Limited thermodynamic driving force in glycolysis of cellulolytic clostridia

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) designing plant cell walls for rapid deconstruction and (2) developing multi-talented microbes or converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. BESC research in biomass deconstruction and conversion targets CBP by studying thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction and manipulating these microorganisms for improved conversion, yields, and biofuel titer. BESC researchers provide enabling technologies in biomass characterization, 'omics, modeling and data management in order to (1) understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion mechanisms.

C. thermocellum and *C. cellulolyticum* are obligate anaerobes capable of converting cellulose into ethanol. The glycolytic pathways of these two microorganisms display unique cofactor utilization, resulting in an energy-efficient sugar catabolism that is thought to generate more usable energy in the form of high-energy phosphate bonds than canonical glycolytic pathways but at the cost of forward thermodynamic driving force.

Here, we have used a combination of ^{13}C-labeling and ^{2}H-labeling to measure absolute metabolite concentrations and fluxes in *C. thermocellum* and *C. cellulolyticum* and experimentally determine changes in free energy (ΔG) at each step in their glycolytic and ethanol fermentation pathways. Our experimental method relies on the fundamental principle that for any reaction, ΔG is log proportional both to a concentration ratio (reaction quotient to equilibrium constant) and to a flux ratio (backward to forward flux), which can be reliably estimated from steady-state labeling data.

We found that the glycolytic and fermentation pathways in these two cellulolytic clostridia are surprisingly fully reversible under normal growth conditions. The overall thermodynamic driving force in the glycolytic pathways of *C. thermocellum* and *C. cellulolyticum* is significantly limited compared to canonical glycolytic pathways in model organisms such as *E. coli* or *S. cerevisiae* or non-cellulolytic thermophilic bacteria such as *T. saccharolyticum*. We also found that forward driving force is dependent on environmental inputs; for example, as ethanol accumulates during fermentation the thermodynamic driving force in glycolysis of *C. thermocellum* becomes even more limited. The limited forward driving force that we observe in the glycolytic and fermentation pathways of cellulolytic clostridia may constitute an evolutionary adaptation to growth in cellulose and the need to produce as much usable energy as possible per glucose.

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