difficult step for hydrogen release is the glycolate dehydrogenase reaction ($E_0' = -92$ mV). Glycolate dehydrogenase, hydrogenase, and ATPase were found to be membrane-bound enzymes. Membrane vesicles produced hydrogen from glycolate only in the presence of ATP; protonophores and DCCD inhibited this hydrogen release. This system provides a suitable model to study reversed electron transport in interspecies hydrogen transfer between fermenting and methanogenic bacteria in methanogenic biomass degradation.

Key words: Interspecies hydrogen transfer; Reversed electron transport; Syntrophy; Biological energy quantum; Fatty acids; Energetics; Glycolate

Introduction

Syntrophy is a special case of symbiotic cooperation of two metabolically different types of bacteria which depend on each other for degradation of a certain substrate, typically for energetic reasons. The term was coined to describe the close cooperation of fatty acid-oxidizing fermenting bacteria with hydrogen-oxidizing methanogens [1], or of phototrophic green sulfur bacteria with chemotrophic sulfur-reducing bacteria [2]. In both cases, the bacteria together perform a reaction which neither one of them could do on its own, i.e. conversion of butyrate to acetate and methane through a hydrogen cycle, or light-dependent assimilation of ethanol and CO_2 through a sulfur cycle.

Of special interest is the pool size of the carrier between both partners: it must be low enough to avoid inhibition or intoxication of the first bacterium in the reaction chain, and high enough to feed the second bacterium sufficiently to allow energy conservation and growth. This is especially true for the type of syntrophy called 'interspecies hydrogen transfer', which plays an important role in the terminal steps of methanogenic biomass degradation in sediments, sewage sludge digesters, and other types of

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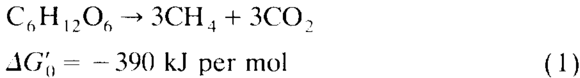
anaerobic reactors producing 'biogas' [3]. The total energy yield of methanogenic biomass degradation is small (with hexose as substrate):



$$C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2$$
$$\Delta G'_0 = -390 \text{ kJ per mol} \qquad (1)$$

and most of this energy is being used up by classical fermenting bacteria ('primary fermenters') which produce acetate, CO_2, and reduced compounds such as alcohols and fatty acids [3,4]. Although the carbon and electron fluxes through these reduced compounds are much smaller in complex methanogenic microbial communities than one would expect from fermentation balances of pure cultures degrading carbohydrates, these fluxes will never be zero because fatty acids of varying chain lengths are formed also in fermentative degradation of proteins and lipids [3]. Such compounds cannot be degraded directly by methanogenic bacteria but need further fermentative conversion to 'methanogenic' substrates such as H_2/CO_2, formate, other C_1-compounds, and acetate.

For these degradations only very little energy is available which has to be shared by at least two different types of bacteria. The amount of energy available to each partner depends on the pool size of the carrier between both, e.g. hydrogen, formate, or acetate, and the way how this energy is being shared by the partners involved is subject of this article.

Energetics of ATP formation

Synthesis of ATP from ADP and P_i according to

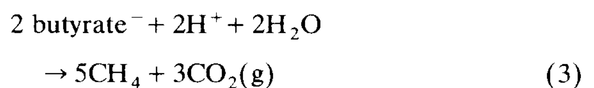
$$ADP + P_i \rightarrow ATP + H_2O \qquad (2)$$

requires +32 kJ per mol under standard conditions [5]. In the living bacterial cell, conditions are quite different from standard conditions (ATP and P_i around 10 mM; ADP around 1 mM in a well supplied, growing *Escherichia coli* culture at high energy charge; [5]), changing the value for physiological ATP synthesis to +49 kJ per mol. Since in all metabolic reaction chains certain irreversible steps are found which release a considerable amount of energy as heat, on average a further 20 kJ are lost in irreversible ATP synthesis by living organisms. With this, the overall energy requirement for ATP synthesis in the living cell comes to +70 kJ per mol, and this value may be only slightly smaller if an organism wastes less energy in heat production, or if it operates at an energy charge considerably lower than that quoted above for well-growing *E. coli* cells. Nonetheless, one cannot expect that the energy requirement for ATP synthesis can go substantially lower than about +60 kJ per mol.

The essential postulate of the Mitchell hypothesis of respirative ATP synthesis is that ATP formation is coupled to a vectorial transport of charged groups, typically protons, across a semipermeable membrane. Today it has been widely accepted that three protons cross the membrane per ATP formed or hydrolyzed, no matter whether bacterial [6] or mitochondrial [7,8] membranes are studied. This bears the consequence that no longer the equivalent of one ATP unit has to be regarded as the smallest quantum of energy a living cell can make use of, but that an energy quantum as small as one-third of an ATP equivalent can still be converted into ATP and, as a result, to metabolically useful energy. Combined with the calculations mentioned above, this means that a bacterium needs from a reaction a minimum of −20 kJ per mol substrate converted to be able to make energetic use of a reaction's free energy change [9,10]. It turns out that this is the range of energy with which syntrophically fatty acid-fermenting bacteria have to deal.

Energy sharing in syntrophic cultures

Fermentation of butyrate to methane and CO_2, according to the equation



$$2 \text{ butyrate}^- + 2H^+ + 2H_2O \rightarrow 5CH_4 + 3CO_2(g) \qquad (3)$$

yields, under standard conditions, −177 kJ per 2 mol butyrate. Under conditions better comparable to those typical of a sludge reactor or sediment (10 μM butyrate, 0.7 bar CH_4, 0.3 bar

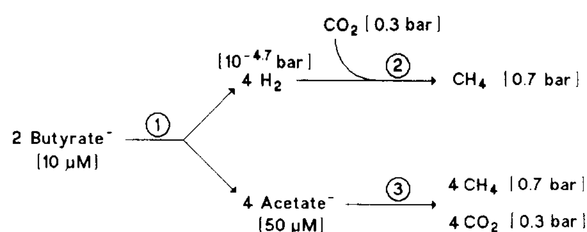


Fig. 1. Scheme of energy sharing among the three metabolic groups of bacteria cooperating in syntrophic butyrate oxidation. Recalculated and redrawn after [4,9]. Reaction 1, conversion of butyrate to acetate and hydrogen; reaction 2, conversion of hydrogen and CO_2 to CH_4; reaction 3, cleavage of acetate to CH_4 and CO_2.

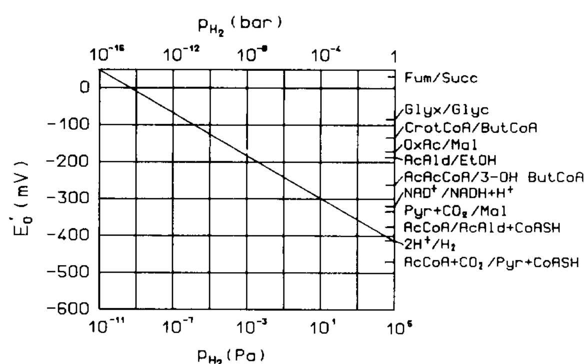


Fig. 2. Graph of dependence of the redox potential on the prevailing hydrogen partial pressure. On the right, standard potentials of redox reactions involved in syntrophic fatty acid and alcohol oxidations are shown. Fum, fumarate; succ, succinate; glyx, glyoxylate; glyc, glycolate; CrotCoA, crotonyl CoA; ButCoA, butyryl CoA; OxAc, oxaloacetate; mal, malate; AcAld, acetaldehyde; EtOH, ethanol; AcAcCoA, acetoacetyl CoA; 3-OH ButCoA, 3-hydroxybutyryl CoA; Pyr, pyruvate; AcCoA, acetyl CoA; CoASH, free coenzyme A.

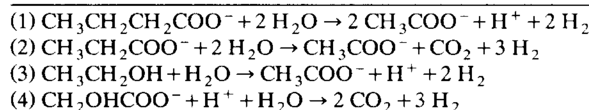
CO_2), this value changes to -145 kJ per 2 mol butyrate. This amount has to feed three different trophic groups of bacteria as pointed out in Fig. 1, and every group has to obtain its energy for growth from this reaction. Assuming that the seven partial reactions involved (reaction 1 runs twice, reaction 2 once, and reaction 3 four times) share the energy to equal amounts (-21 kJ per mol), the concentrations of the intermediates acetate and hydrogen can be calculated and turn out to be in the range of those observed in sediments or sludge digesters (50 μM acetate, $10^{-4.7}$ bar H_2, [3]).

Therefore, the assumption that energy is shared this way to equal parts appears to be basically justified. The two methanogenic partners catalyze their reactions with energy yields in the range of one-third of an ATP unit (-21 kJ per mol reaction) as indicated by growth yield determinations under optimal conditions [3,11].

The question arises how the butyrate-fermenting bacterium manages its energy metabolism. The degradation pathway has been elucidated with *Syntrophomonas wolfei*. It proceeds through classical β-oxidation via acetoacetyl CoA and includes formation of one ATP through substrate level phosphorylation (acetate kinase [4,12]). Since only a fraction of an ATP equivalent is available

Table 1

Hydrogen release reactions involved in syntrophic oxidation of fatty acids and alcohols, as treated in this article


(1) $CH_3CH_2CH_2COO^- + 2\,H_2O \rightarrow 2\,CH_3COO^- + H^+ + 2\,H_2$
(2) $CH_3CH_2COO^- + 2\,H_2O \rightarrow CH_3COO^- + CO_2 + 3\,H_2$
(3) $CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2\,H_2$
(4) $CH_2OHCOO^- + H^+ + H_2O \rightarrow 2\,CO_2 + 3\,H_2$

Reaction No.	$\Delta G'_0$ (kJ (mol reaction^{-1})	No. of electron pairs	E'_0 of electron-releasing redox reaction (mV)
1	+48.3	2	−125, −250
2	+76.0	3	+30, −176, −470
3	+9.6	2	−190, −375
4	+19.3	3	−92, −331, −470

to the bacterium under natural conditions (see above), part of this energy has to be re-invested into some further reaction in primary metabolism.

In Fig. 2, redox potentials in equilibrium with various hydrogen partial pressures are compared with standard redox potentials of the redox reactions involved in fatty acid oxidation (Table 1; Fig. 2). It becomes obvious that oxidation of 3-hydroxybutyryl CoA to acetoacetyl CoA can be coupled to proton reduction at a hydrogen partial pressure close to 10^{-5} bar, similar to the value calculated in Fig. 1. In contrast, proton reduction with electrons from butyryl CoA oxidation would require a hydrogen partial pressure around 10^{-10} bar, a value which is far lower than ever observed in such cultures, and which cannot be maintained by hydrogen-oxidizing methanogens at all: hydrogen-dependent CO_2 reduction reaches thermodynamic equilibrium at $10^{-5.7}$ bar H_2 (with 1 bar CO_2). For this reason, it was postulated earlier [13] that a reversed electron transport system is involved in this step which shifts electrons to a lower redox potential suitable for proton reduction, at the expense of energy derived from ATP hydrolysis.

Reversed electron transport

Butyrate oxidation

Experimental evidence of such reversed electron transport has been provided recently in our lab. In butyrate-grown cells of *S. wolfei*, high activities of membrane-bound ATPase were detected [14]. 3-Hydroxybutyryl CoA dehydrogenase was found exclusively in the cytoplasmic cell fraction, whereas butyryl CoA dehydrogenase and hydrogenase were for a significant part (> 15%) membrane-associated. Hydrogen production from butyrate by intact cells of *S. wolfei* was checked in the presence of bromoethane sulfonate to inhibit methanogenic hydrogen oxidation. Hydrogen accumulated to 6×10^{-4} bar, with concomitant acetate accumulation. This accumulation could be prevented by addition of the protonophor CCCP (carbonylcyanide-*p*-chloro-phenyl hydrazone) or the ATPase inhibitor DCCD (*N*,*N'*-dicyclohexyl carbodiimde), indicating that a membrane-bound, ATP-dependent step was involved in this process. In control experiments with crotonate as substrate both compounds showed no effect, thus proving that the critical, sensitive step is the oxidation of the butyryl to the crotonyl residue.

Hydrogen accumulated in these experiments to a concentration which corresponded to a leftover free energy of −23 kJ in the total system, just the amount of energy we defined above as the minimum amount necessary to sustain energy metabolism by these bacteria. Obviously, they do not burn down their 'battery' to zero before they stop, but leave a minimum tension from which they can resume work as soon as hydrogen is removed again.

We have no information as yet on how the components of such a reversed electron transport system are arranged in these bacteria. Copper chloride has been used repeatedly in the recent past to obtain information on the orientation of hydrogenases in the cytoplasmic membrane [15,16]. Hydrogenase activity of intact cell suspensions of *S. wolfei* could be inhibited to a large extent by copper chloride, indicating that the active site of this enzyme faces the periplasmic space. Hydrogenase activity of the partner bacterium *Methanospirillum hungatei* was measurable with Benzyl viologen only in cell-free extract, not in cell suspensions of intact cells, indicating that the above-mentioned inhibition effect refers only to the hydrogenase activity of the butyrate-forming fermenting bacterium. A menaquinone and also traces of a *c*-type cytochrome were found in *S. wolfei* cells; however, the latter to a higher extent in non-syntrophically grown cells [17]. Other syntrophic butyrate oxidizers such as *Syntrophospora bryantii* do not contain cytochromes at all [14]; obviously, they are therefore not essential carriers in such systems. It appears evident that butyrate dehydrogenase operates on the cytoplasmic surface of the cell membrane. Depending on whether the active site of the hydrogenase enzyme faces the cytoplasmic or the periplasmic space of the cells, the quinone has to act in this electron transport chain as an active proton transducer (Fig. 3a) or not (Fig. 3b). In the latter case, a very simple system for re-

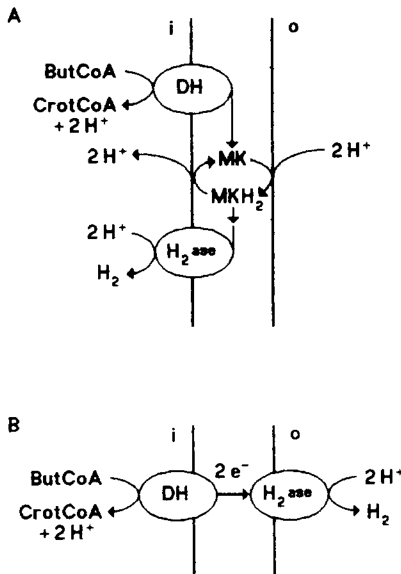


Fig. 3. Hypothetical scheme of organization of enzymes and redox carriers in the cytoplasmic membrane of the butyrate-degrading bacterium in the co-culture *Syntrophomonas wolfei*. i, inside; o, outside; DH, dehydrogenase; H_2 ase, hydrogenase; MK, menaquinone; ButCoA, butyryl CoA; CrotCoA, crotonyl CoA. (**A**, **B**) Two different concepts (see text).

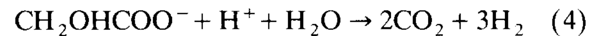
versed electron transport driven by the proton gradient can be proposed which does not involve electron carriers at all between the two enzyme proteins. Further studies are in progress to verify either one of these suggestions.

Propionate oxidation

For syntrophic propionate oxidation, a scheme similar to that in Fig. 1 can be drawn, leaving again a free energy change in the range of −22–−23 kJ per mol reaction to the syntrophic propionate oxidizer [4,18]. The pathway of propionate oxidation in such bacteria is basically a reversal of classical fermentative propionate formation, including methylmalonyl CoA, succinate, malate, pyruvate, and acetyl CoA as intermediates [4,19–21]. Of the redox reactions involved, succinate oxidation and malate oxidation are the most difficult ones to couple to proton reduction (Table 1; Fig. 2): hydrogen partial pressures of 10^{-8} or 10^{-15} bar would be required, respectively, which are again far lower concentrations than a methanogen can maintain (see above). The enzymes and electron transfer components involved in propionate oxidation by *Syntrophobacter wolinii* [22] and other new isolates of syntrophically propionate-oxidizing bacteria were studied in our lab. Studies of this kind became possible after binary mixed cultures with only one methanogenic bacterium and even pure cultures of syntrophic propionate oxidizers became available [14]. Of the enzymes involved, at least the succinate dehydrogenase activity appeared to be firmly bound to the membrane, as well as ATPase and part of the hydrogenase activity [14]. In similar experiments as described above for butyrate oxidation, hydrogen formation from propionate in bromoethanesulfonate-inhibited cultures was strictly suppressed by addition of CCCP and DCCD. This indicates again that an intact (proton?) motive force maintained by ATP hydrolysis is required for hydrogen release, probably at least in the first oxidation step. A menaquinone as well as cytochromes *b* and *c* were detected in these bacteria. Their respective localization, orientation, and physiological role in the electron transport system need still to be elucidated in detail before a concept of electron transport organization in the cytoplasmic membrane can be proposed.

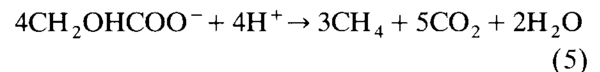
Glycolate oxidation

Glycolate, although not a fatty acid, is oxidized syntrophically by homoacetogenic and methanogenic cocultures to two CO_2, and hydrogen is the electron carrier between the fermenting and the hydrogen-oxidizing partner bacterium [23]. The primary fermentation is an endergonic process under standard conditions, and needs coupling to e.g. a methanogenic bacterium:



$$CH_2OHCOO^- + H^+ + H_2O \rightarrow 2CO_2 + 3H_2 \quad (4)$$

$\Delta G'_0 = +19.3$ kJ per mol glycolate



$$4CH_2OHCOO^- + 4H^+ \rightarrow 3CH_4 + 5CO_2 + 2H_2O \quad (5)$$

$\Delta G'_0 = -78.7$ kJ per mol glycolate

The degradation pathway includes oxidation of glycolate to glyoxylate, condensation of glyoxylate with acetyl CoA to malyl CoA, ATP formation in a malyl CoA synthetase reaction, malate oxida-

tion and decarboxylation, and oxidative decarboxylation of pyruvate to acetyl CoA, thus closing the chain for a new reaction cycle [23]. Three redox reactions are involved in this oxidation, catalyzed by glycolate dehydrogenase, malic enzyme, and pyruvate ferredoxin oxidoreductase. Proton reduction with electrons from these three oxidation steps poses a major problem only with the first reaction (Table 1; Fig. 2): direct proton reduction would require a hydrogen partial pressure at 10^{-11} bar, which cannot be maintained by e.g. methanogenic bacteria. Again a reversed electron transport system has to be postulated, therefore, which has to be fuelled by partial hydrolysis of the ATP formed in substrate level phosphorylation.

Indirect evidence of such a reversed electron transport has been obtained from hydrogen accumulation experiments similar to those reported above on butyrate and propionate: hydrogen production from glycolate by intact cells was inhibited by CCCP (Friedrich, unpublished). With carefully prepared membrane vesicle suspensions, a Methylene blue-reducing glycolate dehydrogenase, as well as ATPase and hydrogenase were found to be membrane-bound for a significant part (25–97%). Direct proof of proton gradient-dependent hydrogen release was provided in experiments with membrane vesicles prepared from the glycolate-fermenting bacterium: such vesicles converted glycolate stoichiometrically to glyoxylate and hydrogen in the presence of ATP. This hydrogen formation was abolished entirely by addition of CCCP and other protonophores, as well as by DCCD. Monensin and other sodium ionophores had no specific effect [24]. Hence, this system is the first case for which proton potential-dependent reversed electron transport to protons to form hydrogen was irrefutably proven.

So far, we do not yet know which electron carriers are involved in this transport system, and how the enzymes involved are organized in the membrane. The bacterium contains menaquinone K_9 and traces of menaquinones K_8 and K_{10}, but no cytochromes. Inhibition experiments with intact cell suspensions revealed that glycolate dehydrogenase and Methylene blue-reducing hydrogenase activity of the glycolate-fermenting bacterium were inhibited by $CuCl_2$ (Table 2), indicating that a copper-sensitive part of both enzymes is accessible from the periplasmic space (Fig. 4c). Depending on the orientation of these enzymes, and on whether or not the menaquinone acts as an active proton transporter in the membrane, three different options of functional organization of the various electron carriers in the membrane can be suggested (Fig. 4a–c). We are presently purifying the enzyme components of this system and hope that reconstitution experiments with phospholipid vesicles will give us a conclusive answer to this question.

Glycolate-dependent hydrogen accumulation

Table 2

$CuCl_2$ inhibition of enzyme activities in the glycolate-degrading co-culture FlGlyM

Enzyme activity	$-CuCl_2$		2 mM $CuCl_2$ Spec. activity		8 mM $CuCl_2$	
	[U/mg]	%	[U/mg]	%	[U/mg]	%
Glycolate/MB DH	2.03	100	1.2	58	0	0
Hydrogenase MB	80.0	100	53.3	67	32.0	40
Hydrogenase MV	11.2	100	10.3	91	8.0	70

Experiments were carried out in anoxic glass cuvettes containing 100 mM potassium phosphate buffer (pH 7.0) and whole cells (5.2 mg protein/ml for glycolate dehydrogenase assay or 1.0 μg protein/ml for hydrogenase assay) under an atmosphere of N_2 or H_2 (0.5 atm). Glycolate dehydrogenase was measured following reduction of Methylene blue (0.1 mM) in the presence of 10 mM glycolate. Hydrogenase was assayed either with Methylene blue (0.1 mM) or with Methyl viologen (5 mM) as electron acceptor; dithionite (approx. 10 nmol) was added to the latter assay mixture to reduce the buffer slightly. Cell suspensions were pretreated with $CuCl_2$ (0, 2, and 8 mM) for 10 min at +4°C prior to start of the reaction under anoxic conditions.
DH, dehydrogenase; MB, Methylene blue; MV, Methyl viologen.

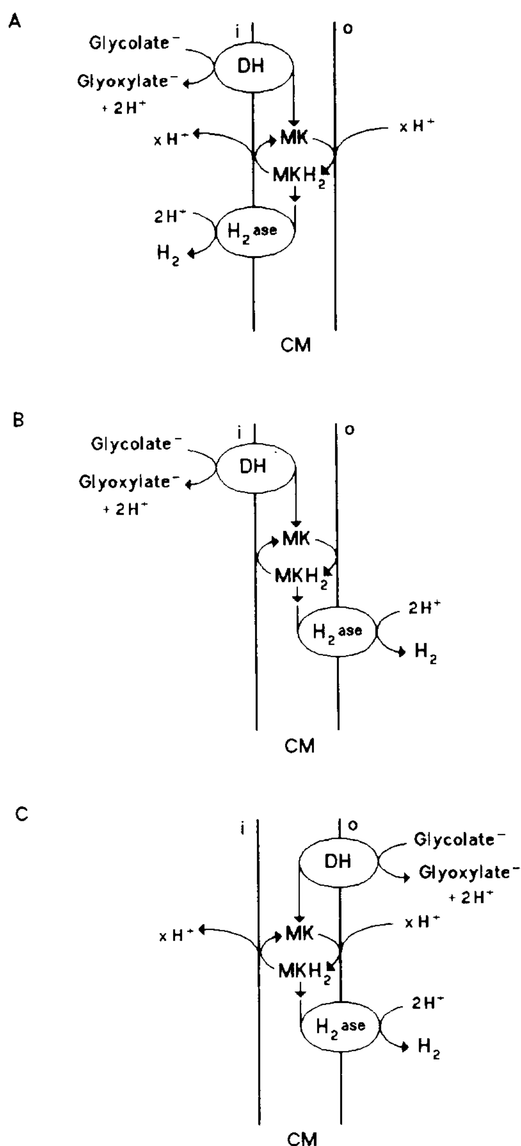
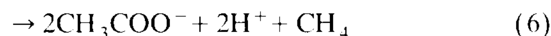


Fig. 4. Hypothetical scheme of organization of enzymes and redox carriers in the cytoplasmic membrane of the glycolate-oxidizing strain FlGlyR. i, inside; o, outside; CM, cytoplasmic membrane; MK, menaquinone; DH, dehydrogenase; H_2 ase, hydrogenase. (**A–C**) Three different concepts (see text).

by membrane vesicles reached an equilibrium value at 3.9×10^{-3} bar H_2 (Friedrich, M., Schink, B. Abstract V 119, Ann. Meetg. VAAM, Leipzig, 1993). This value, together with the respective glycolate (6.7 mM) and glyoxylate (2.3 mM) concentrations, corresponds to an energy expense in this step of +46 kJ per mol glycolate, indicating that about two-thirds of an ATP unit has to be spent to reach the observed steady-state conditions.

Ethanol oxidation

Methanogenic ethanol degradation was actually the first case of syntrophic interspecies hydrogen transfer studied, but the energetics of this system are far from clear. The total fermentation



$$2CH_3CH_2OH + CO_2 \rightarrow 2CH_3COO^- + 2H^+ + CH_4 \quad (6)$$

yields −112 kJ per 2 mol ethanol under standard conditions. On the side of the ethanol oxidizer, e.g. the 'S-strain' of '*Methanobacillus omelianskii*' [25] or other fermenting anaerobes with similar metabolic capacities such as *Pelobacter acetylenicus* [26], ethanol dehydrogenase, acetaldehyde ferredoxin oxidoreductase (acetyl CoA-forming), phosphotransacetylase, and acetate kinase are involved, forming one ATP per ethanol through substrate level phosphorylation. Since the methanogenic hydrogen oxidizer requires at least one-third of an ATP unit for growth (−20 kJ per reaction run; Eq. 6), only an amount of about 45 kJ is available to the ethanol oxidizer per mol ethanol oxidized, which is far too little energy to form one ATP. It has to be postulated, therefore, that again part of the energy bound in ATP has to be re-invested somewhere to 'pull' the overall reaction. Energetically, the most difficult reaction in ethanol oxidation is the ethanol dehydrogenase reaction (Table 1; Fig. 2), but so far we have no reliable proof of involvement of a reversed electron transport system in this step. An alternative option could be as well an electrogenic acetate anion export which would remove acetate actively from the cytoplasmic space and thus 'pull' the overall reaction.

Similar problems as with ethanol oxidation have to be expected with syntrophic oxidation of primary amines. Defined co-cultures of strictly anaerobic bacteria degrading cadaverine and ethanolamine are now in our hands and ready for biochemical checks analogous to the above experiments.

Interspecies formate versus hydrogen transfer

So far, we have discussed the energetics of syntrophic oxidations only with hydrogen as the electron carrier transferred between the partner bacteria in play. The above considerations demonstrate that at least the electron transfer systems discussed here can be modelled sufficiently on the basis of this assumption. However, a possible alternative involvement of formate in such electron transfer processes has been considered from the very beginning [1,25], since nearly all partners in syntrophic cooperations can metabolize both hydrogen and formate. Exclusive participation of hydrogen as electron carrier has been proven so far only with the butyrate-oxidizing coculture *Syntrophomonas wolfei* (Wofford, N.O., McInerney, M.J., Abstract I 31, Annu. Meetg. ASM, New Orleans, 1989) and the thermophilic, syntrophically acetate-fermenting strain THF [27,28]: both strains exhibit high hydrogenase and very little formate dehydrogenase activity. Nonetheless, a formate/CO_2 shuttle could replace hydrogen transfer as well, and this idea has been revived recently on the basis of experiments with undefined floc cultures [29] and with pure cultures [30,31], and of theoretical considerations [32,33]. Energetically, there is no basic difference between both carriers: at 0.3 bar CO_2 as typical of e.g. sewage sludge, a hydrogen partial pressure of 10^{-4} bar is equivalent to a formate concentration of 10 μM [34]. Moreover, transport of the charged formate anion across cytoplasmic membranes (as opposed to free diffusion of hydrogen) and the implications of varying CO_2 pressures in different natural environments pose further problems which need to be considered in this context. Since hydrogenases and formate dehydrogenases usually couple with the same low-potential electron carriers (ferredoxin?) it is extremely difficult to assay for the relative importance of either one in syntrophic cultures, and both carriers may even be used simultaneously [4]. Therefore, formate should be kept in mind as a possible alternative electron carrier, and formate pools and turnovers should be checked for in those cases in which overall process energetics cannot be matched with hydrogen pools and fluxes alone.

Conclusions and outlook

The syntrophic fatty acid and alcohol-oxidizing processes treated in this article represent exciting examples of energy metabolism operating with the smallest energy quantum that can be exploited by living cells at all. The minimum amount of energy which can be converted into ATP in the living cell is in the range of -20 kJ per reaction run, and exactly this is the amount of energy available to the respective partners in these degradation processes. Models of metabolic cooperation and energy sharing between syntrophic partners have been proposed which require reversed electron transport systems for hydrogen release to balance the energy requirements of all bacteria involved.

Experimental evidence of participation of such reversed electron transport in hydrogen release has been provided recently with fatty acid and glycolate-oxidizing fermenting bacteria; in the latter case, the components were shown to be membrane-associated, and operation of the whole system could even be demonstrated in vitro in cell-free membrane preparations. A lot of work is still required until we reach an understanding of the operation of such electron transport systems at the molecular level. In any case, these studies reveal that syntrophic fatty acid oxidizers – which are usually considered rather 'primitive' or 'archeal' types of bacteria – are actually admirable creatures from the point of view of energy conservation and efficient energy utilization. Most of these bacteria grow in plain mineral media and synthesize all their cellular components on the basis of only the minimum quantum of energy which can be exploited by living cells at all: they are spectacular examples to demonstrate how diligently nature has organized the components of global energy flux down to its least conspicuous children.

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