The regulation of PKM2 by acetylation, studied using synthetic biology-based tools

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The glycolytic enzymes are tightly regulated by various mechanisms, including post-translational modifications (PTMs). Growing evidence suggests that the reversible addition of an acetyl group to lysine side chains, PTM called acetylation, may dramatically alter the structure and function of proteins. Recent proteomic studies showed that the M2 isoform of pyruvate kinase (PKM2), the enzyme that catalyses the final step of glycolysis, is being acetylated on multiple sites. However, the biological significance of most identified acetylations is yet to be defined. Here we show that the enzymatic activity of human PKM2 directly depends on the acetylation of specific lysine residues. Using genetic code expansion technology, we produced site-specifically acetylated full-length PKM2 by genetically encoding the incorporation of Nε-acetyl lysine into ribosomally-expressed PKM2. We identified specific acetylation sites that modulate V_{max} and K_M, as well as the thermal stability of PKM2, without significant effect on the three-dimensional structure of the protein. In addition, by monitoring the deacetylation of site-specifically acetylated PKM2, we identified the enzymes that regulate the acetylation state of specific PKM2 lysine residues. We expect these data to expand our understanding of the regulation of the glycolytic pathway by acetylation. Moreover, since PKM2 is the overexpressed pyruvate kinase isoform in most proliferating cancer cells, uncovering possible relations between acetylation and metabolic disorders is of high importance.