In vivo thermodynamic analysis of glycolysis in *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum* using ^{13}C and ^{2}H tracers

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Project Goals: This project will integrate thermodynamic analysis with advanced mass spectrometry, computational modeling, and metabolic engineering to develop an approach for *in vivo* determination of Gibbs free energies (ΔG) in metabolic networks. This project will also investigate how the thermodynamics of biosynthetic pathways in microbial biofuel producers change dynamically as substrates are depleted or products accumulate. This research will result in the construction of computational models that quantitatively define trade-offs between energy efficiency of biosynthetic pathways and their overall catalytic rates. The approach developed in this project will be useful for identifying thermodynamic bottlenecks in native and synthetic pathways and pinpoint the enzymes whose expression levels will have the largest effect on production rates and final product yields.

Abstract: *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum* are thermophilic anaerobic bacteria with complementary metabolic capabilities that utilize distinct glycolytic pathways for the conversion of cellulosic sugars to biofuels. We integrated quantitative metabolomics with ^{2}H and ^{13}C metabolic flux analysis to investigate the *in vivo* reversibility and thermodynamics of the central metabolic networks of these two microbes. We found that the glycolytic pathway in *C. thermocellum* operates remarkably close to thermodynamic equilibrium, with an overall drop in Gibbs free energy 5-fold lower than that of *T. saccharolyticum* or anaerobically-grown *E. coli*. The limited thermodynamic driving force of glycolysis in *C. thermocellum* could in large part be attributed to the small free energy of the phosphofructokinase reaction producing fructose bisphosphate. The ethanol fermentation pathway was also substantially more reversible in *C. thermocellum* compared to *T. saccharolyticum*. These observations help explain the comparatively low ethanol titers of *C. thermocellum* and suggest engineering interventions that may be used to increase its ethanol productivity and glycolytic rate. In addition to thermodynamic analysis, we used our isotope tracer data to reconstruct the *T. saccharolyticum* central metabolic network, revealing exclusive use of the Embden-Meyerhof-Parnas (EMP) pathway for glycolysis, a bifurcated tricarboxylic acid (TCA) cycle, and a sedoheptulose bisphosphate bypass active within the pentose phosphate pathway.

References/Publications

1. Jacobson TB, Korosh TK, Stevenson DM, Foster C, Maranas C, Olson DG, Lynd LR, Amador-Noguez D. 2020. *In vivo* thermodynamic analysis of glycolysis in *C. thermocellum* and *T. saccharolyticum* using ^{13}C and ^{2}H tracers. mSystems 5:e00736-19. doi: 10.1128/mSystems.00736-19.

This material is based upon work supported by the DOE Early Career Research Program under Award Number DE-SC0018998.