Amino Acids Are Preferred Over Glucose and Other Sugars in *Escherichia Coli* by a Novel Mechanism of Carbon Catabolite Repression

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Project Goals: The goal of this project is to determine how protein acetylation affects metabolism in engineered microorganisms. Lysine acetylation is a common post-translational modification that eukaryotes, archaea, and bacteria employ to regulate protein activity. Multiple studies have recently shown that lysine acetylation predominantly targets metabolic enzymes – in fact, most metabolic enzymes are subject to lysine acetylation. We hypothesize that bacteria employ lysine acetylation as a global mechanism to regulate metabolism in response to their energy and redox status. Our previous work suggests that lysine acetylation may be an attractive and innovative target for metabolic engineering. We are investigating how lysine acetylation affects fuel production in engineered microorganisms. The significance of this work is that it will address a fundamental gap in our understanding of bacterial metabolism and identify new approaches for overcoming the problems associated with the production of advanced biofuels.

When we measure protein acetylation in *E. coli*, we routinely grow the cells in tryptone broth (pH 7) supplemented with glucose prior to analysis by liquid chromatography. During the course of these experiments, we observed that consumption of glucose is delayed. In particular, we found that the cells reached an OD600 of ~1 before they started to consume the glucose. Subsequent mass spectrometry analysis demonstrated that *E. coli* consumes multiple amino acids (serine, aspartate, and threonine) before it begins to consume glucose. Similar results were also observed with lactose, arabinose, and glycerol, where again sugar consumption is delayed by amino acids. Of the carbon sources tested, only pyruvate consumption is not delayed.

The unique carbon source selection provides adequate ratios of nutrients for growth. All of the preferred amino acids (serine, aspartate, and threonine) enter metabolism through pyruvate, leading to gluconeogenic growth. This mode of growth appears to inhibit the metabolism of glucose (and other sugars) initially, and allows simultaneous access to carbon and nitrogen. In support of this mechanism, we found that pyruvate also inhibits the uptake of glucose. Curiously, adding ammonium (the preferred nitrogen source) to the growth medium does not affect the preference for amino acids.

Phosphoenolpyruvate (PEP) would be synthesized. PEP initiates the phosphotransferase system (PTS), the major pathway of sugar transport. However, PEP has been shown to inhibit glycolytic enzymes such as phosphofructokinase. Feedback to glycolysis would reduce sugar flux in this case. The PEP:pyruvate ratio indirectly controls sugar uptake through the PTS. Accumulation of pyruvate from the preferred amino acids would lead to less active PTS enzymes to transport sugars. Finally, the TCA intermediate α-ketoglutarate (aKG) would accumulate. aKG has been shown to reduce sugar uptake via inhibition of the PTS and likely contributes to the delay in sugar uptake.

In conclusion, we have serendipitously identified a new fact of *E. coli* physiology that may translate to other species of bacteria. The results are significant as glucose is normally thought of as the preferred carbon source for *E. coli*. However, our results demonstrate that easily consumed amino acids can be preferred over glucose. Furthermore, they demonstrate that metabolic regulation in *E. coli* is more complex than previously thought.

References

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