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Proton Flux Dependence on Glucose Concentration in *E. coli* Hyd-1 and Hyd-4 Mutants During Fermentation

Tatevik Nazaryan[1,2,3*], Liana Vanyan[1,2], Karen Trchounian[1,2,3]

[1] Department of Biochemistry, Microbiology and Biotechnology, Faculty of Bilogy, Yerevan State University, Yerevan, Armenia,

[2] Research Institute of Biology, Yerevan State University, Yerevan, Armenia

[3] Microbial Biotechnologies and Biofuel Innovation Center, Yerevan State University, Yerevan, Armenia

ABSTRACT

This study examines the effects of *E. coli* hydrogenase-1 (Hyd-1) and hydrogenase-4 (Hyd-4) on proton flux during fermentation of glucose with different concentrations (2 g L^{-1} and 8 g L^{-1}). During research, proton flux was (J_{H+}) measured in the *E. coli* BW25113 wild-type strain and mutants *hyaA-F* and *hyfA-R* using 0.2 mM *N,N*-dicyclohexylcarbodiimide (DCCD) to evaluate F_OF_1 ATP synthase activity. Cells were grown separately in the absence of both glucose concentrations and during the experiments, either 2 g L^{-1}or 8 g L^{-1} glucose was supplemented. Our findings indicate that in the *hyaA-F* mutant, where Hyd-1 is absent, proton flux and F_OF_1-ATPase activity are differently affected depending on glucose concentrations (2 g L^{-1} and 8 g L^{-1}). Under low glucose conditions (grown in 2 g L^{-1} and added 2 g L^{-1}), total J_{H+} decreased by 55%, both DCCD sensitive and remaining fluxes were decreased as well by 60%. The contribution of F_OF_1-ATPase remained similar, as in wild type. High glucose addition (grown in 2 g L^{-1} and added 8 g L^{-1}) leads to a 30% decrease in total J_{H+} and F_OF_1-ATPase contribution also decreases by 25%. When cells were grown in the presence of 8 g L^{-1} glucose total J_{H+} decreased by 40% and 50% when 2 g L^{-1} and 8 g L^{-1} were supplemented, respectively. Meanwhile DCCD-sensitive J_{H+} decreased 50% and 80%, respectively. The absence of Hyd-1 significantly reduces total and DCCD-sensitive proton fluxes ATP synthesis. In the *hyfA-R* mutant, where Hyd-4 is absent and F_OF_1-ATPase activity was significantly dependent on glucose concentration (2 g L^{-1} and 8 g L^{-1}). Under low glucose conditions (2 g L^{-1} growth and 2 g L^{-1} addition), total J_{H+} decreased by 60%, but the contribution F_OF_1-ATPase was not affected. High glucose addition reduces total J_{H+} by 50%, but F_OF_1-ATPase contribution increases by 40%. In conditions grown with 8 g L^{-1} glucose total J_{H+} decreased by 10% and 40% when 2 g L^{-1} and 8 g L^{-1} were supplemented, respectively, while F_OF_1-ATPase contribution increased by 30% and 80%. The absence of Hyd-4 has a more significant impact on activity of F_OF_1-ATPase under high glucose conditions, indicating Hyd-4's critical role in regulating *E. coli*'s energy metabolism.

Keywords: glucose concentration, proton flux, Hyd-1 and Hyd-4

References:

1. Vanyan, L.; Trchounian, K. Glucose concentration is determinant for the functioning of hydrogenase 1 and hydrogenase 2 in regulating the proton and potassium fluxes in *Escherichia coli* at pH 7.5, *Energies* **2022**, *15*, 5935. DOI:10.3390/en15165935

***Corresponding Author:**

Tatevik Nazaryan, Faculty of Biology, Yerevan State University, 1 Alex Manoogian str., Yerevan, 0025, Armenia.

Email: tatev.nazaryan.2001@gmail.com