

Reaction Mechanism in Dextransucrase from *Streptococcus bovis* -Acceptor Specificity on the Dextran Synthesis-

*Streptococcus bovis*のDextransucraseの反応機構 — アクセプター特異性 —

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Abstract

Glucosyl transfer from sucrose to acceptors in dextransucrase of *Streptococcus bovis* 148 was investigated by using various saccharides.

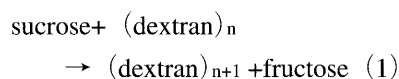
It was shown that the most effective acceptors were maltose and isomaltose. Maltose molecule showed no indication of its influence on the molecular size of glucan produced, and be not a acceptor on the dextran synthesis.

HPLC analysis showed that the degraded dextran of *S. bovis* 148 was able to act as an acceptor on the dextran synthesis. Acceptor specificity on the dextran synthesis was examined by using dextrans of different structure, and was indicated to be presence for the first time. It was suggested that glucosidic bond over 60% is necessary to become the acceptor on dextran synthesis.

Keywords: *Streptococcus bovis*, Dextransucrase, Acceptor reaction, Specificity, Reaction mechanism

Introduction

Dextransucrase catalyzes a glucosyl transfer reaction from sucrose to acceptor dextran and produces a high molecular weight dextran as shown in equation (1) .



It is apparent that structures of dextran produced by each bacterium are different. The specificity of glucan structure between each bacterium should be explained by more extensive studies. To better understand the reaction mechanism of the dextransucrase reaction, it should be evaluated by some experimental approaches of acceptor reaction.

It is important on the investigation to produce a high molecular weight dextran. Koepsell et al. reported oligosaccharides production by transglucosylation of dextransucrase from *L. mesenteroides* to several acceptors¹⁾. A detailed study of the correlation between the enzyme and acceptor molecules on the synthesis of high molecular

weight dextran has not been reported for either *Leuconostoc* or *Streptococcus* enzyme. The dextransucrase of *S. bovis* 148 is appropriate for enzymological studies because this enzyme induced by glucose contains no saccharide. Hayashi et al. studied the influence of maltose as acceptor on glucosyl transfer reaction of the dextransucrase from *S. bovis* 148, and indicated that the degree of polymerization of oligosaccharides produced by the acceptor reaction depend on the molar ratio of sucrose and maltose²⁾.

In this paper, the author described the specificity of the acceptor reactions on the dextran synthesis by *S. bovis* 148 dextransucrase.

Materials and methods

Materials

The five strains, *L. mesenteroides* NRRL B-512F, B-1149, B-1298, B-1415 and B-1424, were provided from Northern Regional Research Center (NRRC, Peoria, Ill, USA) . All other chemicals were of reagent grade and commercially available.

Enzyme preparation

The dextransucrase of *S. bovis* 148 was purified, in accordance with a report described earlier³⁾. The enzyme activity was determined by measuring the amount of reducing sugar released from sucrose during the enzyme catalysed reaction according to the methods of Somogyi and Nelson.

HPLC analysis

The specimens were filtered through a DISMIC-25cs filter (pore size, 0.45 μ m; Advantec Toyo, Tokyo, Japan). Glucan sizes synthesized enzymatically were estimated with a HPLC system (TOSOH) by using a TSKgel GMPWXL column (7.8mm of inner diameter \times 30cm; TOSOH). Commercial dextrans (the series from T-10 to 2000, Pharmacia LKB Biotechnology, upsala, Sweden) were used to estimate molecular size of glucan. A TSKgel G-Oligo-PW column (7.8mm of inner diameter \times 30cm; TOSOH) was used to analyze sugar ranges of oligosaccharides. Carbohydrate peaks were registered with a refraction index detector.

Production and preparation of dextrans

The medium which was used for the production of dextran produced by all strains of *L. mesenteroides*, consisted of 12g of sucrose, 0.05g of yeast extract, 0.1g of Polypepton (Nihon Pharmaceutical Co. Ltd, Tokyo, Japan), 0.5g of K₂HPO₄, 0.1g of NaCl and water to make 100ml. The pH was adjusted to 7.4. The bacteria were cultured at 25°C for 24h.

A culture liquor was centrifuged at 20,000 \times g for 20min. Two volumes of cold ethanol were added to the supernatant. After incubation at 0°C for 2h, the supernatant was centrifuged at 10,000 \times g for 15min to obtain dextran produced as the precipitate. The dextran was washed three times with cold 80% ethanol and dissolved in water.

Production and purification of the dextran produced by *S. bovis* 148 have been described in the report before³⁾.

Each dextran was degraded with endo-dextranase from *Penicillium* sp. (Sigma), and low molecular weight glucans (about 10,000) were fractionated by a TSKgel GMP-WXL column.

Sugar content of each fraction was estimated by a phenol-sulfuric acid procedure.

Estimation of saccharide synthesized enzymatically

The glucosyl transfer reaction catalyzed by the dextransucrase of *S. bovis* 148 was investigated using sucrose as a

donor. The reaction was started by adding 1.0ml of the enzyme in 50mM phosphate buffer (pH 6.0) containing 0.02% sodium azide to 1.0ml of 90mM sucrose and 1.0ml of 270mM several saccharides. Reaction mixture was incubated at 40°C for 24h. The products synthesized enzymatically were investigated by HPLC.

[Glucosyl transfer reaction from sucrose to maltose]

To investigate the glucosyl transfer mechanism with maltose as acceptor molecule the author performed experiments as follows. The synthesis of glucosyl transfer from sucrose to maltose at various molar ratio of sucrose and maltose was done. A reaction mixture containing 1.0ml of 90mM sucrose, 1.0ml of maltose, and 1.0ml of the enzyme in 50mM phosphate buffer (pH 6.0). Maltose concentration was established to be from 0.1 to 10 against 1 of sucrose concentration.

[Glucosyl transfer from sucrose to glucan-acceptors]

To investigate the acceptor specificity of dextran on the dextran elongation the author performed experiments as follows. A reaction mixture of 2.5ml containing 1.6mM sucrose, the enzyme and various concentrations of the degraded dextran from *S. bovis* 148 as acceptor in 50mM phosphate buffer, pH 6.0, were incubated at 40°C for 24h.

Results

Glucosyl transfer from sucrose to several acceptors

[Oligosaccharide]

Oligosaccharides produced by the glucosyl transfer reaction were identified by HPLC analysis on a TSKgel G-Oligo-PW column, but the resolution possible with the column limits the detection of large products. The degree of polymerization (DP) of the largest products was different at every saccharide tested, and the products were grouped into three types based on the DP: Type A, 5 of DP; type B, 4; type C, 3 (Fig. 1). It was suggested that the DP number of transfer products of acceptors was so large as the saccharide to be a stronger acceptor, and strength of acceptor is as follows; type A > B > C. The enzyme had a broad acceptor specificity on acceptor reaction against saccharides tested. The transfer products of acceptors (α -methylglucoside, cellobiose, lactose, melezitose, raffinose, palatinose, maltose, and isomaltose) were detected, and the most effective acceptors were maltose and isomaltose, placed in type A. Arabinose, ribose, xylose galactose, sor-

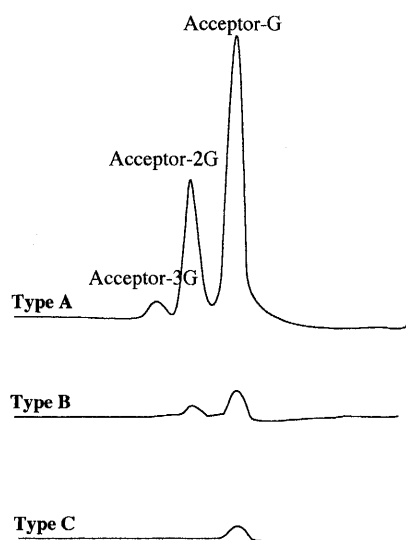


Fig.1 Estimation of the acceptor reaction using saccharides by the dextranucrase from *S.bovis* 148.

Type A; Isomaltose, Maltose
 Type B; Palatinose
 Type C; α -methylglucoside, Lactose, Cellobiose, Merezitose
 Raffinose

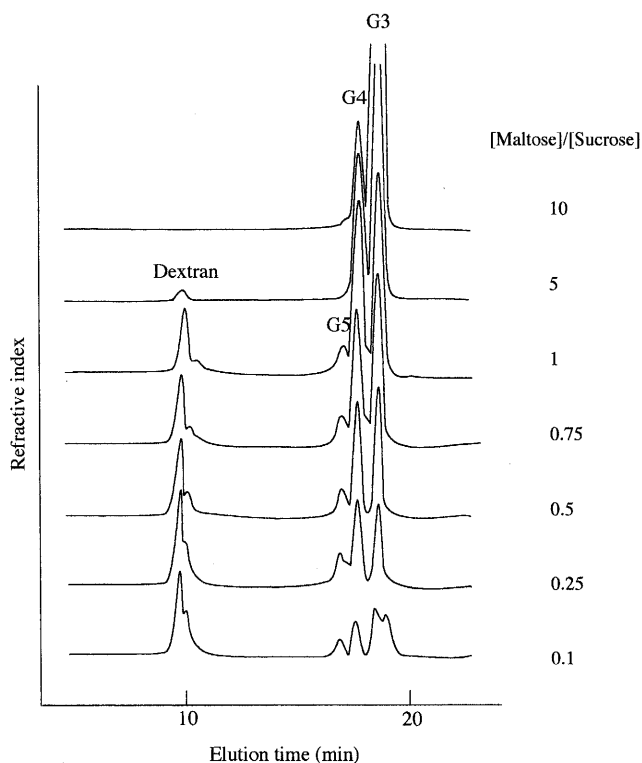


Fig.2 Influence of maltose as a glucosyl acceptor on saccharide production by the dextranucrase of *S. bovis* 148.

bose, trehalose, and rhamnose were not effective, indicating that these substances probably can not participate as acceptors in glucosyl transfer from sucrose.

[Glucosyl transfer reaction from sucrose to maltose]

The results are shown in Fig. 2. Thus, the DP did not elongate up to polysaccharide when the molar ratio was 0.1. Amount of dextran synthesized was inversely proportional to amount of oligosaccharides produced, and in a

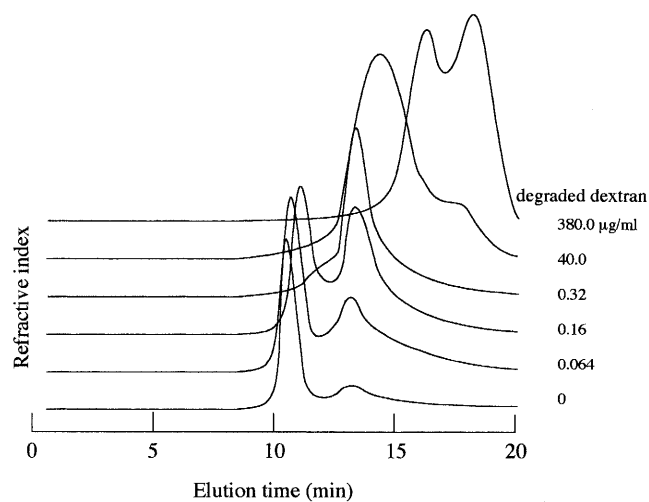


Fig.3 Effect of degraded dextran from *S. bovis* 148 on the dextran synthesis.

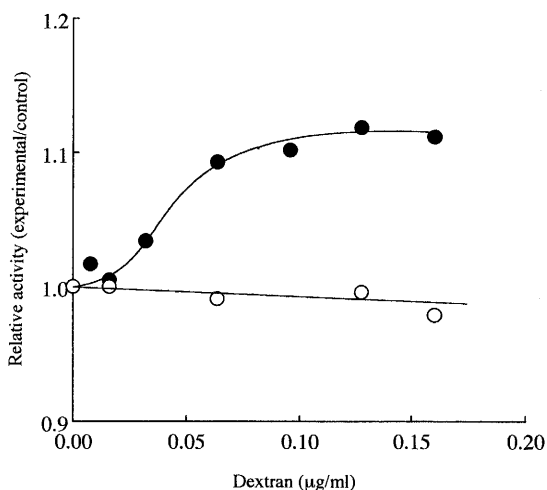


Fig.4 Effect of dextran on the dextranucrase activity. Symbols, ○ and ● indicate dextran T-10 and the degraded dextran from *S. bovis* 148, respectively.

maltose to sucrose molar ratio of 10, the dextran was not synthesized at all.

[Glucosyl transfer from sucrose to glucan-acceptors]

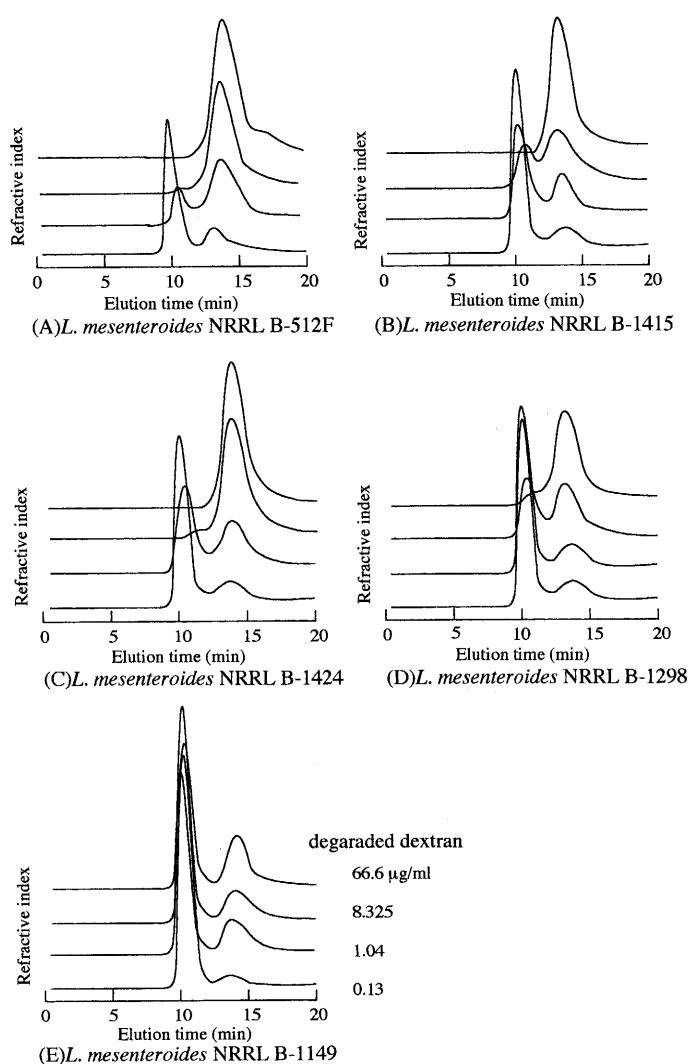
The degraded dextran of *S. bovis* 148 (M.W. 10,000) was indicated by HPLC analysis that the glucan synthesized enzymatically was changed by adding the degraded glucan (Fig. 3). Further, the dextranucrase was assayed in the presence of glucan from 0 to 1 mg/ml. The dextranucrase activity was slightly activated 1.1-fold by low-molecular weight dextran (M.W. 10,000) of *S. bovis* 148, but not activated by dextran T-10 and T-40 (Fig. 4).

The author researched the acceptor specificity on the dextran synthesis using five dextrans of different structure produced by *L. mesenteroides*. The glucan structure of five dextrans analyzed by Jeane et al. are shown in Table 1.

A reaction mixture of 3.1ml containing 6.45mM sucrose, the enzyme and various concentrations of the

Table.1 Structure of dextrans used for acceptor specificity

Strain	Linkage	(%)
<i>L. mesenteroides</i> NRRL B-512F	α -1,6-bond branch	95 5
<i>L. mesenteroides</i> NRRL B-1415	α -1,6-bond branch	89 11
<i>L. mesenteroides</i> NRRL B-1424	α -1,6-bond branch	72 28
<i>L. mesenteroides</i> NRRL B-1298	α -1,6-bond branch	64 36
<i>L. mesenteroides</i> NRRL B-1149	α -1,6-bond branch	52 48

Data from Jeane et al.⁹⁾Fig.5 Influence of degraded dextrans from *L. mesenteroides* on the dextran synthesis.

degraded dextran from *L. mesenteroides* as acceptor in 50mM phosphate buffer, pH 6.0, were incubated at 40°C for 24h. Changes on the molecular weight distribution of product dextrans were investigated by HPLC system on a TSKgel GMPWXL column. Degraded dextrans (M.W. 10,000) of *L. mesenteroides* NRRL B-512, B-1298, B-1415, and B1424 made molecular weight of glucan syn-

thesized change. It was clear that each glucan added in reaction mixture was able to act as acceptor on the dextran synthesis. On the other hand, the degraded dextran of *L. mesenteroides* NRRL B-1149, having 52% of α -1,6-glucosidic bond, did not (Fig. 5). Dextrin having α -1,4-glucosidic bond was not also able to act as acceptor on the dextran synthesis.

Conclusion

Glucosyl transfer from sucrose to acceptors was investigated by using various substrates. The most effective acceptors were maltose and isomaltose in all commercial oligosaccharides used in this study. Hayashi et al. suggested that acceptor molecule like maltose correlated with the dextran synthesis (the molecular weight) of the dextran-sucrase because saccharides produced enzymatically in the presence of maltose depended on the molar ratio of sucrose and maltose²⁾. The author also investigated the concentration dependency of maltose on the molecular weight of the saccharides produced. Maltose molecule showed no indication of its influence on the molecular size of glucan produced.

In general, dextransucrase has a high affinity with kinds of glucan with α -1,6-glucosidic linkage. The author performed experiments to investigate the acceptor specificity of dextran on the dextran elongation. The degraded dextran of *S. bovis* 148 (M.W. 10,000) was indicated by HPLC analysis that the glucan synthesized enzymatically was changed by adding the degraded glucan. It was indicated that glucan of *S. bovis* 148 was able to act as acceptor on the dextran synthesis. Furthermore, the author researched the acceptor specificity on the dextran synthesis using dextrans of different structure. It was indicated that a correlation between the glucan structure and the strength acting as acceptor on the dextran synthesis exists. It was suggested that glucosidic bond over 60% is necessary to become the acceptor on dextran synthesis.

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*Streptococcus bovis*のDextransucraseの反応機構 —アクセプター特異性—

高木 勝広

要 約

Streptococcus bovis 148のdextransucraseのアクセプター試験は少糖類を用いて行われ、その結果、マルトースとイソマルトースが最も効果的なアクセプターであることがわかった。そこで、デキストラン合成におよぼすマルトース濃度の影響の検討を行った。マルトースは酵素合成されたデキストランの分子量に影響を与えないことが示され、デキストラン合成のアクセプターにはならないことが示された。

部分分解された*S. bovis* 148由来のデキストランは、デキストラン合成におけるアクセプターとなることが確認された。そこで、構造の異なるデキストランを用いてアクセプター特異性が調べられ、他の菌株が生成するデキストランが、*S. bovis*のデキストラン合成におけるアクセプターとなることが確認された。反応中に添加されたグルカンが酵素に取り込まれるには約60%以上の α (1,6)結合が必要であることが示唆された。

キーワード：*Streptococcus bovis*, dextransucrase, アクセプター反応, 特異性, 反応機構