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KEY WORD: CYCLODEXTRIN GLYCOSYLTRANSFERASE/*Bacillus sp.* A11 /IMMUNOAFFINITY CHROMATOGRAPHY

PORNCHAI KIM : PURIFICATION OF CYCLODEXTRIN GLYCOSYLTRANSFERASE BY IMMUNOAFFINITY CHROMATOGRAPHY. THESIS ADVISOR : ASSOC. PROF. PIAMSOOK PONGSAWASDI, Ph.D., THESIS CO-ADVISOR : ASSIST. PROF. TIPAPORN LIMPASENI, Ph.D., SOMPORN KAMOLSIRIPICHAIPORN, Ph.D., 106 PP. ISBN 974-634-945-7

A polyclonal antibody prepared against cyclodextrin glycosyltransferase (CGTase) was purified from rabbit antiserum by ammonium sulfate precipitation with a 45 % saturation and DEAE-cellulose ion exchange chromatography. Fractions were tested for the presence and purity of IgG by SDS-polyacrylamide gel electrophoresis. The result demonstrated that antibody against CGTase of high purity was obtained by the described purification method and the antibody titer was determined to be $1:2^8$ by Ouchterlony immunodiffusion.

The purified antibody was linked to CNBr-activated Sepharose 4 B and used for immunoaffinity purification of CGTase. The bound enzyme was eluted with 3.5 M sodium thiocyanate in 50 mM ammonium hydroxide, pH 10.5, at a flow rate 0.1 millilitre/minute at room temperature. The specific activity of the purified CGTase was increased 155 folds and about 45 % of the total activity was recovered. The prepared enzyme was separated into two bands after non denaturing-polyacrylamide gel electrophoresis, but showed only a single band in SDS-polyacrylamide gel electrophoresis. The molecular weight of the protein band was estimated to be 72,000 dalton.

ภาควิชา..........

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