Novel assay system for transketolase activity using xylulokinase from *Saccharomyces cerevisiae*

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Abstract

Transketolase is generally assayed with two substrates, ribose 5-phosphate and xylulose 5-phosphate, by measurement of decrease in the observance of OD_{340}. However one of the substrate, xylulose 5-phosphate, is not commercially available. Thus there is a requirement for novel assay system to determine the activity of transketolase. In this context, recently, few reports described an assay system that excessively used a single substrate ribose 5-phosphate in the reaction mixture, but single substrate assay system has not been generally employed due to low sensitivity and reproducibility. In this study an efficient protocol for the detection of transketolase activity was established by coupling xylulokinase. An ATP-dependent xylulokinase from Saccharomyces cerevisiae. converted xylulose into xylulose 5-phosphate, and it further served as real substrate for transketolase. These reaction carried out simultaneously or sequentially in a tube and showed relatively high sensitivity and reproducibility, about 100 fold higher than those of single substrate assay system currently used. To minimize the interference with contaminant chemical or enzymes, xylulokinase was cloned and one-step purified to apparent homogeneity, and then used for reaction.

References

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