e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2023; 13(8); 315-320

Original Research Article

Changes in Escherichia Coli's Cellular Physiology Due to Impaired Oxidative Phosphorylation Caused by Faulty F1-ATPase

Santosh Singh[1], Lipika Jena[2], Diptimayee Jena[3], Kundan Ku Sahu[4]

[1]Professor, Department of General Medicine, IMS & SUM Hospital, Bhubaneshwar, Odisha, India
[2]Assistant Professor, Department of Microbiology, IMS & SUM Hospital, Bhubaneshwar, Odisha, India
[3]Assistant Professor, Department of Biochemistry, Shri Jagannath Medical College, Puri, Odisha, India
[4]Professor, Department of Microbiology, IMS & SUM Hospital, Bhubaneshwar, Odisha, India

Received: 11-05-2023 / Revised: 12-06-2023 / Accepted: 25-07-2023
Corresponding Author: Lipika Jena
Conflict of interest: Nil

Abstract

The cellular physiology of *Escherichia coli* undergoes significant alterations when oxidative phosphorylation is impaired due to defects in the F1-ATPase enzyme. This work examines the physiological and metabolic alterations in the F1-ATPase-deficient mutant HBA-1 in contrast to the wild-type strain W1485. To maintain the same growth rates, both strains were cultivated in a chemostat that was glucose limited. In comparison to the parent strain, the mutant showed a 60% reduction in biomass production and noticeably raised specific rates of respiration (170%) and glucose consumption (169%). The mutant's improved glucose metabolism resulted in a rise in glycolytic flux but only an 18% elevation in TCA cycle activity, mostly because of excretion of acetate. The results of transcriptome analysis showed that gene expression had changed somewhat, with respiratory chain components being upregulated and TCA cycle enzymes being significantly downregulated. These results were supported by enzyme activity assays, which revealed lower TCA cycle enzyme activities and elevated pyruvate dehydrogenase complex and acetate kinase activities. Along with increased respiration rates, the mutant also showed elevated activities of terminal oxidases and NADH dehydrogenases, including NDH-2 and cytochrome *bd* oxidase. Structural mutations in the F1-ATPase subunits, such as Gly-48 to Asp in the epsilon subunit, inhibited ATPase activity and disrupted ATP-dependent proton translocation. These findings highlight the extensive physiological changes resulting from F1-ATPase defects, emphasizing the enzyme's critical role in cellular energy metabolism and its potential as a target for antibacterial strategies. This study provides comprehensive insights into the adaptive metabolic strategies of *E. coli* under conditions of impaired oxidative phosphorylation, revealing a complex interplay between metabolic fluxes, gene expression, and enzyme activities.

Keywords: F1-ATPase enzyme, TCA, NADH

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

The cellular physiology of *Escherichia coli* undergoes significant alterations when oxidative phosphorylation is impaired due to defects in the F1-ATPase enzyme. This impairment can arise from mutations affecting various subunits of the enzyme, leading to decreased ATP synthesis and changes in metabolic flux.

1. Growth and Metabolic Rates: Mutations in the F1-ATPase can lead to decreased growth yields and raised specific rates of glucose consumption and respiration. For instance, an F1-ATPase-defective mutant of *E. coli* showed a 60% decrease in growth yield and a 168% increase in glucose consumption rate [1].
2. Glycolysis and TCA Cycle Alterations: The impaired ATP synthesis shifts the metabolic flux. Glycolysis rates can increase significantly, while the TCA cycle activity may not proportionally increase, often leading to the excretion of metabolic byproducts like acetate. This phenomenon has been observed in mutants with increased glycolytic flux but only an 18% rise in the TCA cycle due to the excretion of acetate [2].

3. Respiratory Chain Components: The respiratory chain's constituents' activities have undergone significant alterations. Even when ATP synthesis is hampered, increased activity of terminal oxidases and NADH dehydrogenases can lead to higher respiration rates. For instance, the overall activity of NADH dehydrogenases and terminal oxidases rose around threefold in the mutants, mostly as a result of elevated NDH-2 enzyme activity and elevated cytochrome bd levels.
4. Membrane ATPase and Proton Pumping: Mutations in the F1-ATPase can also affect the binding and functional coupling between F1 and F0

sectors. For instance, a mutation in the alpha-subunit causing impaired oxidative phosphorylation resulted in decreased membrane ATPase activity and altered proton permeability [3].

5. Structural and Functional Mutations: Specific amino acid substitutions in the F1-ATPase subunits can further elucidate the role of these residues in enzyme function and stability. Mutations such as Gly-48 to Asp in the epsilon-subunit can inhibit ATPase activity and disrupt ATP-dependent proton translocation [4].

Defects in the F1-ATPase enzyme of *E. coli* led to extensive physiological changes, affecting growth, metabolic pathways, and respiratory functions, highlighting the critical role of this enzyme in cellular energy metabolism.

Methodology

Bacterial Strains and Culture Conditions

We used two strains of *E. Coli*: the wild-type strain W1485 (ATCC 12435) and an F1-ATPase-defective mutant, HBA-1 (*atpA401 bgl*+). To create the mutant, strain W1485 was transduced with the faulty gene atpA401, which codes for the α subunit of F1-ATPase, using P1kc. This particular mutation causes the phosphorylation linked to electron transport to become uncoupled; it was first identified as uncA401 in 1971. Further examinations identified this mutation in the F1-ATPase α subunit, exposing a single nucleotide alteration that causes Phe to replace Ser 373. Even with the same component structure and organisation, the mutant F1-ATPase exhibits almost negligible ATPase activity. It has been proposed that this mutant maintains proton impermeability by binding to membranes depleted of ATPase.

AN718bgl-7 was used as the donor strain in order to cotransduce atpA401 with bgl+ in order to achieve effective transduction. The ATPase activity of strain HBA-1 was significantly lower than that of the parent strain.

The two strains were cultivated in modified M9 minimum media supplemented with trace elements within a glucose-limited chemostat. For the purpose of maintaining ideal dissolved oxygen levels, temperature regulation, and pH control, the continuous chemostat culture was kept at a dilution rate of 0.2 h^{-1} under certain aeration and stirring conditions.

Fermentation Analysis

Spectrophotometry was used to track growth, and the glucose oxidase method was used to measure the concentration of glucose. Liquid chromatography under high pressure was used to analyse organic acids. Using a dissolved oxygen analyzer, the respiration rate during chemostatic culture was determined.

Flux Analysis

Metabolic fluxes were estimated using a stoichiometric approach, by-product formation, glucose consumption, considering growth rate, and biomass production.

RNA Extraction and Analysis

Total RNA was extracted, treated with DNase, and its concentration and quality determined. *E. coli*-specific cDNA synthesis was performed for DNA array and real-time PCR analyses.

DNA Array Analysis

cDNA probes were synthesized and hybridized to *E. coli* gene arrays, followed by data analysis to determine differential gene expression.

Northern Blot Analysis

For the purpose of assessing gene expression, total RNA was isolated using agarose gel electrophoresis, deposited onto membranes, and hybridised with certain probes.

Real-time PCR Analysis

Reverse transcription followed by real-time PCR was conducted to quantify gene expression levels.

Measurement of Enzymes

Enzyme activities in central carbon metabolism and the respiratory chain were determined spectrophotometrically, with specific assay conditions described for each enzyme.

Immunoblot Analysis

Protein abundance of terminal oxidases was assessed via immunoblot analysis using specific antibodies.

Result

Chemostat Cultures Reveal Enhanced Glucose Metabolism in the F1-ATPase-Defective Mutant HBA-1

The HBA-1 mutant and its parental strain, W1485, were grown in M9 minimal media within glucose-limited chemostats at identical growth rates (D = 0.2 h^{-1}) in order to assess the effect of an F1-ATPase deficiency on glucose metabolism. Analysis was done on the fermentation factors for both strains. In comparison to the parent strain, the mutant showed decreased biomass production (60%) and higher specific rates of respiration (170%) and glucose intake (169%). The mutant strain exhibited a notable increase in acetate excretion, while the original strain did not exhibit any acetate excretion. These results, which are in line with earlier findings in a variety of *atp* mutants, unequivocally showed improved glucose metabolism in the F1-ATPase-defective mutant.

Flux Analysis

Stoichiometric analysis was used to estimate metabolic fluxes within central metabolic pathways based on the observed fermentation parameters. The distribution of glycolytic flux in HBA-1 was roughly double that of W1485; however, the mutant's distribution within the TCA cycle was only marginally larger than that of the parent. This discrepancy was ascribed to HBA-1's excretion of acetate, which decreased the amount of acetyl-CoA that entered the TCA cycle. Another *atp* mutant cultured in glucose minimum medium under batch culture conditions showed similar observations regarding elevated carbon flux through the TCA cycle and glycolysis, combined with enhanced excretion of acetate.

Transcriptome Analysis

By comparing the genomic expression patterns of the parent and mutant strains using DNA array analysis, it was possible to determine if changes in glucose metabolism were associated with changes in gene expression. Overall, variations in the levels of gene expression were negligible (less than twofold) and mostly unimportant. When changes were detected, the majority of genes showed reduced expression in the mutant. Interestingly, even though the mutant had improved glucose metabolism, no genes related to glycolysis were present. On the other hand, there was a notable decrease in the expression levels of multiple genes that encode the TCA cycle enzymes, including *gltA*, which codes for citrate synthase. Furthermore, upregulated patterns were noted in *aceE* and *aceF*, which encode PDH complex enzymes, possibly supporting the redirection of glycolytic flux towards the production of acetate. Additionally, some genes encoding respiratory chain enzymes showed enhanced expression levels, indicating transcriptional regulation in conjunction with enhanced respiration in the mutant.

Table 1: Comparison of multiple gene expression ratios as ascertained by real-time PCR, Northern blot, and DNA array analysis.

Gene	Expression ratio (HBA-1/W1485) determined by indicated analysis		
	DNA array (a)	Northern blot (c)	Real-time PCR (c)
ndh	1.28 (b)	ND	2.36
cyoA	1.26	ND	1.63
cydA	1.30 (b)	ND	1.34
hns	0.48 (b)	0.64	ND

The Function of Enzymes in Central Carbon Metabolism

Complementing fermentation and transcriptome analyses, enzyme activities pertinent to central carbon metabolism were determined to validate observed metabolic changes, particularly acetate production in the mutant. PDH complex activity in the mutant was approximately double that of the parent, while activities of select TCA cycle enzymes were approximately halved. These findings aligned with transcriptome analysis results, corroborating metabolic alterations at the enzyme activity level. Additionally, acetate kinase activity, although not reflected in transcriptome analysis, was notably elevated in the mutant, further supporting increased acetate production.

Overall NDH and Terminal Oxidases Activities

Given the observed respiratory rate enhancement and transcriptional upregulation of select respiratory genes in the mutant, total activities of NDHs and terminal oxidases were measured. Both NDH and terminal oxidase activities were significantly elevated in the mutant, correlating with enhanced respiration. Moreover, analyses revealed a striking shift in NDH isozyme proportion, with NDH-2 activity predominantly increased in the mutant.

Examination of NDH and Terminal Oxidases Proportions

Immunoblot analysis of terminal oxidases indicated an increase in the *bd*-type oxidase concentration relative to the *bo*-type oxidase in the mutant, consistent with transcriptional upregulation of *cydA*. These findings collectively underscore substantial metabolic and regulatory adaptations in response to F1-ATPase deficiency, shedding light on the intricate interplay between cellular bioenergetics and gene expression.

Discussion

In this investigation, the comprehensive alterations occurring in an *E. coli* K-12 cell due to a mutation affecting oxidative phosphorylation caused by an F1-ATPase defect were explored. The findings shed light on how metabolic shifts, leading to improved glucose consumption, can arise in response to energy deficiencies caused by the F1-ATPase mutation.

The F1-ATPase-defective mutant and its parent strains were cultured in a glucose-limited chemostat, which enabled accurate assessment of their exponential growth at a similar pace ($D = 0.2\ h^{-1}$). Flux analysis provided additional evidence for the mutant's improved glucose metabolism, which was also indicated by the fermentation characteristics.

The mutant was shown to have a twofold increase in flux across the glycolytic pathway. The results of the DNA array study, however, did not point to the transcriptional activation of glycolysis-related genes. Consequently, it is hypothesised that the enhanced glycolytic flow was brought about by the allosteric activation of two important enzymes in this pathway, namely pyruvate kinase II (activated by AMP) [6] and phosphofructokinase I (activated by ADP) [5], in response to the decreased ATP/ADP ratio.

It's interesting to note that the mutant's flux through the TCA cycle was only 18 percent higher than the parent's, mostly because the flux was diverted into acetate. This suggests that strict metabolic regulation is needed to keep glycolytic flux out of the TCA cycle. Enzymatic activity results showed that various TCA cycle enzymes, involving citrate synthase, had decreased activity whereas the PDH complex and acetate kinase had increased activity. Citrate synthase controls the TCA cycle flux in *E. coli* by using NADH as a negative effector to prevent feedback [7]. This inhibition, along with noted changes in citrate synthase, PDH complex, and acetate kinase activities, may direct glycolytic flow into acetate because the rate of NADH generation in the mutant with improved glucose metabolism would be greater than that in the parent. From an energy perspective, the ATPs produced by the acetate route (production at the acetate kinase reaction), or the TCA cycle are similar when it comes to substrate-level phosphorylation from the metabolism of acetyl-CoA. Thus, substrate-level phosphorylation in the acetate route is probably not the physiological relevance of glycolytic flux redirection into acetate; rather, it has to do with something else. The F1-ATPase-defective mutant has already produced excess NADH through increased glycolysis, therefore the most likely explanation is the prevention of additional NADH generation during the TCA cycle. Three genes (*icdA, sucA*, and *mdh*) that code for dehydrogenases that produce NADH are among the downregulated genes encoding TCA cycle enzymes. These modifications appear to lessen the synthesis of NADH by reducing the metabolism of acetyl-CoA via the TCA cycle, in conjunction with lower citrate synthase activity. Additionally, downregulation of the glyoxylate shunt enzyme-encoding genes (*aceA* and *aceB*) was noted. This could potentially decrease the formation of NADH via malate dehydrogenase, while maintaining acetyl-CoA for substrate-level phosphorylation in conjunction with the formation of acetate (acetate kinase) or the TCA cycle (succinyl-CoA synthetase).

This work shows that in order to induce improved glucose metabolism in the F1-ATPase-defective mutant, it is required to simultaneously reduce the flow of the TCA cycle and modify the components of the respiratory chain to raise the rate of respiration [3]. For the F1-ATPase-defective mutant, preferential rise in NDH-2 activity and cytochrome *bd* oxidase content were seen in the respiratory chain. *E. coli's* respiratory chain has four different NDH and terminal oxidase isozymes: NDH-1 (2H+/e−), NDH-2 (0H+/e−), *bo*-type oxidase (2H+/e−), and bd-type oxidase (1H+/e−). These isozymes demonstrate varied efficiency in creating the electrochemical gradient of protons coupled with electron transfer [8, 9]. Because the enlarged respiratory chain's components are less bioenergetically efficient, the mutant can recycle extra NADH produced by its improved central metabolism and prevent excessive proton-motive force generation. Indeed, measurements have shown that the *atp* deletion mutant has a 20% greater membrane potential [10]. Thus, from a bioenergetic perspective, this modification in respiratory components appears beneficial in preserving the mutant's homeostasis. The changes in respiratory chain components that have been found are a new discovery of an adaptive response in the F1-ATPase-defective mutant (probably shared by all *atp* mutants), and they are consistent with the metabolic redirection strategy that has been discussed previously to restrict the synthesis of NADH in the TCA cycle. The mechanism for NDH-2 and bd-type oxidase transcriptional increase in response to the *atp* mutation is another fascinating feature. Fnr, the product of the *fnr* (fumarate nitrate reduction) gene, represses the expression of *ndh*, which codes for NDH-2, in anaerobic environments [11]. Under aerobic conditions, transcription from the *ndh* promoter is modulated in a growth phase-dependent manner by Fis (the *fis* gene product; a factor for inversion stimulation). Fis represses *ndh* expression during the early logarithmic growth phase, when Fis expression is at its peak, guaranteeing effective NDH-1 utilisation. When Fis expression falls during the stationary phase, this repression is released. Thus, when cellular energy is sufficient, NDH-2 appears to be fully expressed [12]. In this particular situation, the mechanism underlying the transcriptional increase of *ndh* in the F1-ATPase-defective mutant is difficult to explain and needs further investigation. On the other hand, the interaction of three global regulatory proteins—Fnr, ArcA (aerobic respiration regulation; the *arcA* gene product), and H-NS—controls the *cydAB* operon, which codes for the *bd*-type oxidase. This guarantees the highest possible expression in microaerobic environments [13–16]. This is crucial physiologically because, due to its high oxygen affinity, bd-type oxidase functions well in microaerobic environments. Because of H-NS repression, the expression of the *cydAB* operon is normally regulated at a low level in aerobic circumstances [14]. On the other hand, even in aerobic conditions, the bd-type oxidase level rises in

the F1-ATPase-defective mutant. Interestingly, H-NS expression in the mutant seems to be half that of the parent. Thus, lower quantities of H-NS protein may be the cause of the rise in *bd*-type oxidase content.

It was discovered that the mutant had downregulated transcription levels of *ompF*, which codes for porin, and seven other genes involved in flagellar biogenesis. Other genes (*flgB, flgC, flgL, fliC*, and *fliD*) are also affected in a similar way by the downregulation of the flhC and flhD genes, which together comprise the master operon that turns on flagellar biogenesis gene expression [20]. Since *hns* mutation causes flagellar loss [12] and H-NS positively regulates the *flhDC* operon in vivo [17], decreased *hns* expression is implicated once more. These reactions could help reduce the cost of biosynthesis [18, 19], particularly in an *atp* mutant with a constrained supply of ATP.

This work clarifies a number of physiological alterations linked to a mutation in *E. coli* that is deficient in F1-ATPase [1]. The mutation affects components of the respiratory chain and the cellular structure in addition to changing central carbon metabolism. These results demonstrate a novel, but conceivable, method that allows *E. coli* to endure energetically demanding conditions brought on by compromised oxidative phosphorylation. The observed qualitative changes in the mutant, notably the overexpression of cytochrome bd oxidase and the downregulation of TCA cycle enzymes, suggest that global control network(s) like the Arc two-component system are likely involved in their operation, even though there is a dearth of experimental data to support this theory. More investigation is required to determine the felt signal by the network(s) and to shed light on the possible role of global control networks in the atp mutant's adaptive response.

Conclusion

In summary, defects in the F1-ATPase enzyme of *Escherichia coli* led to significant alterations in cellular physiology, characterized by increased glucose consumption, enhanced glycolytic flux, and altered TCA cycle activity. The mutant strain exhibits notable changes in respiratory chain components and enzyme activities, highlighting adaptive mechanisms to manage energy production and maintain cellular homeostasis. These findings underscore the critical role of F1-ATPase in cellular energy metabolism and reveal potential targets for antibacterial strategies.

References

1. Li, L., Wada, M., & Yokota, A. A comparative proteomic approach to understand the adaptations of an H+-ATPase-defective mutant of Corynebacterium glutamicum ATCC14067 to energy deficiencies. *PROTEOMICS*. 2007.
2. Sawada, K., Kato, Y., Imai, K., Li, L., Wada, M., Matsushita, K., & Yokota, A. Mechanism of increased respiration in an H+-ATPase-defective mutant of Corynebacterium glutamicum. *Journal of Bioscience and Bioengineering*, 2012; 113(4): 467-473.
3. Wada, M., Hijikata, N., Aoki, R., Takesue, N., & Yokota, A. Enhanced valine production in Corynebacterium glutamicum with defective H+-ATPase and C-terminal truncated acetohydroxyacid synthase. *Bioscience, Biotechnology, and Biochemistry*, 2008; 72: 2959-2965.
4. Boecker, S., Slaviero, G., Schramm, T., Szymanski, W., Steuer, R., Link, H., & Klamt, S. Deciphering the physiological response of *Escherichia coli* under high ATP demand. *Molecular Systems Biology*. 2021.
5. Koebmann, B., Westerhoff, H., Snoep, J., Solem, C., Pedersen, M., Nilsson, D., & Jensen, P. R. The extent to which ATP demand controls the glycolytic flux depends strongly on the organism and conditions for growth. *Molecular Biology Reports*, 2004; 29: 41-45.
6. Stevenson, B., Liu, J., Kuchel, P., & Ollis, D. Fermentative glycolysis with purified *Escherichia coli* enzymes for in vitro ATP production and evaluating an engineered enzyme. *Journal of Biotechnology*, 2012; 157(1): 113-123.
7. Alekseev, K., Dubina, M., & Komov, V. P. Molecular-genetic and biochemical characteristics of citrate synthase from the citric-acid producing fungus Aspergillus niger. *Applied Biochemistry and Microbiology, 2016; 52*: 810-817.
8. Bergé, M., Pezzatti, J., González-Ruiz, V., Degeorges, L., Rudaz, S., & Viollier, P. Bacterial cell cycle control by citrate synthase independent of enzymatic activity. *eLife, 2019; 9*.
9. Mall, A., Sobotta, J., Huber, C., Tschirner, C., Kowarschik, S., Bačnik, K., Mergelsberg, M., Boll, M., Hügler, M., Eisenreich, W., & Berg, I. Reversibility of citrate synthase allows autotrophic growth of a thermophilic bacterium. *Science, 2018; 359*: 563-567.
10. Jung, T., & Mack, M. Interaction of enzymes of the tricarboxylic acid cycle in Bacillus subtilis and Escherichia coli: a comparative study. *FEMS Microbiology Letters, 2018; 365.*
11. Jia, B., Jia, X., Kim, K. H., & Jeon, C. Integrative view of 2-oxoglutarate/Fe(II)-dependent oxygenase diversity and functions in bacteria. *Biochimica et biophysica acta. General subjects, 2017;1861*: 323-334.
12. Brandi, A., Giangrossi, M., Giuliodori, A., & Falconi, M. An interplay among FIS, H-NS, and

guanosine tetraphosphate modulates transcription of the *Escherichia coli* cspA gene under physiological growth conditions. *Frontiers in Molecular Biosciences, 2016;3*.
13. Kroner, G., Wolfe, M. B., & Freddolino, P. *Escherichia coli* Lrp regulates one-third of the genome via direct, cooperative, and indirect routes. *Journal of Bacteriology, 2018; 201*.
14. Gawade, P., Gunjal, G., Sharma, A., & Ghosh, P. Reconstruction of transcriptional regulatory networks of Fis and H-NS in *Escherichia coli* from genome-wide data analysis. *Genomics*. 2020.
15. McQuail, J., Matera, G., Gräfenhan, T., Bischler, T., Haberkant, P., Stein, F., Vogel, J., & Wigneshweraraj, S. Global Hfq-mediated RNA interactome of nitrogen-starved *Escherichia coli* uncovers a conserved post-transcriptional regulatory axis required for optimal growth recovery. *Nucleic Acids Research*. 2023.
16. Huang, H.-T., Bobst, C. E., Iwig, J., Chivers, P., Kaltashov, I., & Maroney, M. Co(II) and Ni(II) binding of the *Escherichia coli* transcriptional repressor RcnR orders its N terminus, alters helix dynamics, and reduces DNA affinity. *The Journal of Biological Chemistry, 2017;293*: 324-332.
17. Fahrner, K., & Berg, H. Mutations that stimulate *flhDC* expression in *Escherichia coli* K-12. *Journal of Bacteriology, 2015; 197*(18): 3087-3096.
18. Kim, E. A., & Blair, D. Function of the histone-like protein H-NS in motility of Escherichia coli: Multiple regulatory roles rather than direct action at the flagellar motor. *Journal of Bacteriology, 2015; 197*(18): 3110-3120.
19. Zhang, Z., Kukita, C., Humayun, M., & Saier, M. Environment-directed activation of the *Escherichia coli* flhDC operon by transposons. Microbiology, 2017; 163(4): 554-569.