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Affinity Immobilization of Dextransucrase on Dextran-based Support and the Production of Leucrose

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Abstract A simple and convenient method of immobilizing dextransucrase via an affinity interaction is described, along with the use of this system to synthesize leucrose. Dextransucrase was produced in sucrose-free medium by fermenting a constitutive mutant of *Leuconostoc mesenteroides* NRRL B-512F and was separated using an ultrafiltration membrane. The purified enzyme was free of dextran polymer, which previously was always found with the sucrose-induced enzyme. Therefore, it was possible to immobilize the enzyme on dextran-based resins using an affinity interaction. Sephadex G-200 was the best resin for immobilizing the dextransucrase and gave a fast flow rate through the packed column. The immobilized dextransucrase retained more than 80% of its specific activity after immobilization (K_m = 18.1 mM and k_{cat} = 450 sec^{-1} vs. 13.1 mM and 640 sec^{-1}, respectively, for the free enzyme). The immobilized dextransucrase showed improved stability over a pH range of 4.0 to 6.5 and at moderately high temperatures over 40°C. When immobilized dextransucrase was used to synthesize leucrose via the transfer reaction with sucrose and fructose, about 74% of the sucrose was converted into leucrose after one day, and the half-life of the enzyme activity was 15 days. Regeneration of the resin by supplementation with dextransucrase enabled the recovery of the initial activity of the system, but both the reaction and the flow rate were lower, probably owing to the accumulation of dextran inside the resin.

Keywords: dextransucrase, *Leuconostoc mesenteroides*, immobilization, leucrose

Introduction

Dextransucrase [EC 2.4.1.5. α-(1,6)-D-glucan: D-fructose 2-glucosyltransferase] catalyzes the synthesis of dextrans, which are glucans linked mainly by α-(1,6) bonds, according to the reaction:

$$\text{Sucrose} + (1,6\text{-}\alpha\text{-D-glucosyl})_n \rightarrow \text{D-fructose} + (1,6\text{-}\alpha\text{-D- glucosyl})_{n+1}$$

The enzyme from *Leuconostoc mesenteroides* NRRL B-512F has been used industrially to produce clinical dextrans and Sephadex (1). Dextrans are commercially important materials produced with the aid of enzymes that transfer the glucosyl residues of sucrose to the growing polymer chain. Apart from reactions using sucrose as a substrate, reactions with many different acceptors lead to a range of products (2, 3). For example, leucrose [5-O-(α-D -glucopyranosyl)-D-fructopyranose] is of industrial interest (4). It has been produced on a pilot scale as a sugar substitute, and high yields can be obtained with immobilized dextransucrase.

The yield of dextransucrase synthesized by *L. mesenteroides* strains is rather low, and immobilized enzyme preparations are preferred to soluble ones. Immobilized dextransucrase has several advantages: re-use of the enzyme, high substrate conversion rates, and the capability of a continuous process. Dextransucrase has been immobilized using several methods, of which alginate is generally recognized as the most efficient material, and the activity of the immobilized enzyme is 1- to 5-fold that of the free form (5-9). However, alginate beads have some defects when used as an immobilization matrix. Enzymes are usually immobilized inside alginate beads by covalent bond linkage using glutaraldehyde in a process which is difficult, and the enzymes often lose their activity during the reaction. In addition, to avoid leakage of the material encapsulated in the alginate, the alginate beads are sometimes coated with polycationic polymers, such as chitosan (10) and DEAE-dextran (11), which form a membrane on the bead surface. Entrapment of the enzyme inside the beads prevents frequent contact of the enzyme with its substrate, thereby decreasing the enzyme activity.

Dextransucrase is produced when *L. mesenteroides* is cultured in sucrose medium. The gene sequence of the dextransucrase of *L. mesenteroides* NRRL B-1299 implies that there is a glucan-binding domain at the C-terminus of the enzyme. This seems to be a structure common to the dextransucrases of *Leuconostoc* sp. and *Streptococci* (12). The enzyme produced by *Leuconostoc* sp. in sucrose medium forms aggregates that include the dextran made by the enzyme. The addition of sodium dodecyl sulfate (SDS) does not completely dissociate the aggregates into monomers, and efforts to remove dextran from the produced dextransucrase have failed (13). These findings imply that there is a strong affinity between the enzyme and dextran polymers, and that their interaction makes it difficult to separate the enzyme from the culture medium.

Kim and Robyt reported success in the preparation of a dextran-free dextransucrase by developing a mutant strain of *L. mesenteroides* that does not require sucrose in the

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medium (14, 15). The dextran-free dextransucrase was observed to aggregate with soluble dextran and to bind Sephadex, a dextran-based matrix. This indicates that the enzyme retains its specific affinity for the polymer. In addition, Robyt *et al.* (16) reported that dextran-bound dextransucrase retained its activity and that the enzyme activity was activated allosterically by the binding of the polymer. This unique property of the enzyme enabled us to develop a simple, enzyme-immobilized reactor.

In this report, we describe the method used for the affinity immobilization of dextransucrase on a dextran-based support, the characteristics of the bound enzyme, and the synthesis of leucrose using this immobilized system.

Materials and Methods

Strain and enzyme preparation Dextransucrase was prepared by culturing a high-producing, constitutive mutant of *L. mesenteroides* B-512FMC in glucose medium (14, 17). Bactopeptone (50 g) and yeast extract (50 g) were dissolved in 10 L of water in a 15-L jar fermenter and autoclaved. Sterile glucose solution (1.5 L, 300 g/L) and 100 mL of sterile salt solution containing $MgSO_4 \cdot 2H_2O$ (20 g/L), NaCl (1 g/L), $FeSO_4 \cdot 7H_2O$ (1 g/L), $MnSO_4 \cdot H_2O$ (1 g/L), and $CaCl_2 \cdot 2H_2O$ (1.3 g/L) were added. The fermentation was started by adding 1.2 L of pre-cultured inoculum, and the pH of the culture was maintained at 6.0 using a pH-controller and the addition of 500 ml of 200 g/L NaOH solution. Fermentation was continued until the pH no longer changed. After removing the cells by centrifugation, Tween-80 (1 g/L) and $CaCl_2$ (1 mM) were added to the culture supernatant. The enzyme was concentrated by passing the culture supernatant through a polysulfone ultrafiltration hollow-fiber cartridge [H5P100-43 (100 kDa cut-off), Amicon, Lexington, MA, USA].

Immobilization of dextransucrase Dextran-based matrices, including Sephadex G-25, G-100 and G-200 and Superdex 200 (Pharmacia, Peapack, NJ, USA), were mixed with the concentrated dextransucrase preparation and agitated gently for 3 h at 4°C. The unbound enzyme in the mixture was removed using a sintered glass funnel, and the matrix was washed with 20 mM Na-acetate buffer (pH 5.2) containing 1 mM $CaCl_2$.

Dextransucrase activity The dextransucrase activity was assayed by measuring the amount of fructose released from the sucrose (125 mM sucrose). Aliquots of the reaction mixture (50 mL) were taken every 5 min for 20 min and were mixed with 50 mL of 10% (v/v) pyridine to terminate the reaction. The solution was diluted if needed, and 1 μL of the diluted solution was put on a Whatman K5F TLC plate. The plate was then irrigated four times with acetonitrile: water (85:15, v/v). Carbohydrates on the TLC plate were detected by dipping the plate in methanol containing 0.3% (w/v) N-(1-naphthyl)-ethylenediamine and 5% (v/v) sulfuric acid followed by heating at 120°C for 10 min. The density of fructose on the TLC plate was measured using an image densitometer (Model GS-670, Bio-Rad, Hercules, CA, USA), and the concentration was determined by comparison with a standard curve. The method gave results identical to those obtained using the conventional procedure with ^{14}C-sucrose substrate (18), at least in the early stage of the reaction. One unit was defined as the amount of enzyme that released 1 μmol of fructose from 125 mM sucrose after 1 min at 25°C.

Reaction conditions for free and immobilized enzymes For the free enzyme, 0.9 mL of buffer solution containing 0.2 U of enzyme was mixed with 0.1 mL of 20 % (w/v) sucrose solution and incubated at 25°C with gentle shaking. For the immobilized enzyme, 0.9 mL of buffer solution containing 30 mg of pre-immobilized wet beads (approx. 3 U) was mixed with 0.1 mL of 20% sucrose and incubated at 25°C with gentle shaking. At intervals, samples were decanted and the fructose concentration was analyzed to measure the retained enzyme activity.

Optimum pH, temperature, and thermostability of the immobilized enzyme The optimum pH of the soluble and immobilized dextransucrase was determined at various pH values after a 15-min incubation in the appropriate buffer. The buffers used to test the optimum pH were glycine-HCl (pH 3), sodium acetate-acetic acid (pH 4 and 5), and phosphate (mono- and dibasic) (pH 7, pH 7.5 and 8). One milliliter of buffer solution at each pH (3.0-8.0) containing 125 mM sucrose and 1 mM $CaCl_2$ was mixed with 20 mg (wet wt) of immobilized dextransucrase or soluble enzyme (0.2 U). The mixtures were incubated at 25°C, and the enzyme activity was measured as described above. The optimum temperature of the enzyme was determined by measuring its activity at various temperatures. For the thermostability analysis, enzymes were kept at various temperatures for 15 min, and the residual activity was measured at 25°C.

Synthesis of leucrose using immobilized dextransucrase To synthesize leucrose, the immobilized enzyme (100 units in 10 mL resin) was mixed with 10% sucrose and 40% fructose in 20 mM Na-acetate buffer (50 mL, pH 5.2) containing 1 mM $CaCl_2$ and 0.02% NaN_3, and was agitated at 25°C in a shaking reactor. At regular intervals, samples were decanted and mixed with 10% pyridine solution to stop the reaction. The sucrose and leucrose contents were analyzed using TLC and the image density analysis, as described above.

Results and Discussion

Preparation of dextran-free enzymes Dextran-free dextransucrase was prepared by culturing a high-producing constitutive mutant of *L. mesenteroides* B-512FMC in a glucose-containing medium (14). The culture supernatant was concentrated using dialysis membrane (MW cut-off 1,200-14,000) and was subsequently used to immobilize the enzyme on various dextran-based beads. The enzyme preparation showed a single band at 190 kDa on SDS-PAGE analysis (data not shown). Dextran-bound dextransucrase was prepared in the same manner, but in a

sucrose-containing medium.

Affinity adsorption of dextransucrase on dextran-based beads In order to examine the affinity and binding capacity of the matrix for dextransucrase, various concentrations of enzyme (20-300 U/mL) were mixed with 10 mg of several types of beads in 20 mM Na-acetate buffer (pH 5.2). The mixtures were equilibrated for 3 h at 4°C with constant agitation and then centrifuged at 5,000 × g for 2 min to precipitate the beads. The enzyme activity remaining in the supernatant was assayed, and the adsorption isotherms were measured (Fig. 1). Of the beads tested, Sephadex G-200 gave the highest adsorption isotherm with dextransucrase, and Sephadex G-25 gave the lowest. This result was expected because G-200 beads have bigger pores and greater surface area than G-25 beads, allowing the enzyme molecules to penetrate throughout the matrix. By contrast, although Superdex 200 and Sephadex G-200 have pores of similar size, Superdex 200 had a lower adsorption isotherm than did Sephadex G-200, owing to the heterogeneous composition of Superdex, which is cross-linked with dextran and agarose polymer.

Tween-80 is frequently added to the culture supernatant of *L. mesenteroides* to prevent enzyme aggregation and to buffer solutions to prevent precipitation of dextransucrase at high concentrations. However, it was found that Tween-80 interferes with the binding between the enzyme and beads, which diminished the affinity isotherm (data not shown). Therefore, when preparing the enzyme for affinity immobilization, the inclusion of a detergent such as Tween in the buffer solution should be avoided.

The dextran-bound dextransucrase of *L. mesenteroides* B512FMC prepared in sucrose medium showed very poor affinity on Sephadex G-200, as Kobayashi *et al.* (19) reported. This clearly shows that endogenous dextran present in the enzyme preparation inhibited the binding of the enzyme to dextran beads, and thus that a dextran-free enzyme would be more effective than the dextran-bound form for affinity immobilization.

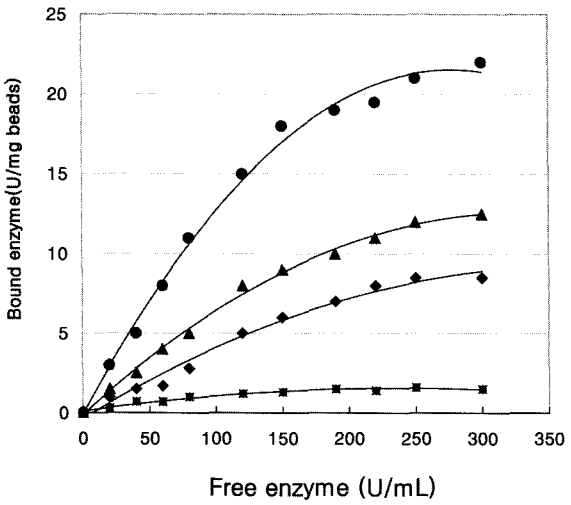

Fig. 1. Adsorption isotherms of dextransucrase to various dextran-based beads. Sephadex G-200 (●), Superdex 200 (▲), Sephadex G-100 (◆), Sephadex G-25 (■)

Characteristics of immobilized dextransucrase The dextransucrase immobilized on Sephadex G-200 was examined to compare its enzymatic characteristics with those of the soluble enzyme in terms of pH, temperature, and thermal stability. The optimum pH of the immobilized enzyme was pH 5.2, which was same as that of the free enzyme (Fig. 2-A), although the overall pH profile of the immobilized enzyme was broader and was shifted slightly toward lower pH values. The optimum temperature of the immobilized dextransucrase was 30°C, which was the same as that of the free enzyme, but the relative activity at each temperature was generally increased by immobiliza-

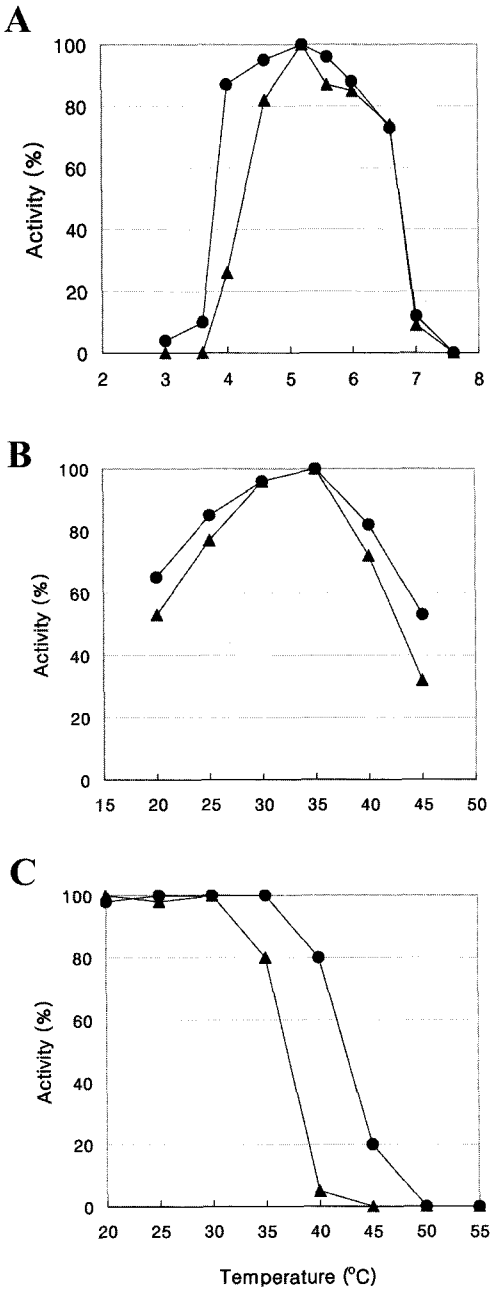

Fig. 2. Characteristics of immobilized (●) and free (▲) dextransucrase. A: activity of the enzyme at various pH values, B: activity of the enzyme at various temperatures, C: thermostability of the enzyme at various temperatures.

tion (Fig. 2-B). The thermal stability of the immobilized enzyme was increased (Fig. 2-C). Overall, the immobilized dextransucrase showed slightly improved stability over the pH range of 4.0 to 6.5 and at moderately high temperature of 40°C. Improved enzyme stability against changes in the environment is usually observed after immobilization because the binding of dextran to the enzyme results in a rigid protein conformation, which helps the enzyme maintain its catalytic activity during environmental changes (20).

The kinetic parameters of the immobilized enzyme were estimated and compared with those of the free enzyme in three independent experiments (Fig. 3). The Michaelis-Menten constant (K_m) and the catalytic reaction rate constant (k_{cat}) were 18.1 mM and 450 sec^{-1} for the immobilized enzyme, respectively, and 13.1 mM and 640 sec^{-1} for the free enzyme, respectively. The k_{cat} for the immobilized enzyme was slightly reduced after immobilization as a result of the conformational rigidity of the polymer-bound protein. The minor difference in the K_m values for free (13.1 mM) and immobilized dextransucrase (18.1 mM) indicated that there was no serious diffusion limitation for the immobilized enzyme, although this does occur in many immobilized systems (21, 22). Therefore, our system was thought to have overcome the major problem of entrapment and surface coating of alginate beads (10, 11). In the solution with high substrate concentration, the reaction rate of the immobilized dextransucrase was as high as that of the free enzyme.

Batch reaction of immobilized dextransucrase to produce leucrose Robyt (23) reported that when fructose is present at high concentration as an acceptor molecule with sucrose in the dextransucrase reaction, dextran synthesis was not observed and leucrose was produced instead. Therefore, the dextransucrase-immobilized beads were mixed with 10% sucrose and 40% fructose, and the leucrose synthesis reaction was carried out in a batch manner. Figure 4 shows the reaction profile of leucrose synthesis. After 24 h, the 10% sucrose was converted into 7.4% leucrose (74% yield), showing a typical Michaelis-Menten reaction pattern. Leucrose was obtained via the transglucosylation reaction from sucrose to fructose. Stodola *et al.* first described this reaction in 1952 (24). Leucrose is of practical interest as its sweetness is similar to that of sucrose, yet it has anti-tooth decay activity. Our dextransucrase-immobilized system should provide an economic enzyme-recycling procedure for the manufacture of leucrose.

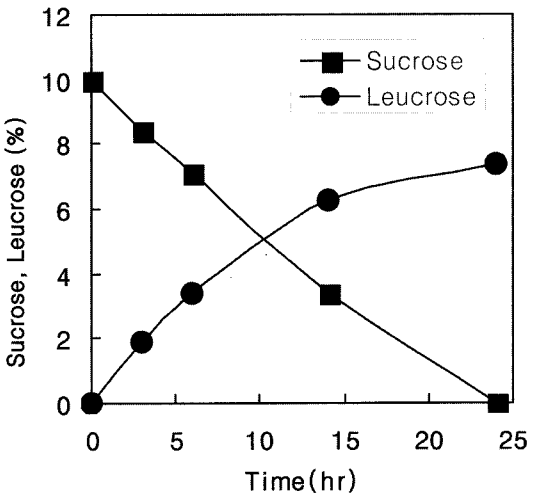

Fig. 4. Conversion reaction profile of sucrose and fructose into leucrose using the immobilized dextransucrase reactor. The immobilized enzyme (100 units in 10 mL resin) was mixed with 10% sucrose and 40% fructose in 20 mM Na-acetate buffer (50 mL, pH 5.2) containing 1 mM $CaCl_2$ and 0.02% NaN_3, and agitated at 25°C in a shaking reactor.

After using the system 11 times over 15 days, the activity of the immobilized enzyme was reduced to one-half. To recycle the beads, purified dextransucrase was added to the beads which were allowed to equilibrate for 3 h at 4°C. The enzyme activity remaining in the supernatant was assayed, and the adsorbed enzyme activity was deduced by subtraction. The regeneration of the resin by supplementation with dextransucrase enabled the recovery of the activity of the system, but the reaction rate was slower than the deduced activity. This reduction in activity after regeneration might have been caused by the synthesis of dextran and its accumulation inside the beads. This appears to be likely because when the flow rates were measured after packing the column, the rate with the regenerated beads was only 70% that of the initial enzyme-immobilized beads. Dextran was always synthesized in experiments with a dextransucrase-immobilized reactor (25, 26), even when the reaction conditions were chosen carefully to avoid the polymerization reaction.

Procedures that use the affinities of biomolecules and ligands to immobilize enzymes are gaining increasing acceptance in biotechnological applications (27). The affinity interaction can be used for protein separation and immobilization owing to its ligand specificity and relatively strong binding strength (28-30). The affinity immobilization of dextransucrase on dextran-based supports has three benefits: one-step immobilization of the enzyme from the culture broth with pre-purification steps; the availability of cheap, widely used matrices like

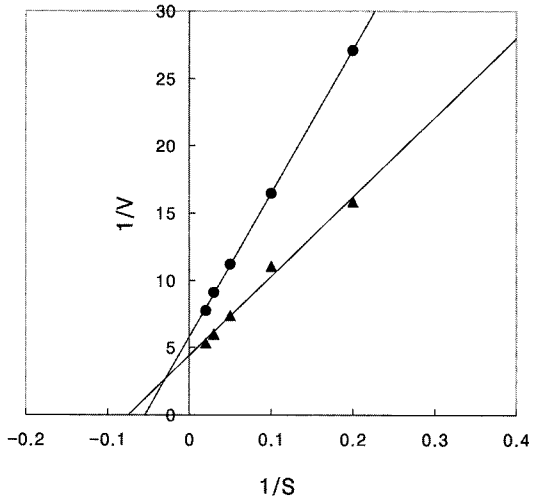

Fig. 3. Kinetic analysis of immobilized (●) and free (▲) dextransucrase through a reciprocal plot of 1/V and 1/S.

Sephadex and Superdex; and the repeated use of the resin by simple supplementation of the enzyme.

Dextran is a relatively inexpensive, chemically inert material and is safe for use in food and pharmaceutical applications. Many dextran matrices are available, including Sephadex, which is the first and most widely used commercialized resin. They are attractive for enzyme immobilization mainly because their versatility in terms of physical properties allows their use with a variety commercial products. The high hydrophilicity of dextran also favors the activity of many enzymes. As dextran-free dextransucrase adsorbs spontaneously to dextran-based supports from almost any solution, no pretreatment of the samples is required before immobilization. Therefore, the immobilization method described here simplifies the process, allowing the purification and immobilization steps to be performed in a single filtration step. In addition, the regeneration of the resin by dextransucrase supplementation recovered the activity of the system, which allowed the resin to be used repeatedly. This simple and convenient immobilization system via the affinity interaction will allow the economic synthesis of oligosaccharides using the acceptor reaction of dextransucrase.

Polymers and polymer-binding domains are generally used as fusion partners, such as starch with starch-binding domains (31), maltose ligand with maltose-binding domains (32), and cellulose with cellulose-binding domains (33), for the separation and immobilization of heterologous proteins. The dextran-binding domain of dextransucrase is found in many oral streptococci and *L. mesenteroides* and consists of a series of tandem amino acid repeats in which aromatic residues (tryptophan, tyrosine-phenylalanine pairs) and glycine are highly conserved (34). Our experimental results imply that, as with other polymer-binding domains, the dextran-binding domain could be used as a fusion partner. This tagging system could be useful for performing biotransformation in the food and pharmaceutical industries, as it provides an immobilized enzyme on a widely used support that can be easily handled in the laboratory.

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References

1. De Belder AN. Dextrans. pp. 399-425. In: Industrial Gums. Whistler RL, BeMiller JN (eds). Academic Press, San Diego, USA (1993)
2. Robyt JF, Walseth TE. The mechanism of acceptor reactions of *Leuconostoc mesenteroides* B-512F dextransucrase. Carbohydr. Res. 61: 433-445 (1978)
3. Robyt JF, Eklund SH. Relative, quantitative effects of acceptors in the reaction of *Leuconostoc mesenteroides* B-512F dextransucrase. Carbohydr. Res. 121: 279-286 (1983)
4. Schwengers D. Leucrose, a ketodisaccharide of industrial design. pp. 183-195. In: Carbohydrates as Organic Raw Materials. Lichtenthaler FW (ed). VCH Weinheim, New York, USA (1991)
5. Kaboli H, Reilly PJ. Immobilization and properties of *Leuconostoc mesenteroides* dextransucrase. Biotech. Bioeng. 22: 1055-1069 (1980)
6. Monsan P, Lopez A. On the production of dextran by free and immobilized dextransucrase. Biotech. Bioeng. 23: 2027-2037 (1981)
7. El-Sayed AMM, Abdul-Wahid K, Coughlin RW. Investigation of production of dextran and dextransucrase by *Leuconostoc mesenteroides* immobilized within porous stainless steel. Biotech. Bioeng. 40: 617-624 (1992)
8. Reischwitz A, Reh K-D, Buchholz K. Unconventional immobilization of dextransucrase with alginate. Enzyme Microb. Technol. 17: 457-461 (1995)
9. Reh K-D, Noll-Borchers M, Buchholz K. Productivity of immobilized dextransucrase for leucrose formation. Enzyme Microb. Technol. 19: 518-524 (1996)
10. Huguet ML, Groboillot A, Neufeld RJ, Poncelet D, Dellacherie E. Hemoglobin encapsulation in chitosan/calcium alginate beads. J. Appl. Polymer Sci. 51: 1427-1432 (1994)
11. Huguet ML, Neufeld RI, Dellacherie E. Calcium-alginate beads coated with polycationic polymers: comparison of chitosan and DEAE-dextran. Process Biochem. 31: 347-353 (1996)
12. Monchois B, Willemot R-M, Remaud-Simeon M, Croux C, Monsan P. Cloning and sequencing of a gene coding for a novel dextransucrase from *Leuconostoc mesenteroides* NRRL B-1299 synthesizing only α(1-6) and α(1-3) linkages. Gene. 182: 23-32 (1996)
13. Funane K, Yamada M, Shiraiwa M, Takahara H, Yamamoto N, Ichishima E, Kobayashi M. Aggregated form of dextransucrases from *Leuconostoc mesenteroides* NRRL B-512F and its constitutive mutant. Biosci. Biotech. Biochem. 53: 776-780 (1995)
14. Kitaoka M, Robyt JF. Large-scale preparation of highly purified dextransucrase from a high-producing constitutive mutant of *Leuconostoc mesenteroides* B-512FMC. Enzyme Microb. Technol. 23: 386-391 (1998)
15. Kim D, Robyt JF. Production and selection of mutants of *Leuconostoc mesenteroides* constitutive for glucansucrases. Enzyme Microb. Technol. 16: 659-664 (1994)
16. Robyt JF, Kim D, Yu L. Mechanism of dextran activation of dextransucrase. Carbohydr. Res. 266: 293-299 (1995)
17. Kim D, Robyt JF. Properties of *Leuconostoc mesenteroides* B-512FMC constitutive dextransucrase. Enzyme Microb. Technol. 16: 1010-1015 (1994)
18. Miller AW, Eklund SH, Robyt JF. Milligram to gram scale purification and characterization of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. Carbohydr. Res. 147: 119-133 (1986)
19. Kobayashi M, Matsuda K. Characterization of the multiple forms and main component of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. Biochem. Biophys. Acta 614: 46-62 (1980)
20. Kang G-J, Kim M-J, Kim J-W, Park KH. Immobilization of thermostable maltogenic amylase from *Bacillus stearothermophilus* for continuous production of branched oligosaccharides. J. Agric. Food Chem. 45: 4168-4172 (1997)
21. Arica, MY, Hasirci V, Alaeddinoglu NG. Covalent immobilization of α-amylase onto PHEMA microspheres: preparation and application to fixed bed reactor. Biomaterials. 13: 704-709 (1995)
22. Dermircioglu H, Beyenal H, Tanyolac A, Hasirci N. Entrapment of urease in glycol containing polymeric matrices and estimation of effective diffusion coefficients of urea. Polymer. 36: 4091-4096 (1995)
23. Robyt JF. Mechanisms in the glucansucrase synthesis of polysaccharides and oligosaccharides from sucrose. Adv. Carbo. Chem. Biochem. 51: 133-168 (1995)
24. Stodola FH, Sharpe ES, Koepsell HJ. The preparation, properties and structure of the disaccharide leucrose. J. Am. Chem. Soc. 78: 2514-2518 (1956)
25. Monsan, P, Paul F, Auriol D, Lopez A. Dextran synthesis using immobilized *Leuconostoc mesenteroides* dextransucrase. Methods Enzymol. 136: 239-254 (1987)
26. Pereira AM, Costa FA, Rodrigues MI, Maugeri F. In vitro synthesis of oligosaccharides by acceptor reaction of dextransucrase from *Leuconostoc mesenteroides*. Biotechnol. Lett. 20: 397-401 (1998)
27. Saleemuddin, M. Bioaffinity based immobilization of enzymes.

Adv. Biochem. Eng. Biotechnol. 64: 203-226 (1999)
28. Schatz PJ. Use of peptide libraries to map the substrate specificity of a peptide-modifying enzyme: a 13 residue consensus peptide specifies biotinylation in *Escherichia coli.* Biotechnology. 11: 1138-1143 (1993)
29. Neri D, De Lalla C, Petrul H, Neri P, Winter G. Calmodulin as a versatile tag for antibody fragments. Biotechnology. 13: 373-377 (1995)
30. Kweon DH, Lee DH, Han NS, Seo J-H. Solid-phase refolding of cyclodextrin glycosyltransferase adsorbed on cationic-exchange resin. Biotech. Prog. 20: 277-283 (2004)
31. Chen LJ, Ford C, Kusnadi A, Nikolov ZL. Improved adsorption to starch of a beta-galactosidase fusion protein containing the starch-binding domain from Aspergillus glucoamylase. Biotechnol. Prog. 7: 225-229 (1991)
32. Cattoli F, Sarti GC. Separation of MBP fusion proteins through affinity membranes. Biotechnol. Prog. 18: 94-100 (2002)
33. Levy I, Shoseyov O. Cellulose-binding domains: biotechnological applications. Biotechnol. Adv. 20: 191-213 (2002)
34. Shah DS, Joucla G, Remaud-Simeon M, Russell RR. Conserved repeat motifs and glucan binding by glucansucrases of oral streptococci and *Leuconostoc mesenteroides.* J. Bacteriol. 186: 8301-8308 (2004)