

Regulation of sugar consumption in *Escherichia coli* by amino acids

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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.

The mechanism is independent of the phosphotransferase system and appears to result from the switch between glycolytic and gluconeogenic 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). In support of this mechanism, we found that pyruvate also inhibits the uptake of glucose.

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*. Our results, however, demonstrate that easily consumed amino acids are the preferred carbon source. Furthermore, they demonstrate that metabolic regulation in *E. coli* is more complex than previously thought.

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