The Mechanism of Dextran Elongation on the Dextran Synthesis of Dextransucrase

Dextransucraseのデキストラン合成におけるデキストラン伸長のメカニズム

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Abstract

In the series of isomaltohomologues from two to seven glucose units, interestingly, those were not able to act as acceptor molecule on the dextran synthesis. To elucidate the smallest acceptor on the dextran synthesis the author investigated using dextran of *S.bovis* 148. The acceptors of degree of polymerization from 3 to 10 were preparated by degrading the dextran with the dextranase. From the results, it was assumed that the glucosidic linkage of α-1,3 or 1,4 was needed for initial process of the polymerization. The author investigated the mechanism of dextran elongation on the dextran synthesis of dextransucrase from *S.bovis* 148. The mechanism of dextran elongation of dextransucrase from *S.bovis* 148 was assumed be single chain elongation.

Keywords: *Streptococcus bovis*, Dextransucrase, Single chain elongation, Acceptor reaction

INTRODUCTION

Dextransucrase catalyzes a glucosyl transfer reaction from sucrose to acceptor dextran and produces a high molecular weight dextran.

Glucosyl transfer from sucrose to acceptors was investigated by using various substrates in a previous paper. The most effective acceptors were maltose and isomaltose. Hayashi *et al.* suggested that acceptor molecule like maltose correlated with the dextran synthesis of the dextransucrase because the saccharides produced enzymatically in the presence of maltose depended on the molar ratio of sucrose and maltose[1]. The author also investigated the concentration dependency of maltose on the molecular weight of the saccharides produced[2]. But, maltose molecule was indicated not to influence on the molecular size of glucan produced.

In this paper, the author describes the mechanism of dextran elongation on the dextran synthesis of dextransucrase from *S. bovis*.

MATERIALS AND METHODS

Materials

All chemicals were of reagent grade and commercially available.

Enzyme preparation

The dextransucrase of *S. bovis* 148 was purified, in accordance with a report described before[3].

HPLC analysis

The specimens were filtered through a filter of DISMIC-25cs (pore size, 0.45 μm; Advantec Toyo, Tokyo, Japan). Glucan sizes synthesized enzymatically were estimated with a HPLC system (TOSOH, Tokyo, Japan) by using a TSKgel GMPW$_{XL}$ column (7.8 mm 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 (7.8 mm of inner diameter $\times$ 30cm; TOSOH) was used to analyze sugar ranges of oligosaccharides. Carbohydrate peaks were registered with an refraction index detector.

Production and preparation of dextrans

Production and purification of the dextran produced by *S. bovis* 148 have been described in the previous report[3].The dextran was degraded with endo-dextranase from *Penicillium* sp. (Sigma, St.Louis, MO, USA), and low molecular weight glucans (about 10,000) were fractionated by a TSKgel GMPW$_{XL}$ column.

RESULTS & DISCUSSION

Acceptor that relates to the synthesis of dextran

The influence of the isomaltoligosaccharide and the panose on the synthesis of the dextran was examined respectively. The structural formula of the isomaltoligosaccharide and the panose is shown in Figure 1.

The isomaltoligosaccharide and the panose did not influence the molecular weight of the dextran synthesized enzymatically (Fig. 2).

The author researched the smallest acceptor on the

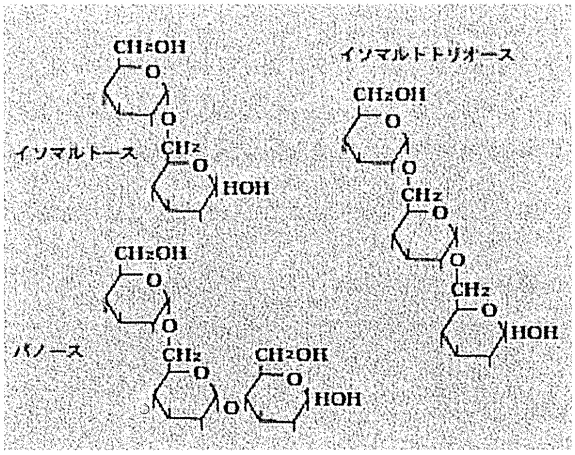


Fig.1 The structual formula of the isomaltoligosaccharide and panose

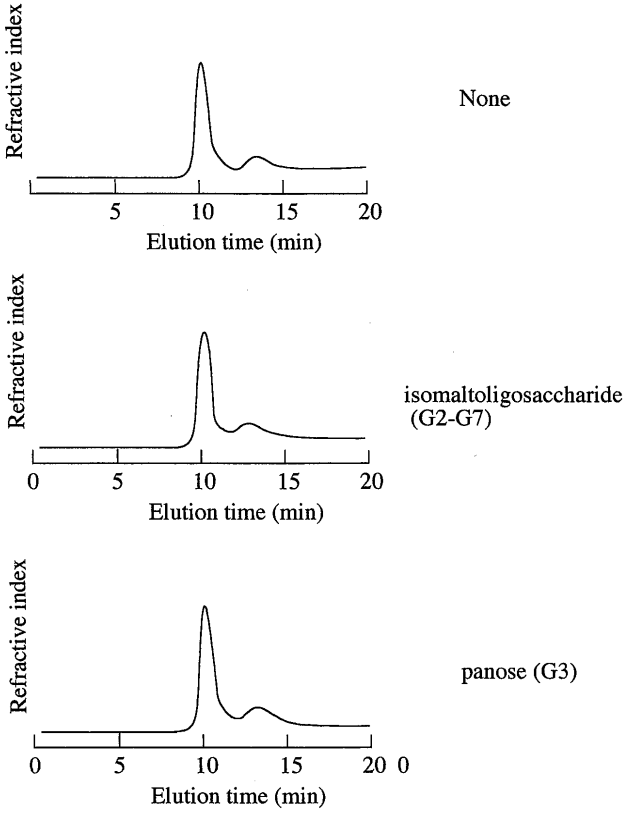


Fig.2 Effect of oligosaccharides on the dextran synthesis

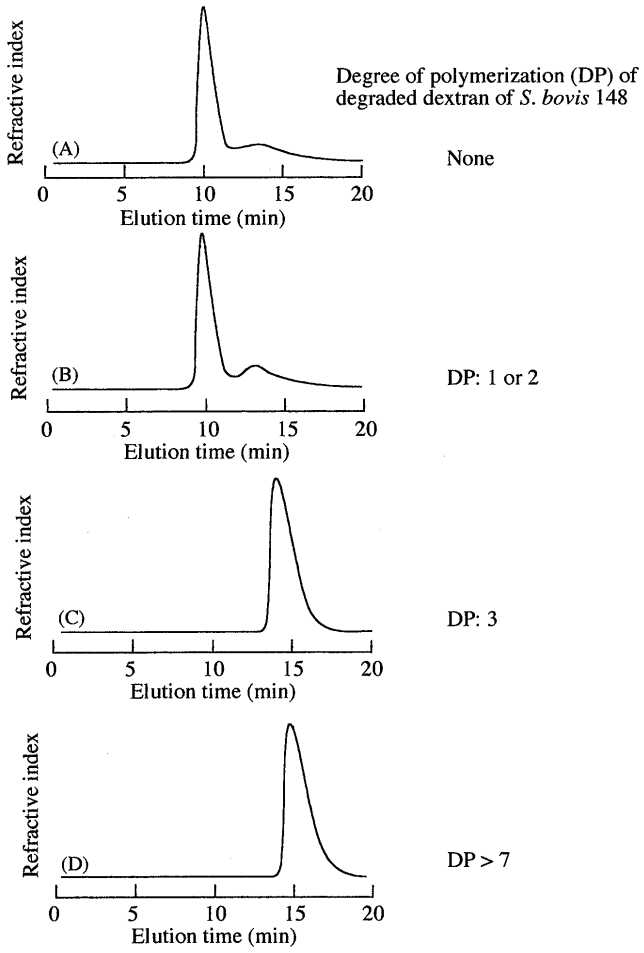


Fig.3 **Elution pattern of glucan produced by structure specificity of acceptors.** Each acceptor was prepared by degrading *S. bovis*-dextran with dextranase. (A) none acceptor, (B) DP: 1 or 2,(C) DP: 3, (D) DP>7.

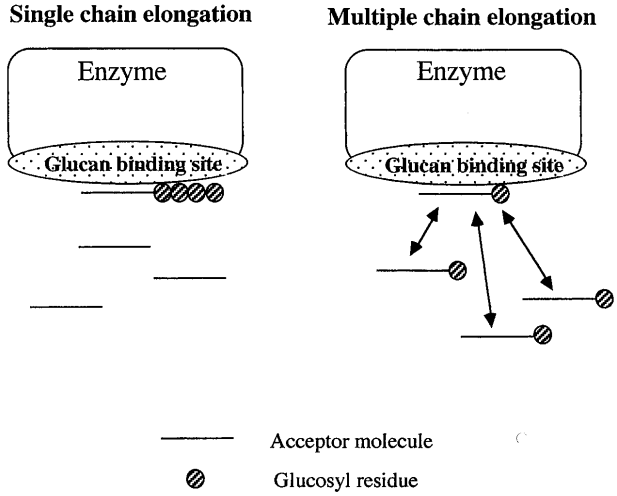


Fig.4 Mechanism of dextran elongation

dextran synthesis. The smallest acceptors were preparated by degrading the dextran of *S. bovis* 148 with the dextrananse and degree of polymerization (DP) from 3 to 10. The results were shown in Fig. 3. Changes on the molecular weight distribution of produced dextrans were observed under the condition of not only 10 of DP but also

3. It was assumed by this phenomenon that the branch of α-1,3 or 1,4 was needed for the initial process of the polymerization.

The mechanism of dextran elongation

Three mechanisms for the elongation of levan may be taken into consideration by Chambert *et al*[4].(Fig. 4): (a) single chain elongation in which the glucosyl residues are added succesively in the same molecule of levan acceptor, (b) multiple chain elongation in which the glucosyl residues are added randomly to all molecules of levans acceptor, or (c) multirepetitive chain elongation in which the glucosyl residues are added ramdomly but in groups of more than one, to a molecule of levan acceptor.

[Single chain elongation mechanism]

If the author assumes the single chain elongation mechanism, the author can compute theoretically the molecular weight of dextran synthesized with respect to the number of glucosyl residues transferred from sucrose to dextran acceptor as follows:

$$Mr(1) = Mr(2) + \frac{[\text{Glucosyl residues transferred}]}{[\text{Enzyme}]} \times Mr(3)$$

Mr(1); the molecular weight of dextran synthesized

Mr(2); the molecular weight of dextran acceptor

Mr(3); the molecular weight of glucosyl residue.

In such a case the expected molecular weight of dextran synthesized must be higher than 2 millions.

[Multiple chain elongation mechanism]

The molecular weight of dextrans synthesized by multiple chain elongation mechanism with respect to the number of glucosyl residues transferred from sucrose to dextran acceptor can be calculated as follows:

$$Mr(1) = Mr(2) + \frac{[\text{Glucosyl residues transferred}]}{[\text{Dextran acceptor}]} \times Mr(3)$$

Mr(1); the molecular weight of dextran synthesized

Mr(2); the molecular weight of dextran acceptor

Mr(3); the molecular weight of glucosyl residue.

In such a case the expected molecular weight of dextran synthesized must be 75,000.

[Multirepetitive chain elongation]

In the case of multirepetitive chain elongation, the author can not compute theoretically the molecular weight of dextrans synthesized with respect to the number of glycosyl moieties added successively per moleucule of dextran acceptor. However, if it is assumed that this number of small, the molecular weight of dextrans synthesized should only be slightly different from the molecular weight of dextran acceptor and the elution patterns on a TSKgel GMPW$_{XL}$ column of such dextrans will be approximately the same as those the author expected assuming a multiple chain elongation.

To investigate the mechanism of dextran elongation the author performed experiments as follows. A reaction mixture of 2.5 ml containing 2.0 mM of sucrose, 885 μM of the enzyme, and 380 mg/ml of the degraded dextran (M.W. 40,000) from S. bovis 148 in 50 mM phosphate buffer, pH 6.0, were incubated for 24 hr at 40℃.

It appeared that the dextran synthesized had more than 2 millions. It is assumed that the chromatographic pattern of such dextrans on a TSKgel GMPW$_{XL}$ column will have the characteristics of single chain elongation mechanism as those the author obtained in the experimental results (Table 1). The author can affirm unambiguously that multiple or multirepetitive chain elongation does not occur in this study's experimental conditions.

Table.1 Analysis of dextran elongation mechanism

Experimental data				Theoretical results expected	
				Single chain elongation	Multiple chain elongation
Sucrose conc.	Dextran conc.	Enzyme. conc	Approximately glucan-M. W.	Expected glucan-M. W.	Expected glucan-M. W.
2 mM	0.01 mM	0.885 mM	> 2 millions	4 millions	75,000

CONCLUSION

The author performed experiments to investigate the mechanism of dextran elongation on the dextran synthesis of dextransucrase from *S.bovis* 148. From a result, the mechanism of dextran elongation of dextransucrase from *S.bovis* 148 was assumed be single chain elongation. *Bacillus subtilis* levansucrase (primer dependent enzyme) had been suggested to be that of multiple or multirepetitive chain elongation mechanism [4].

The author assumed the enzyme characteristics between single and multiple chain elongation mechanisms as follows;

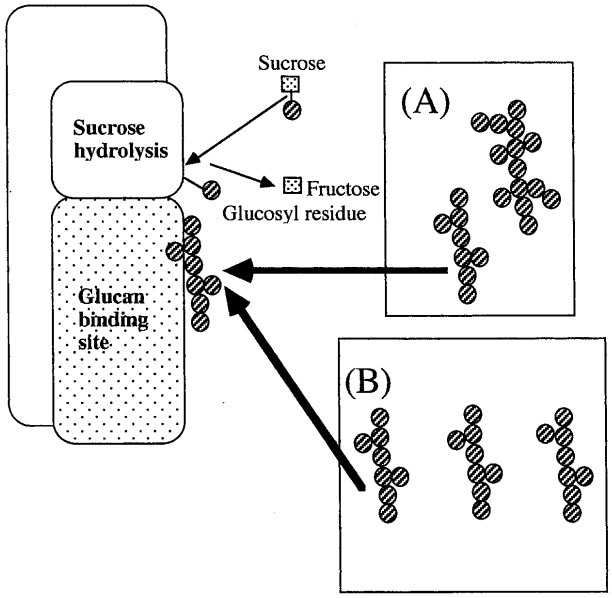
[Single chain elongation]: The enzyme activity (the glucan yield) advancing polymarization reaction in this way may be given no effect by added dextran as acceptor, and the glucan synthesized by this enzyme must have small numbers of branches.

[Multiple chain elongation]: The enzyme activity may be given much effect by added dextran as acceptor, and the

glucan synthesized by this enzyme have large numbers of branches.

The author postlates that the primer independent enzyme is that of single chain elongation mechanism and the dependent enzyme is multiple.

Finally, the author proposes the acceptor specificity of *S.bovis* dextransucrase against glucans added in the reaction mixture to Fig. 5.



(A) Acceptor specificity on dextran synthesis
(B) Single chain elongation

Fig.5 Proposed characteristics of glucan binding site of dextransucrase

References

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Dextransucraseのデキストラン合成におけるデキストラン伸長のメカニズム

高木　勝広

要　約

デキストランの構成単位であるイソマルトオリゴ糖（G2からG7）用いて，アクセプター試験を行った．イソマルトオリゴ糖はデキストラン合成のグルコシルアクセプターにならないことが明らかとなった．そこで，*S.bovis* 148のデキストランを酵素分解により調整したオリゴ糖（G2からG10）を調整し，検討を行った．その結果，G3以上のオリゴ糖は本酵素のデキストラン合成におけてアクセプターとなることが確認された．デキストラン合成の初期段階において，本酵素のアクセプターとなるためにはα（1，6）結合ほかにα（1，3）または（1，4）などの分岐が必要であることが推定された．

デキストランの伸長メカニズムを検討した．その結果，本酵素のアクセプターに対するグルコース転移反応は“single chain elongation”であり，スクロースからのデキストラン合成もまた同様のメカニズムで進むことが推察された．

キーワード：*Streptococcus bovis*, Dextransucrase，デキストランの伸長機構，アクセプター反応