DEPARTMENT OF CHEMICAL ENGINEERING
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Metabolic flux analysis of photosynthetic organisms

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105 Shillman
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Photoautotrophic metabolism is the major process by which plants and other photosynthetic organisms use solar energy to fix available CO2 into complex organic molecules. This process is the primary source of all food on earth as well as raw materials for bio-based production of fuels and chemicals. The ability to perform quantitative studies using isotope tracers and metabolic flux analysis (MFA) is critical for deciphering flux regulation in these organisms. Although 13C is the preferred isotope tracer for studying central carbon metabolism in heterotrophic systems, photoautotrophs assimilate carbon solely from CO2 and therefore produce a uniform steady-state 13C-labeling pattern. However, transient measurements of isotope incorporation following a step change from unlabeled to 13C labeled CO¬2 can be used to estimate fluxes successfully with newly developed techniques of isotopically nonstationary MFA (INST-MFA). This computational tool now permits comprehensive flux analysis of photoautotrophic metabolism, complementing previous studies which were constrained to heterotrophic or mixotrophic conditions.

We have applied the INST-MFA approach to study the metabolism of Synechocystis sp. PCC 6803, a model photosynthetic organism, under autotrophic conditions using both GC/MS and LC/MS/MS to quantify the trajectories of metabolite labeling that result from introduction of 13C-labeled bicarbonate. The INST-MFA flux map was compared to values predicted by flux balance analysis (FBA) that requires only few experimental measurements, but does assumes optimal growth. Although FBA predicts no carbon flux through the oxidative pentose phosphate pathway, the experimental results indicate that around 10% of the fixed carbon is lost via this pathway. Due in part to these losses, 142 12 moles of CO2 must be fixed to yield a net gain of 100 C-moles of biomass. This is significantly more than the 111 moles of CO2 predicted by the FBA model, indicating that growth is suboptimal with respect to carbon utilization. Another finding is the oxygenation reaction of RuBisCO is insignificant, as expected due to the potent CO2-concentrating mechanism in cyanobacteria. We will also discuss efforts to apply this technique to metabolically engineered cyanobacteria.

Prof. Morgan obtained his Ph.D. from Rice University where he studied quantifying metabolic fluxes to secondary metabolites in plants. For his postdoctoral training, Dr. Morgan worked on improving enzymes in organic solvents. He is presently a Professor and Director of Graduate Studies in the School of Chemical Engineering at Purdue University. Prof. Morgan's research program is currently directed at the quantitative understanding of metabolic pathways enabling the production of valuable compounds in photosynthetic organisms.