Engineered Regulation-resistant Fructose-1,6-bisphosphatase for Deregulation of Gluconeogenesis in *Escherichia coli*

서상우[1], 양재성[2], 장성호[1], 김상욱[3,2], 정규열[1,2,*]
[1]포항공과대학교 화학공학과;
[2]포항공과대학교 시스템생명공학부;
[3]포항공과대학교 생명과학과
(gyjung@postech.ac.kr*)

Pentose phosphate pathway generates reducing equivalents, NADPH, and 5-carbon sugars. The ribose 5-phosphate produced by this pathway can be recycled into glucose 6-phosphate (G6P) by transketolase, transaldolase, and some of the enzymes of the gluconeogenic pathway. Consequently, complete oxidization of 1 mole of G6P to CO2 can generate 12 moles of NADPH. However, in most cases, glycolysis is much more activated than gluconeogenesis to generate energy sufficient for cell survival. Moreover, glycolysis and gluconeogenesis are reciprocally regulated so that they do not take place simultaneously in the same cell to a significant extent. Therefore, it is important to deregulate gluconeogenic pathway to fully oxidize G6P for generation of NADPH. Fructose-1,6-bisphosphatase (FBPase) is a key enzyme that participate in the regulation of gluconeogenesis. It is allosterically inhibited by both AMP and G6P. Here, we engineered FBPase to be deregulated by AMP and G6P based on the 3D-structure. Variants of FBPase purified by Ni-NTA column were characterized in terms of kinetic parameters.