S6-5

# Potential Industrial Applications and Evolution of Carbohydrolases and Glycansucrases

Doman Kim[1,2,5], Eun-Seong Seo[1,3], Jin-Ha Lee[1,4], Hee-Kyoung Kang[1,4], and Jae-Young Cho[1,3]

*[1]Laboratory of Functional Carbohydrate Enzymes and Microbial Genomics; [2]School of Biological Sciences and Technology; [3]Department of Materials and Biochemical Engineering; [4]Engineering Research Institute Chonnam National University, Gwang-Ju, 500-757, Korea; [5]Biology Research Center for Industrial Accelerators in Dongshin University, Naju, Jeonnam, 520-714, Korea*

## Abstract

Dextrans make up a class of polysaccharides that are D-glucans of various structures with contiguous α-1→6 glycosidic linkages in the main chains and α-1→2, α-1→3, or α-1→4 branch glycosidic linkages, depending on the specificity of the particular dextransucrase. Glucansucrases that catalyze glucans synthesis from sucrose. When other carbohydrates, in addition to sucrose, are present in the enzyme digest, the enzyme transfers glucose to the carbohydrate acceptors in the secondary reaction that diverts some of the glucose from incorporation into glucan. Many carbohydrate acceptors have been recognized and the products that result are dependent on the particular enzyme and the structure of the particular acceptor. Because of these unique catalytic characteristics, various dextransucrases have many important industrial and medical uses. To improve the understanding of their action mode and extend their applications, this study describes mechanism of glucan synthesis and potential industrial uses of dextransucrases, and our recent findings on the structural, functional organization and directed evolution of the glucansucrases to offer for designing glucansucrases with improved properties.

### Dextrans and Glucansucrase

A dextran is now defined as a glucan that is glucopyranose units in the main chains. All known dextrans are branched. This is one of the important characteristics that distinguish the different kinds of dextrans. Branch linkages have been found to be α-1→2, α-1→3, and α-1→4 and branches can be single D-glucose units and/or chains of α-1→6 linked D-glucose units (7). Some strains of *L. mesenteroides* produce one type of glucansucrase and one type of dextran, whereas others produce multiple types of glucansucrases and dextrans (glucans). Differences in the proportions of types are evident from both heterogeneity in glucan solubility profiles and electrophoretic band patterns of glucansucrases (2). Various dextransucrases have many important industrial and medical uses (4). Each enzyme is also important because of its theoretical and practical aspects in understanding the mechanism of glucan synthesis (5) and in its ability to synthesize a wide variety of oligosaccharides by glucosyl transfer reactions to acceptors (6). Also, different structure dextrans could have applications in food preparations, pharmaceuticals, and drug or pesticide delivery

systems. Before dextran, alternan, and/ or other branched dextrans, however, can be marketed for food and pharmaceutical applications, technologies for mass producing them must developed; such as the isolation of hyper glucansucrase producing strains, the development of relatively simple purification methods, and the development of continuous processes for polysaccharide production using enzyme reactors.

**Dextran Molecular Size and Degree of Branching as a Function of Sucrose Concentration, pH, and Temperature of Reaction of *Leuconostoc mesenteroides* B-512FMCM Dextransucrase (1)**

We have studied the size distribution and the degree of branching of dextrans synthesized by *L. mesenteroides* B-512FMCM dextransucrase, using a relatively broad concentration of sucrose, with different pH values, and different temperatures (Table 1). The optimum conditions for the synthesis of very high MW dextran in high yields would be obtained at relatively low sucrose concentrations (0.1 M - 0.3 M), high pH values of 5.5 - 6.0, and high temperature of 37 °C - 45 °C. The optimum conditions for the synthesis of low MW dextrans would be high sucrose concentrations of 3.0 M - 4.0 M, low pH (4.5), and intermediate temperatures of 23 °C - 28 °C. The data clearly shows that as the concentration of sucrose was increased from 0.1 M to 4.0 M, the percent of branching is significantly increased from 5.0 % to 16.6 %. As the temperature was increased, the degree of branching was increased (Table 1). This study could be expanded, using a more complete combinatorial study of sucrose concentration and temperature to give a pattern that could be used to select a wide range of molecular weights and degrees of branching. This would lead to better controlling and selecting dextrans of different molecular weights, with different degrees of branching.

**Table 1. Dextranase/TLC determination of the degree of branching of dextrans synthesized by *L. mesenteroides* B-512FMCM dextransucrase at various concentrations of sucrose, pH, and temperatures**

| Sucrose (M)[b] | Mono-Saccharide [% M] | Isomalto-dextrins [% IMD] | Branched Isomaltodextrins [% BIM] | Unhydrolyzed Dextran [% UHD] | Ratios (R) [BIM+UHD] / [M+IMD] | Percent Branching[a] |
|---|---|---|---|---|---|---|
| 0.1 | 31.3 | 48.8 | 12.5 | 7.3 | 0.247 | 5.0 |
| 0.3 | 33.9 | 44.9 | 14.6 | 6.6 | 0.269 | 5.4 |
| 1.0 | 30.8 | 43.2 | 21.9 | 4.1 | 0.351 | 7.1 |
| 1.5 | 31.5 | 39.7 | 24.9 | 4.1 | 0.404 | 8.2 |
| 2.0 | 25.4 | 42.3 | 30.0 | 2.3 | 0.477 | 9.7 |
| 3.0 | 16.7 | 44.8 | 36.8 | 1.7 | 0.626 | 12.7 |
| 4.0 | 18.4 | 36.6 | 37.5 | 7.5 | 0.818 | 16.6 |
| pH[c] | [% M] | [% IMD] | [% BIM] | [% UHD] | [BIM+UHD] / [M+IMD] | Percent Branching[a] |
| 4.5 | 35.2 | 40.1 | 18.9 | 5.7 | 0.326 | 6.6 |
| 5.0 | 29.4 | 44.3 | 20.0 | 6.3 | 0.357 | 7.1 |
| 5.2 | 28.5 | 43.2 | 21.9 | 6.2 | 0.403 | 8.2 |
| 5.5 | 27.1 | 44.3 | 21.8 | 6.8 | 0.401 | 8.1 |
| 6.0 | 34.5 | 41.1 | 17.5 | 6.9 | 0.323 | 6.5 |
| ℃[d] | [% M] | [% IMD] | [% BIM] | [% UHD] | [BIM+UHD] / [M+IMD] | Percent Branching[a] |
| 4 | 23.4 | 57.6 | 14.5 | 4.5 | 0.235 | 4.8 |
| 15 | 19.8 | 59.3 | 16.0 | 4.9 | 0.265 | 5.3 |
| 23 | 17.9 | 57.6 | 18.5 | 6.0 | 0.325 | 6.6 |
| 28 | 16.4 | 58.3 | 18.9 | 6.4 | 0.339 | 6.9 |
| 37 | 16.5 | 53.1 | 20.7 | 9.7 | 0.437 | 8.8 |
| 45 | 8.7 | 49.2 | 26.6 | 15.5 | 0.727 | 14.7 |

[a]Branching for dextran synthesized from 0.1 M sucrose was assumed to be 5 % from refs. 24&25. A branching conversion factor was obtained by dividing 5 % by the ratio (R), 0.247, giving 20.24. The percent branching for the other dextrans was then obtained by multiplying their ratios (R) by the conversion factor, 20.24. [b]Reactions were conducted at pH 5.5 and 28℃. [c]Reactions were conducted at 1.5 M sucrose and 28℃. [d]Reactions were conducted at 1.5 M sucrose and pH 5.5.

**Directed Evolution of a Dextransucrase for Increased Constitutive Activity and the Synthesis of a Highly Branched Dextran (3)**

Kim *et al.* elaborate a novel extracellular dextransucrase gene (*dsrB742ck*) after ultrasoft X-ray irradiation, producing a dextransucrase of increased activity and synthesis of a highly branched dextran. The purified DSRB742CK dextransucrase showed 2.3-times higher activity per mg protein, compared to that of the parent clone, DSRB742 dextransucrase. The nucleotides sequence of the *dsrB742ck* dextransucrase gene showed seven DNA base differences comparing to that of *dsrB742* dextransucrase gene; three nucleotide deletions and one nucleotide substitution. Therefore, the start codon appears at 30 amino acids downstream of DSRB742 dextransucrase. There are two nucleotide sequence differences at the promoter region and one nucleotide sequence difference at the RBS. The dextran synthesizing activity of DSRB742CK dextransucrase was 2.3 times higher than that of DSRB742 dextransucrase based on Western blot analysis using anti-dextran antibody. DSRB742CK dextran contained 15.6% branching and showed 2.7-times higher resistance to *Penicillium* endodextranase hydrolysis compared to that of DSRB742 dextran (Table 2).

**Table 2. Dextranase/TLC determination of the degree of branching of dextrans synthesized by DSRB742 and DSRB742CK dextransucrases***

| | DSRB742 dextran | DSRB742CK dextran |
|---|---|---|
| Mono-saccharide (M, %) | 35.4 | 29.5 |
| Isomaltodextrins (IMD, %) | 42.2 | 27.1 |
| Branched Isomaltodextrins (BIM, %) | 19.1 | 23.8 |
| Unhydrolyzed Dextran (UHD, %) | 3.3 | 19.6 |
| [BIM+UHD] Ratio | 0.29 | 0.77 |
| [M+IMD] Percent Branching** | 5.9% | 15.6% |

* Hydrolysis reactions were conducted at 37° C for 3 hrs.

** Branching for dextran synthesized from 0.1 M sucrose was assumed to be 5% from refs. 23 & 24. A branching conversion factor was obtained by dividing 5% by the ratio, 0.247, giving 20.24. The percent branching for DSRB742 and DSRB742CK dextrans was obtained by multiplying their ratio by the conversion factor.

**Modified Oligosaccharides as Potential Dental Plaque Control Materials**

Metabolic acids produced by oral pathogens demineralize tooth surfaces leading to dental caries. Insoluble glucosyltransferase produced by Mutans streptococci are the key factor in this process. We synthesized various modified oligosaccharides and tested them for their inhibitory effects on glucosyltransferase activity. Modified oligosaccharides were produced using a mixed-culture fermentation of *Lipomyces starkeyi* and *Leuconostoc mesenteroides* and then modified as iron- and sulfate-oligosaccharides. Iron- and sulfate-oligosaccharides reduced glucosyltransferase activity of *Streptococci* from 17 to 43% and prevented the formation of insoluble biomass on the surface of glass vials or stainless steel wires in the presence of sucrose. They also reduced the growth and acid productions of oral pathogens including *S. mutans, S. sobrinus, Eikenella corrodens, Prevotella intermedia* and *Actinobacillus actinomycetemcmitans.*

## References

1. Kang, H. K., E. S. Seo, J.F. Robyt and D. Kim. 2003. Directed evolution of a dextransucrase for increased

constitutive activity and the synthesis of a highly branched dextran. *J. Mol. Catal. B* **26**: 167-176.

2. Kato, C. and H. K. Kuramitsu. 1990. Carboxy-terminal deletion analysis of the *Streptococcus mutans* glucosyltransferase-I enzyme. *FEMS Microbiol. Lett.* **72**: 299-302.
3. Kim, D., J. F. Robyt, S. Y. Lee, J. H. Lee and Y. M. Kim. 2003. Dextran molecular size and degree of branching as a function of sucrose concentration, pH, and temperature of reation of *Leuconostoc mesenteroides* B-512FMCM dextransucrase. *Carbohydr. Res.* **338**: 1183-1189.
4. Mitsuya, H., D. Looney, S. Kuno and R. Ueno *et al.* 1988. Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4+ cells. *Science* **240**: 646 - 649.
5. Robyt, J. F., B. K. Kimble, and T. F. Walseth. 1974. The mechanism of dextransucrase action. Direction of dextran biosynthesis. *Arch. Biochem. Biophysics* **165**: 634 - 640.
6. Robyt, J.F. and S.H. Eklund. 1983. Relative, quantitative effects of acceptors in the reaction of *Leuconostoc mesenteroides* B-512F dextransucrase. *Carbohydr. Res.* **121**: 279 - 286.
7. Robyt, J. F. 1996. Mechanism and action of glucansucrases, pp.1-22. *In* Park, K. H., Robyt, J. F. and Choi, Y. D. (ed.), Progress in Biotechnology, Elsevier, **12**:1-22.