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

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Project Goals: To develop experimental-computational approaches for *in vivo* genome-scale determination of Gibbs free energies (ΔG) in metabolic networks.

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*. The use of a near equilibrium glycolytic pathway, with potentially increased ATP yield, by *C. thermocellum* may represent an evolutionary adaptation to growth on cellulose but it has the drawback of being highly susceptible to product feedback inhibition. The results of this study will facilitate future engineering of high-performance strains capable of transforming cellulosic biomass to biofuels at high yields and titers.