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Note

Mechanism of dextran activation of dextransucrase ☆

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Germaine et al. [1–3] showed that the addition of dextran to *Streptococcus mutans* dextransucrase digests increased the rate of dextran synthesis. They found that the rate was dependent on the size of the dextran chain and reached a maximum when the average size of the added dextran was 30 glucose residues. The glucansucrases elaborated by *Streptococci* are constitutive and are produced by the organisms when grown in glucose, fructose, or mannitol media [4]. Sucrose is not required in the medium for elaboration of the enzyme(s) and hence dextran is not produced along with the dextransucrase in the culture supernatant.

Kobayashi and Matsuda [5,6] also reported that purified dextransucrases elaborated by both *Leuconostoc mesenteroides* B-512F and *Streptococcus sp.* were stimulated by the addition of dextran, although both enzymes could synthesize dextran without the addition of dextran to the digests. The rate of dextran synthesis in dextran-free digests was accompanied by a lag-period that could be eliminated by the addition of exogenous dextran.

Both Germaine et al. [1–3] and Kobayashi and Matsuda [7] interpreted their data as evidence for a primer-based mechanism for dextran synthesis. This mechanism had been assumed for the synthesis of dextran in the 1940's, 50's and early 60's [8–11] and was based primarily by analogy with the studies of Cori and Cori [12] and Swanson and Cori [13] on glycogen phosphorylase and Hanes [14] on potato starch phosphorylase. In these studies it was shown that glucose residues were added from α-D-glucose 1-phosphate to the nonreducing glucose residues of glycogen and starch. The reaction did not take place unless a glycogen primer chain or a starch primer chain was present. It, thus, resulted that a primer

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was an absolutely *required* constituent in the enzyme digest to obtain chain elongation. The phosphorylase reaction, however, was later shown not to be the mechanism for either glycogen or starch chain elongation, but to be a degradative process in which phosphorylase catalyzed the reaction of inorganic phosphate with the nonreducing glucose residue of the glycogen or starch chains to give α-D-glucose 1-phosphate [15].

A significant difference in the synthesis of dextran by dextransucrase and the chain elongation of glycogen and starch by phosphorylase, however, exists in that even though dextransucrase is stimulated by the addition of exogenous dextran, it can still synthesize dextran in the absence of any added dextran. A number of other problems developed for the primer mechanism for dextran synthesis. Robyt et al. [16,17], using pulse and chase techniques with [^{14}C]sucrose and Bio-Gel P2-immobilized dextransucrase, showed that glucose and dextran were covalently attached to the enzyme during synthesis and that the glucose is added to the reducing end of the growing dextran chain by a two-site insertion mechanism. In this mechanism, a primer is not necessary for the synthesis to occur. The two-site insertion mechanism has been verified by a number of subsequent studies [18–23]. In addition, Robyt and Corrigan [24] found that dextran T17.7 and T72.6 modified by a blocking (triisopropylbenzenesulfonyl, tripsyl) group on the C-6 hydroxyl of the nonreducing-end glucose residue, increased the rate of dextran synthesis by *S. mutans* dextransucrase equally as well as unmodified dextran. The modified dextran could not participate in a priming reaction as the requisite site for the addition of glucose, the C-6 hydroxyl of the nonreducing-end glucose residue, was blocked by a tripsyl group. This showed that the added dextran was not stimulating the reaction by acting as a primer but by some other mechanism.

We report here the investigation of the mechanism of dextran activation of dextransucrase by studying the initial velocity of the synthesis of dextran as a function of the concentration of added dextran, using two dextransucrases elaborated by *S. mutans* 6715 and *L. mesenteroides* mutant (B-512FMC) that is constitutive for dextransucrase [25].

1. Experimental

Enzymes.—*Streptococcus mutans* 6715 dextransucrase (EC 2.4.1.5, GTF-S) was obtained by the method of Ciardi et al. [4] and purified and separated from GTF-I by the method of Robyt and Martin [17]. *Leuconostoc mesenteroides* B-512FMC dextransucrase was obtained by the method of Kim and Robyt [25].

Rate studies.—The initial velocities of the enzyme–sucrose digests, containing variable amounts of dextran T-40 (M_n 26 500, Pharmacia Fine chemicals, Uppsala, Sweden) from 0–300 μg/mL, were obtained using [^{14}C]sucrose digests. The GTF-S digests (200 μL final volume) contained 100 mM [U-^{14}C]sucrose (0.57 μCi) buffered at pH 6.5 with 25 mM imidazolium chloride. The reaction was initiated at 37°C in the various digests by the addition of 40 mIU of enzyme. One IU is the amount of enzyme that incorporates one μmol of glucose into dextran per min at the desired temperature and pH [21]. Aliquots (25 μL) were taken at four times between 10 and 40 min. The aliquots were placed onto 1.5×1.5 cm Whatman 3MM paper and immediately placed into 300 mL of MeOH to quench the reaction and precipitate the polysaccharide on the paper. The papers were washed two more

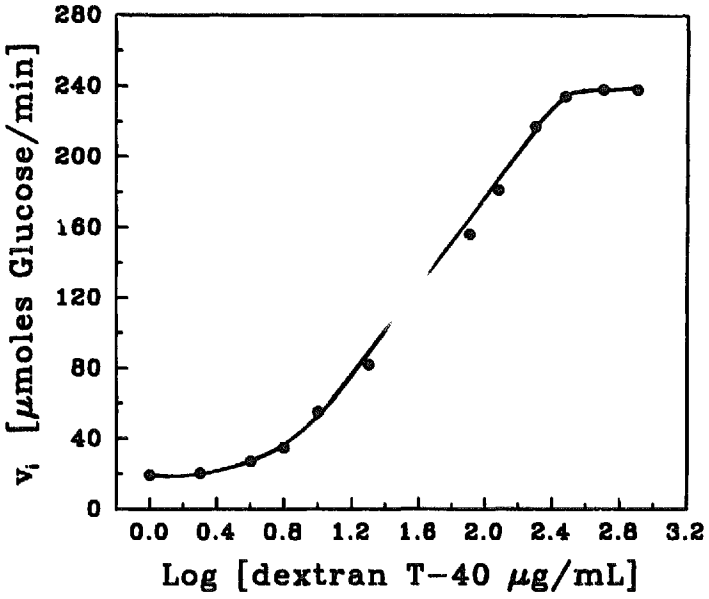

Fig. 1. Activity of *Streptococcus mutans* 6715 dextransucrase (GTF-S) as a function of the concentration of exogenous dextran. Digests (200 μL) containing 100 mM [U-^{14}C]sucrose (0.57 μCi), 25 mM imidazolium chloride buffer (pH 6.5), and 40 mIU of enzyme were reacted in the presence of various amounts of dextran T-40 at 37°C; 25-μL aliquots were taken at 4 times (10–40 min) and the μmol of glucose incorporated into dextran were determined by liquid scintillation counting using a filter paper assay. The initial velocities were determined from plots of the μmol of glucose incorporated into dextran vs. time (min).

times with 300 mL of MeOH, dried, and counted in a toluene cocktail using a liquid scintillation spectrometer [21]. The initial velocities were obtained by a linear least-squares fit of the amount of glucose incorporated into dextran vs. the time of reaction.

A similar reaction protocol was used for *L. mesenteroides* B-512FMC dextransucrase, but at a pH of 5.2 using 25 mM acetate buffer and 60 mM [U-^{14}C]sucrose. The reaction was initiated at 30°C in two different experiments by adding 20 and 75 mIU enzyme, respectively.

2. Results and discussion

The results of the experiments are shown in Figs. 1–3 in which the initial velocities (μmol of glucose incorporated into dextran/min) are plotted against the log of the concentration (μg/mL) of added dextran T-40. Fig. 1 is a plot of the *S. mutans* GTF-S enzyme in which may be seen a sigmoidal-shaped curve as the concentration of added dextran is increased. The rate of dextran synthesis was determined as an initial velocity and hence the amount of dextran synthesized in the digests can be neglected in comparison with the amount of added dextran. There is a slow increase in the initial velocity when 1 and 4 μg/mL of dextran is added (a lag-phase), and then a more rapid, nearly linear increase occurs until the dextran reaches 300 μg/mL, where the initial velocity becomes constant and maximum. This represents a 12.3-fold activation of the enzyme activity by the added dextran.

The *L. mesenteroides* B-512FMC dextransucrase reaction also gave sigmoidal curves as a function of the concentration of added dextran. Fig. 2 gives the results of a digest containing

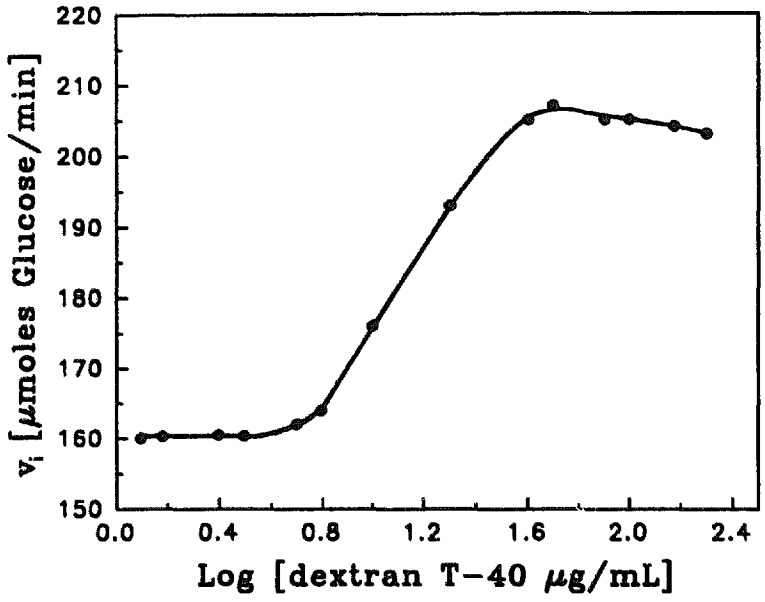

Fig. 2. Activity of *Leuconostoc mesenteroides* B-512FMC dextransucrase as a function of the concentration of exogenous dextran. Digests (200 μL) containing 60 mM [U-^{14}C]sucrose (0.57 μCi), 25 mM acetate buffer (pH 5.2), 20 mIU of enzyme, and various amounts of dextran T-40 were reacted at 30°C. The μmol of glucose incorporated into dextran and the initial velocities were determined as in Fig. 1.

20 mIU of enzyme. It had an initial velocity lag between 1.25 and 3.12 μg/mL of exogenous dextran. Thereafter, the initial velocity increased up to an exogenous dextran concentration of 40 μg/mL, where a constant, maximum velocity was attained. In this digest, the initial velocities decreased slightly with an additional increase in the concentration of exogenous dextran.

Fig. 3 shows a second *L. mesenteroides* B-512FMC dextransucrase reaction in which there was 3.8 times more enzyme than in the digest of Fig. 2. This reaction was similar to the other two experiments, giving a sigmoidal curve. It had an initial velocity lag between 1.25 and 1.52 μg/mL of exogenous dextran, diminished from the size of the lag observed in the experiment of Fig. 2. The digests with 2.5 and 10.0 μg/mL exogenous dextran had significantly higher initial velocities than the equivalent digests of Fig. 2. The initial velocity of Fig. 3 maximized at 40 μg/mL of exogenous dextran as it also did for the experiment of Fig. 2. In both of the *Leuconostoc* dextransucrase experiments, the maximum amount of activation was 1.28 times that of the digest without any added dextran. This was considerably less than the amount of activation (12.3-fold) observed for the *S. mutans* dextransucrase.

The initial activity of the *L. mesenteroides* B-512FMC dextransucrase was proportional to the amount of enzyme. The amount of enzyme used in the experiment of Fig. 3 was 3.75-times the amount of enzyme used for the experiment of Fig. 2. The initial activity, without exogenous dextran, of Fig. 3 was exactly 3.75-times the initial activity of Fig. 2. Further, the initial activity of *L. mesenteroides* B-512FMC dextransucrase, without exogenous dextran (Fig. 2), was significantly higher, by a factor of 8, than the initial activity of *S. mutans* dextransucrase (Fig. 1.). Yet, the final activities, after maximum stimulation of the two enzymes by the addition of dextran, were relatively close, 208 vs. 232 μmol of glucose incorporated into dextran per min.

The plots of Figs. 1–3 are Michaelis–Menten type-plots that usually give hyperbolic curves. The sigmoidal curves obtained are not that of usual Michaelis–Menten kinetics.

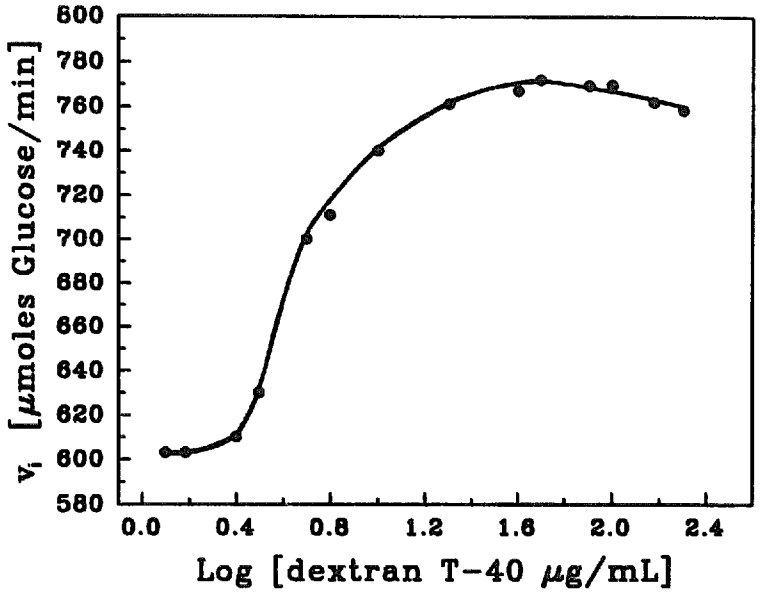

Fig. 3. Activity of *Leuconostoc mesenteroides* B-512FMC dextransucrase as a function of the concentration of exogenous dextran. The conditions were the same as in Fig. 2 but with 75 mIU of enzyme.

Sigmoid curves are particularly characteristic of a cooperative binding of ligands (in this case dextran) to enzymes that have multiple binding sites [26]. The sigmoidal increase in the reaction rate with increasing ligand concentration can be interpreted as due to the binding of the ligand at a noncatalytic site or allosteric site to give a more active enzyme. This positive allosteric effect often involves the joining together of subunits [26].

This is very likely the mechanism of exogenous dextran activation of the dextransucrases. Mooser et al. [27,28] found that *S. mutans* dextransucrase has a dextran-binding domain that is distinct and some distance from the active-site domain. Further, both the *S. mutans* dextransucrases [29–31] and the *L. mesenteroides* B-512F(MC) dextransucrases [25,32] bind to lightly cross-linked dextran (Sephadex G-200). The binding is inhibited when exogenous dextran is present. This noncatalytic, dextran-binding domain, is very likely an allosteric site to which the dextran binds and induces a favorable conformation for the synthesis of dextran from sucrose.

Further, there is evidence that the favorable conformation involves the formation of multiple subunit complexes. This is suggested by a number of experimental observations. The molecular weights of the dextransucrases have been reported to range from 185 KDa [5] to 64 KDa [6]. Very high-molecular-weight aggregates also have been observed, especially when the enzymes are elaborated in a sucrose medium where dextran is synthesized [33,34]. These aggregates often precipitate from the culture supernatant and they do not migrate on polyacrylamide gel electrophoresis [25,34]. This indicates that the presence of dextran in relatively large amounts, with a high molecular weight, acts as a cross-linking matrix that can join many enzyme subunits together.

Kobayashi and Matsuda [5] have reported that *L. mesenteroides* B-512F dextransucrase had a subunit molecular weight of 64 KDa. This subunit, however, had very low enzyme activity [6] and most probably was an inactive subunit that either partially reassembled in the presence of sucrose to give some active enzyme containing more than one subunit, or it contained a small amount of active, multisubunit enzyme that was not detected in the

molecular-weight analysis. Kim and Robyt [25] recently found that purified dextransucrase from the constituent mutant, *L. mesenteroides* B-512FMC, had a molecular size of 185 KDa that was composed of three subunits, two identical subunits with molecular weights of 63 KDa and one subunit with a weight of 59 KDa. They found that these subunits were inactive. A small amount of active enzyme with a molecular size of 126 KDa (two 63 KDa subunits) was observed, indicating that a minimum of two 63 KDa subunits are required for enzyme activity.

The decrease in the amount of lag from the experiment of Fig. 2 to the experiment of Fig. 3, when the amount of enzyme was increased 3.75-fold, also indicates that the activation of dextransucrase is by the association of subunits induced by the binding of dextran. As the concentration of enzyme is increased per unit volume, the concentration of inactive or less active subunits is increased, giving a higher probability of association. Thus, a lower concentration of dextran is required to induce the association into active units and give activation. The maximum degree of activation, however, remains the same as the relative proportion of subunits that can associate remains the same.

In summary, the addition of dextran to dextransucrase digests affects the initial velocity of dextran synthesis in a sigmoidal manner that is characteristic of an allosteric binding of dextran to the enzyme. This is supported by the presence of a dextran binding site that is not part of the active site. The *L. mesenteroides* B-512F(MC) dextransucrase has been shown to be composed of three subunits having a molecular size of ca. 63 KDa. A minimum of two subunits appears to be required for dextran synthesis. Enzyme aggregates are formed when dextran is synthesized, and it is proposed that the allosteric activation of dextransucrase by exogenous dextran occurs when the dextran binds to the dextran binding-site where it acts as a matrix to join or induce the subunits to associate into an active form.

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