CHARACTERISATION OF THERMOSTABLE DEXTRANASES FROM MICRO-ORGANISMS FOR COMMERCIAL APPLICATION

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Contents

ABSTRACT

Currently, when dextranase is used in the sugar factory, modifications are required to normal factory operating conditions in order to achieve cost effective dextran 'removal'. The major change involves the decrease in primary heater temperature from 75°C to about 60°C. A number of processing problems result from this temperature change. Therefore, it was proposed to develop a thermostable dextranase which was active and stable at 75°C. The use of a high temperature dextranase would also overcome the problems associated with operating the primary heater at the lower temperature.

Existing culture collections, thermal environments and sites within raw sugar factories were used as the sources of microbial isolates screened for thermostable dextranase producers. Based on the amount of enzyme produced at elevated temperatures, five bacterial strains (SRI 2125, SRI 2128, AB11A, RT364 and DP17) were selected as the most promising sources of thermostable dextranases. These isolates were grown to pure strain using standard microbial techniques. Thereafter, broth culture of these strains was undertaken to produce sufficient crude extracellular dextranase for liquid assay. However, compared to fungal isolates, the amount of dextranase produced by the bacterial isolates was low.

Several assays to measure micro-quantities of dextranase activity were developed or modified to assess the thermal stability of the enzymes. Temperature and pH profiles of the crude extracts were determined using the PAHBAH assay to obtain a rapid and sensitive measure of optimal conditions required for enzymic activity. The subsequent development of the micro-haze test allowed the thermal stability and activity to be reliably assessed under simulated factory conditions.

Both new and existing enzymic assays were utilised to determine the activity and thermal stability of the most cost-effective commercial dextranase currently available *i.e.* the *C. gracile* dextranase. This enzyme was found to exhibit optimum activity at 55-60°C (pH 5.0) under all assay conditions. However, thermal stability at temperatures above 65°C was very low. A comparative assessment of the commercial potential of new thermostable dextranases was possible using the *C. gracile* dextranase as the 'bench mark'.

In addition, detailed studies on the *C. gracile* dextranase were performed to determine the physico-chemical characteristics of the enzyme(s) in the commercial preparation. The dextranase was separated into five distinct components by electrophoresis of the native enzyme. However, each of these components were found to possess a similar (if not identical) molecular size. Partial separation of these components was achieved using chromatofocusing. Each fraction obtained exhibited endo-dextranase activity.

Crude preparations of the five bacterial dextranases were found to exhibit optimal activity between 63 and 75°C (depending on the assay conditions and the method employed for activity measurement). The specific activities of these crude dextranase preparations were very low (0.021 to 0.68 μmole min^{-1} mg^{-1}) compared to the crude extracts obtained from fungal sources such as *C. gracile*. The presence of endo-dextranase activity was confirmed by determining the activity against cane dextran under simulated factory conditions.

The dextranase produced by the RT364 isolate was selected for purification investigations as it was the most active in the crude form. Several standard techniques for protein purification were utilised and the purity of the resulting preparations established by electrophoretic analysis. The active dextranase in the fraction of highest purity and activity was shown to represent in excess of 50 per cent of the protein in the preparation. Using this estimate, a final specific activity for the purified thermostable dextranase from RT364 was calculated to be about 20 μmole min^{-1} mg^{-1}. At this level, the specific activity of the RT364 enzyme is approximately 150 times less than that of the commercial *C.gracile* dextranase measured its optimum at temperature of 55°C. On this basis, the commercial potential of the thermostable dextranase from the RT364 isolate appears to be limited.

To date, the specific activities of purified forms of the remaining four thermostable dextranases have not been determined. It is possible that one of these isolates may be the source of a thermostable dextranase with greater commercial potential. However, further investigations with these enzymes to establish their commercial viability would require high developmental costs in relation to the very small market (the Australian sugar industry) currently available for these enzymes.

FOREWORD

The program of study described in this thesis formed an important part of a joint project between the Sugar Research Institute, the University of Queensland, and Griffith University to develop a high-temperature dextranase for use in the sugar industry.

The initial phases of the project required the development of screening methods for dextranase producing microorganisms and screening of microbial isolates from many and varied sources, in particular thermal environs. The microbiological aspects of the research were carried out by microbiologists and associated staff at their respective research institutions.

This thesis describes the biochemical characterisation of thermostable dextranases produced by the microbial isolates identified in the earlier microbial study. Initial assessment of the temperature optima, thermal stability and pH optima of the dextranases present in crude fermentation extracts of both aerobic and anaerobic thermophiles was carried out under defined conditions. Subsequently, methods were developed to allow micro-scale assessment of the crude dextranases under simulated factory conditions. A detailed physico-chemical characterisation of the most promising of the thermostable enzymes was undertaken in conjunction with a comparative analysis of the best dextranase available commercially. Preliminary investigations were also carried out to develop a purification scheme for a promising enzyme. These studies are ongoing in our group.

Future work will be aimed towards selection of dextranase-producing microorganisms which exhibit the potential for further development to the commercial stage. The selected enzyme(s) will be purified and characterised prior to cloning and expression in suitable microbial hosts. These proposed investigations would be carried out by researchers at the Centre for Molecular Biology and Biotechnology (UQ).

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

Chapter 6

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 4

CHAPTER 5

LIST OF ABBREVIATIONS

AS	-	ammonium sulphate
ATCC	-	The American Type Culture Collection
CM	-	carboxymethyl
DEAE	-	diethylaminoethyl
EDTA	-	ethylenediaminetetra acetic acid (disodium salt)
HPLC	-	high performance liquid chromatography
ME	-	2-mercaptoethanol
$\bar{M}_n$	-	number average molecular weight
MW	-	molecular weight
NCIB	-	The National Collection of Industrial and Marine Bacteria
NRRL	-	The United States Department of Agriculture Culture Collection
PAGE	-	polyacrylamide gel electrophoresis
PAHBAH	-	*p*-hydroxybenzoic acid hydrazide
ppm on brix	-	parts per million on brix
RI	-	refractive index
SDS	-	sodium dodecyl sulphate
Tris	-	tris (hydroxymethyl) aminoethane

DEFINITIONS

In the course of this thesis certain terms have been used which are peculiar to the technology of the sugar industry, *e.g.* brix as a measure of solution concentration. Short definitions of some of these terms follow.

Brix : An appropriate measure of dissolved solids content derived from measurement of the density or the refractive index of the sample expressed as the percentage by weight of sucrose in a solution which has the same specific gravity (brix or °bx) or refractive index (Refractometer brix) as the sample.

Mud Filter : Mixed cane is clarified to remove suspended or insoluble impurities prior to evaporation to syrup. The 'mud solids' obtained are removed from process using a rotary vacuum filter (the mud filter).

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The work described in this thesis was carried out at the Sugar Research Institute, Mackay, Queensland during the period February, 1991 to July, 1992, with exception of some investigations on the *C. gracile* dextranase (Chapter 3). This work was performed prior to this time as part of the appraisal of commercial enzymes for the sugar industry.

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PUBLICATIONS

A list of publications to date, arising from this study.

(i) Lucas, S.R., Wynter, C., Brown, C.F.*, Dawson, M., Inkerman, P.A, Patel, B., de Jersey, J., Hamilton, S. (1990). "Screening procedures for high-temperature dextranase from thermophilic bacteria". Poster Session, Aust. Biotech. Conf.

(ii) Wynter, C.V.A., Chang, M., Hamilton, S., de Jersey, J., Galea, C. and Inkerman, P.A. (1992). "Purification and properties of a thermostable dextranase". Proc. Aust. Soc. Biochem. and Molecular Biol., 24.

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* Author's maiden name was Brown.