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Immobilization of dextransucrase on regenerated benzoyl cellulose carriers

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Abstract

The regeneration of the benzoyl cellulose carrier was accomplished by treatment by Triton X-100, sodium dodecyl sulfate (SDS), or alkyldimethylbenzyl-ammonium chloride (ADMB) detergent solutions, or by NH_4SCN as chaotropic agent. It was found that Triton X-100, as a more hydrophobic substance, was linked to the carrier, eluting the protein from the carrier. Regenerated in this way, the carrier very efficiently binds new amounts of proteins, including dextransucrase, without regarding the fact that Triton X-100 was washed out by alcohol, or remained linked to the carrier. Dextransucrase immobilization at this regenerated carriers yielded high values for the immobilized activity, total preserved activity and immobilization efficiency.
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Keywords: Dextransucrase; Benzoyl cellulose; Enzyme immobilization; Carrier regeneration

1. Introduction

Dextransucrase [EC 2.4.1.5; sucrose: 1,6-α-D-glucan 6-α-D-glucosyltransferase] from *Leuconostoc mesenteroides* B-512F catalyses the formation of the soluble dextran by a transfer of the D-glucosyl moieties of sucrose to a growing polymer chain. Dextran is a polysaccharide in which more than 95% of the D-glucosyl units are α-1,6 linked, with less than 5% α-1,3-branch linkages. The reaction takes place via an insertion mechanism [1]. The prospects of dextransucrase application are significant in the synthesis of dextran for use as an essential component in a blood volume expander [2], as well as for the production of leucrose and other oligosaccharides [3,4]. Recently, a review on the dextransucrase in food enzymology was published [5].

Different methods have been proposed to immobilize dextransucrases, based on adsorption [6], entrapment in polymers [7–9], or covalent binding [7,10,11]. The immobilization methods, which rely to the covalent and ionic binding result in a considerable enzyme denaturation and low immobilization efficiencies. When phenoxyacetyl cellulose was used as a carrier for immobilizing dextransucrase by hydrophobic interaction, high immobilized activities with high immobilization efficiencies were obtained [12]. Entrapment in calcium alginate, showed good results for dextransucrase concerning activity, yield, and operation stability [8]; however, applications of this type of immobilization is restricted to oligosaccharides production [3].

Butler and coworkers found that benzoyl cellulose, even with a low degree of acylation (0.43 mequiv./g), binds proteins by weak hydrophobic interaction [13,14]. The influence of the degree of acylation of benzoyl cellulose and time of duration of the immobilization on the immobilized activity and immobilization efficiency of dextransucrase on benzoyl cellulose, as well as some properties of immobilized enzyme was studied [15].

In this work, the possibilities of benzoyl cellulose carrier regeneration for the immobilization of dextransucrase from *L. mesenteroides* B-512F were investigated. Detergents Triton X-100, sodium dodecyl sulfate and alkyldimethylbenzyl-ammonium chloride, and NH_4SCN (as chaotropic agent) were used as regeneration agents. Additionally, we investigated the regeneration of benzoyl cellulose carrier by Triton X-100 in detail.

2. Materials and methods

2.1. Materials

The Merthiolate, Coomasie Brilliant Blue G 250, SDS and bovin serum albumine (BSA) used in this study were from Sigma Chemical Co., Triton X-

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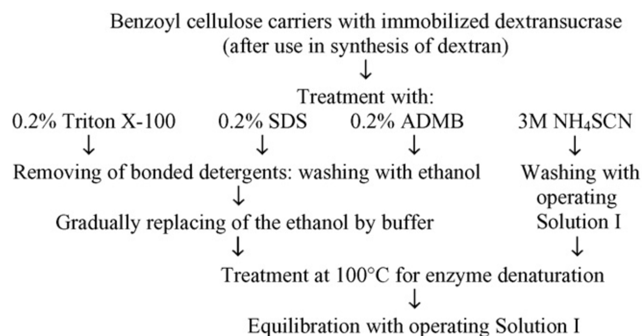

Scheme 1. First regeneration and preparation of carriers for first immobilization.

Table 1
Treatment of benzoyl cellulose carrier with 0.2% solution of Triton X-100 in acetate buffer (Solution II)

Treatment no.	Carrier quantity (g)	Solution II quantity (mL)	Treatment duration (min)
1	6.0	120	885
2	6.0	120	30
3	6.0	120	55
4	5.4	120	45
5	5.4	100	40
6	5.4	100	40

100 from Fluca AG and Avicel PH 102 from Selectchemie AG. Avicel PH 102 represents the microcrystalline cellulose with average size of particle 100 μm. All the other chemicals were p.a. quality.

Dextransucrase was obtained in crude form by cultivating *L. mesenteroides* B-512F as described in [16] with the pH maintained in the range 6.6–6.7. The supernatant after cell removal, pH adjustment to 5.2 and adding Merthiolate in an amount of 0.005% was used as the enzyme solution. The enzyme solution was stored at +4 °C until use.

Solutions: I (pH 5.4), 0.5 M solution of NaH_2PO_4 with 0.005% Merthiolate; II (pH 5.2), 0.2% (v/v) solution of Triton X-100 in 75 mM acetate buffer with 5 mM $CaCl_2$ and 0.005% Merthiolate; III (pH 5.4), 0.5 M solution of NaH_2PO_4 with 1 M $(NH_4)_2SO_4$, 5 mM $CaCl_2$ and 0.005% Merthiolate; IV (pH 5.4), 0.5 M acetate buffer with 5 mM $CaCl_2$ and 0.005% Merthiolate.

2.2. Carrier synthesis

Benzoyl cellulose with the acylation degree of 2.53 mequiv./g was synthesized according to previously published protocol [13,15], and used as a carrier for immobilization and regeneration.

2.3. First regeneration of carriers

First regeneration and preparation of carriers for first immobilization were presented in Scheme 1. The regeneration was carried out with 0.20 g samples of each carrier that had already been used for the synthesis of dextran. The residual activity of immobilized enzyme was 0.76 U/g. The carriers in the cuvettes were treated four times with 4 mL 0.2% solution of Triton X-100, SDS or ADMB, or 4 mL of 3 M solution of NH_4SCN. The individual treatments consisted in 10 min shaking on the vortex and subsequent centrifuging. In order to remove the bonded detergents, the first three carriers were washed five times with 6 mL of ethanol each (the carrier treated with SDS was additionally heated in water bath for better SDS dissolving). After that, the alcohol was gradually replaced by the buffer. Samples were rinsed with 5 mL of the following solutions: ethanol (95%), ethanol (70%), ethanol (45%), 10 mM acetate buffer (pH 5.4) with 25% ethanol, 50 mM acetate buffer with 10% ethanol and 0.2 M acetate buffer (pH 5.4). Finally, the carriers were equilibrated with 5 mL of operating Solution I. The carrier treated with NH_4SCN was immediately rinsed five times with 5 mL of operating Solution I. Before the final rinse and equilibration with the Solution I, the regenerated carriers, as well as two samples of 0.20 g each of non-regenerated carrier (control samples) were placed for 4 min in the boiling water bath for reliable residual active enzyme denaturation.

2.4. First dextransucrase immobilization

Dextransucrase was immobilized on benzoyl cellulose carriers by a batch procedure using an enzyme solution obtained by simple removal of cells from the fermentation broth [15]. The first immobilization was carried out with samples of regenerated carriers obtained in first regeneration, 0.20 g each, and 7.45 mL enzyme samples containing 1 mmol/mL $(NH_4)_2SO_4$ at 23 °C and pH 5.4. The duration of the process was 125 min. The Solution I was used as the operating solution for carrier equilibration and for rinsing after immobilization.

Immobilization efficiencies represents the fraction of immobilized activity in the total disappearance of activity from solution during immobilization [11] and it was calculated by the following equation:

$$\text{immobilization efficiencies}\,(\%) = \frac{\text{immobilized activity}}{\text{activity before immobilization} - \sum \text{activity in supernatant and washing solutions}} \times 100.$$

2.5. Second regeneration of carriers

The second regeneration was carried out using benzoyl cellulose carrier with the immobilized enzyme obtained after previous synthesis of dextran, which have residual immobilized dextransucrase activity of 2.49 U/g. A carrier sample of 0.36 g was left for control tests, and 6 g of carrier was treated three times with 120 mL of cold Triton X-100 solution in buffer (Solution II) at +4 °C. Three samples of 0.20 g each (A_1S, A_2O, and A_2S) were taken from the precipitate, and the rest of the carrier was treated with Solution II three more times. The abbreviations (A, B, 1, 2, O, S, and MA) are listed in Table 2. The treatment times and quantities of Solution II used are given in Table 1. After each treatment the precipitate was separated by centrifuging (3 min at 3000 rpm). The eluted activity in the supernatant and absorption at 280 and 260 nm were determined.

Table 2
Treatment of carriers for dextransucrase immobilization after treatment with Triton X-100 (Solution II)

Carrier[a]	Treatment procedure				
	1	2	3	4	5
MA	*t*-BuOH 6× 25 mL	Ethanol 2× 40 mL	a	b	c
Control	nt	nt	nt	b	c
A_2O	nt	Ethanol 6× 6 mL	a	b	c
B_1O	nt	nt	d	b	c
A_2S	nt	Ethanol 6× 6 mL	a	b	c
A_1S	nt	nt	d	b	c
B_1S	nt	nt	d	b	c

a: replacement ethanol by buffer (see Methods). b: keep the carrier in boiling water bath (4 min for 0.20 g samples, and 20 min for 5 g MA carrier). c: MA, control and . . .O carriers equilibrated with the Solution I, and . . .S carriers with Solution III. d: treatment six times with 6 mL of the same buffer used for later equilibration. nt: no treatment.

[a] Signs and abbreviations: A, first series samples taken from main amount of precipitate (after first three treatment with Triton X-100), B, second series samples taken from main amount of precipitate (after next three treatment with Triton X-100), 1, without *t*-BuOH and ethanol treatment, 2, ethanol treatment, O, immobilization at lower ionic strength, S, immobilization at higher ionic strength and MA, main amount.

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