BIOTRANS
LA ROCHELLE 2023

N°936 / OC / PC
TOPIC(s) : Enzyme discovery and engineering / Biocatalytic cascade reactions

NEW REACTIVITIES FOR TRANSALDOLASE FROM THERMOPLASMA ACIDOPHILUM

AUTHORS
Massimo DI MASCIO / UNIVERSITÄT GÖTTINGEN, GINSTERWEG 2, GOETTINGEN

PURPOSE OF THE ABSTRACT
The enzyme transaldolase from Thermoplasma acidophilum (TacTAL) is a thermostable protein that physiologically converts D-fructose 6-phosphate (F6P, the donor) and D-erythrose 4-phosphate (E4P, the acceptor) into D-sedoheptulose 7-phosphate (S7P) and D-glyceraldehyde 3-phosphate (G3P) [1]. It is possible to convert the enzyme from a transaldolase to an aldolase by mutating two amino acids; in fact, while the wild-type TacTAL cannot split the F6P in G3P and dihydroxyacetone, the double mutant E60Q/F132Y can do so [2]. In this study, the affinity of TacTAL (wild-type and “aldolase” variant) for different 5-carbon sugars as substrates was studied using different techniques such as steady-state kinetics, HPLC, mass spectrometry and circular dichroism. Using protein crystallography, the way the enzyme binds these compounds was studied, and the interactions between the substrates and some important residues were identified. The enzymatic production of various 8- and 7-carbon-atom sugars from F6P and/or 5-carbon-atom sugars was demonstrated by GC-MS. Finally, an enzymatic cascade process was developed to maximise the yield of the transaldolase reactions, in which F6P is used as a donor substrate. In this process, glyceraldehyde-3-phosphate (generated when F6P is cleaved) is converted to glycerol-3-phosphate using NADH and the two enzymes: triose-phosphate isomerase (TIM) and sn-glycerol-3-phosphate:NAD+ 2-oxidoreductase (G3PDH). This reaction converts NADH into NAD+. Finally, NAD+ is regenerated using aldehyde dehydrogenase (ADH) from Saccharomyces cerevisiae and isopropanol as substrate. In this last step, ADH converts isopropanol to acetone and NAD+ to NADH by regenerating the cofactor. The results of this study provide a better understanding of how TacTAL works. In the future, it will be possible to improve the enzyme's reactivity towards the compounds tested in this research using rational design. Finally, by optimising the cascade reaction for the production of sugars with 8- and 7-carbon atoms, it might be possible to scale up the reaction increasing the final quantity of the desired product.

FIGURES

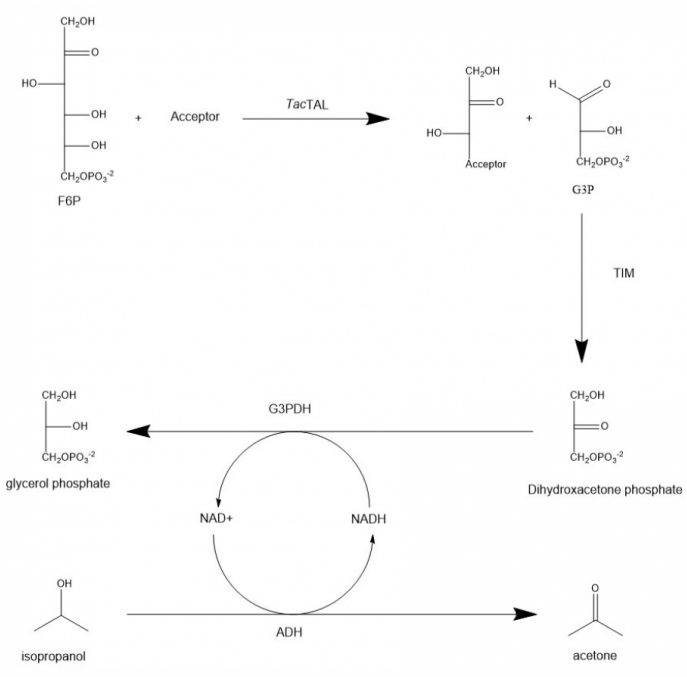

FIGURE 1

Cascade reaction

Cascade reaction, the cofactor NADH needed to eliminate Dihydroxyacetone phosphate is regenerated by the ADH reaction using isopropanol.

FIGURE 2

KEYWORDS

cascade reaction | phosphosugars

BIBLIOGRAPHY

[1] A. Lehwess-Litzmann, P. Neumann, C. Parthier, S. L□dtke, R. Golbik, R. Ficner, K. Tittmann, N. Chem. Bio, 2011, 7, 678-684.

[2] V. Sautner, M. Friedrich, A. Lehwess-Litzmann, K. Tittmann, Biochemistry, 2015, 54, 4475-4486.