

## Effects of Amino Acid Mutations in *Escherichia coli* DcuD Transporters in Proton Flux at pH 5.5

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### ABSTRACT

*Escherichia coli* relies on Dcu transporters to facilitate succinate transport during fermentation, with DcuD being one of three members of this transporter family primarily involved in C4 -dicarboxylate exchange. This study investigated the role of amino acid mutations in *E. coli* DcuD transporters in proton flux at pH 5.5. In this study proton flux (JH<sup>+</sup>) was measured in *E. coli* BW25113 wild type strain and *dcuD* mutants with specific amino acid defects. JH<sup>+</sup> was determined using selective pH electrode. To evaluate the contribution of F<sub>o</sub>F<sub>1</sub>-ATPase, 0.2 mM *N,N*-dicyclohexylcarbodiimide (DCCD) was used. When glucose was added in *E. coli* BW25113 assay total JH<sup>+</sup> was ~0.81 mmol min<sup>-1</sup> per 10<sup>9</sup> cells in 1 unit and when cells were treated with DCCD JH<sup>+</sup> was ~0.11 mmol min<sup>-1</sup> per 10<sup>9</sup> cells. In mutant with defect of Arg67 total JH<sup>+</sup> was 1.9-fold lower, compared to the wild type, and the DCCD has effect, thus contribution of F<sub>o</sub>F<sub>1</sub>-ATPase to the proton export pathway has role. In mutant with defect in Glut64, the total JH<sup>+</sup> was similar to Arg67. In mutant with defect of Glut324 total JH<sup>+</sup> was ~2.7-fold lower compared to wild type. However, in mutant with defect of Lys328 no significant differences were observed compared to wild type. These findings suggest that specific amino acids within the DcuD transporter influence proton flux mechanisms. Overall, the results provide insights into the functional roles of key amino acids in DcuD-mediated proton transport and proton efflux is primarily associated with of F<sub>o</sub>F<sub>1</sub>-ATPase.

**Keywords:** DcuD transporter, proton flux, membrane potential

### References:

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