ELSEVIER

Process Biochemistry 40 (2005) 1101–1111

PROCESS BIOCHEMISTRY

www.elsevier.com/locate/procbio

# Purification and characterization of cyclodextrin glucanotransferase from alkalophilic *Bacillus* sp. G1

Ho Kek Sian[a], Mamot Said[b], Osman Hassan[b], Kamarulzaman Kamaruddin[c], A. Fauzi Ismail[a], Roshanida A. Rahman[a], Nik Azmi Nik Mahmood[a], Rosli Md. Illias[a,*]

[a]*Faculty of Chemical Engineering and Natural Resources Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia*
[b]*School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan, Malaysia*
[c]*Bioprocess and Chemical Technology Centre, SIRIM Berhad, P.O. Box 7035, Section 2, 40911 Shah Alam, Malaysia*

Received 25 November 2003; received in revised form 15 March 2004; accepted 28 March 2004

**Abstract**

A cyclodextrin glucanotransferase (CGTase) was successively purified by ammonium sulphate precipitation, and affinity chromatography on α-CD (epoxy)-Sepharose 6B column. The specific activity of the CGTase was increased approximately 2200-fold, from 8.43 U/mg protein to 18,866 U/mg protein. SDS-PAGE showed that the purified CGTase was homogeneous and the molecular weight of the purified CGTase was about 75 kDa. The molecular weight of the enzyme that was estimated by gel filtration under native condition was 79 kDa. This has indicated that *Bacillus* sp. G1 CGTase is a monomeric protein. The isoelectric point (p*I*) of the enzyme was about 8.8. Characterization of the enzyme exhibited optimum pH and temperature of 6.0 and 60 °C, respectively. The enzyme was stable from pH 7.0 to 9.0 and retained its high activity up to 60 °C. However, in the presence of 20 mM $Ca^{2+}$, the purified CGTase is able to prolong its thermal stability up to 70 °C. CGTase was strongly inhibited by $ZnSO_4$, $CuSO_4$, $CoCl_2$, $FeSO_4$, $FeCl_3$ and EDTA. $K_m$ and $V_{max}$ for the purified enzyme were 0.15 mg/ml and 60.39 mg β-cyclodextrin/(ml min), respectively, with soluble starch as substrate. In cyclodextrin production, tapioca starch was found to be the best substrate used to produce CDs. The enzyme produced γ- and β-CD in the ratio of 0.11:0.89 after 24 h incubation at 60 °C, without the presence of any selective agents.
© 2004 Elsevier Ltd. All rights reserved.

*Keywords:* Cyclodextrin; Cyclodextrin glucanotransferase

* Corresponding author. Tel.: +607-5535564; fax: +607-5581463.
*E-mail address:* r-rosli@utm.my (R.Md. Illias).

0032-9592/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.procbio.2004.03.018