168. Conservation of absolute cellular metabolite concentrations

Junyoung O. Park1,2* (jopark@princeton.edu), Sara A. Rubin,3 Yi-Fan Xu,1,3 Daniel Amador-Noguez,1 Jing Fan,1,3 and Joshua D. Rabinowitz1,3

1Lewis-Sigler Institute for Integrative Genomics; 2Department of Chemical and Biological Engineering; and 3Department of Chemistry, Princeton University, Princeton, N.J.

Project Goals: Absolute metabolite concentrations control enzyme binding site occupancies and the thermodynamics of metabolic reactions. Accordingly, they are critical to both metabolic modeling and metabolic engineering. Here we aim to apply a combination of direct mass spectrometry measurements and thermodynamic constraints to determine absolute metabolite concentrations across E. coli, yeast, and mammalian cells. We also aim to elucidate design principles underlying the observed abundances. To quantitate metabolite abundances, we grew organisms on isotope-labeled nutrients to label cellular metabolites, which were then extracted in the presence of unlabeled metabolite standards of known concentrations. Subsequent liquid chromatography-mass spectrometry (LC-MS) analysis yielded the ratio of labeled endogenous metabolites to unlabeled standards, which we in turn converted (after correcting for any unlabeled endogenous compound, which we measured independently) into absolute cellular metabolite concentrations. This approach, which we have described previously,[1] works well for abundant and stable metabolites with commercially available standards. Some key intermediates cannot, however, be measured directly. To evaluate concentrations of these intermediates, we used isotope tracers to determine the relative forward and backward flux through reversible reactions such as triose-phosphate isomerase. The ratio of forward to backward flux determines the reaction free energy $\Delta G'$ in the cell. Given direct measurement of all but one of the substrates/products, we can then determine the missing concentration based on the fundamental equation: $\Delta G' = \Delta G^{\circ\prime} + RT \ln Q$. We directly validated that the associated reaction free energy $\Delta G'$ approaches zero with increasing enzyme expression. Through this approach, we were able to measure 10 previously unknown absolute concentrations of central carbon metabolites and also to refine the concentrations of many others.

Here we report the absolute concentrations of 117, 82, and 93 metabolites in E. coli, yeast, and the cultured mammalian cell line iBMK. We compare the resulting metabolite concentrations to substrate and effector binding affinities in the BRENDA[2] database. We find that, as previously observed for E. coli,[1] most substrate concentrations exceed the associated enzyme binding site Km, i.e., enzyme active sites are saturated. In contrast, we find that inhibitor concentrations are typically not saturating. These trends hold true across all three organisms. Perhaps most surprisingly, despite marked differences in central metabolic fluxes, absolute metabolite concentrations were substantially conserved across the three organisms. A potential explanation is that only a finite set of metabolite concentrations simultaneously meet global thermodynamic requirements while producing sufficiently high metabolite levels to result in enzyme active site saturation and thus efficient enzyme utilization. Apparently, due to such design constraints, a wide variety of cell types from prokaryotes to mammals operate with absolute metabolite concentrations within a similar range.

References

1. Bennett, B. D. et al. Absolute metabolite concentrations and implied enzyme active site occupancy in Escherichia coli. Nat Chem Biol 5, 593-599, doi:Doi 10.1038/Nchembio.186 (2009).
2. Schomburg, I. et al. BRENDA in 2013: integrated reactions, kinetic data, enzyme function data, improved disease classification: new options and contents in BRENDA. Nucleic Acids Res 41, D764-D772, doi:Doi 10.1093/Nar/Gks1049 (2013).

Funding: DOE DE-SC0012461