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Input of hydrogenases in H₂ cycling and proton motive force generation in *Escherichia coli* during fermentation

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*Escherichia coli* has the capacity to encode four membrane bound [Ni-Fe] hydrogenases (Hyd) catalyzing the redox reaction of 2H⁺+2e⁻ → H₂. All hydrogenases are reversible: depending on pH and carbon source they can work in H₂ uptake or producing mode. It has been proposed that the activity of Hyd enzymes and their working direction also depend on H₂ cycling through the membrane. Hyd enzymes form H₂ cycle during glucose or glycerol fermentation at pH of 5.5-7.5. Moreover, it was shown that deletion of three of the Hyd enzymes disturbs H₂ cycling. Probably, Hyd enzymes by forming H₂ cycle and redox chain to the proton F₀F₁ ATPase are working towards neutralizing the end acids, equilibrating the cytoplasmatic pH and involved in proton motive force generation.

It was shown that at pH 7.5 during glycerol fermentation in *hypF* mutant (all Hyd enzymes are absent) proton motive force generated is higher (122 mV) compared to wild type (99 mV). In contrast at pH 5.5 in *hypF* mutant it was lower than in wild type by ~20 mV. In addition, Hyd-1 and Hyd-2 activity has been shown to depend on the activity of F₀F₁ ATPase at extreme pHs (pH 5.5 and pH 7.5) during glucose or glycerol fermentation. Taken together it might be concluded that besides F₀F₁ ATPase Hyd enzymes also are involved in proton motive force generation during fermentation. H₂ cycling as new membrane-associated cycle contributes towards regulation of cytoplasmatic pH by producing or uptaking H₂. It is suggested that Hyd enzymes might be one of the main H⁺ sensing systems in the cell during fermentative conditions.