

APPLICATION NOTE

Removal of Contaminants from Partially Purified Glucose Oxidase (*A.niger*) using Mimetic Orange 3 A6XL

Introduction

Glucose oxidase is an enzyme which converts glucose to gluconic acid and hydrogen peroxide. Considered to be commercially important, glucose oxidase is used in food processing, production of gluconic acid, quantitative determination of glucose in fermentation processes and medical diagnostics.

Removal of contaminants from partially purified glucose oxidase *Aspergillus niger* (*A.niger*) by affinity chromatography on Mimetic Orange 3 A6XL is outlined below.

Experimental Conditions

A 4ml Mimetic Orange 3 A6XL affinity chromatography column (ProMetic BioSciences Ltd, UK) was loaded with partially purified *A.niger* glucose oxidase in 20 mM sodium phosphate, pH 6 at a flow rate of 0.8 ml/min. Elution was undertaken using 20 mM sodium phosphate /0.5 M NaCl buffer, pH 6.0.

Results and Conclusions

Glucose oxidase bound to Mimetic Orange 3 A6XL affinity chromatography column and was eluted using 0.5 M NaCl (Fig. 1).

The specific activity of the glucose oxidase recovered in the elution was 120 units/ml-1. Recovery of glucose oxidase in the elution fraction was 92%. The glucose oxidase was concentrated 12.6 fold.

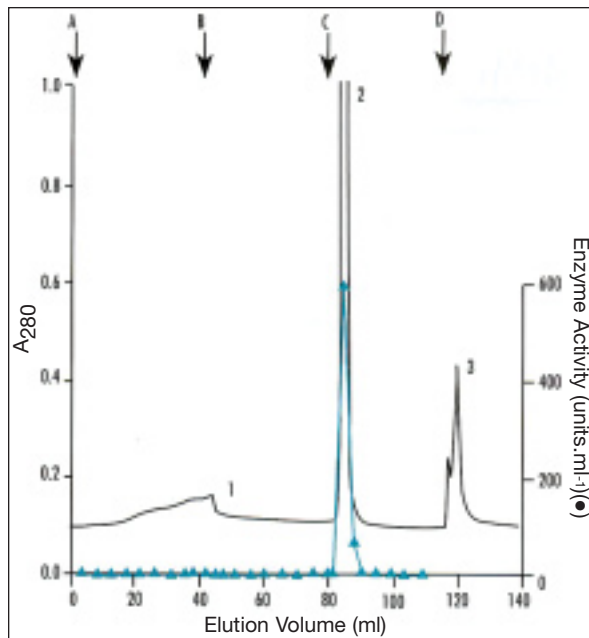


Fig. 1: An FPLC trace of the removal of contaminants from partially purified glucose oxidase using Mimetic Orange 3 A6XL. (A) Partially purified *A. niger* glucose oxidase in 20 mM sodium phosphate, pH 6 (40 ml; 0.4 mg/ml of protein; specific activity 100 units/mg-1) (B) 20 mM sodium phosphate, pH 6.0; (C) 20 mM sodium phosphate /0.5 M NaCl, pH 6.0; (D) 1 M NaOH. Eluted peaks: (1) unbound contaminants, (2) glucose oxidase and (3) residual contaminants.

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