Modulating the Synthesis of Dextran with the Acceptor Reaction Using Native and Encapsulated Dextranucrases

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Summary

Dextranucrases are glucansucrases with broad applications in the food, cosmetics and pharmaceutical industries. Using sucrose as the glucosyl donor, they synthesize both high molecular weight (HMW) dextrans and potential prebiotic oligosaccharides. The process selectivity can be modulated by varying the reaction conditions. When no other molecule is present in the reaction, only dextrans are synthesized. In the presence of methyl α-D-glucopyranoside, the synthesis of methyl polyglucosides takes place, diminishing the transfer of glucose molecules to form dextran. In this work, the formation of HMW soluble dextran and methyl polyglucosides was studied with dextranucrases from Leuconostoc mesenteroides, strains B-512F and B-1299. The amount of dextran formed with dextranucrase B-512F was reduced up to 4 % with respect to the control in absence of acceptor, using a mass ratio of sucrose:methyl α-D-glucopyranoside of 1:4. The encapsulation in alginate retains the dextran inside the beads, causing a distortion of the biocatalyst and finally releasing the polysaccharides into the reaction medium.

Key words: glucosyltransferases, immobilization, dextran, acceptor, prebiotics

Introduction

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products (5): dextran from \textit{L. mesenteroides} B-512F contains 95% of \(\alpha(1\rightarrow6)\) linkages and 5% of \(\alpha(1\rightarrow3)\) branch-point linkages; the insoluble form of dextransecurase (IGT) from \textit{L. mesenteroides} B-1299 catalyses the synthesis of an insoluble dextran that contains 63% of \(\alpha(1\rightarrow6)\), 27% of \(\alpha(1\rightarrow2)\) and 8% of \(\alpha(1\rightarrow3)\) linkages, and of a soluble dextran that contains 68% of \(\alpha(1\rightarrow6)\), 29% of \(\alpha(1\rightarrow2)\) and 3% of \(\alpha(1\rightarrow3)\) linkages (6). Molecular mass of dextran can also vary considerably depending on the synthesis conditions (7) as well as on the producing enzyme (8–11). Dextran enterprises various applications in the food industry and as gel-permeation matrices for laboratory chromatography (12). They also have various clinical applications, including their use as blood volume expanders. For these applications dextran are produced in both Europe and the USA using dextransecurases obtained from \textit{L. mesenteroides} B-512F and are later partially hydrolysed.

The mechanism of dextran synthesis is still unclear (13), although a two-site insertion pathway has been proposed (14–17). When other sugars are added to the reaction mixture, dextranase can also transfer glucosyl residues from sucrose to the non-reducing end of these molecules forming the so-called acceptor products (18). Maltose is the best acceptor for dextranase due to its capacity of diverting the synthesis of dextran to the formation of acceptor products (19). Several acceptor products obtained with dextranase can be considered as prebiotics (20), because they present high resistance to glycolytic digestive enzymes and are specifically fermented by bifidobacteria (21,22). Both dextrans and prebiotic oligosaccharides synthesized with dextranase are interesting from an industrial point of view.

For the industrial application of dextransecurases, the search for efficient immobilization methods is required. Up to now, enthainment has been the most successful method for immobilization of soluble and insoluble dextransecurases (23–26), with activity recoveries close to 90% and a notable operational stability. Enthapsulation in alginate beads or fibres is the most popular method. This technique was first applied to the immobilization of whole cells due to the mild conditions required and the easy diffusion of substrates and products through the wide pores of the matrix (27). The effect of the production of dextran on the stability of the support has not been evaluated yet.

In the work reported here, we compared the production of high molecular weight (HMW, >10^5) dextran using two dextransecurases from different strains of \textit{L. mesenteroides}, B-512F and B-1299. We also studied the effect of the addition of the acceptor methyl \(\alpha\)-D-glucopyranoside on dextran production by both free and alginate-immobilized enzyme.

Materials and Methods

Reagents

\textit{Leuconostoc mesenteroides} NRRL B-512F dextranase (dextransecurase B-512F) was obtained and purified as described (28). \textit{L. mesenteroides} NRRL B-1299 dextranase (dextransecurase B-1299) was produced as reported (4). The insoluble fraction (IGT) was used for this work as a lyophilized powder. Dextranase (E.C. 3.2.1.11) from \textit{Penicillium} sp. (grade 1200, 500 U/mg of protein), methyl \(\alpha\)-D-glucopyranoside and dextran standard (MW=144 000) were from Sigma. Other dextran standards (MW=180, 504, 830, 4400, 9900, 21 400 and 43 500) were from Phomenex and from Amershan Biosciences (MW=70 000 and 500 000). Alginate SG-300 (rich in guluronic acid) was provided by System Bio-Industries. Merck supplied sucrose and fructose. All other reagents and solvents were of the highest available purity and used as purchased.

Assay of dextranase activity

Dextranase activity was determined by measuring the initial rate of fructose production using the di-nitrosalicylic acid (DNS) method (29). The reaction was carried out at 30 °C in a flask containing 20 mM sodium acetate buffer (pH=5.4), CaCl\(_2\) 0.05 g/L and sucrose 100 g/L. The absorbance at \(A_{420\text{nm}}\) was measured using a Kontron 930 spectrophotometer. One unit of dextranase activity was defined as that corresponding to the formation of 1 \(\mu\)mol fructose per min under the conditions described above.

Entrapment of native dextranase in calcium alginate beads

A 4% mass per volume ratio of sodium alginate solution (15 g) was mixed with 15 mL of one of the two dextranase suspensions (5 U/mL) prepared in 10 mM sodium acetate buffer (pH=5.4). The alginate-enzyme mixture was dropped into a stirred solution (250 mL) of 50 mM sodium acetate buffer (pH=5.4), containing 0.2 M CaCl\(_2\). The formed beads were further stirred in the solution for 2 h to allow them to harden. The diameter of the beads obtained was around 2 mm. The biocatalyst was stored at 4 °C in 50 mM acetate buffer (pH=5.4) containing 0.2 M CaCl\(_2\) and 0.02 % sodium azide.

Soluble dextran synthesis

Dextran synthesis was assayed at 30 °C in a medium (10 mL) containing sucrose 100 g/L, CaCl\(_2\) 0.05 g/L and 20 mM sodium acetate buffer (pH=5.4). The dextranase (3 U for B-512F and B-1299, 2 U for the enzymes immobilized in alginate) was added. Several amounts of acceptor (methyl \(\alpha\)-D-glucopyranoside) were added to different flasks (0, 0.5, 1 or 4 g). At several intervals, 500-\(\mu\)L aliquots were extracted from the reaction mixture. The reaction was stopped by incubation at 60 °C for 10 min. The mixture was then centrifuged for 5 min at 6000 rpm using eppendorfs with a 0.45-\(\mu\)m Durapore™ membrane (Millipore), and analysed by HPLC.

HPLC analysis

The molecular weight distribution of the dextran formed was analyzed by size exclusion chromatography (SEC) with a pump SP 8810 (Spectra-Physics Inc.) coupled to a precolumn BioSep-SEC S-2000 (75 x 7.8 mm) and a column BioSep-SEC S-2000 (300 x 7.8 mm). The mobile phase was 20 mM acetate buffer (pH=5.4) containing 0.05 g/L of CaCl\(_2\), at a flow rate of 0.7 mL/min. The temperature of the column was kept constant at 40 °C. A Waters 2410 refraction index detector was used at 45 °C.
The data obtained were analyzed using the Varian Star Software. The standards used for calibration were in the range between 180 (glucose) and 500 000 Da. The quantification of the HMW dextran produced was estimated by a calibration curve obtained with dextran 500 000.

Results and Discussion

Production of HMW dextrans by dextransucrase

Dextransucrase is able to synthesize both high molecular weight dextrans and low molecular weight compounds. The use of size-exclusion chromatography (SEC) allowed us to analyse the molecular weight distribution of the compounds formed.

The production of soluble HMW dextrans with dextransucrases B-512F and B-1299 was compared (Fig. 1). The formation of dextrans was detected from the initial stages of the reaction. The production of soluble HMW dextran was 10-fold higher with the dextransucrase B-512F than with that of B-1299. After 24 h of reaction the concentration of HMW dextran reached 45.7 g/L with the former enzyme, whereas it reached only 3.75 g/L with the latter enzyme.

Effect of the acceptor (methyl α-D-glucopyranoside) on the production of HMW dextran

When methyl α-D-glucopyranoside was added to the reaction it acted as an acceptor, which is a consequence of the mechanism of action of dextransucrase, causing the synthesis of low molecular weight compounds called methyl polyglucosides. The scheme of the reaction is shown in Fig. 2. Methyl α-D-glucopyranoside also modulated the amount of dextran that was produced (Fig. 3). The higher the acceptor concentration, the lower the amount of dextran that was synthesized. However, there was only a slight change in the molecular weight distribution of the formed dextran. When the mass ratio of sucrose to acceptor was 1:1, the mass of dextran formed after total consumption of sucrose was only 19 % of the mass formed in a control reaction carried out in the absence of the acceptor. When the mass ratio of sucrose to acceptor was 1:4, the mass of dextran formed fell further still, being only 2 % of the mass formed in the control. This effect was observed with both enzymes although with dextransucrase B-1299, in the range of concentrations tested, the decrease in the mass of dextran formed was not as pronounced. The dextran formed with this enzyme was approximately 82 % of the control for a sucrose:acceptor mass ratio of 1:1 and 36 % of the control for a sucrose:acceptor mass ratio of 1:4.

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Effect of exogenous dextran on the production of HMW dextran

In order to know if dextransucrase was able to use an HMW molecule as an acceptor for further elongation, we studied the synthesis of dextran in the presence of an exogenous dextran with an average molecular weight of 40,000 (Fig. 4). The dislocation of the HMW peak to the left in Fig. 4A, i.e., to higher molecular weights, shows that the enzyme was able to increase the molecular weight of this added compound progressively, forming a dextran with a very high MW, at a much higher concentration than that obtained in the absence of exogenous dextran (Fig. 4B). The successful elongation of added dextran in this experiment opens the possibility of obtaining dextran preparations of particular molecular weight distributions from lower molecular weight preparations. Thus, we could be able to obtain the desired molecular weight of dextran, using a single step process.

Production of dextran with the immobilized enzyme

Dextransucrase was successfully immobilized by encapsulation in alginate beads as shown in Table 1. The recovered activity was around 90 % for dextransucrase B-512F and about 60 % for dextransucrase B-1299. The entrapment was quite efficient for dextransucrase B-512F. Lower recovery obtained with dextransucrase B-1299 might be due either to the presence of cells or to the homogenisation method used for this enzyme, as was reported elsewhere.

When the alginate-immobilized enzyme is used in the acceptor reaction, the internal diffusion of the oligosaccharides formed is not restricted. In contrast, the dextran produced by the synthesis reaction is retained inside the alginate matrix. Fig. 5 shows the production of HMW dextran, using dextransucrases B-512F and B-1299 encapsulated in the alginate. In the first 6 h, a negligible amount of dextran was detected in the reaction medium. The appearance of dextran in the solution...
dextran, dextransucrase B-512F is more efficient than dextransucrase B-1299. However, dextransucrase B-1299 is more convenient for the synthesis of prebiotic oligosaccharides, because the enzyme is able to produce compounds with resistant α(1→2) bonds and the formation of dextran diminishes with respect to B-512F. Immobilized dextransucrase can be used for the production of prebiotic oligosaccharides; however, the use of the entrapped biocatalyst should be avoided for the synthesis of dextran compounds due to the swelling and breakdown of the support.

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References

Moduliranje sinteze dekstrana s akceptorskom reakcijom koristeći prirodne dekstransukraze i one u kapsulama

Sažetak

Dekstransukraze su glukansukraze s velikom primjenom u prehrani, kozmetici i farmaceutskoj industriji. Koristeći saharozu kao donor glukozilnog ostatka, one sintetiziraju dekstrane velike molekularne mase i potencijalne prebiotske oligosaharide. Mijenjajući reakcijske uvjete može se modulirati selektivnost procesa. Kada u reakciji nema drugih molekula, sintetiziraju se samo dekstrani. U prisustvu metil-D-glukopiranozida dolazi do sinteze metilpoliglukozida smanjujući prijenos molekula glukoze za stvaranje dekstrana. U radu je istraživano stvaranje visokomolekularnog topljivog dekstrana i poliglukozida koristeći dekstransukraze iz sojeva B-512F i B-1299 *Leuconostoc mesenteroides*. Količina nastalog dekstrana s dekstransukrazom B-512F smanjena je za 4% u usporedbi s kontrolnim uzorcom koji nije sadržavao akceptor. U sintezi je upotrijebljen maseni udjel saharoze:metil-α-D-glukopiranozid u omjeru 1:4. Enzim u kapsuli alginate zadržava dekstran unutar kapsule uzrokujući distorziju biokatalizatora uz konačno otpuštanje polisaharida u reakcijoski medij.