Glycolysis Balances Enzyme Efficiency and Metabolic Adaptivity

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Project Goal: To track thermodynamic changes in glycolysis using isotope tracers

Rapid glycolysis during slow growth is a desirable feature for industrial biofuel production. In practice, however, glycolysis tends to slow down together with growth. Here we set out to develop and apply, in fast and slow-growing cells, isotope tracer methods for measuring the Gibbs energy of reaction (ΔG) of glycolytic reactions.

To this end, we selected glucose tracers harboring either ^2H at the fifth carbon, [5-$^2H_1$], or ^{13}C at the first two carbons, [1,2-$^{13}C_2$]. These tracers generate labeling patterns across glycolytic intermediates that depend on the pathway's forward-to-backward flux ratio (J^+/J^-) and therefore $\Delta G = -RT \ln(J^+/J^-)$. Quantitative methods for integrating data from the two tracers to reveal reaction thermodynamics will be described.

Using these tracers, we show that rapid upregulation of glycolysis in *Escherichia coli* is accomplished by increasing the pathway's thermodynamic driving force. Specifically, in fast-growing cells, we observed $\Delta G < -2$kJ/mol (i.e. forward flux > 2.2× backward flux) for most glycolytic reactions, reflecting efficient enzyme usage with enzymes mainly catalyzing the forward productive reaction. On the other hand, in nitrogen-limited cells with reduced glycolytic flux, in lower glycolysis, we observed $\Delta G \sim 0$ (forward flux ≈ backward flux). Such a near-equilibrium situation is energy-efficient but enzyme-inefficient. The likely evolutionary benefit of inefficient enzyme usage became manifest upon nitrogen upshift: by shifting from reversible to forward-driven thermodynamics, rapid glycolysis and growth rate were restored within minutes, without requiring increased enzyme levels or activity. Thus, nutrient-rich cells can run glycolysis at near maximal enzyme capacity, whereas nutrient-limited cells sacrifice enzyme efficiency for fast adaptation. The ^2H- and ^{13}C-tracer methods developed here should be broadly useful for understanding glycolytic thermodynamics and regulation across strains, species, and environmental conditions.

References

1. Park, J. O., Rubin, S. A., Xu, Y. F., Amador-Noguez, D., Fan, J., Shlomi, T., and Rabinowitz, J. D. (2016). Metabolite concentrations, fluxes and free energies imply efficient enzyme usage. *Nature Chemical Biology* 12(7): 482-489.
2. Park, J. O., Wei, M. H., Tanner, L. B., Amador-Noguez, D., Li, S., and Rabinowitz, J. D. (to be submitted). Glycolysis balances enzyme efficiency and metabolic adaptivity.

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