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New role of *Escherichia coli* hydrogenase 4 during glucose fermentation

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Escherichia coli encodes four [Ni-Fe]-hydrogenases (Hyd) catalyzing the redox reaction: H_2 to $2H^+$: H_2<->$2H^+ + 2e^-$ [1]. Hyd-1 and Hyd-2 are reversible Hyd enzymes depending on carbon source [1]. Hyd-3 with formate dehydrogenase H (FDH-H) forms formate hydrogen lyase (FHL-1) complex, whereas Hyd-4 with FDH-H comprises FHL-2 complex [1]. Hyd-3 and Hyd-4 are mainly H_2 producing Hyd enzymes upon glucose fermentation but they might operate in reverse mode during glycerol fermentation. Moreover, it has been shown that glucose can inhibit *hyf* operon expression and Hyd-4 activity [2].

Hyd enzyme activity and H_2 production by *E. coli* wild type and Hyd-4 mutant strains during glucose (0.2% and 0.8%)fermentation at pH 7.5 were investigated. When wild type cells were grown in the presence of 0.2% glucose H_2 production rate was ~2 fold higher than the cells grown on 0.8% glucose. In Hyd-4 single mutants H_2 production rate was either similar to wild type or ~1.7 fold higher. Interestingly, the same results were obtained when the cells were grown in the presence of 0.8% glucose. These data would suggest that in both cases Hyd-4 is functioning as uptake Hyd enzyme. But when *hyfA-B* or *hyfB-R* mutants were grown on 0.2% glucose H_2 production rate was decreased ~5 fold compared to wild type. Moreover, no any differences were found when the mutants were grown on 0.8% glucose.

Taken together it can be concluded that during glucose fermentation at pH 7.5 Hyd-4 main function is not to uptake or produce H_2 but to translocate protons from proton ATPase to Hyd-3 or other membrane proteins for H_2 evolution. In single mutants no any effect was observed, as one subunit is compensating the other's function and only the deletion of several subunits disturb the translocating function, thus decreasing the H_2 production.

1 K. Trchounian, A. Poladyan, A. Vassilian, A. Trchounian, Crit. Rev. Biochem. Mol. Biol. 2012, 47, 236-49.
2 K. Trchounian, A. Trchounian, Int. J. Hydrogen Energy 2014, 39, 16914-8.