DEVELOPMENT AND MINIATURIZATION OF METHODS FOR BETA-GLUCOSIDASE CHARACTERIZATION

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ABSTRACT

Our objective was purification of recombinant β-glucosidases and measurement of their kinetics on natural substrates using a novel kinetic method.

Recombinant β-glucosidase Zm-p60.1 was expressed from plasmid pRSET A, using the expression system Escherichia coli BL21(DE3)pLysS-T1R. The enzyme was purified by affinity chromatography on a HisTrap HP column and subsequently by gel filtration on a Superdex 200 column. Concentration of enzyme was determined by the Bradford method. Densitometric analysis of bands of polyacrylamide gels was used to verify purity of the enzyme, which was over 98%.

We improved a method to measure enzyme kinetics where concentration of glucose was determined after the reaction. β-D-glucopyranose is oxidized in the presence of glucose oxidase, releasing hydrogen peroxide. This hydrogen peroxide then oxidizes Amplex Red in the presence of horseradish peroxidase to form the fluorescent resorufin. This method was used for the characterization of β-glucosidase Zm-p60.1 on the natural substrate trans-zeatin-O-β-D-glucopyranoside.

In contrast to the existing method(s) of kinetic measurements, the novel procedure is the result of partial miniaturization, as the volume of the reaction mixture as well as the detected amount of sample was reduced. This is very important as natural substrates are very expensive.

Key words: β-glucosidase, glucose oxidase, peroxidase, Amplex Red, resorufine, fluorescence, purification

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