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REVIEW

Structural and Functional Features of Formate Hydrogen Lyase, an Enzyme of Mixed-Acid Fermentation from *Escherichia coli*

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Abstract—Formate hydrogen lyase from *Escherichia coli* is a membrane-bound complex that oxidizes formic acid to carbon dioxide and molecular hydrogen. Under anaerobic growth conditions and fermentation of sugars (glucose), it exists in two forms. One form is constituted by formate dehydrogenase H and hydrogenase 3, and the other one is the same formate dehydrogenase and hydrogenase 4; the presence of small protein subunits, carriers of electrons, is also probable. Other proteins may also be involved in formation of the enzyme complex, which requires the presence of metal (nickel-cobalt). Its formation also depends on the external pH and the presence of formate. Activity of both forms requires F_0F_1-ATPase; this explains dependence of the complex functioning on proton-motive force. It is also possible that the formate hydrogen lyase complex will exhibit its own proton-translocating function.

Key words: formate hydrogen lyase complex, formate dehydrogenase H, hydrogenase 3, hydrogenase 4, F_0F_1-ATPase, mixed-acid fermentation, *Escherichia coli*

FORMATE HYDROGEN LYASE IS AN ENZYME OF MIXED FERMENTATION IN BACTERIA

Mixed fermentation and energy transformation. Fermentation is a process of partial oxidation of hydrogen-rich organic compounds such as sugars under anaerobic conditions in the absence of exogenous electron acceptors. It is widely distributed in many bacteria. However, only a small proportion of energy released during fermentation is utilized for ATP synthesis; hydrogen formed reduces substances required for various biosyntheses, whereas end products of partial oxidation of organic compounds are released by cells into the external medium.

Several types of fermentation have been recognized depending on the end products. Homoenzymatic fermentation yields only acid (e.g., lactate) or alcohol (e.g., ethanol), whereas heteroenzymatic fermentation results in formation of a mixture of lactate, acetate, formate and other organic acids, and alcohol. *Escherichia coli* cells and some other bacteria demonstrate mixed fermentation, which results in formation of a mixture of organic acids and release of gaseous molecular hydrogen.

During sugar fermentation degradation of glucose proceeds via the Embden–Meyerhof–Parnas pathway: at the stage of phosphoenolpyruvate some intermediates may be used for succinate formation, whereas all other end products are formed from pyruvate (Fig. 1). The ratio of the forming products is variable and it depends on various factors [1, 2] such as concentration of a fermenting substrate, pH, redox potential, etc. Production of molecular hydrogen reduces organic acid formation and therefore represents an important factor in regulation of cytoplasmic pH in bacteria.

Under anaerobic conditions oxidation of organic acids in bacteria involves a chain of sequential chemical reactions (Fig. 1) catalyzed by sequential fermentation enzymes. One of these steps includes formate hydrogen lyase.

Formate hydrogen lyase catalyzes the reaction of formate oxidation (one of the fermentation products) to carbon dioxide and molecular hydrogen [3].

There are several recent reviews summarizing properties of formate hydrogen lyase and its hydrogenases [4-9]. However, the terminal stages of fermentation are still intensively studied in many laboratories including ours. Therefore, it is necessary to summarize recent data and discuss new hypotheses.

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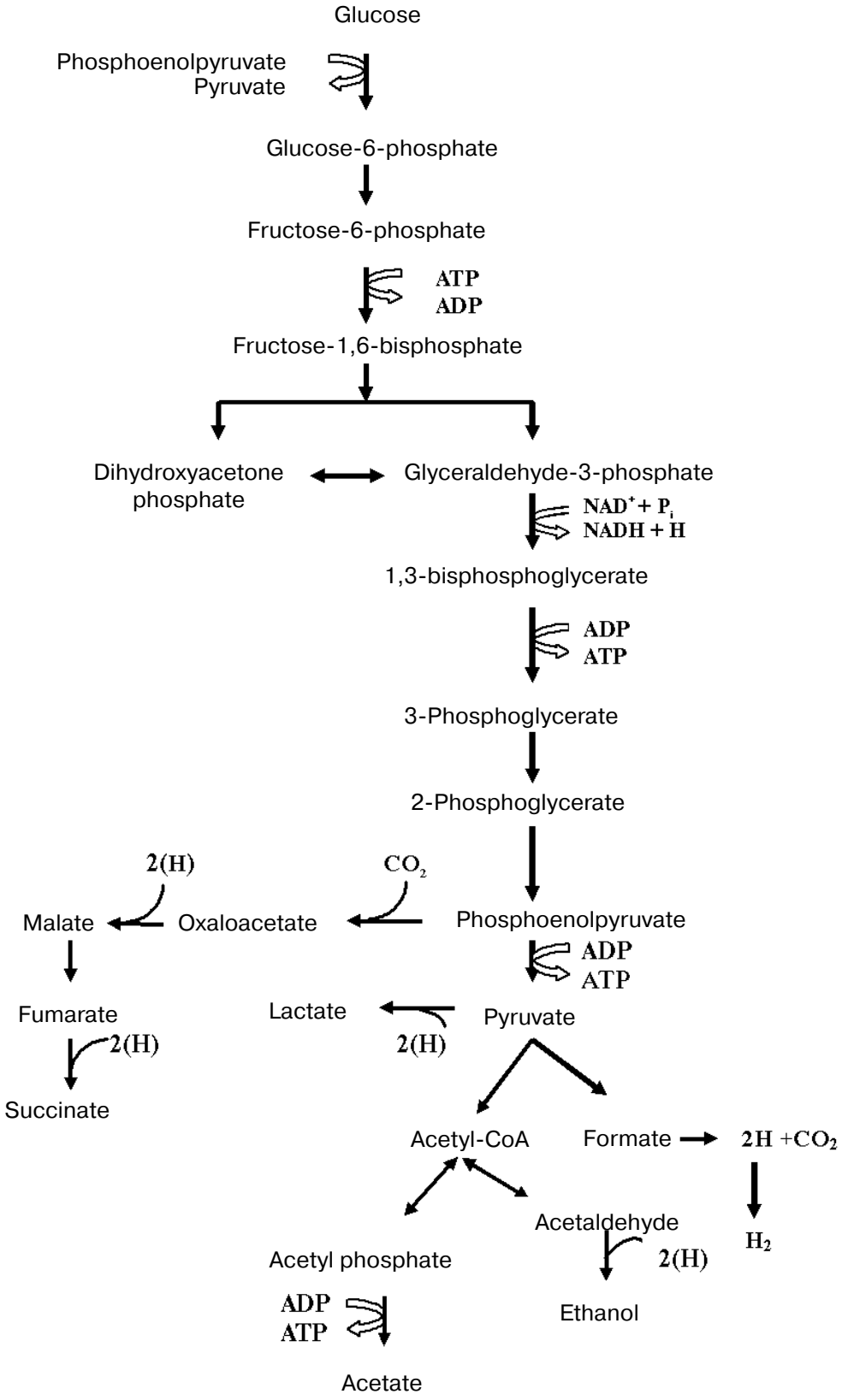


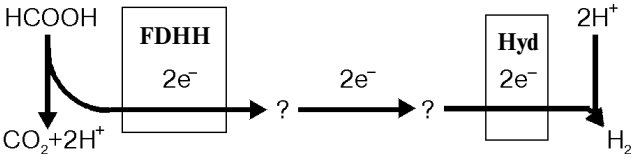
Fig. 1. Scheme of mixed fermentation in *E. coli*. Glucose oxidation follows via the Embden–Meyerhof–Parnas pathway accompanied by formation of the fermentation products – lactate, formate, acetate, succinate, and ethanol; formate oxidation results in (stepwise) formation of molecular hydrogen and carbon dioxide. Substrates involved in ATP formation are shown.

In the present review we summarize results of studies of formate hydrogen lyase activity using artificial electron acceptors (benzyl viologen, methyl viologen, methylene blue) in reaction with formate or molecular hydrogen under experimental conditions and measurements of intensity of molecular hydrogen release by intact cells and membrane vesicles. Data on the structure of proteins exhibiting such activity were obtained by means of known molecular-biological experiments on cloning, analysis of nucleotide sequence and deletions in genes encoding corresponding subunits, and also using traditional methods of purification and analysis of proteins. Hydrogenases constituting formate hydrogen lyase were analyzed by means of crystallography.

It should be noted that various methods for isolation and purification of corresponding proteins gave various results. Apparent discrepancy may be attributed to possible loss of some subunits during isolation of these membrane-bound proteins and different cultivation conditions of bacteria, which may also contribute to appearance of certain differences in the composition of these proteins.

STRUCTURE AND MOLECULAR GENETICS OF FORMATE HYDROGEN LYASE

Structure of the enzyme complex and the role of subunits. In *E. coli*, formate hydrogen lyase is a complex that consists of two enzymes, formate dehydrogenase H (FDHH) and hydrogenase (Hyd), and electron carriers responsible for formic acid oxidation (HCOOH) to CO_2 and H_2 according to the generally accepted scheme [10]:



Within this scheme formic acid acts as the actual electron donor, whereas protons are the only terminal acceptor. This means that both components of formate hydrogen lyase should contain redox centers and electron transfer from FDHH to hydrogenase requires some carriers. Their nature is not finally elucidated, but it is suggested that hydrogenase subunits may act as these carriers.

Formate dehydrogenase H is a selenocysteine and molybdenum-containing peripheral membrane protein located at the cytoplasmic side. It consists of several subunits denominated as Fdh (table). The large subunit, FdhF, exhibits catalytic properties [11] and has a molecular mass of 79 kD [12]. Selenocysteine is located at position 140 [13, 14] and is directly involved in active site formation [15].

Formate dehydrogenase H contains iron-sulfur clusters [16, 17]. Certain evidence exists that the large subunit contains one [4Fe-4S] cluster. It catalyzes oxidation of formic acid to CO_2 and a couple of redox equivalents 2 $(H^+ + e^-)$ which involve NAD^+ formed in the fermentation process:

$$HCOOH \rightarrow CO_2 + 2\,(H^+ + e^-). \quad (1)$$

Decrease in pH value from 7.5 to 6.0 is accompanied by reduction of formate dehydrogenase H activity [16, 17], which is also decreased in the presence of nitrate or azide [16]. Other forms of formate dehydrogenase are usually recognized in the presence of exogenous electron acceptors such as oxygen or nitrate.

Hydrogenase of the formate hydrogen lyase complex exists in two forms, Hyd-3 [18, 19] and Hyd-4 [20], each of which consists of many subunits. Some of these subunits represents polytopic membrane proteins.

Hyd-3 is a protein complex that consists of several large and small subunits, denominated as Hyc (table). This complex is rather stable. One of the most interesting large subunits is nickel-containing iron-sulfur HycE protein with molecular mass of 65 kD [21-23]; in some cases nickel may be substituted by zinc or other metals [24]. This peripheral membrane protein located at the cytoplasmic side is responsible for equilibrium of the reaction of proton reduction:

$$NADH + H^+ \leftrightarrow NAD^+ + H_2, \quad (2)$$

coupled to electron transfer from reaction (1) to reaction (2) (see also the scheme above). The nature of natural electron donors and acceptors involved in the hydrogenase reaction remains to be clarified. It is suggested that small Hyd-3 subunits with molecular masses of 21-33 kD may participate in such electron transfer [19]. Some of these subunits possessing α-helical sites are tightly bound to the membrane. It is also suggested that these subunits contain iron-sulfur clusters [4Fe-4S], which are involved in intramolecular electron transfer between redox centers. Interestingly, amino acid sequences of some of these small subunits share homology with components of the electron transport chains of bacteria, mitochondria, and plastids (e.g., NADH-ubiquinone-oxidoreductase, Complex I) [20, 22]. This suggests involvement of hydrogenase subunits in formation of various electron transport chains.

The existence of Hyd-4 was postulated only recently [20]. It is possible that Hyd-4 is also a nickel-containing protein that includes iron-sulfur clusters (table). Some Hyd-4 subunits (denominated as Hyf) are homologous to Hyd-3 subunits; they have not been isolated and studied yet. It is important to determine conditions required for maximal induction of this hydrogenase.

Thus, Hyd-3 and Hyd-4 are nickel-iron-containing hydrogenases forming one group of hydrogenases. The

Comparative characteristics of formate hydrogen lyase components, protein products of the *fdh*-, *hyc*-, and *hyf*-operons [16, 17, 19, 20, 22]

Protein product	Molecular mass, kD*	Number of α-helices	Homology** of protein products and their possible role
FdhD	30.5	?	protease
FdhE	32.0	?	binding to membrane
FdhF	79.1	0	selenium-molybdenum-containing iron-sulfur protein, large subunit of FDHH, formate-benzyl viologen reductase
HycA	17.6	?	regulatory protein
HycB	21.8	?	iron-sulfur protein, bacterial ferredoxin
HycC	64.1	9	subunit IV of NADH-ubiquinone oxidoreductase (Complex I) of electron transport chain, role is unclear
HycD	33.0	6	subunit I of Complex I, role is unclear
HycE	65.0	0	nickel-containing iron-sulfur protein, large subunit of Hyd-3
HycF	20.3	?	iron-sulfur protein, membrane binding
HycG	28.0	?	iron-sulfur protein, large subunit of Hyd-3
HycH	15.5	0	regulatory protein
HycI	?	?	protease
HyfA	22.2	0	HycB (50), iron-sulfur protein, bacterial ferredoxin
HyfB	72.5	16	HycC (37), proton translocation
HyfC	34.3	8	HycD (51), subunit XII of complex I, role is unclear
HyfD	51.8	14	HycC (17), proton translocation
HyfE	23.4	7	role is unclear
HyfF	56.8	14	HycC (22), proton translocation
HyfG	53.4	0	HycE (73), nickel-iron protein, subunit of Hyd-4, initial part is subunit III of Complex I
HyfH	20.2	0	HycF (44), iron-sulfur protein, bacterial ferredoxin
HyfI	28.1	0	HycG (63), iron-sulfur protein, subunit of Hyd-4
HyfJ	15.6	0	HycH (47), role is unclear
HyfR	75.3	0	FhlA (46), formate-sensitive regulatory protein
FocB	30.6	6	formate permease

* Deduced from coding nucleotide sequence of corresponding gene.
** In brackets identity of amino acid sequence in percent is shown.

other group of these enzymes includes hydrogenases which contain only iron [9].

Formate hydrogen lyase containing Hyd-3 is considered as formate hydrogen lyase 1 [12, 19, 22, 25], whereas the enzyme containing Hyd-4 is considered as formate hydrogen lyase 2 [20].

Besides Hyd-3 and Hyd-4 involved in functioning of formate hydrogen lyase complex, *E. coli* cells contain two other hydrogenases, Hyd-1 and Hyd-2, which also participate in hydrogen metabolism [21]. These hydrogenases are not related to formate hydrogen lyase [18]. Certain evidence exists that they preferentially oxidize hydrogen (see reaction (2)) and operate under different conditions [26]. Hyd-2 is sensitive to inhibition by formate and alkaline pH [27]; it is also sensitive to oxygen [26].

Hyd-1 is a multicomponent protein comprised of subunits with molecular masses of 64, 31, and 29 kD; these subunits form Hyd-1 tetramer with the stoichiometric ratio 2 : 1 : 1 [28]. Activity of Hyd-1 is not well studied. Hyd-2 contains a large subunit with molecular mass of 56-62 kD [27] that oxidizes molecular hydrogen to protons (see reaction (2)) and is probably involved in their translocation.

Genes encoding subunits of formate hydrogen lyase and their expression. Enzyme complex formation. Components of formate hydrogen lyase are encoded by various genes. FDHH is encoded by *fdh* genes; *fdhF* encodes the large subunit of formate hydrogen lyase. This is a monocistronic gene. It is suppressed by oxygen and nitrate [29]. Protein products of *fdhD* and *fdhE* are required for catalytic activity of FDHH. The former is suggested to be a protease involved in formation of mature large subunit of FDHH, whereas the latter is essential for membrane binding (table).

Since FDHH is a selenocysteine protein, selenium incorporation into the synthesizing protein includes its transfer by some special transport system. Incorporation of molybdenum into the protein is a rather complex process that involves at least 13 genes located at 5 separate loci of the chromosome. Four proteins encoded by the *fdh*-operon obviously form molybdenum-binding and transport systems.

Subunits of Hyd-3 are encoded by the *hyc*-operon, which consists of nine genes [23, 30]; the first of these genes, *hycA*, is a promoter for the whole operon and its protein product, HycA, antagonizes expression of other genes, particularly *fdhF* [19]. The effect of HycA is possibly realized via binding to a formate sensitive protein. The large subunit of Hyd-3 is encoded by the fifth gene, *hycE* [22] (table), whereas the second, the sixth, and the seventh genes (*hycB*, *hycF*, and *hycG*) encode small subunits [19]. It is possible that *hycB* causes pleiotropic effects. Its protein product is suggested to be the small subunit of FDHH. This interesting hypothesis of Sawers [5] was supported by results of our studies [31, 32]. We demonstrated that the activity of formate hydrogen lyase 2 (including FDHH and Hyd-4) requires the presence of the protein product of this gene. Oxygen and nitrate suppress both *hycB* and *fdhF* expression [29]. One of the other protein products of this operon, HycH, is not a constituent part of Hyd-3, but it is required for formate hydrogen lyase formation [19]. The latter protein product of this operon forms a protease required for large subunit formation [23]. Formate and pH influence *hyc*-operon functioning [30] (see below).

Hyd-4 is encoded by the *hyf*-operon formed by 12 genes [20]. Nine of these genes encode subunits that are homologous to seven Hyd-3 subunits (table). Genes *hyfG* and *hyfI* encode large and small subunits, respectively. The gene *hyfR* encodes a formate sensitive regulatory protein, and the terminal gene *focB* encodes formate permease. Protein products of three genes (*hyfD*, *hyfE*, and *hyfF*) probably represent integral membrane proteins that lack analogs in Hyd-3. Perhaps these products might underline characteristic functions of this hydrogenase if they actually exist. The comparative characteristics of protein products of *hyc*- and *hyf*-operons are listed in the table.

Thus, both operons include structural genes encoding small and large subunits of hydrogenase and the whole set of genes encoding regulatory proteins required for "maturation" of this enzyme. These operons share high identity. It is suggested that these operons encoding hydrogenases were derived from a common predecessor; they represent ancestor genes that may exhibit some interaction [9].

It should be noted that gene expression, synthesis of subunits, and formation of formate hydrogen lyase complex depend on many proteins encoded by other genes. They may be important for incorporation of molybdenum and nickel into corresponding subunits of this enzyme complex [33] or for repression of genes encoding components of the electron transport chains under aerobic or anaerobic conditions in the presence of, say, nitrate or oxygen. *E. coli* cells contain various systems responsible for transduction of corresponding signals (e.g., the presence of exogenous electron donors or acceptors), such as the Arc-system [34, 35]. However, their relationship to formate hydrogen lyase remains poorly understood.

Models of the enzyme complex in the membrane. Based on structure and membrane localization of the enzyme and expression of genes encoding its subunits, several models of the enzyme complex location in the membrane have been proposed.

Figure 2a shows the model of formate hydrogen lyase complex one proposed by Sauter et al. [19]. According to this model, two catalytic components, FDHH and large subunit of Hyd-3, associated with the cytoplasmic side of membrane, interact with other Hyd-3 subunits in the membrane. These Hyd-3 subunits are responsible for H^+ and electron transfer from FDHH to the large subunit of Hyd-3. This model requires experimental validation

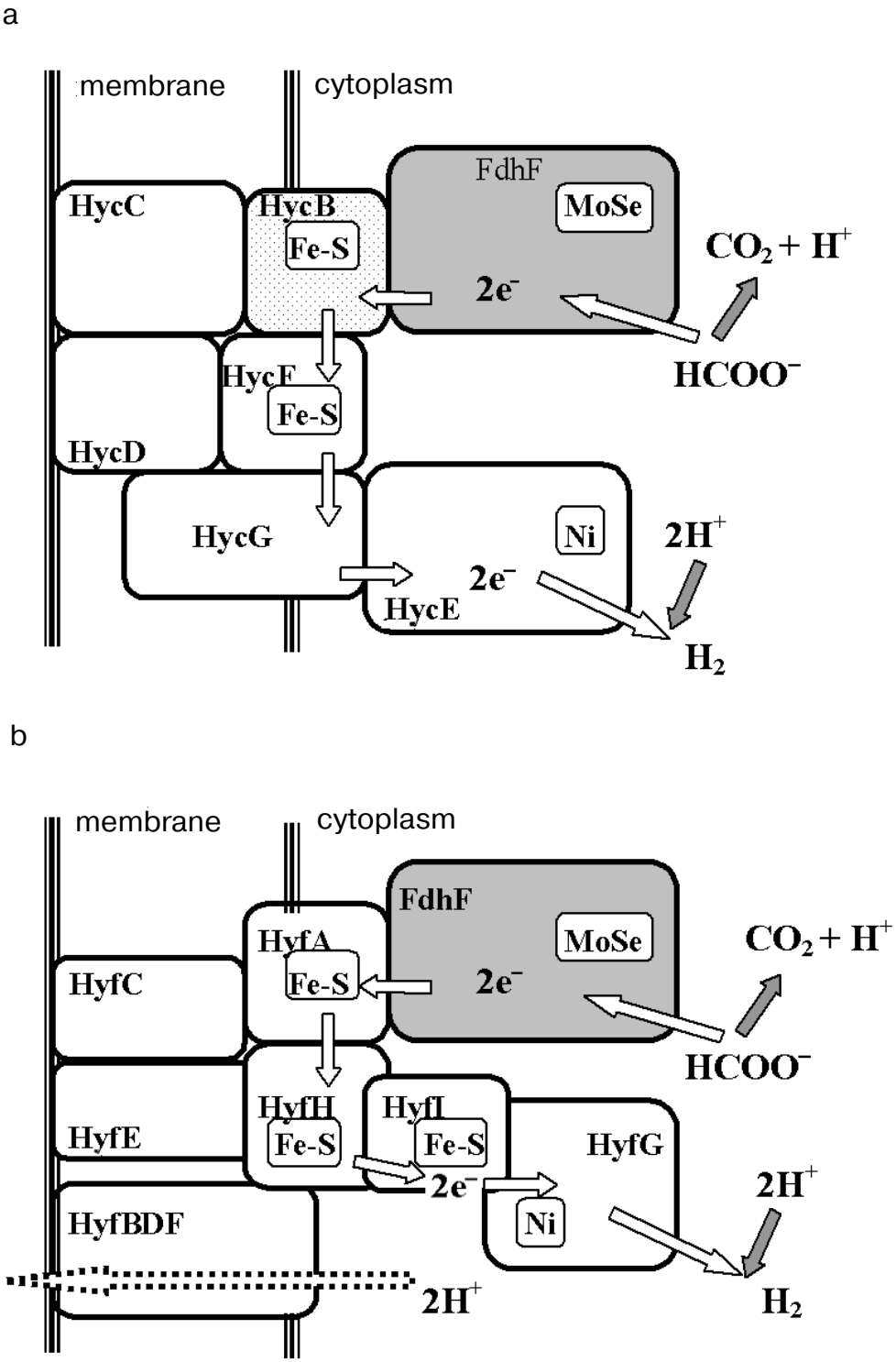


Fig. 2. Putative models of arrangement of formate hydrogen lyase complex formed by formate dehydrogenase H and hydrogenases 3 (a) or 4 (b), proposed by Sauter et al. [19] and Andrews et al. [20], respectively. Hyd-3 and Hyd-4 are presented by Hyc- and Hyf protein products, respectively. The protein products HycE and HycG (a) represent large subunits, whereas others are small subunits. Formate is the electron donor for FDHH, the protein product HycB acts as the direct electron acceptor from FDHH, and protons are terminal acceptor of electrons. Protein products HyfB, HyfD, and HyfF (b) may be involved in proton translocation. Open arrows show electron transfer through subunits of formate hydrogen lyase. Other explanations are in the text.

because of the little information about small subunits and precise pathways of redox equivalent transfer within formate hydrogen lyase. It is possible that functionally competent formate hydrogen lyase complex includes other proteins.

In spite of limited information about Hyd-4 subunits, a model of formate hydrogen lyase 2 (Fig. 2b) proposed by Andrews et al. [20] also suggests cytoplasmic location of FDHH and large Hyd-4 subunit, which bind to membrane via other subunits of Hyd-4. It is assumed that three Hyd-4 subunits provide transmembrane proton translocation. This model is rather speculative because of limited experimental data.

TWO FORMS OF FORMATE HYDROGEN LYASE AND THEIR RELATION TO MOLECULAR HYDROGEN PRODUCTION

Two forms of formate hydrogen lyase. In *E. coli* cells formate hydrogen lyase exists in two forms, which differ mainly by the presence of hydrogenase forms Hyd-3 and Hyd-4, respectively [20]. Since Hyd-3 and Hyd-4 are encoded by genes of different operons and are characterized by different subunit composition (table) it is reasonable to assume that these forms are functionally active under different conditions and therefore they play distinct roles in *E. coli* cells.

Kobayashi [36] believes that multiplicity of bacterial membrane systems is important for adaptation, when different systems are preferentially active under different conditions. For example, *E. coli* cells possess two constitutive systems of potassium uptake, which are characterized by moderate affinity and distinct rates of ion transport [37]. One system, TrkA, operates under alkaline conditions whereas the other one, Kup, is active in acidic medium [38, 39]. Moreover, TrkA may function in different modes at highly and weakly alkaline pH values [40].

Factors determining functional activity of multiple membrane systems in bacteria include environmental pH, concentration of substrate(s) and/or product(s) of fermentation, the presence of some exogenous electron acceptors, ratios of end products, etc. These conditions probably influence synthesis of different forms of formate hydrogen lyase, formation of functionally active membrane complex; it is also possible that different forms of formate hydrogen lyase function at different pH values (e.g., in acidic or alkaline media) [32]. Formate concentration may also act as a regulatory factor: various forms of formate hydrogen lyase are active at different formate concentrations, at micromolar or at relatively high millimolar (≥30 mM) concentrations. The end product of fermentation, succinic acid (Fig. 1), may also regulate activity of formate hydrogen lyase: addition of succinate together with glucose caused 2-fold increase in molecular hydrogen production [41].

Methods for detection of molecular hydrogen formation. Molecular hydrogen production is studied by various methods, which need some analysis for correct comparison of results obtained in different laboratories.

The use of test-tubes for determination of molecular hydrogen is the simplest method [42]. During bacterial sugar fermentation accumulated hydrogen bubbles could be easily seen without any special equipment. Production of molecular hydrogen by bacteria may be determined by chemical reaction of color disappearance during interaction of potassium permanganate solution with molecular hydrogen [43]:

$$2\ KMnO_4 + H_2 + 3\ H_2SO_4 \rightarrow K_2SO_4 + 2\ MnSO_4 + 4\ H_2O.$$

Quantitative assays of molecular hydrogen usually employ a gas chromatography method [44]. The use of a couple of redox electrodes (platinum and titanium-silicate) also has certain advantages. In contrast to platinum, titanium-silicate electrode data are not affected by the presence of molecular hydrogen (or oxygen) in the medium; this allows differentiation of molecular hydrogen in the experimental medium under anaerobic conditions [31, 32, 43, 45].

However, quantitative evaluation of molecular hydrogen formation using the above mentioned method gives equivocal results. Chemical detection and gas chromatography of molecular hydrogen require gas extraction and calculation of its solubility in a liquid medium. So, potentiometric determination of molecular hydrogen using the couple of redox electrodes gives more accurate quantitative results [31, 32]. Transformation of potentiometric data into quantity (concentration) of molecular hydrogen has certain difficulties, but it allows monitoring of processes on the surface of the bacterial membrane.

REGULATION OF ACTIVITY OF FORMATE HYDROGEN LYASE AND ITS INTERACTION WITH F_0F_1-ATPase

Role of formate and pH in induction of the enzyme. Formate plays a clear role in regulation of *E. coli* formate hydrogen lyase under conditions when it is the substrate determining (together with one of the products of formate hydrogen lyase reaction) pH of the medium. Increase in fermentation derived formate concentration as well as the presence of exogenous formate induced formate hydrogen lyase 1 [30, 46]. Although acidification of the medium also promoted induction of this enzyme [30], such induction was observed in weakly alkaline medium [46]. What is the mechanism of this phenomenon?

Increase in concentration of fermentation products, organic acids, causes a decrease in pH; this is accompanied by an increase in formate concentration in cytoplasm. This also may be attributed to transport of formic

acid from an external medium into cells via formate permease, which functions by a proton-symport mechanism [30]. Inside cells formate may bind to specific FhlA protein, which interacts with the transport permease [47] and is susceptible to activation by some formate-sensitive protein [48]. Interestingly, FhlA exhibits ATPase activity that is stimulated by formate [46, 49]. This protein subsequently activates expression of corresponding genes encoding components of formate hydrogen lyase. Induction of *fdhF* and *hycB* gene expression by formate [29] involves FhlA [30, 50, 51]. In all cases the presence of formate inside cells is ultimately required for expression of corresponding genes and synthesis of subunits forming formate hydrogen lyase complex.

Proteins required for synthesis of hydrogenases are encoded by genes organized into the *hyp*-operon [52, 53], in which gene encoding so-called "alternative σ-factor" acts as promoter of this operon [52]. Its expression depends on formate and FhlA [52, 54]. It is suggested that HypA protein is required for Hyd-3 synthesis and *fhlA* gene is the promoter of the *hyp*-operon and FhlA synthesis is self-regulated. HypC forms a complex with the large subunit of Hyd-3 [55, 56]. Interestingly, this complex formation involves cysteine residues of both proteins [57], which form disulfide bonds. It is also suggested that HypB and other protein products of this operon are required for incorporation of nickel (and then iron) into Hyd-3 [55]. They probably participate in maturation of other hydrogenases as well [53]. Regulation of *fdhF* gene expression and *hyc*-operon also involves molybdenum, which may be transported into cells [48, 58]. It is also possible that formate can also induce expression of corresponding genes [59].

Although information on regulation of synthesis of components of formate hydrogen lyase complex by formate becomes increasingly available, a general mechanism for this process still has not been proposed. It should be noted again that formate hydrogen lyase is sensitive to inhibition by exogenous electron acceptors such as oxygen or nitrates. However, the presence of nitrates induces nitrate reductase activity of this complex [30].

F_0F_1-ATPase requirement for enzyme functioning. We have obtained experimental evidence for the requirement of F_0F_1-ATPase for molecular hydrogen production by *E. coli* formate hydrogen lyase. First, H_2 production by *E. coli* cells was sensitive to dicyclohexylcarbodiimide (DCCD), a known F_0F_1-ATPase inhibitor [43, 60-62]. It should be noted that DCCD is the nonspecific inhibitor of F_0F_1-ATPase; it can also inhibit other systems involved in proton translocation [63]. However, a point mutation in the *atp*-operon encoding F_0F_1-ATPase resulted in tolerance of transmembrane proton translocation to DCCD [64]. The latter suggests selective inhibition of F_0F_1 by DCCD. Transmembrane proton translocation was insensitive to inhibition by DCCD during aerobic growth of *E. coli* cells [65] or under anaerobic conditions but in the presence of nitrate [66]. Molecular hydrogen production was not detected in *atp*-mutants lacking functionally competent F_0F_1-ATPase [32, 60-62]. Only *atp*-mutants possessing functionally active F_0F_1-ATPase were able to produce molecular hydrogen, but characteristics of this process were different [61, 62]. Production of molecular hydrogen sensitive to inhibition by DCCD was also found in protoplasts (with increased membrane permeability) in the presence of ATP and formate [62]. In the presence of arsenate and protonophores [43] decreasing proton-motive force, molecular hydrogen was not produced by intact cells.

Production of molecular hydrogen was expectedly not observed in *fdhF* and *hyf*-mutants of *E. coli* lacking the large subunit of FDHH [61, 62] or Hyd-4 subunits [32, 33], respectively. Interestingly, production of molecular hydrogen was detected in various *hyc*-mutants lacking large and small subunits of Hyd-3, but it was not formed in *hycB*-mutant, lacking corresponding protein product [32, 45, 61]. These results suggest that production of molecular hydrogen by *E. coli* in weakly alkaline medium involves formate hydrogen lyase 2, formed by FDHH and Hyd-4 [20]. Requirement of HycB subunit suggests that it represents an important constituent of FDHH.

The requirement of ATPase for molecular hydrogen production is supported by results of independent studies on *Salmonella typhimurium* [67]. Barrett's group demonstrated that DCCD-inhibited production of molecular hydrogen was not observed in *atp*-mutants lacking functionally active F_0F_1-ATPase [67].

How can we explain such requirement for F_0F_1-ATPase? It is possible that under fermentation conditions this ATPase couples ATP hydrolysis with transmembrane proton translocation and therefore it provides transformation of energy accumulated in ATP into proton motive force [68] required for formate hydrogen lyase functioning. Such possibility is quite reasonable if we take into consideration probability of formation of functionally active membrane complex at certain value of proton motive force or at transmembrane proton translocation by this enzyme, which contributes to proton motive force. This does not contradict the hypothesis by Andrews et al. [20] that Hyd-4 includes subunits responsible for transmembrane proton translocation. Although proton translocation by Hyd-4 has not been demonstrated, such assumption seems to be plausible.

Results obtained using *atp*-mutants [31, 32, 60-62] also suggest direct involvement of proton ATPase, which might interact with formate hydrogen lyase 2 and form some multienzyme assembly. In acidic medium when molecular hydrogen is formed due to activity of formate hydrogen lyase 1, F_0F_1-ATPase is also necessary [31, 32].

The role of formate hydrogen lyase in regulation of cytoplasmic pH. In *E. coli* cells formate hydrogen lyase is involved into regulation of cytoplasmic pH value. Such

hypothesis proposed by Bock and Sawers [6] is based on recent observations. First of all, formate hydrogen lyase catalyzes decomposition of formic acid to carbon dioxide and molecular hydrogen [3, 10], i.e., the enzyme neutralizes acidic product of fermentation. This process depends on pH value and formate concentration in the cell [30]. Formic acid is a weak acid that may act as an uncoupling factor dissipating proton motive force [69]. Decrease in pH value potentiates such effect of acids formed during fermentation.

It becomes clear why *E. coli* cells possess two forms of formate hydrogen lyase [20, 31, 32, 45] and why decrease in pH increases expression of genes encoding components of formate hydrogen lyase 1 [30]. However, even in this case formate hydrogen lyase is also involved in regulation of cytoplasmic pH. Some authors suggest that a decrease of pH also increases expression of genes encoding subunits of Hyd-4 that constitute formate hydrogen lyase 2 [20]. Although such expression has not yet been demonstrated, it is hard to assume that both forms of formate hydrogen lyase are simultaneously activated in response to decrease in pH value.

Hyd-4, a component of formate hydrogen lyase 2, may be involved in regulation of cytoplasmic pH due to putative proton translocation [20].

Interaction with other enzymes and formation of multienzyme assemblies. It seems unlikely that formate hydrogen lyase catalyzing terminal reaction of mixed fermentation in *E. coli* cells operates independently from other fermentation enzymes.

This viewpoint can be supported by the following arguments. First, formate hydrogen lyase functioning requires catalytically competent F_0F_1-ATPase. According to our data, formate hydrogen lyase may interact with the main system of potassium uptake in *E. coli*, TrkA. Lack of molecular hydrogen production during potassium depletion was demonstrated long ago [43] and this phenomenon could be explained in various ways. Mutations in this system are accompanied by changes in molecular hydrogen production under weakly alkaline pH [61, 62]. Under these conditions the TrkA system forms membrane proton-potassium pump in the complex with F_0F_1-ATPase [70]; energy transfer from this ATPase to TrkA involves reversible thiol-disulfide transition [32, 62, 71], which requires redox equivalents [72]. According to our model (Fig. 3), FDHH may provide these equivalents that are further utilized by Hyd-4 for molecular hydrogen formation. Such a multiprotein complex also provides maintenance of high gradient of potassium ions between cytoplasm and the external medium; this gradient is important for fermentation [69, 70]. It is also possible that formate hydrogen lyase activity and molecular hydrogen production depend on intracellular concentration of potassium ions and therefore in potassium ions-depleted cells molecular hydrogen production is blocked. Another scenario of fermentation impairment in response to decreased intracellular potassium ion includes reduction of activity of phosphofructokinase and pyruvate kinase [73], which catalyze earlier stages of fermentation (Fig. 1), and this also blocks molecular hydrogen production.

Interaction of formate hydrogen lyase with other enzymes involved into fermentation may be well explained within the concept on assembly of glycolytic enzymes in different cell types [74]. However, arrangement of pathways of sugar metabolism, especially terminal fermentation reactions in bacteria (Fig. 1), includes formation of functional membrane-bound complexes including formate hydrogen lyase; this is very important from structural and energetic viewpoints for regulation under anaerobic conditions.

DO WE KNOW MUCH ABOUT FORMATE HYDROGEN LYASE?

Formate hydrogen lyase is a membrane bound multienzyme complex. It is characterized by the presence of both hydrophobic subunits (which are integral membrane proteins) and hydrophilic subunits (which are peripheral membrane proteins). Subunits contain cysteine ligands in iron-sulfur clusters and redox centers. Formate hydrogen lyase is a nickel-iron dependent enzyme.

Formate hydrogen lyase shares some similarity with Complex I [20, 22, 75] of electron transport chains and, perhaps, exhibits proton-translocating function.

The enzyme is characterized by a complex mechanism of formation and binding to the membrane. Its functioning requires F_0F_1-ATPase, which may also be important for formation of multienzyme assembly. Such assembly plays an important role in generation of proton motive force, detoxification of formate formed during fermentation, and neutralization of the cytoplasm.

Formate hydrogen lyase activity has been found in various bacteria inhabiting different biotopes, under aerobic and anaerobic conditions [7, 67, 76-78]. In these organisms molecular hydrogen is the most important intermediate during degradation of organic compounds in an anaerobic environment. Bacteria living under such conditions accumulate energy during fermentation and use hydrogen production to utilize excess reducing equivalents; pyruvate and formate are main sources of reduced equivalents (Fig. 1). The amount of molecular hydrogen formed by certain bacteria in the human and animal gut or in sediment of reservoirs is not very high because of activity of other bacteria utilizing this hydrogen. Habitants of freshwater reservoirs (e.g., methanogenic archeae) utilize hydrogen for carbon dioxide reduction to methane, and this looks like interspecies transfer of molecular hydrogen. Its formation and utilization involves various hydrogenases.

In all cases formate hydrogen lyase represents an important link in molecular hydrogen production by bac-

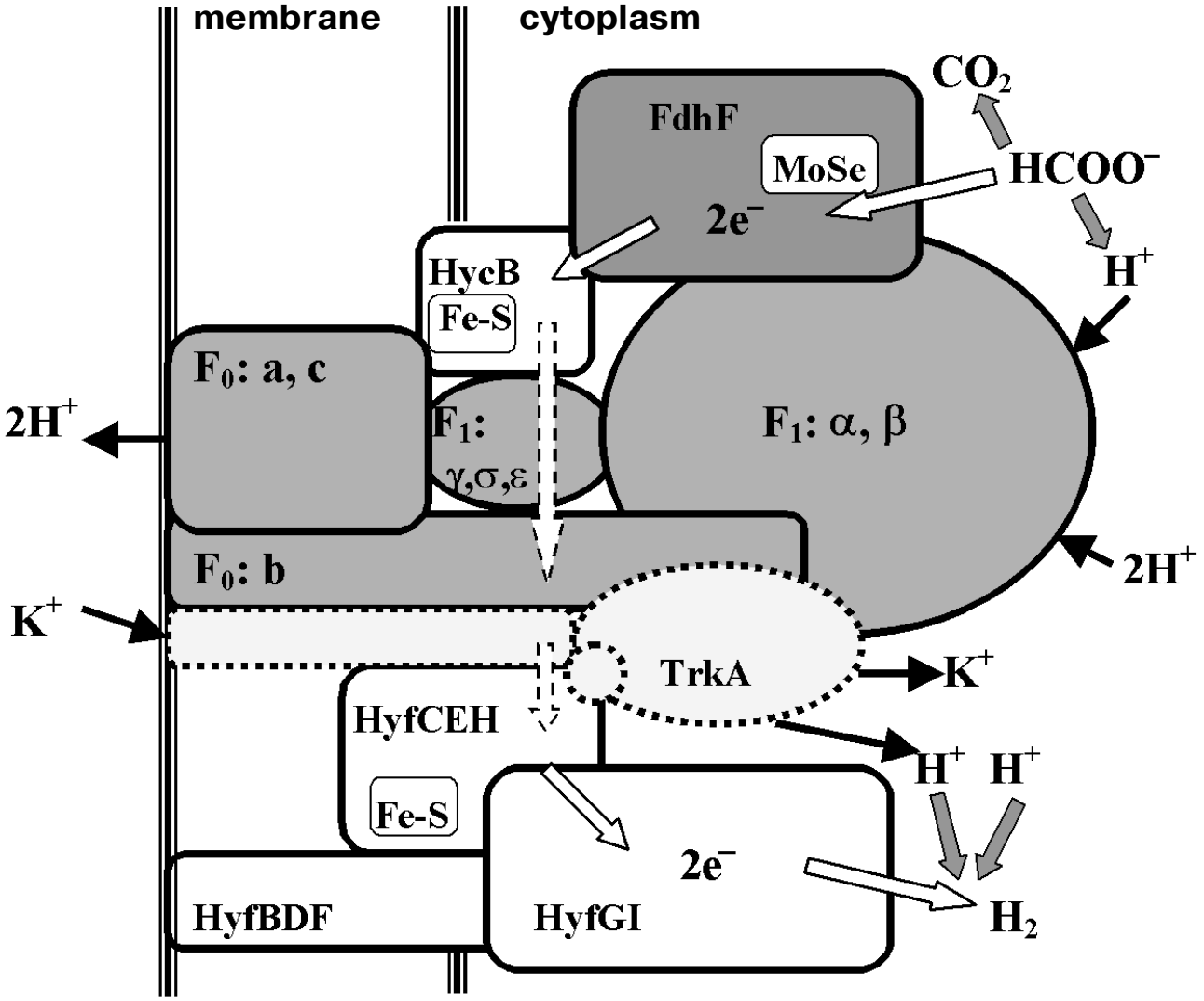


Fig. 3. Putative model of formate hydrogen lyase 2 interaction with F_0F_1-ATPase in membrane of *E. coli* cells. FDHH and the protein product HycB are responsible for supply of reducing equivalents from formic acid. These equivalents are used for energy transfer from F_0F_1-ATPase to potassium transport system TrkA due to thiol-disulfide transition [32, 43, 62, 71] (dashed arrows show the transfer though F_0F_1-ATPase and TrkA) and subsequently they enter Hyd-4 for molecular hydrogen production. F_0F_1-ATPase and TrkA (which consists of three proteins [37, 70]) are shown schematically. Large subunits of F_1 that interact with F_0 via small subunits are shown separately. Two F_0 subunits (*a* and *c*) are localized in membrane and subunit *b* has an extramembrane part that possibly interacts with large subunits of F_0. F_0F_1-ATPase transfers three rather than two protons, one of them is from formic acid. HyfCEH and HyfGI designate corresponding three and two protein products forming Hyd-4. Other explanations are in the text.

teria and this is interesting object for biotechnological hydrogen production.

Although mechanisms of terminal stages of fermentation are rather clear, our knowledge on structure and function of formate hydrogen lyase in *E. coli* and other bacteria is not exhaustive. Subsequent studies of this enzyme would clarify the role of formate hydrogen lyase in regulation of cytoplasmic pH and adaptation of bacteria to media with wide range of pH values, energy transformation in membranes, binding to other transport and enzymatic systems, formation of functional multienzyme assemblies in membrane, and their effectiveness in catalysis of fermentation reactions. If we find clear answers to these questions we will definitely find effective ways to regulate the fermentation process for solution of biotechnological and medical problems.

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