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ՊՐՈՏՈՆԱՅԻՆ F_0F_1-ԱԵՖԱԶԻ ԵՎ ՄՐՋՆԱԹԹՈՒՋՐԱԾԻՆԼԻԱԶԻ ՓՈԽԱԶԴԵՑՈՒԹՅՈՒՆԸ ԱԾԽԱԾՆԻ ՏԱՐԲԵՐ ԱՂԲՅՈՒՐՆԵՐԻ ԵՎ ԴՐԱՆՑ ԽԱՌՆՈՒՐԴՆԵՐԻ ԽՄՈՐՄԱՆ ԸՆԹԱՑՔՈՒՄ

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կենսաբանական գիտությունների թեկնածուի
գիտական աստիճանի հայցման ատենախոսության

ՍԵՂՄԱԳԻՐ

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MINISTRY OF EDUCATION, SCIENCE, CULTURE AND SPORTS OF RA
YEREVAN STATE UNIVERSITY

GEVORGYAN HEGHINE KHAZHAK

THE INTERACTION BETWEEN PROTON F_0F_1-ATPASE AND FORMATE HYDROGEN LYASE DURING FERMENTATION OF DIFFERENT CARBON SOURCES AND THEIR MIXTURES

SYNOPSIS

of dissertation for conferring of science degree of
Candidate of Biological Sciences
In the specialty of 03.00.04-Biochemistry

YEREVAN 2021

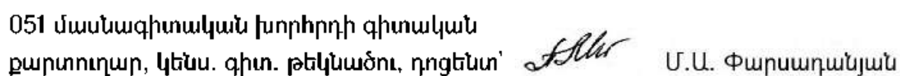
Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում

Գիտական ղեկավար՝	կենս. գիտ. դոկտոր, պրոֆեսոր Կ. Ա. Թռչունյան
Պաշտոնական ընդդիմախոսներ՝	կենս. գիտ. դոկտոր, պրոֆեսոր Կ. Բ. Ենկոյան կենս. գիտ. դոկտոր, պրոֆեսոր Ա. Զ. Փեփոյան
Առաջատար կազմակերպություն՝	ՀՀ ԳԱԱ Հր. Բունիաթյանի անվան կենսաքիմիայի ինստիտուտ

Ատենախոսության պաշտպանությունը տեղի կունենա 2021թ. սեպտեմբերի 17-ին ժամը 14[00]-ին, Երևանի պետական համալսարանում գործող ՀՀ ԲՈԿ-ի Կենսաֆիզիկայի 051 մասնագիտական խորհրդի նիստում (0025, Երևան, Ալեք Մանուկյան փ. 1, ԵՊՀ, կենսաբանության ֆակուլտետ):

Ատենախոսությանը կարելի է ծանոթանալ Երևանի պետական համալսարանի գրադարանում:

Ատենախոսության սեղմագիրն առաքված է 2021թ. օգոստոսի 3-ին:

051 մասնագիտական խորհրդի գիտական
քարտուղար, կենս. գիտ. թեկնածու, դոցենտ՝  Մ.Ա. Փարսադանյան

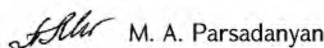
The theme of dissertation has been approved at Yerevan State University

Academic advisor:	Dr. of Biological Sciences, Professor K. Trchounian
Official opponents:	Dr. of Biological Sciences, Professor K. Yenkoyan Dr. Of Biological Sciences, Professor A. Pepoyan
Leading organization:	H. Buniatian Institute of Biochemistry of NAS RA

The defense of the dissertation will be held on 17th September, 2021, at 14:00, at the session of 051 Scientific Specialized Council on Biophysics of SCC of RA at Yerevan State University (0025, Yerevan, Alex Manoogian str. 1, YSU, Faculty of Biology).

The dissertation is available at the library of Yerevan State University.

The synopsis has been sent on 3rd August, 2021.

Scientific Secretary of 051 Specialized Council,
PhD., Associate Professor  M. A. Parsadanyan

INDRODUCTION

Topic's significance. The application areas of the fermentation process are gradually expanding, which makes it possible to obtain the desired end-products through the usage of different substrates and appropriate organisms (Chen at al., 2013). *Escherichia coli* is able to utilize a wide range of carbon sources and perform mixed-acid fermentation. These bacteria assimilate different carbon sources not only separately, but also in mixtures (Trchounian & Trchounian, 2013). The study of the co-fermentation of the latter is important not only from a fundamental, but also from a practical point of view in large-scale production of H_2. H_2 as a biofuel has many advantages: it is renewable, has a high efficiency of 142 kJ g^{-1}, it is not toxic, no air pollution occurs during combustion (Trchounian & Trchounian, 2015). H_2 is produced by four membrane-bound hydrogenases (Hyd). These enzymes produce or oxidize H_2 depending on the fermentation conditions, in particular the pH of the growth medium and the fermenting substrate (Trchounian at al., 2012). To improve H_2 production, the study of the interaction of enzymes involved in H_2 metabolism with other membrane proteins is a priority. As a result of the activity of the Hyds, a hydrogen cycle is formed through the membrane, which is associated with the proton cycle (Trchounian & Sawers, 2014). This interaction takes place between the proton F_0F_1-ATPase and the Hyds involved in the production of H_2, which is aimed to generate proton motive force, thus to maintain bacterial viability. Previous studies have shown that K^+ ions have a significant effect on proton ATPase activity. In particular, Hyd-4, the K^+ transporting Trk system and F_0F_1-ATPase interact during glucose fermentation at pH 7.5. Currently, many studies are aimed at studying the transfer of energy as a result of the interaction of proteins and enzymes, which form complexes in the membrane, and the role of dithiol-disulfide exchange in the mentioned process.

Thus, the determination of biochemical and bioenergetic characteristics during the fermentation of mixed carbon sources (glucose, glycerol, formate) will be applicable to the improvement and regulation of biomass production and H_2 large-scale generation when using different wastes as a mixture of different carbon sources.

Research goals and tasks. The main purpose of this study is to reveal the interaction mechanisms between Formate Dehydrogenases, Hydrogenases and F_0F_1-ATPase during fermentation of mixed carbon sources (glucose, glycerol and formate) at pHs 5.5, 6.5 and 7.5, and role of the mentioned metabolic cross-talk in bacterial growth properties and proton motive force generation.

Constituted tasks of the research were:

- to investigate the role of interaction between proteins involved in the Formate-hydrogen lyase complex and proton F_0F_1-ATPase on the *E. coli* bacterial growth properties and H_2 production at pH 5.5, 6.5 and 7.5 during fermentation of mixed carbon sources.
- to study co-fermentation of glucose, glycerol and formate, generation of fermentation end-products and those variation during bacterial growth.

- to reveal the role of dithiol-disulfide interchange on the mechanisms of intramembrane interaction between FHL components and F_0F_1-ATPase during co-fermentation of mixed carbon sources at pHs 5.5 and 7.5.
- to determine the role of FhlA regulatory protein on the proton F_0F_1-ATPase activity, metabolism and proton motive force generation during the growth at alkaline pH

Scientific novelty and practical value of the study. The results obtained have shown, that *E. coli* is able to growth utilizing and co-fermenting the mixture of glucose, glycerol and formate at pHs 5.5, 6.5 and 7.5. Furthermore, pH 7.5 was optimal for the bacterial growth. It was shown that FHL components have vital role for the growth. These results provide important information about the biomass production. It was revealed that utilization of formate during the lag phase did not oxidize to H_2 and have impact on appropriate enzymes operons transcription and FHL pathway synthesis. Glycerol utilization rate was higher with the glucose co-fermentation at pH 7.5. F_0F_1-ATPase, FDH-H and Hyds interact during fermentation of glucose, glycerol and formate at pH 7.5 and 5.5. It was shown, the potassium transport system has important role for the interaction between FHL components and proton ATPase. Data received demonstrate, that the interactions mentioned occurred via dithiol-disulfide interchange at pH 5.5. Investigation of this mechanism is vital for the regulation of mixed carbon sources fermentation. Data collected using *fhlA* mutant illustrate, that FhlA regulatory protein has a role on the proton ATPase activity and ΔpH regulation during the 20 and 72 h of the growth. Moreover, FhlA regulate substrate utilization and fermentation end-product generation. Data obtained are important to understand how bacteria maintain their viability during co-fermentation of the mixture of carbon sources. Results are applicable for the optimization of biomass production and large-scale H_2 generation using wastes as the mixture of different carbon sources. Furthermore, monitoring and regulation of the biochemical and bioenergetic parameters will provide a development of process of fermentation and increase the output of the desired end-products.

Main points to present at the defense.

1. *E. coli* is able to co-ferment the mixture of glucose, glycerol and formate at pH 5.5, 6.5 and 7.5. Moreover, formate is utilized during the initial phase of the growth.
2. Cells regulate ratio of acids generated during the co-fermentation to balance ΔpH. ΔpH regulation occurs via formate/lactate/acetate at pH 7.5.
3. Interaction between proton F_0F_1-ATPase and FDH-H occurs through SH or thiol groups via dithiol-disulfide interchange (HS-SH -> –S-S-) at pH 5.5.
4. The activity of proton ATPase is higher at 72 h of the growth. Although, the promoted effect of formate on the proton F_0F_1-ATPase activity is not dependent on cell growth phase.
5. FhlA protein regulates the ratio of fermentation end-products and generation of proton motive force' affecting on the proton F_0F_1-ATPase activity.

Work approbation. Main results of the dissertation were discussed at seminars in Department of Biochemistry, Microbiology and Biotechnology, Biology Faculty of Yerevan State University, and at scientific conferences: ASM Microbe 2018 (Atlanta, GA, 2018), 43rd FEBS Congress (Prague, Czech Republic, 2018), 44th FEBS Congress (Krakow, Poland, 2019), "Modern Trends in Biochemistry and Space Biology: The Great Sissakian and the Importance of His Research" International Conference (Yerevan, Armenia, 2019), V International Conference of Biotechnology and Health (Virtual, Yerevan, Armenia, 2020), EuroMicropH 1st Open Meeting (Lisbon, Portugal, 2020), ASM Microbe 2020 (Chicago, USA, 2020), Microbial Stress 2020 meeting (Virtual, Rome, Italy, 2020), 7th International Renewable and Clean Energy Conference (Virtual, Yerevan, Armenia, 2020), EFB 2021 Virtual Conference (Virtual, Barcelona, Spain, 2021), XI International Scientific Conference «Microbial biotechnologies: fundamental and applied aspects» (Virtual, Minsk, Belarus, 2021), World Microbe Forum (Virtual, Online Worldwide, 2021).

Publications. According to experimental data observed in dissertation, 20 papers, including 5 articles in peer-reviewed journals and 15 abstracts were published.

Volume and structure of dissertation. The dissertation contains following chapters: introduction, literature review (Chapter 1),experimental part (Chapter 2), results and discussion (Chapter 3), concluding remarks, conclusions and cited literature (total 185 papers and books).The dissertation consists of 122 pages,7 tables and 29 figures.

MATERIALS AND METHODS

All experiments were done using *E. coli* BW25113 or MC4100 wild type (WT) and appropriate mutants (Table 1).

Table 1. Charachteristics of *E. coli* strains used in this study

Strain	Genotype	Appropriate absent or defective proteins	Reference
BW25113	*rrnB ΔlacZ4787 HsdR514 Δ(araBAD)567 Δ(rhaBAD)568 rph-1 (old genotype: lacIq rrnBT14 ΔlacZW116 hsdR514 ΔaraBADAH33 Δrha BADLD78)*	Wild type	Baba et al., 2006
JW 2701*	BW25113 *ΔfhlA*	FHl activator	Maeda et al., 2007
JW4040*	BW25113 *ΔfdhF*	Formate dehydrogenase H	Baba et al., 2006
KT 2110	BW 25113 *ΔhyaB ΔhybC ΔselC*	Large subunits of Hyd-1 and Hyd-2 and $tRNA^{sec}$	Trchounian et al 2012
MC 4100	*araD139 ΔlacU169 rpsL thi fla*	Wild type	Casadaban et al., 1979
FM 460*	MC 4100 Δ*selC*	$tRNA^{sec}$	Soboh et al., 2011
DHP-F2	MC 4100 *ΔhypF*	All four Hyd enzymes	Trchounian et al., 2012

* Resistant to Kanamycin

Bacterial cultivation and growth conditions. *E. coli* were grown at 37°C for 18-20 h in anaerobic conditions by direct transfer from nutrient agar surface in Petri dish into high buffered liquid peptone growth medium containing 20 g/l peptone, 15 g/l K_2HPO_4, 1.08 g/l KH_2PO_4, 5 g/l NaCl **(pH 7.5)**, 20 g/l peptone, 7.4 g/l K_2HPO_4, 8.6 g/l KH_2PO_4, 5 g/l NaCl **(pH 6.5)** or 20 g/l peptone, 15 g/l KH_2PO_4, 1.08 g/l K_2HPO_4, 5 g/l NaCl **(pH 5.5)**. 10 g/l glycerol, 2 g/l glucose and 0.68 g/l formate were added.

Determination of specific growth rate. The bacterial growth was monitored measuring the optical density (OD_{600}) spectrophotometrically. Specific growth rate (μ) was determined by regular measurement of optical density (OD) until the stationary phase with doubling time as described before (Trchounian et al., 2013). DCCD with 0.2 mM final concentration was used as indicated.

Analytical methods. Utilization of substrates and generation of fermentation end-products were determined by the HPLC with a refractive index detector (set on positive polarity and optical unit temperature of 55°C) (Trchounian et al., 2021). Using Agilent OpenLAB CDS data processing was carried out. C18 column (250 x 4.6 mm) was selected for analysis. The column was purged with acetonitrile/ water (80/20) at 60°C overnight and then regenerated with a mobile phase (5mM sulfuric acid in ddH_2O). The total analysis time of a single injection was 42 min, injection volume was 10 μl. Column temperature was kept at 60°C using a thermostatically controlled column compartment. Analyts were eluted at 0.4 ml min^{-1}. 5mM H_2SO_4 was added into each sample. The substrate utilization rate was calculated as difference of decreasing concentration of substrate per hour, and end-product generation rate was determined as difference of increasing concentration of product per hour and expressed in mmol h^{-1}.

Determination of ATPase activity in membrane vesicles. Bacteria cells were harvested at 20 and 72 hours during growth on mixed carbon sources. Membrane vesicles (MV) were isolated from bacteria treated with lysozyme and ethylenediaminetetraacetic acid by the osmotic lysis of spheroplasts (Konings and Kaback, 1973). ATPase activity was determined by the amount of inorganic phosphate (P_i) produced in the reaction of MV with 5 mM ATP (pH is respective with the growing environment) in 50 mM Tris–HCl buffer (pH is respective with the growing environment) at 37 °C. The ATPase activity was expressed in nmol P_i (min μg protein)$^{-1}$. P_i was determined spectrophotometrically (Taussky & Shorr, 1953). Protein levels were measured by the method of Lowry (Lowry et al., 1951) using bovine serum albumin as a standard. To determine F_OF_1-ATPase activity DCCD with 0.2 mM of final concentration was used. F_OF_1-ATPase activity was calculated as a difference between activities in the absence and in the presence of the inhibitor. To study the effect of potassium ions and external formate in the assays 100 mM KCl and 10 mM sodium formate were added when indicated.

Determination of extracellular and intracellular pH, membrane potential and calculation of proton motive force. For the measurement of external pH (pH_{ex}) during bacterial growth pH-meter with selective pH-electrode was used.

Intracellular pH (pH_{in}) was determined using 9-aminoacridine fluorescent dye (9-AA, with excitation at 390 nm and emission at 460 nm) (Puchkov et al., 1983). 9-AA is distributed across the membrane according to ΔpH. The uptake of 9-AA by bacterial cells was determined from the quenching of fluorescence. ΔpH was calculated as the difference of pH_{in} and pH_{ex} as described elsewhere (Slonszewski et al., 2009).

Membrane potential (ΔΨ) inside negative was measured determining tetraphenylphosphonium cation (TPP^+) distribution between the bacterial cytoplasm and the external medium at a steady state level, as described elsewhere (Zakharyan & Trchounian, 2001). The changes in the TPP^+ concentration in external medium were determined by using a TPP^+-selective electrode. The absorption of TPP^+ on the bacterial cell surface was determined for boiled (during 3 min) cells. Δp was calculated as a sum of ΔΨ and ΔpH according to $\Delta\mu_{H+}/F = \Delta\Psi - Z\Delta pH$ (negative value in mV), where Z is RT/F equal to 61.1 mV at 37 °C.

Measurement of redox potential and determination of hydrogen production. Redox potential (E_h) in bacterial biomass was determined using two different redox, titanium-silicate (Ti-Si) and platinum (Pt) glass electrodes (Trchounian & Trchounian, 2009). The Ti-Si-electrode measures the overall E_h, whereas the Pt-electrode is responsive to H_2 under anaerobic conditions. H_2 production rate (V_{H2}) was calculated as the difference between the initial rates of decrease in Pt- and Ti-Si-electrodes readings and expressed in mV of E_h per min per mg dry weight of bacteria.

Accessible SH-groups assays. Accessible SH-groups were determined by the reaction with Ellmann's reagent, as described (Riddles et al., 1983) using glutathione as a standard. Membrane vesicles were treated with the reagent until the latter was fully reacted, and the optical density became constant. The level of SH-groups was expressed in 10^{-4} mol L^{-1} per mg protein.

Data processing. The average data obtained from 3 independent assays were represented, and standard deviation of values did not exceed 3 %. For the differences between different series of experiments, Student validity criteria (p) were determined using Microsoft Excel 2016; the difference was valid if $p<0.05$.

RESULTS AND DISCUSSION

Effect of DCCD and different concentrations of formate on growth of *E. coli* wild type and mutant strains during mixed carbon sources fermentation at pH 7.5, 6.5, 5.5.

E. coli is able to utilize the mixture of glucose, glycerol and formate anaerobically, as shown before (Mirzoyan at al., 2018). The SGR of *E. coli* bacteria was higher at pH 7.5, than at pH 6.5 and 5.5, which was shown during fermentation of other carbon sources, previously (Blbulyan & Trchounian, 2015).

To understand the role of proton ATPase in SGR, the effect of DCCD was studied at pH 7.5 (Fig. 1). It was shown, that the SGR in the presence of DCCD was decreased in all strains. DCCD suppressed the SGR of wt by ~ 35%, ~ 36% and ~ 47% with addition of 1, 5 and 10 mM formate, respectively. Whereas, overwhelming effect of DCCD was increased in *hypF* and *selC* mutant strains in the presence of respective

amounts of formate. Moreover, effect of DCCD was severe in *fhlA* mutant strain as SGR was decreased by ~ 89%, ~ 89% and 86% when 1, 5 and 10 mM formate was added in the growth medium.

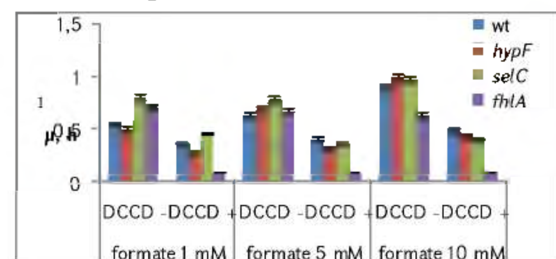


Fig. 1. SGR of *E. coli* wt and mutant strains during fermentation of mixed carbon sources at pH 7.5. DCCD was added to the growth medium with 0.2 mM final concentration. For mutant strains see Table 1. For growth condition and other details see Methods.

Overwhelming effect of DCCD on SGR highlights the essential role of F_OF_1-ATPase, particularly in the lack of Hyd and FDH enzymes, when H_2 generation was absent and H^+ outflow and intracellular pH regulation occurred via F_oF_1 pump were not active. Mentioned membrane-bound enzymes are in interaction and this interplay has a function in bacteria growth and is not dependent on external formate concentration. On the other hand, FhlA protein has its own ATP binding and ATPase activity (Sanchez-Torres et al., 2009), and when the *fhlA* mutant lacks the FhlA protein and the proton ATPase is suppressed, it is suggested that the energy required for bacterial growth is provided less. Formate has a positive effect on bacteria growth during utilization with glucose and glycerol at pH 7.5. The promoting effect of external formate concentration was mentioned in *hypF* and *selC*, but not in *fhlA* mutant strain. It is suggested that the effect of external formate on the growth of bacteria is mediated by FhlA regulatory protein at pH 7.5.

The effect of DCCD was less at pH 6.5, than it was at pH 7.5, and was expressed only in addition of 10 mM formate. The inhibitory influence of DCCD on bacterial growth was shown in the mutant strain absent of FDH enzymes and this effect is dependent on formate concentration at pH 5.5. DCCD weak inhibitory effect might be explained by that F_OF_1-ATPase possibly has such a conformation and position in the membrane that inhibitors, such as DCCD are not able to bond. Another point of view is availability of mechanisms to regulate $\Delta\mu_{H+}$ which are targeted to resist at acidic pH.

Utilization of mixed carbon sources and generation of fermentation end-products at pHs 7.5, 6.5 and 5.5.

The utilization of the glucose, glycerol and formate, and production of fermentation end-products were studied. Externally added formate (10 mM) is utilized by bacteria in the amount of ~2.6 mM during bacterial growth lag phase. This fact demonstrates previous assumptions, that external formate can pass into the cell and have an impact on H_2 production (Trchounian & Trchounian, 2014). After 2 h glucose is starting to be utilized. As a result, one part of the synthesized formate is converted to H_2 and CO_2 by FHL complex (Mirzoyan et al., 2018) and the second part – accumulated in the bacterial cell and exported to the external environment by

specific channels (Hakobyan et al., 2018) (Fig. 2). The main utilization and metabolism of glucose was performed between 2-24 h with utilization rate of 0.39 mM/h. At the mentioned growth period the production rates of formate (~ 0.11 mM/h), acetate (~ 0.42 mM/h), succinate (~ 0.14 mM/h), lactate (~ 0.073 mM/h) and ethanol (~ 0.8 mM/h) were detected. The utilization of glycerol started after 6 h and lasted until 192 h. That is why after 192 h H_2 was not produced. The glycerol utilization rate was 0.712 mM/h. During glycerol fermentation the following end products were generated: acetate (~ 0.22 mM/h), succinate (~ 0.03 mM/h), lactate (~ 0.014 mM/h), ethanol (~ 0.58 mM/h).

The fermentation process is also determined during the utilization of mixed carbon sources at pH 6.5. The amount of glucose was decreased in the growth medium starting from 2 h. The main utilization of glucose was within 2-24 h with 0.45 mM/h rate. ~1.43 mM of external formate imported into the cells during bacterial growth at first hours (Fig. 2). Formate transportation into the cells decreased by 1.8 fold compared with pH 7.5, which regards not only external pH but also with intracellular pH and is suggested to regulate H_2 production and membrane potential. During the glucose fermentation formate (0.13 mM/h) acetate (~ 0.32 mM/h), succinate (~ 0.06 mM/h), lactate (~ 0.29 mM/h), ethanol (~ 0.51 mM/h) were produced with respective production rates. Glycerol utilization started from 3 h and prolonged till 408 h at pH 6.5. However, the main utilization was performed between 3-240 h with 0.027 mM/h rate (Fig. 2). During this period of growth, it was determined the production rates of acetate (~ 0.07 mM/h), succinate (~ 0.014 mM/h) and ethanol (~ 0.29 mM/h). As at pH 7.5, during bacterial growth cells utilized both glucose and glycerol simultaneously at pH 6.5. As the data demonstrate, acetate is the permanent end-product during fermentation all pHs studied. It is due to the formation of additional ATP and NADH + H^+ during acetate generation (Thomas & Marvin, 1970). Co-utilization of glucose and glucerol in the presence of external formate was detected at pH 5.5. Formate transported to the cytoplasm ~ 0.36 mM, which was decreased comared to pH 7.5 and 6.5. This is due to the low pH of extracellular pHand is aimed to regulate ΔpH. Glucose was utilized from 1 h of the growth with 0.3 mM/h rate (1-24 h). Glycerol fermentation was observed from 6 h with 0.25 mM/h rate. Although glycerol was in fermentation, H_2 generation lasted until 48 h. These results prove previously discovered fact, that during the glycerol utilization, H_2 production was absent.

ATPase activity of *E. coli* wild type and mutants membrane vesicles at different pHs

To understand how the cells are surviving during fermentation of mixture of glucose, glycerol and formate, activity of the key enzyme of bioenergetic relevance (the F_0F_1-ATPase) has been investigated. For this, total and DCCD-inhibited ATPase activity at different pH values was determined. Especially, the highest total ATPase activity in *E. coli* BW25113 wt cells membrane vesicles was detected at pH 7.5 resulting in ~182 nMol P_i/min/μg protein (Fig. 2) compared to pH 6.5 and 5.5 where it was decreasing ~1.7 and ~2 fold, respectively (Figs. 2).

It was shown that at all tested pHs in *E. coli* wt DCCD inhibited total ATPase activity by ~ 80-90 % (Fig. 2) which shows that mainly the total ATPase activity is formed due to the F_0F_1-ATPase. The data suggest that during mixed carbon fermentation the F_0F_1-ATPase is always active.

In *hypF* mutant MV, where Hyd enzymes are absent, total ATPase activity was decreased at pH 7.5 and pH 5.5 ~27% and ~45%, respectively, compared to wt. But interestingly no significant differences were detected at pH 6.5. At pH 7.5 DCCD inhibited ATPase activity ~80% in *hypF* mutant which was less compared to wt. This might be due to that Hyd enzymes and the F_0F_1-ATPase are interacting together and there is a metabolic cross talk in between.

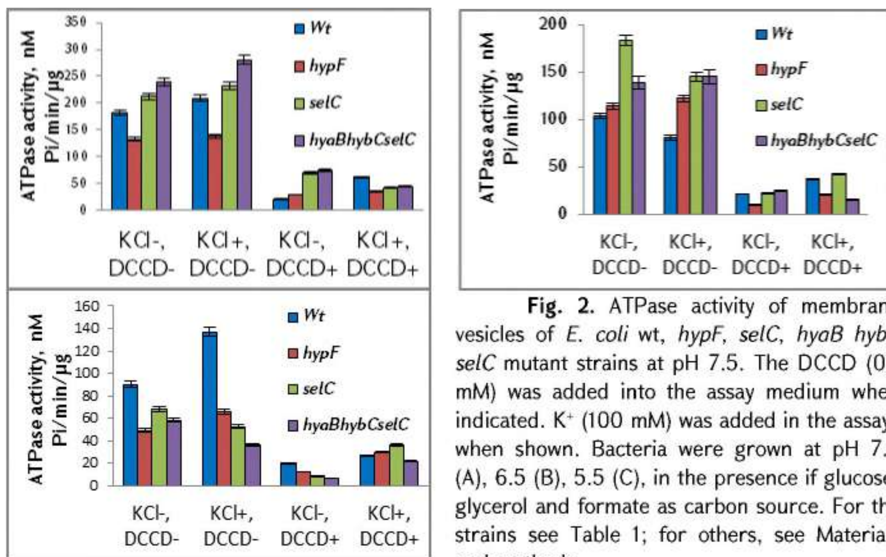


Fig. 2. ATPase activity of membrane vesicles of *E. coli* wt, *hypF*, *selC*, *hyaB hybC selC* mutant strains at pH 7.5. The DCCD (0.1 mM) was added into the assay medium when indicated. K^+ (100 mM) was added in the assays when shown. Bacteria were grown at pH 7.5 (A), 6.5 (B), 5.5 (C), in the presence if glucose, glycerol and formate as carbon source. For the strains see Table 1; for others, see Materials and methods

Interestingly, at pH 7.5 in *selC* and *hyaB hybC selC* mutants the total ATPase activity is higher by ~17% and ~32%, respectively, compared to wt. This suggests that besides Hyd enzymes, FDHs interact with the F_0F_1-ATPase. But DCCD inhibited ATPase activity ~68 % in these two mutants, which was less compared to the wt and *hypF* mutants. These data might be explained as external formate must be neutralized by FDH-H and further transferred to the F_0F_1-ATPase and then to Hyd for H_2 production but in the absence of FDH formate import into the cell does not occur, as there is no FDH which can neutralize formate and thus the F_0F_1-ATPase is less active. Furthermore, it seems that there is direct link between Hyd, FDH and the F_0F_1-ATPase.

At pH 5.5, in all tested mutants total ATPase activity was decreased significantly. But DCCD-inhibited one was stronger in the mutants where FDHs were absent. This suggests that at low pH the role of FDHs is more important than Hyd enzymes. The data are also confirmed by the change of external pH in FDH absent mutants.

DCCD inhibited ATPase activity and number of SH groups at pH 7.5 in *E. coli* wt and FDH deficient mutants

As it was mentioned above, *E. coli* can ferment not only single, but also mixtures of carbon sources (Mirzoyan et al., 2018). Addition of formate in the assays increased the DCCD-inhibited ATPase activity by ~28%. The latter can be explained if formate has direct effect on ATPase or can act via FDH. Interestingly, in the presence of K^+ no effect was determined as had been shown before when the cells where grown during glucose only fermentation (Trchounian & Vassilian, 1994).

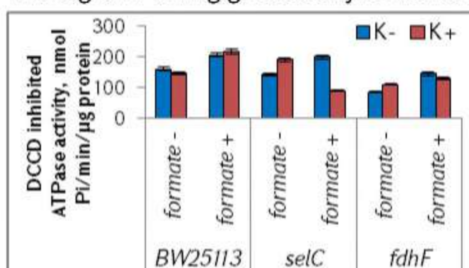


Fig.3 DCCD inhibited ATPase activity of membrane vesicles of *E. coli* wt, *selC*, *fdhF* mutant strains at pH 7.5. The DCCD (0.1 mM) was added into the assay medium. Pota zssium ions (K^+ 100 mM) were added in the assays when indicated as K+. Formate (10 mM) added in the assays indicated as formate+. For the strains see Table 1; for others, see Materials and methods.

To understand if formate could directly interact or affect proton ATPase, mutants that encode FDHs have been used. Especially, in *fdhF* mutant (see Table 1) DCCD-inhibited ATPase activity was ~50% less than in wt suggesting that the absence of FDH disturbs the proton ATPase activity or has conformational changes where DCCD cannot inhibit the enzyme (see Fig. 3). In formate assays DCCD-inhibited ATPase activity increased significantly ~70% compared to the assays without formate.

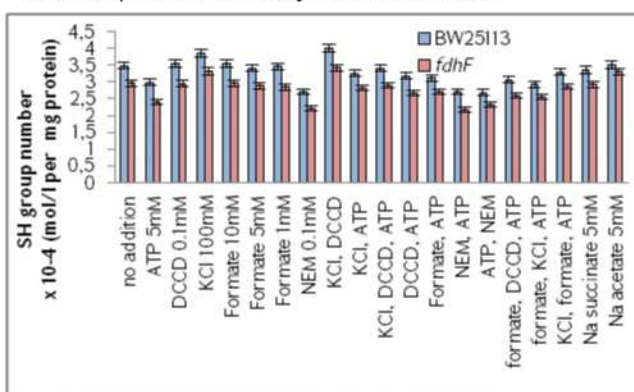


Fig.4 Accessible SH groups number of *E. coli* wt and *fdhF* mutant strains at pH 7.5. For the assays ATP (5mM), K^+ (100 mM), DCCD (0.1 mM), formate (1-10 mM), NEM (0.1mM) was added in the assays. When several reagents were present in the assay the concentration was used as single ones. For others see or Materials and methods.

Suggesting that formate, as a weak acid, can enter into the cell and cause directly conformational changes of proton ATPase which can be more accessible for DCCD in the absence of FDH-H. But such unexpected data were not determined with K^+ clearly showing that K^+ had no effect on FDH and further on proton ATPase. Besides direct interaction, it was shown before that formate increases accessible SH groups and via thiol groups energy is transferred to solute secondary systems from proton ATPase (Trchounian, 2004). To test whether formate is indirectly affecting thiol groups and interacting with membrane-bound systems, accessible SH or thiol groups were measured at this

pH (see Fig. 4). In *fdhF* mutant compared to wt cells accessible SH groups were less by 17%. In the assays addition of K^+, DCCD or formate had no effect in the change of SH groups number.

DCCD inhibited ATPase activity and number of SH groups at pH 5.5 in *E. coli* wt and FDH deficient mutants

In wt cells DCCD-inhibited ATPase activity was ~55% less at pH 5.5 than at pH 7.5 (Fig. 5) which suggests that proton ATPase activity is higher generally at pH 7.5. But in formate assays DCCD-inhibited ATPase activity was significantly decreased ~60% compared to assays without formate (Fig. 5).

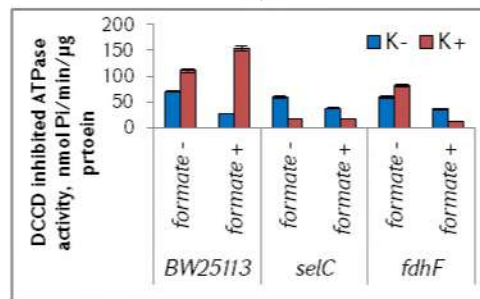


Fig.5 DCCD inhibited ATPase activity of membrane vesicles of *E. coli* wt, *selC*, *fdhF* mutant strains at pH 5.5. For other see legends to Fig. 3.

At low pH (pH 5.5) K^+ showed completely different characteristics and stimulated DCCD-inhibited ATPase activity in the absence/presence of formate ~1.6 fold and ~5.7 fold, respectively. The data clearly demonstrate that K^+ itself affect proton ATPase and secondly via interacting with enzymes of formate metabolism further effect proton ATPase. It can be concluded that K^+ transporting system and proton ATPase can interact together for partially neutralizing formate, in addition to FHL complex, and maintaining ΔpH and, thus, proton motive force. It was shown that in *selC* or *fdhF* single mutants DCCD-inhibited ATPase activity was similar to each other and less ~15% compared to wt (see Fig. 5). But opposite effects were detected in these mutants with K^+. It could not be unanimously explained the effect of K^+ on all FDHs but it is suggested that *selC* gene product is related to K^+ transporting system. The same relationship was shown for pH 7.5. In formate assays K^+ had the same effect and the same values clearly demonstrating that FDH-H, K^+ transporting system and proton ATPase are related together.

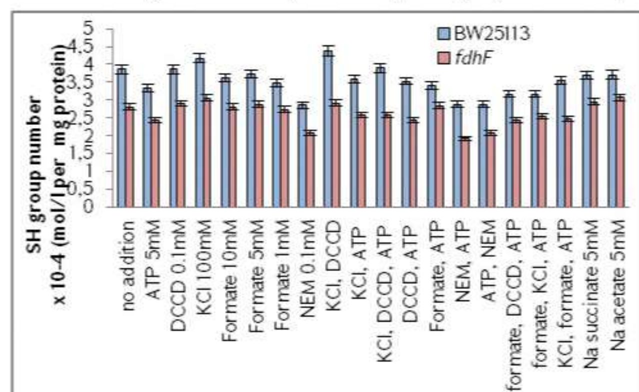


Fig. 6. Accessible SH groups number of *E. coli* wt and *fdhF* mutant strains. Bacteria were grown at pH 5.5. For others see legends to Fig. 4 or Materials and methods

Determination of accessible SH groups showed that in wt cells it was approximately the same as at pH 7.5 but in *fdhF* mutant accessible SH groups were 28% less than in wt (see Fig. 6). Addition of ATP, DCCD and K^+ had the same influence as at pH 7.5. But addition of 1 mM formate decreased the SH groups on ~10% which was not determined in *fdhF* mutant. Also DCCD in the presence of K^+ increased the accessible SH groups by ~15% and no such effect occurred in *fdhF* mutant. The data might indicate that proton ATPase activity can be regulated indirectly via formate and K^+ through dithiol-disulfide interchange between FDH-H and proton ATPase, as suggested ((Trchounian, 2004). And this reaction takes place only when formate and K^+ are present (see Fig. 6).

The assays with K^+, formate and DCCD or ATP clearly show the difference of accessible SH groups in wt and *fdhF* mutant. The results state that F_0 is important for the interaction with other membrane-bound enzymes via SH groups such as FDH-H, otherwise in the assays without DCCD but K^+ no such effects were seen. At pH 5.5 formate decreases the accessible SH groups number, and thus the main regulatory mechanism of interaction of formate with proton ATPase and K^+ transport system is the change in number of accessible SH groups.

It is important to mention that the role of thiol groups for interaction of proton ATPase and Hyd enzymes is activated when K^+ transporting enzymes are related to proton ATPase and Hyd like at pH 7.5, and thus at pH 5.5 in our current conditions most probably the cells have unique mechanism of regulation of energy transfer and proton motive force via or from proton ATPase through FDH or Hyd, and this suggests that proton ATPase, FDH and Hyd are forming complex for regulation of proton gradient when needed for the cell (e.g. energy limited conditions).

Substrates utilization and fermentation end-products generation during fermentation of mixed carbon sources in wt and *fhlA* mutant strain

In all catabolic processes the first and the last steps are the translocations of the substrates into, and of the end products out, of the cell. Formate externally added did not enter into the *fhlA* mutant cells during in the initial stages of growth in contrast wt. Unlike the wt, the assimilation of glucose in the mutant started at 1 hour with 0.48 ± 0.03 mmol h^{-1} rate (1-24 h) at pH 7.5. Synthesized formate from glucose converted to H_2 and CO_2 through FHL complexes (Trchounian at al., 2012) or passed to external environment through membrane FocA/B formate channels in wt (Hakobyan et al., 2018). As a result, cells regulate the external formate concentration (~ 9-10 mM, 3-72 h) in a relatively stable manner (Fig. 7A). Nevertheless, in the mutant strain with defect of FhlA regulatory protein, formate concentration raised up to ~ 30-50 mM (3-216 h) (Fig. 7B). Note, that external added formate did not oxidize to H_2 (H_2 generates after 3 hours, when glucose is fermented (Mirzoyan et al., 2018)). Formate can have impact on appropriate enzymes operons transcription and FHL pathway synthesis as K_m of FhlA for formate is 5 mM (Sawers, 2005). The absence of absorbance of external formate by *fhlA* mutant cells is presumably due to

the lack of enzymes that break down the formate, and the cells did not assimilate what could not be oxidized or is bioenergetically non-favorable.

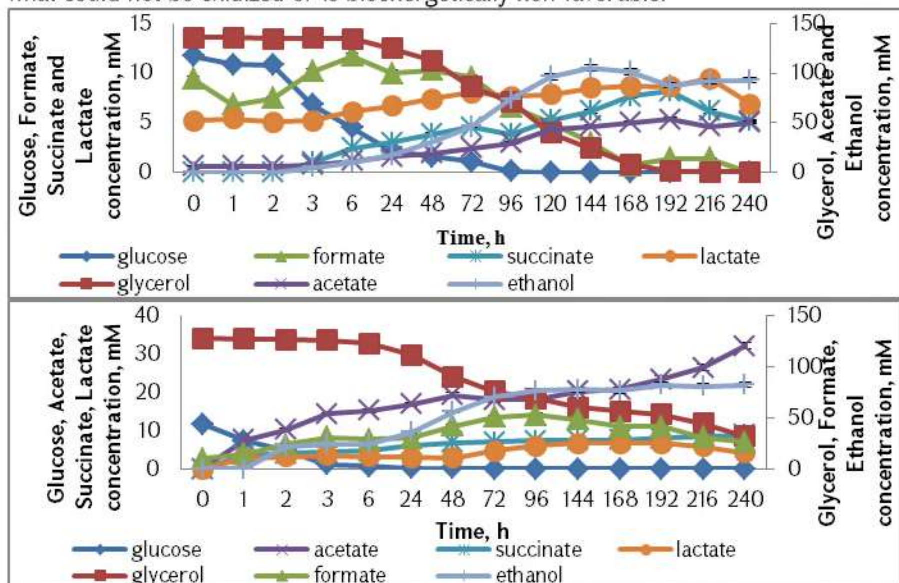


Fig. 7 Utilization of substrates and generation of fermentation end-products by *E. coli* wt (A) and *fhlA* mutant strain (B) grown at pH 7.5. For others see Materials and Methods.

In *fhlA* mutant strain utilization of glycerol started from 2 hours with ~ 0.68 ± 0.03 mmol h^{-1} rate till 24 h, when glucose utilization is observed, and with ~ 0.34 ± 0.03 mmol h^{-1} rate (24-192 h), when glycerol is sole carbon substrate (See Fig. 7AB). As glycerol fermentation extended reasonably later in wt, the quantitative and qualitative difference between end-products from glucose and glycerol at pH 7.5 reported being apparent. The reduced utilization rate of glycerol in *fhlA* mutant cells after 24 h is explained by the redox state of medium (Poladyan et al., 2012). ORP value was – 350 to + 72 ±10 mV (24-192 h) in fhlA, which is not high reducing condition for glycerol utilization, compared to wt (-536 ±10 mV). As glycerol is more reduced substrate, ethanol, succinate and NADH + H^+ generation during utilization of glycerol is more, compared to glucose utilization (44). In this case, combined with the formation of ethanol, NADH + H^+ to NAD^+ exchange is efficient, which stimulate glycolysis continuity (Dharmadi et al., 2006). It can interpret glucose higher utilization rate in mutant strain. From the data it is suggested new regulation and role of FhlA: glycerol assimilation promote glucose utilization rate during co-fermentation in the presence of external formate, when FHL complexes are not functional at pH 7.5. However, in *fhlA* mutant acetate generation rate became lower after 48 h (0.28 ± 0.03 mmol h^{-1}). At the same time formate concentration became higher.

Acetate concentration became lower by 5 - 30 ± 0.03 mM (72-240 h) compared with wt. It unites with ATPase activity distinction between 20 and 72 hours at pH 7.5. Moreover, variation of the ratio of ethanol/acetate (24-72 h) in wt was coupled with ORP reduction (-536 to -584 ± 10 mV), as suggested before (Riondet et al., 2000). Meanwhile, the increase of the ethanol/acetate ratio during the growth was not ORP dependent in *fhlA* mutant strain. This phenomenon might be described by high concentration of formate, which can serve as a source of redox equivalents for ethanol synthesis. On the other hand, decrements of acetate amount compensated formate high concentration as weak acid. Afterwards, formate concentration was decreased starting at 96-144 h. Despite other acids (acetate, succinate, but not lactate) have continued to emerge, the pH_{ex} was gradually alkalizing (by ~ 0.23 unit). It suggests the essential role of formate and lactate on ΔpH, and consequently on Δp at pH 7.5. This is persuasive evidence for that lactate distribution across the membrane is a function of the pH_{in} and pH_{ex} (Harold et al., 1974).

Proton ATPase activity during fermentation of mixed carbon sources in wt and *fhlA* mutant strain

It was investigated the overall (data are not shown) and proton ATPase activity at 20 and 72 hours during mixed carbon sources fermentation assuming that the change in the ratio of assimilated substrates at different hours of growth would lead to a change in the activity of proton F_0F_1-ATPase (Fig. 8). As a result, proton ATPase activity was increased by ~ 50 % in wt and was decreased by ~ 70 % in *fhlA* mutant strain at 72 h compared to the values of enzyme activity at 20 h of growth at pH 7.5 (Fig. 8). Increased proton ATPase activity at 72 h (compared to 20 h) at pH 7.5 in wt is probably due to the acceleration of metabolic processes, which in turn requires the hydrolysis of the high-energy molecules, such as ATP. The same way, acids generated during fermentation acidified intracellular medium, which lead to release acids or protons to external environment to regulate ΔpH (Fig. 9) and balance acids distribution on both sides of the membrane.

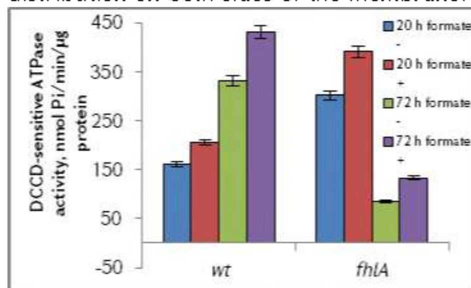


Fig. 8 Proton ATPase activity of membrane vesicles of *E. coli* wt and *fhlA* mutant strain at pH 7.5 during the growth at 20 and 72 hours in the presence of mixture of glucose, glycerol and formate. For others, see Materials and methods.

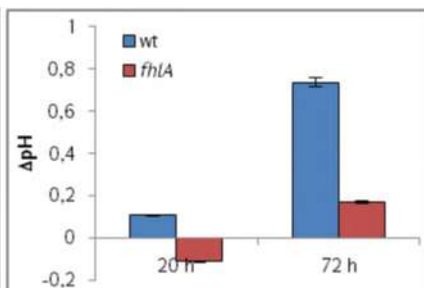


Fig. 9 The values of ΔpH of *E. coli* wt and *fhlA* mutant strain at pH 7.5 during the growth at 20 and 72 hours in the presence of mixture of glucose, glycerol and formate. For others, see Materials and methods.

In *fhlA* mutant strain glycerol was remained as carbon substrate at 72 h, which utilization rate, thereby acids generation was lower (see Fig. 7B). Under these conditions, the translocation of protons was suggested quite limited, which is shown by ΔpH value at 72 h. Indicated alterations caused reduction of proton ATPase activity. Formate added in the assays had promoted effect on proton ATPase activity both at 20 and 72 hours (Fig. 8) at pH 7.5, suggesting that formate effect is not dependent on cell growth phase at slight alkaline pH. The increase of F_oF_1-ATPase activity in the presence of external formate supposes that F_oF_1 is formate-dependent and formate can directly effect on F_o conformation.

Proton motive force generation during fermentation of mixed carbon sources in wt and *fhlA* mutant strain

As discussed, process of fermentation, transport of acids and activity of proton F_oF_1-ATPase 'work and collaborate' together to maintain ΔpH and thus Δp. pH_{in} was 6.770 ± 0.02 in wt at 20 h of growth. It was low in *fhlA* mutant strain by 0.195 unitsFurthermore, the negative ΔpH value was shown only in *fhlA* mutant strain at 20 h. It was mentioned alkalization of pH_{in} at 72 h of growth by 0.497 units in wt, in comparison to 20 h growing. The alkalization of pH_{in} was detected in *fhlA* mutant strain, too, The excretion of acids acidified the pH_{ex} by 0.143 units.

In *fhlA* mutant strain the pH_{in} alkalization is relatively low, compared with wt, which is explained by formate accumulation and lack of the ability for its neutralization. Furthermore, FHL complexes have a role in proton consumption and are responsible for pH sensitivity (Harold et al., 1974). The negative ΔpH in *fhlA* mutant strain at 20 h at pH 7.5 testifies that glycolysis was at high speed and extrusion of generated acids was not completely decreasing pH_{in} and maintains ΔpH. *fhlA* mutant strain maintained ΔpH value near zero: either positive or negative during the growth (Fig. 9). Besides, the role of components in FHL complexes is proposed in ΔpH generation during fermentation of glucose, glycerol and formate at pH 7.5, which was discussed above based on data of μ and F_oF_1-ATPase activity.

ΔΨ was -150 ± 5 mV and -158 ± 5 mV in wt and FhlA lacking cells, respectively, during fermentation of glucose glycerol and formate at pH 7.5. The ΔΨ value did not change either the cells were grown for 20 or 72 h in wt or mutant strain. Thus, wt cells generated Δp of -144 ± 5 mV during the 20 h of growth. It was decreased by 30% after 72 h of growth reaching to -105 ± 5 mV. Essential changes of Δp were not mentioned in *fhlA* mutant cells during the fermentation. ΔΨ is constant during the growth of *E. coli* bacteria. Moreover, FhlA regulatory protein does not have significant role in the generation of ΔΨ. Thus, variation of Δp is conditioned by the changes of chemical component - ΔpH. The high value of Δp in *fhlA* strain is conditioned by F_oF_1-ATPase high activity in 20 h of growth. Besides, elevated concentration of acids (especially formate) generated during the fermentation have stimulatory impact on Δp generation.

CONCLUDING REMARKS

Summarizing the data received it is significant to emphasize, that *E. coli* utilize the mixture of glucose, glycerol, formate anaerobically. Moreover, pH 7.5 is the optimum condition for *E. coli* bacterial growth during the co-fermentation of glucose and glycerol in the presence of formate. Furthermore, the influence of DCCD is considerable at pH 7.5. The mediated role of FhlA in the promotion of bacterial growth in the presence of external formate is suggested. It is demonstrated FhlA regulatory protein compensatory effect on SGR in the terms of proton ATPase inhibition by DCCD at pH 7.5 and 6.5 with addition of 10 mM formate. Moreover, the SGR is formate concentration dependent at all pHs. Mainly, acetate is always generated during co-fermentation for supplying high-energetic molecules. Ethanol and succinate are synthesized in relatively high amounts to balance redox state. It can be concluded, that fermentation substrates force the cells to survive via balancing end products formation through choosing bioenergetically favorable pathways.

The results obtained clearly demonstrate mainly the role of F_0F_1-ATPase at pH 5.5 and pH 7.5 and its relationship with FDHs and Hyd enzymes. Moreover, effects of potassium ions were revealed depending on the mutant and pH. Correlation between external pH and role of FDHs in the F_0F_1-ATPase activity were detected. Especially, higher effect of FDHs was shown when external pH decreased. Moreover, it seems that in all conditions the F_0F_1-ATPase and Hyd enzymes compensate each other in terms of H^+ transport and previously proposed relationship of H_2 and H^+ cycles (Trchounian & Sawers, 2014) might be the reason why in Hyd deficient mutant the medium is acidified and affects the F_0F_1-ATPase activity.

It was shown that during mixed carbon fermentation at low pH proton ATPase activity depends on the presence of formate and potassium ions. Moreover, it was experimentally shown that proton ATPase and FDH-H might interact together via accessible SH or thiol groups through dithiol-disulfide (-SH-HS to –S-S) interchange. The unique mechanism of interaction of proton ATPase, potassium transporting enzymes and enzymes of formate metabolism such as formate dehydrogenases might be important for understanding how the bacterial cells survive at acidic pH.

The effect of FhlA regulatory protein on fermentative utilization of mixed carbon sources (glucose, glycerol, formate), generation of end-products and Δp, F_0F_1-ATPase activity during growth (at 20 h and 72 hours) was observed. It was shown the interplay between amounts of generated acids and F_0F_1-ATPase activity and this interaction was aimed to regulate ΔpH and consequently Δp. $\Delta\Psi$ is constant during the growth of *E. coli* bacteria. Furthermore, FhlA regulatory protein does not have significant role in the generation of $\Delta\Psi$. It was noticed stimulated glucose utilization in the presence of glycerol at pH 7.5. It is significant to mention that utilized external formate during lag phase of growth did not form H_2, and may have regulatory influence for H_2 metabolism enzymes. The ratio of produced acids suggested that bacterial cells maintain ΔpH via acetate-formate-lactate exchange at pH 7.5. It was demonstrated the essential role of FhlA regulatory protein on F_0F_1-ATPase activity and ΔpH regulation, assuming the involvement of component of FHL complexes in Δp generation.

Finally, it is important to mention that the investigation of fermentation of mixtures of carbon sources and identification of enzyme activities and their link with each other is very important for applications in different fields of industry. Thus, our researches will

continue to reveal the probable interaction between DCCD-sensitive ATPase and channels of generated acids during mixed carbon fermentation. Moreover, the discovering of exact component involved in bacterial growth and ΔpH maintenance is also an exploration object.

CONCLUSIONS

The following conclusions were made based on experimentally obtained results:

1. pH 7.5 is the optimal condition for the *E. coli* bacterial growth, metabolism and proton ATPase activity during fermentation of mixed carbon sources.
2. *E. coli* is able to co-ferment the mixture of glucose and glycerol in the presence of external formate at all pHs studied. Moreover, utilization of the glycerol has promoting effect on the glucose consumption. Acetate is generated as the main end-product to provide with high-energetic molecule.
3. Proton ATPase and potassium transport system interact with FHL components for formate neutralization and consequently intracellular pH regulation at pH 7.5 and 5.5.
4. The promoting effect of formate and K^+ ions on the proton ATPase activity occurs indirectly via decrease of available thiol groups and dithiol-disulfide interchange between FDH-H and F_0F_1 at pH 5.5.
5. The important role of the FhlA regulatory protein in bacterial growth characteristics, proton ATPase activity, but not in the generation of ΔΨ has been identified. Its effect on the change of Δp is due to the change of the chemical component ΔpH.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC

1. **Gevorgyan Kh. H.**, Vassilian V. A., Trchounian A. K. (2021) The role of proton ATPase specific inhibitor *N,N'*-dicyclohexylcarbodiimide and external formate concentration on *E. coli* growth during mixed carbon sources fermentation at different pHs. ***PROCEEDINGS OF THE YSU, Chemistry and Biology*** 55 (1), 67-74,
2. **Gevorgyan H.**, Khalatyan S., Vassilian A., Trchounian K. (2021) The role of *Escherichia coli* FhlA transcriptional activator in generation of proton motive force and F_0F_1-ATPase activity at pH 7.5, ***IUBMB Life*** 73 (6): 883-892,
3. **Gevorgyan H.** (2020) The utilization of carbon sources mixture (glucose, glycerol and formate) and generation of fermentation end-products by *Escherichia coli*, ***PROCEEDINGS OF THE YSU, Chemistry and Biology*** 54 (1), 55-62,
4. **Gevorgyan H.**, Trchounian A., Trchounian K. (2019) Formate and potassium ions affect *Escherichia coli* proton ATPase activity at low pH during mixed carbon fermentation, ***IUBMB Life*** 72, 915-921,
5. **Gevorgyan H.**, Trchounian A., Trchounian K. (2018) Understanding the role of *Escherichia coli* hydrogenases and formate dehydrogenases in the F_0F_1-ATPase activity during the mixed acid fermentation of mixture of carbon sources, ***IUBMB Life*** 70 (10), 1040–1047,
6. **Gevorgyan H.**, Poladyan A., Trchounian K. (2021) The role of formate neutralization and molecular hydrogen generation in the metabolic flux in *Escherichia coli* during fermentation of mixed carbon sources, ***Proceedings of the XII International Scientific***

Conference «Microbial biotechnologies: fundamental and applied aspects», ISBN 978-985-08-2731-9, p.12-13

7. **Gevorgyan H.**, Trchounian A., Trchounian K. (2021) The effect of molecular hydrogen generation on the metabolic network formation during fermentation of mixed carbon sources in *Escherichia coli* at pH 7.5, ***Proceedings of the 7th International Renewable and Clean Energy Conference***, 7, ISBN 978-9939-1-1257-2, p.60-64
8. Khalatyan S., **Gevorgyan H.**, Trchounian K. (2021) Role of FhlA Regulatory Protein in the Assimilation of Substrates and Generation of fermentation end-products in *Escherichia coli* at acidic pH, ***World Microbe Forum meeting abstracts***, WMF 21-1483.
9. Khalatyan S., **Gevorgyan H.**, Trchounian K. (2021) The Role of FhlA Regulatory Protein in *Escherichia coli* ATPase Activity at pH 5.5 During Fermentation of Mixed Carbon Sources, ***World Microbe Forum meeting abstracts,*** WMF 21-2033.
10. **Gevorgyan H.**, Trchounian K. (2021) The Role of Hydrogenases on the Interplay Between Potassium Transport System and F_0F_1-ATPase During the Co-fermentation of Mixed Carbon Sources in *E. coli* at pH 7.5, ***World Microbe Forum abstracts***, WMF21-1521
11. **Gevorgyan H.**, Trchounian K. (2021) The Role of Molecular Hydrogen Generation in the Metabolic Flux During Co-utilization of Mixed Carbon Sources in *Escherichia coli* at pH 6.5, ***World Microbe Forum meeting abstracts,*** WMF 21-2029
12. **Gevorgyan H.**, Khalatyan S., Trchounian A., Trchounian K. (2020) Study of Co-Fermentation of Glucose and Glycerol in the Presence of External Formate in *Escherichia Coli* Bacteria at pH 6.5. The Role of Fhla Regulatory Protein, ***V INTERNATIONAL CONFERENCE OF BIOTECHNOLOGY AND HEALTH, Book of Abstracts,*** 63-64
13. **Gevorgyan Kh. H.** (2020) The significant role of FhlA protein in *Escherichia coli* growth, proton ATPase activity and ΔpH regulation during mixed carbon fermentation at pH 6.5, ***II International Book Edition of the countries of the Commonwealth of Independent States «BEST YOUNG SCIENTIST– 2020»,*** 160-163
14. **Gevorgyan H.**, Trchounian A., Trchounian K. (2020) pH homeostasis in *Escherichia coli* at acidic pH during fermentation of glucose and glycerol in the presence of external formate, ***Microbial Stress 2020 Abstract book***, 50
15. Trchounian K., Trchounian A., **Gevorgyan H.**, Vassilian A. (2020) Simultaneous Utilization of Glucose and Glycerol in the Presence of External Formate by *E. coli* at Slightly Alkaline Ph, ***ASMMicrobe2020 Abstracts,*** MBP06
16. **Gevorgyan H.**, Trchounian A., Trchounian K. (2020) Interaction of membrane-bound enzymes related to proton transport and hydrogen production in *Escherichia coli* at acidic pH, ***EuroMicropH 1st Open Meeting 2020 BOOK OF ABSTRACTS***, 37
17. **Gevorgyan H.**, Trchounian A., Trchounian K. (2019) Investigation of *Escherichia coli* bacteria growth during fermentation of mixed carbon sources at different external formate concentration, **Conference proceedings** "***Modern Trends in Biochemistry and Space Biology: The Great Sissakian and the Importance of His Research***", ISBN 978-9939-50-460-5, p. 48-51.
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19. **Gevorgyan H.**, Poladyan A., Trchounian A., Trchounian K. (2018) The relationship of *Escherichia coli* Hyd enzymes with the F_0F_1-ATPase during fermentation of mixture of carbon sources, ***FEBS OPEN BIO 8***, 358-358
20. **Gevorgyan H.**, Vassilian A., Sawers G., Trchounian A, Trchounian K. (2018) Effect of hydrogenases on the F_0F_1-ATPase activity in *Escherichia coli* during fermentation of glucose, glycerol and formate, ***ASM Microbe 2018 Abstracts,*** 3633

ԳԵՎՈՐԳՅԱՆ ՀԵՂԻՆԵ ԽԱԺԱԿԻ

ՊՐՈՏՈՆԱՅԻՆ F_0F_1-ԱԵՖԱԶԻ ԵՎ ՄՐՋՆԱԹԹՈՒՋՐԱԾԻՆԼԻԱԶԻ ՓՈԽԱԶԴԵՑՈՒԹՅՈՒՆԸ ԱԾԽԱԾՆԻ ՏԱՐԲԵՐ ԱՂԲՅՈՒՐՆԵՐԻ ԵՎ ԴՐԱՆՑ ԽԱՌՆՈՒՐԴՆԵՐԻ ԽՄՈՐՄԱՆ ԸՆԹԱՑՔՈՒՄ

Ամփոփագիր

Հանգույցային բառեր՝ *Escherichia coli*, ածխածնի աղբյուրների խառնուրդների համախմորում, պրոտոնային F_0F_1-ԱԵՖազ, մրջնաթթու, հիդրոգենազներ (Հիդ), մրջնաթթուջրածինլիազ համալիրներ, ներթաղանթային փոխազդեցություն, պրոտոնաշարժ ուժ, FhlA կարգավորիչ սպիտակուց:

Այս աշխատանքը նվիրված է *E. coli*-ում ածխածնի խառն աղբյուրների՝ գլյուկոզի, գլիցերոլի և մրջնաթթվի խմորման պայմաններում մրջնաթթուդեհիդրոգենազների, Հիդ ֆերմենտների և F_0F_1-ԱԵՖազի փոխազդեցության մեխանիզմների ուսումնասիրմանը միջավայրի pH-ի 7.5, 6.5, 5.5 արժեքներում, ինչպես նաև այդ փոխազդեցության նշանակության բացահայտմանը բակտերիաների աճման և պրոտոնաշարժ ուժի առաջացման գործընթացում: Ուսումնասիրվել է նաև տարբեր թթուների և խառը խմորման այլ վերջնանյութերի առաջացումը, դրանց հարաբերակցության փոփոխությունը բակտերիաների աճման ընթացքում: Հետազոտվել է FhlA կարգավորիչ սպիտակուցի դերը պրոտոնային F_0F_1-ԱԵՖազային ակտիվության, նյութափոխանակության և պրոտոնաշարժ ուժի առաջացման գործընթացում աճման ընթացքում միջավայրի հիմնային պայմաններում:

Ստացված տվյալները ցույց են տվել, որ *E. coli*-ն համախմորում է գլյուկոզի և գլիցերոլի խառնուրդը մրջնաթթվի ներկայությամբ ուսումնասիրված բոլոր pH-ներում: Բացահայտվել է, որ pH 7.5-ը ամենաբարենպաստ պայմանն է *E. coli* բակտերիաների աճման և պրոտոնային ԱԵՖազի ակտիվության համար ածխածնի խառն աղբյուրների խմորման պայմաններում:

Աճման սկզբնական ժամերին բակտերիաները յուրացնում են մրջնաթթուն: Քացախաթթուն առաջանում է՝ որպես հիմնական վերջնանյութ բջջին էներգիայով հարուստ մոլեկուլով ապահովման համար: Դեռ ավելին, բջիջները կարգավորում են համախմորման արդյունքում առաջացող թթուների հարաբերակցությունը, ինչն ուղղված է ΔpH-ի կարգավորմանը: Բացահայտվել է, որ pH 7.5-ում ΔpH-ի կարգավորումն իրականանում է մրջնաթթու/կաթնաթթու/քացախաթթու փոխանակման միջոցով:

E. coli Հիդ-ների և ՄԴՀ-ների սինթեզն ու կարգավորումն իրականացնող սպիտակուցների գենետիկական խանգարումներով տարբեր մուտանտների օգտագործմամբ պարզվել է, որ պրոտոնային ԱԵՖազը և K^+ իոններ տեղափոխող համակարգը փոխազդում են ՄՋԼ համալիրի բաղադրիչների հետ pH 7.5 և 5.5 արժեքներում: Ցույց է տրվել, որ pH 5.5-ում մրջնաթթվի և K^+ իոնների խթանիչ ազդեցությունը պրոտոնային ԱԵՖազի ակտիվության վրա

անուղղակի է և իրականանում է ՄԴՀ-H-ի ու F_oF_1-ի միջև երկթիոլ-երկսուլֆիդ փոխանակման միջոցով: Մինչդեռ, հիմնային pH-ում նման ազդեցությունը միջնորդված չէ թիոլ խմբերի վերօքս կարգավիճակով:

Նկատվել է պրոտոնային ԱԵՖազի ակտիվության արժեքի մեծացում բակտերիաների աճման 72-րդ ժամում: Այնուամենայնիվ, մրջնաթթվի խթանիչ ազդեցությունը F_oF_1-ԱԵՖազային ակտիվության վրա կախված չէ աճման ժամանակից:

Ցույց է տրվել, որ FhlA կարգավորիչ սպիտակուցն ունի կարևոր նշանակություն բակտերիաների աճման բնութագրերի և պրոտոնային ԱԵՖազային ակտիվության վրա: Այս սպիտակուցի ազդեցությունը Δp-ի փոփոխության վրա պայմանավորված է քիմիական բաղադրիչի՝ ΔpH-ի փոփոխությամբ:

Ստացված տվյալները կարևոր են հասկանալու, թե ինչպես են բակտերիաները պահպանում իրենց կենսունակությունը ածխածնի աղբյուրների խառնուրդի համախմորման պայմաններում: Տվյալները կիրառելի են հետագայում թափոնների՝ որպես ածխածնի խառն աղբյուրների յուրացման պայմաններում բակտերիաների կենսազանգվածի ստացման և H_2-ի մեծածավալ արտադրության բարելավման համար: Դեռ ավելին, կենսաքիմիական և կենսաէներգետիկական բնութագրերի կարգավորումը կնպաստի խմորման գործընթացի բարելավմանը և ցանկալի վերջնանյութի ելքի մեծացմանը:

ГЕВОРГЯН ЭГИНЕ ХАЖАКОВНА

ВЗАИМОДЕЙСТВИЕ ПРОТОННОЙ F_oF_1-АТФАЗЫ И ФОРМИАТ-ВОДОРОД-ЛИАЗЫ ПРИ ФЕРМЕНТАЦИИ РАЗЛИЧНЫХ ИСТОЧНИКОВ УГЛЕРОДА И ИХ СМЕСЕЙ

РЕЗЮМЕ

Ключевые слова: *Escherichia coli*, ферментация смесей источников углерода, протонная F_oF_1-АТФаза, формиат, гидрогеназы (Гид), формиат-водород-лиаза комплексы, внутримембранное взаимодействие, протон-движущая сила, регуляторный белок FhlA

Эта работа посвящена изучению механизмов взаимодействия формиат-дегидрогеназы, Гид ферментов и F_oF_1-АТФазы при ферментации смешанных источников углерода: глюкозы, глицерина и формиата при pH 7.5, 6.5, 5.5, а также выявлению значения этого взаимодействия в процессе роста бактерий и генерации протон-движущей силы. Также были изучены образование различных кислот и других конечных продуктов смешанного брожения, изменение их соотношения в процессе роста бактерий. Была исследована роль регуляторного белка FhlA в развитии активности протонной F_oF_1-АТФазы, метаболизме и генерации протон-движущей силы во время роста при слабощелочном pH.

Результаты показали, что *E. coli* способна осуществлять смешанное сбраживание глюкозы и глицерина в присутствии экзогенного формиата при всех изученных pH. Было обнаружено, что pH 7.5 является оптимальным условием для роста бактерий *E. coli* и активности протонной АТФазы при сбраживания смешанных источников углерода.

Бактерии утилизировали формиат на ранних стадиях роста. Уксусная кислота образуется в качестве основного конечного продукта, обеспечивающего клетку высокоэнергетической молекулой. Более того, клетки регулируют соотношение кислот, образующихся при брожении, что направлено на регулирование ΔpH. Было обнаружено, что регулирование ΔpH при pH 7.5 достигается за счет обмена формиат/лактат/ацетат.

Использование различных мутантов *E. coli* в генетических нарушениях белков исполняющих регуляцию и синтез Гид и ФДГ, показало, что протонная F_0F_1-АТФаза и система транспорта ионов K^+ взаимодействуют с компонентами комплекса ФВЛ при pH 7.5 и 5.5. Было показано, что стимулирующее действие формиата и ионов K^+ на активность протонной АТФазы при pH 5,5 осуществляется опосредованно путем обмена дитиол-дисульфида между ФДГ-Н и F_0F_1. Однако при pH 7.5 такой эффект не опосредован окислительно-восстановительным статусом тиоловых групп.

Увеличение активности протонной АТФ-азы наблюдалось в течение 72-го часа роста бактерий. Однако стимулирующее действие формиата на активность F_0F_1-АТФазы не зависит от времени роста.

Было показано, что регуляторный белок FhlA играет важную роль в характеристиках роста бактерий и активности протонной АТФазы. Влияние этого белка на изменение Δp обусловлено изменением химического компонента ΔpH. Эти данные можно использовать, чтобы понять, как бактерии поддерживают свою жизнеспособность при сбраживании смешанных источников углерода.

Данные могут быть использованы для производства бактериальной биомассы и дальнейшего улучшения производства H_2 при использовании отходов в качестве смешанных источников углерода. Более того, регулирование биохимических и биоэнергетических характеристик улучшит процесс ферметации и увеличит выход желаемого конечного продукта.