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THE FORMATION OF α-D-(1→3) BRANCH LINKAGES BY AN EXOCELLULAR GLUCANSUCRASE FROM *Leuconostoc mesenteroides* NRRL B-742*

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ABSTRACT

Leuconostoc mesenteroides NRRL B-742 produces two exocellular α-D-glucans, namely, fraction L, which consists of an α-D-(1→6) backbone with α-D-(1→4) branch-points, and fraction S, which consists of an α-D-(1→6) backbone with α-D-(1→3) branch-points. It was found that the percentage of α-D-(1→3) branch-points in fraction S glucan is variable, depending on the conditions under which it is synthesized from sucrose by the exocellular glucansucrase, and that α-D-(1→3) branch formation by this glucansucrase occurs by acceptor reactions in which α-D-glucosyl groups are transferred from sucrose to OH-3 groups on α-D-(1→6)-linked D-glucan chains. Thus, any change in reaction conditions that affects the rate of acceptor reactions relative to chain elongation also affects the degree of branching in B-742 fraction S dextran. It was also found that this glucansucrase is capable of modifying other dextrans, such as B-512F and B-742 fraction L, by transferring D-glucosyl groups to OH-3 of D-glucosyl residues in these dextrans as well.

INTRODUCTION

In order to understand further the biosynthesis of secondary D-glucosidic linkages in dextrans, we have undertaken the study of such D-glucans that contain high relative proportions of α-D-(1→3)-glucosidic linkages, both at the branch points and in linear positions of the polysaccharide chains. We have previously described enzymes that are capable of forming both types of α-D-(1→3) linkage[1–3]. In the present work, we discuss the formation of α-D-(1→3) branch linkages by an exocellular glucansucrase from *Leuconostoc mesenteroides* NRRL B-742. As in the *L. mesenteroides* B-1355 system, this strain also produces two exocellular α-D-glucans from sucrose, namely, fraction L, which is precipitated at an ethanol concentration of 39%, and fraction S, precipitated at an ethanol concentration[4] of 45%. This particular strain was first isolated and described by Hucker and Pederson[5] in

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TABLE I

METHYLATION DATA OF SEYMOUR AND CO-WORKERS[16]

Dextran	*Mole % of each methylated* D-*glucose*[a]				
	2,3,4,6	*2,3,4*	*2,4,6*	*2,3*	*2,4*
B-742 S	45.4	4.4	—	—	50.2
B-742 L	14.4	72.5	0.7	12.4	—

[a]Numbers refer to positions of *O*-methyl groups on D-glucose

1930. Subsequent workers described the growth of the bacteria and the production of exocellular D-glucan in detail[6-15], as well as some of the structural characteristics of the two dextrans.

Seymour *et al.*[16-24] described their structural analyses of a number of D-glucans, including those produced by *L. mesenteroides* B-742. According to their data (see Table I), B-742 dextran fraction S consists of a linear chain of α-D-(1→6)-linked D-glucopyranosyl residues, each bearing a single α-D-glucopyranosyl group linked to O-3, to give a "comb-like" polymer. Nearly all of the D-glucosyl residues in the backbone chain would be substituted in this way, with approximately one in ten, or twenty, lacking a D-glucosyl group on O-3. It would be assumed that such a dextran would be completely resistant to hydrolysis by most endodextranases, such as that produced by *Penicillum funiculosum*. However, we have found that B-742 S dextran may not always be so highly branched. Our investigation has shown that branch formation and the degree of branching in this dextran can vary, depending on the conditions of synthesis. We have also found that branch formation by *L. mesenteroides* B-742 S dextransucrase can occur by acceptor reactions with less highly branched dextran moleules, such as B-512F[a] dextran, to give a much more highly branched product. Branch formation by acceptor reactions can vary not only from one strain of dextran-producing bacteria to another, but can also vary depending on the reaction conditions.

EXPERIMENTAL

Organism. — A lyophilized sample of *Leuconostoc mesenteroides* strain B-742 was obtained from the Northern Regional Research Center of the U.S. Dept. of Agriculture. The culture conditions were the same as those described by Hehre[25].

Crude enzyme-mixture — A preparation of crude-culture supernatant-fluid was used that had been dialyzed, and concentrated, as previously described for *Leuconostoc mesenteroides* B-512F (ref. 26) and B-1355 (ref. 1). This preparation contained 0.6 IU/mL of glucansucrase as measured by a radiochemical assay[26,27],

[a]The dextran produced by *L. mesenteroides* B-512F has 5% of α-D-(1→3) branches

and will be referred to as CSDC (culture supernate, dialyzed, and concentrated). It also contained 25 mg of carbohydrate per mL.

Chromatography. Molecular-size exclusion-chromatography was conducted with Bio-Gel P-6 and P-10 and A-15m (Bio-Rad, Richmond, CA) in columns that were eluted with 0.02% sodium azide at room temperature.

Thin-layer chromatograms were obtained either on plates of glass-backed, Whatman K5 silica gel, or plastic-backed plates of E. Merck silica gel. Two different solvent-systems were employed: *A*, 4:1 (v/v) acetonitrile–water, and *B*, 3:1:1 (v/v/v) ethanol–nitroethane–water. Visualization was achieved by charring with sulfuric acid for unlabeled compounds, and by autoradiography for ^{14}C-labeled material. (Phenoxyacetyl)cellulose (PA-cellulose) chromatography was performed as previously described[1].

Hydrolysis by dextranase. — *Penicillum funiculosum* endodextranase was purchased from Sigma Chemical Co., St. Louis, MO. Hydrolyses of dextrans were conducted at room temperature in 20mM acetate buffer, pH 5.5. The extent of hydrolysis of radioactive dextran was determined by measuring the amount of labeled material that was rendered soluble in methanol, similarly to the filter-paper assay for formation of D-glucan[26,27]. Hydrolysis was monitored in this way until no further decrease in methanol-insoluble, radioactive material was observed.

Synthesis of samples of D-*glucan.* — Two different sets of conditions were employed for preparing various D-glucan samples. The first, the usual method, consisted in incubating a glucansucrase preparation with sucrose at an initial concentration of 0.2M sucrose in 20mM pyridine acetate buffer, pH 5.4, containing 2mM calcium chloride and 0.02% of sodium azide. The reaction was allowed to proceed at room temperature until no sucrose remained, as detected by t.l.c. in solvent *A*. The D-glucans were then fractionally precipitated by slowly adding ethanol[4]. The precipitated D-glucans were redissolved in water, refractionated, and dried *in vacuo* for ~12 h at 40°.

An alternative method for synthesizing glucan was to place the glucansucrase in dialysis tubing (~3–5 mL), which was then placed in a vessel containing stirred, buffered, 0.2M sucrose (1.5 L). The material inside the tubing was removed when it reached a pasty, gel-like consistency. This material was diluted with water, and the D-glucans were fractionated by precipitation with ethanol[4] as just described. These two methods will be referred to as the usual method and the dialysis-bag method. As will be shown, the resulting D-glucans differ significantly. An authentic sample of NRRC B-742 fraction S dextran was kindly supplied by Dr. Morey E. Slodki of the Northern Regional Research Center (NRRC) of the U.S.D.A. (Peoria, IL).

^{13}C-Nuclear magnetic resonance spectroscopy. — Polysaccharides were analyzed by ^{13}C-n.m.r. spectroscopy in order to determine the relative amounts of different linkage types, in a manner similar to that of Seymour *et al.*[17]. Dry polysaccharide (~50–150 mg) was dissolved in deuterium oxide (2 mL) in a 10-mm, glass, n.m.r. tube. Spectra were recorded at 80° with a JEOL FX-90Q, F.t.-n.m.r. spectrometer (22.5 MHz) operated in the proton-decoupled, ^{13}C mode.

RESULTS

It was found that, on hydrolysis by endodextranase, fraction S dextran that had been produced under the usual conditions by our B-742 CSDC gave large quantities of oligosaccharide products, and the solution, initially opalescent, became clear. When a sample of B-742 S dextran supplied by the NRRC was subjected to hydrolysis with endodextranase under the same conditions, only slight traces of oligosaccharides were detected by t.l.c., and the solution remained opa-

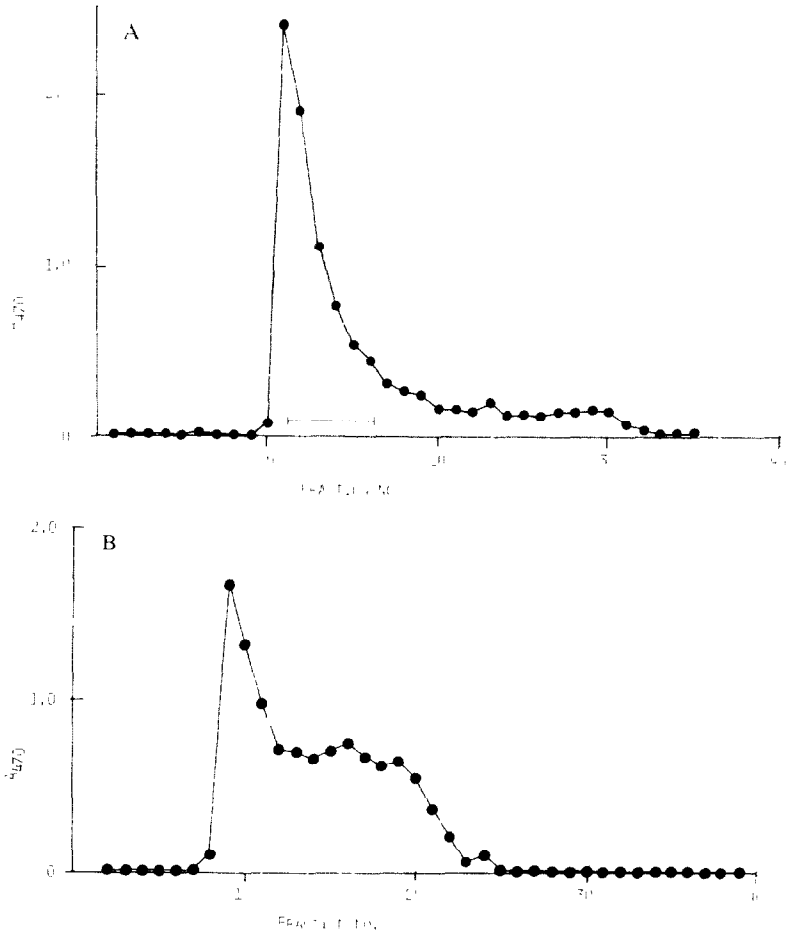


Fig. 1. A. Bio-Gel P-6 elution-profile of endodextranase-treated NRRC B-742 fraction S dextran. [Column was 1 × 45 cm, fractions were 1.5 mL each. A_{470} was determined after phenol–sulfuric acid assays of fractions, each fraction (50 μL) was mixed with H_2O (0.45 mL) + 5% phenol (0.5 mL), followed by the rapid addition of sulfuric acid (2.5 mL). The horizontal bar indicates the fractions that were pooled and concentrated for Bio-Gel P-10 chromatography.] B. Bio-Gel P-10 elution-profile of void-volume material from the P-6 column in Fig. 1A. [Column was 1 × 45 cm, fractions were 1.5 mL each. A_{470} was determined after phenol–sulfuric acid assays as in Fig. 1A.]

TABLE II

PRODUCT DISTRIBUTION OF DEXTRANASE-HYDROLYZED ^{14}C-DEXTRANS

Product	*Radioactivity (% of total)*	
	B-742 L	*B-742 S*
D-Glucose	3.7	4.6
Isomaltose	23.2	19.8
Isomaltotriose	4.8	0.6
B_4[a]	2.4	7.8
B_5	1.9	4.0
B_6	6.4	6.0
B_7–B_9	17.2	14.4
Origin	40.5	42.8

[a] B_n compounds are isomalto-oligosaccharides having n D-glucosyl residues containing α-D-(1→4) or α-D-(1→3) branch linkages.

lescent, indicating that high-molecular-weight dextran was not broken down.

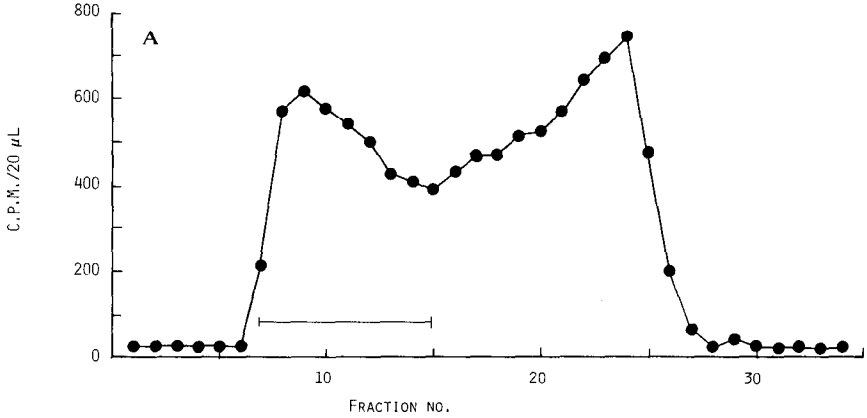
After exhaustive treatment with endodextranase, the NRRC sample of B-742 S dextran gave the chromatographic-elution patterns in Fig. 1; these showed that endodextranase hydrolyzes only a small proportion of the NRRC B-742 S dextran.

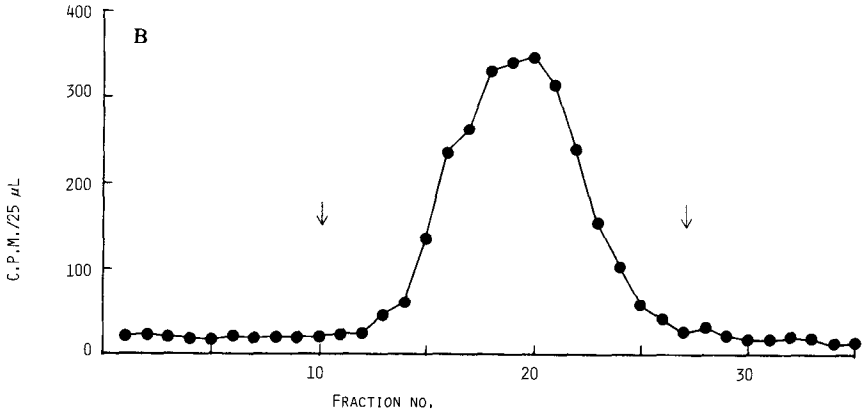
On the other hand, fraction S dextran produced by our B-742 CSDC under the usual conditions was hydrolyzed to a much greater degree by endodextranase. When a ^{14}C-labeled sample of our fraction S dextran was subjected to the action of endodextranase, 49% of the ^{14}C-labeled material was rendered methanol-soluble. A ^{14}C-labeled sample of our fraction L was also hydrolyzed by endodextranase, to give 42% of the labeled material in methanol-soluble products.

These results were somewhat surprising, considering the values obtained by Seymour *et al.* when these dextrans were analyzed by methylation and g.l.c.–m.s.[16]. Their results (see Table I) showed that the NRRC sample of B-742 S contains at least 45–50% of α-D-(1→3) branches, whereas fraction L contains only 12–15% of branched residues, although the branch linkages in L are α-D-(1→4).

Further to determine the nature of the dextranase breakdown-products of our ^{14}C-dextrans, they were separated by thin-layer chromatography on a plastic-backed, t.l.c. plate, using two ascents in solvent *B*. Spots containing radioactive material, located by autoradiography, were cut out, and counted for ^{14}C content by liquid scintillation spectrometry. The results, shown in Table II, revealed the presence of considerable amounts of oligosaccharides arising from both D-glucans. The two showed similar quantities of material at the origin, as well as similar amounts of glucose and isomaltose. The most notable differences appeared in the relative amounts of material in isomaltotriose and in the branched oligosaccharides having four and five D-glucosyl residues.

The products of dextranase hydrolysis were also studied by gel-permeation chromatography. After exhaustive hydrolysis with endodextranase, the product mixture was passed through a column of Bio-Gel P-6. The portion that was eluted


A
800
600
400
200
0
C.P.M./20 μL
10
20
30
FRACTION NO.


B
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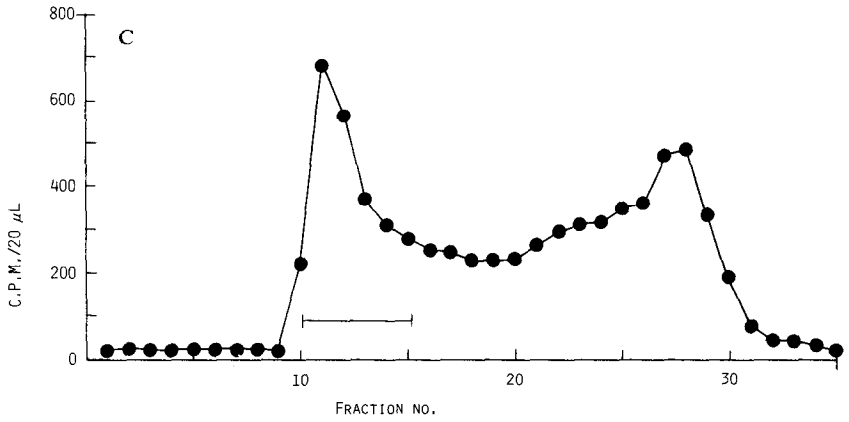
300
200
100
0
C.P.M./25 μL
10
20
30
FRACTION NO.


C
800
600
400
200
0
C.P.M./20 μL
10
20
30
FRACTION NO.

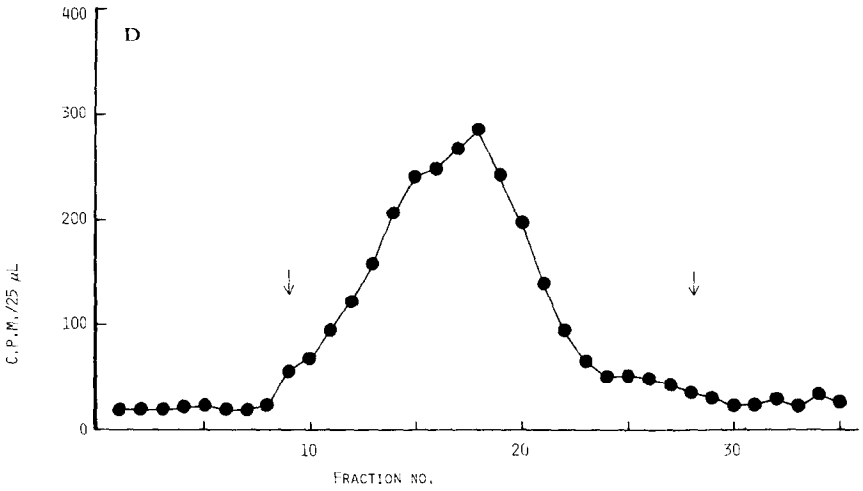


Fig. 2. A. Bio-Gel P-6 elution-profile of endodextranase-hydrolyzed, ^{14}C-labeled, CSDC-produced, B-742 fraction S dextran. [Column was 1 × 45 cm, fractions were 1.5 mL each. The horizontal bar indicates those fractions pooled and concentrated for subsequent, Bio-Gel P-10 chromatography.] B. Bio-Gel P-10 elution-profile of Bio-Gel P-6 void-volume material from column described for Fig. 2A. [Column was 1 × 45 cm, fractions were 1.5 mL each Arrows indicate void volume and included volume.] C. Bio-Gel P-6 elution-profile of endodextranase-hydrolyzed, ^{14}C-labeled, CSDC-produced, B-742 fraction L dextran. [Column was 1 × 45 cm, fractions were 1.5 mL each Horizontal bar indicates those fractions pooled and concentrated for subsequent, Bio-Gel P-10 chromatography.] D. Bio-Gel P-10 elution-profile of Bio-Gel P-6 void-volume material from column described for Fig. 2C. [Column was 1 × 45 cm and fractions were 1.5 mL each. Arrows indicate void volume and included volume.]

in the void volume of the P-6 column was concentrated, and then chromatographed on Bio-Gel P-10. The results (see Fig. 2) showed that, besides the oligosaccharides identified by t.l.c., a high-molecular-weight portion remains after dextranase hydrolysis. This dextranase-resistant fragment is equivalent in size to a globular protein in the range of molecular weight of 6,000 to 20,000, and is polydisperse over this range. This high-molecular-weight fraction is still a good deal smaller than the original dextran (before dextranase treatment). Before hydrolysis, both the L and the S dextrans migrated in the void volume of a column of Bio-Gel A-15m.

^{13}C-N.m.r. spectra of these three dextrans (*i.e.*, the NRRC sample of B-742 S and our CSDC-synthesized B-742 fractions S and L) are shown in Figs. 3 and 4. The important features to note are the relative peak-intensity ratios of the two anomeric-carbon resonances in the spectra of the S fractions (peaks 1 and 2 in Figs. 3 and 4A). The peak-intensity ratio is a reliable measure[17] of the relative contributions due to anomeric carbon atoms linked to O-3 (peak 1) and O-6 (peak 2). In Fig. 3, the ratio is 0.8, whereas, in Fig. 4A, it is only 0.6. This indicates ~45% of branching at O-3 for the NRRC sample of B-742 S, as compared to ~30–35% of branching for our CSDC-prepared fraction S.

On the other hand, the spectrum of our CSDC-prepared B-742 L dextran (Fig. 4B) is almost identical to that obtained by Seymour *et al.*[17] for an NRRC sam-

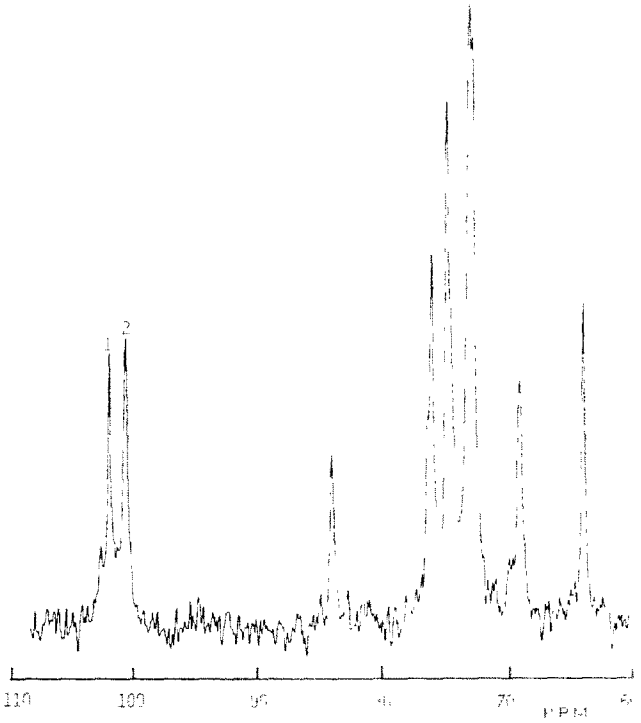


Fig. 3. Proton-decoupled, ^{13}C-n.m.r. spectrum of B-742 fraction S dextran obtained from NRRC. [Chemical shift in p.p.m. with respect to tetramethylsilane.]

ple of B-742 L, indicating that our B-742 fraction L does not differ significantly from that supplied by the NRRC and described by Seymour *et al.*[17]. The ^{13}C-n.m.r. spectrum of the Bio-Gel P-6 void-volume portion of dextranase-hydrolyzed, CSDC-prepared B-742 S dextran was nearly identical to that depicted in Fig. 3, showing that the proportion of branch points in this dextranase-resistant fragment is higher than that in the original polysaccharide.

In contrast to the S glucan produced by the CSDC under the usual conditions, the D-glucan produced in the dialysis bag had a ^{13}C-n.m.r. spectrum identical to that shown in Fig. 3 for the NRRC sample, indicating that the fraction S glucan formed in the dialysis bag is considerably more branched than that formed under the more usual conditions.

To determine if the higher degree of branching produced by CSDC in the dialysis bag could be attributed to increased acceptor-reactions with less highly branched dextran, the following experiments were performed: ^{14}C-labeled B-742 S dextran (30 mg) produced under the usual conditions was mixed with B-742 CSDC (3 mL) and 2 mL of acetate buffer, pH 5.4, in a dialysis bag; the sealed, dialysis bag was then placed in a vessel containing stirred 0.2M sucrose in buffer (1.5 L). after 2.5 days, the gel that had formed inside the bag was removed, and diluted with

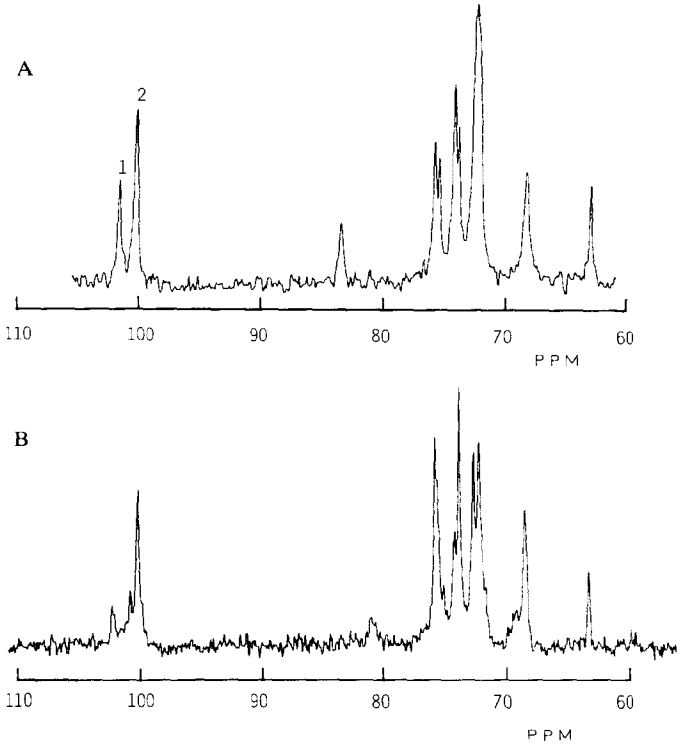


Fig. 4. A. Proton-decoupled, ^{13}C-n.m.r. spectrum of our CSDC-produced fraction S dextran. [Chemical shift in p.p.m. with respect to tetramethylsilane.] B. Proton-decoupled, ^{13}C-n.m.r. spectrum of our CSDC-produced B-742 fraction L dextran. [Chemical shift in p.p.m. with respect to tetramethylsilane.]

water, and the L and S glucans were fractionated by precipitation with ethanol. The S fraction was then subjected to hydrolysis by endodextranase, whereupon it was found that 10% of the ^{14}C-labeled material had been rendered methanol-soluble. Chromatography of this dextranase-hydrolyzed material on Bio-Gel P-6 gave results similar to those shown in Fig. 1. The ^{13}C-n.m.r. spectrum of this S fraction was identical to that in Fig. 3.

An experiment similar to that just described was also performed in which the ^{14}C-labeled acceptor added was the L dextran produced by our B-742 CSDC under the usual conditions. When the products were removed from the dialysis bag, and fractionated by two successive, ethanol precipitations, it was found that 85% of the ^{14}C label was in the L fraction, and 15% in the S fraction. When the L fraction underwent hydrolysis by endodextranase, ~20% of the ^{14}C-labeled material became methanol-soluble. The ^{13}C-n.m.r. spectrum of fraction S was, as before, identical to that in Fig. 3. However, the ^{13}C-n.m.r. spectrum of the L fraction had, in addition to a peak arising from a C-4 atom in an α-linkage, an additional resonance due to a C-3 atom in an α-linkage (see Fig. 5). The anomeric region also reflected the

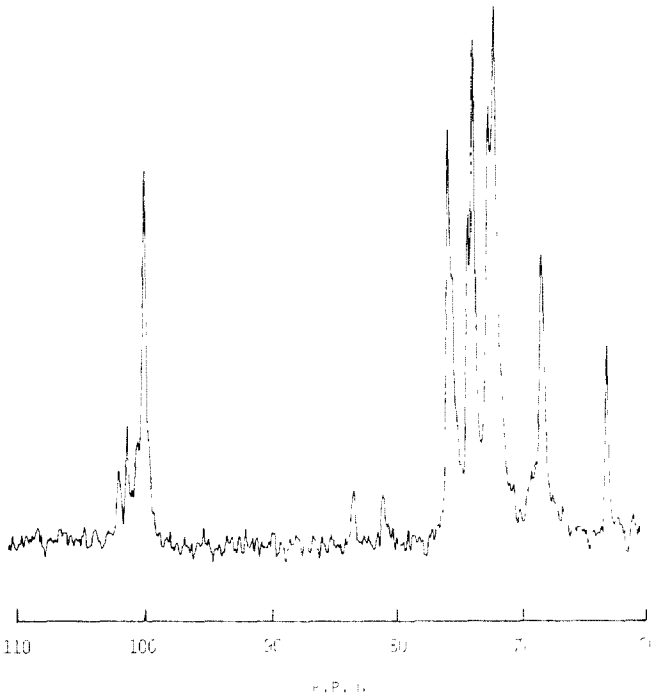


Fig. 5. Proton-decoupled, ^{13}C-n.m.r. spectrum of B-742 fraction I from dialysis-bag synthesis with CSDC and ^{14}C-labeled B-742 L acceptor dextran [Chemical shift in p.p.m. with respect to tetramethylsilane.]

presence of both linkage types. Apparently, some of the L dextran can act as an acceptor in the formation of α-D-(1→3) branch linkages under these conditions, and give rise to an S fraction (15%).

In light of these findings, further work was performed in order to determine whether such a D-glucan as B-512F dextran, which contains 95% of α-D-(1→6) linkages and 5% of α-D-(1→3) branch linkages, could undergo further branching by acceptor reactions catalyzed by B-742 S dextransucrase.

A reaction mixture consisting of 0.06 IU of our B-742 CSDC enzyme mixture, ^{14}C-labeled B-512F dextran (2 mg), and sucrose (30 μmol) in 0.3 mL of 20mM pyridine acetate buffer, pH 5.4, with 2mM calcium chloride and 0.01% of sodium azide was incubated at room temperature until all of the sucrose had been consumed. Ethanol (2 vol.) was then added, and the precipitated polysaccharide was redissolved in buffer. This mixture of D-glucans was treated with endodextranase, and the solubility of the ^{14}C-labeled product in methanol was monitored as previously described. It was found that 50% of the labeled material was hydrolyzed, to give methanol-soluble products. In contrast, the original, labeled B-512F dextran was hydrolyzed to give >90% of the label in methanol-soluble products (see Table III). These results indicated that the B-742 CSDC is capable of so modifying the B-512F dextran as to make it much more resistant to hydrolysis by en-

TABLE III

MODIFICATION OF ^{14}C-LABELED B-512F DEXTRAN BY B-742 ACCEPTOR REACTIONS

Enzyme preparation used to modify ^{14}C B-512F dextran	*% of ^{14}C remaining methanol-insoluble after dextranase hydrolysis*
None	7 ±2
B-742 CSDC	50 ±3
B-742 L dextransucrase	10 ±2

dodextranase. Bio-Gel P-6 chromatography of the dextranase products arising from the modified, and unmodified, ^{14}C-labeled B-512F dextrans also supported these findings.

Because the possibility existed that either of the two (or more) enzymes present in the B-742 CSDC could have been responsible for the modification of the B-512F dextran, it was necessary to isolate one, or both, of them free from contamination by the other. It was found that chromatography on (phenoxyacetyl)cellulose could be used to isolate the B-742 L dextransucrase in the same manner that it had previously been used[1] to isolate alternansucrase from a CSDC preparation from *Leuconostoc mesenteroides* B-1355.

When a sample of B-742 CSDC was chromatographed on PA-cellulose, there was immediately eluted a non-binding portion that contained all of the polysaccharide present in the CSDC, but the only glucansucrase activity present in this fraction was that which synthesized fraction L dextran; this was shown by ethanol fractionation (no new S glucan was synthesized) and by the ^{13}C-n.m.r. spectrum of the D-glucan produced by this fraction. The only new D-glucan produced from sucrose by this fraction had a ^{13}C-n.m.r. spectrum identical to that in Fig. 4B. Additional glucansucrase could be eluted from the PA-cellulose column by buffer containing Triton X-100, Tween 80, or sodium taurocholate (see Fig. 6). This detergent-eluted fraction produced both L and S dextrans, with L preponderating, although the ratio was variable. Additional glucansucrase remained bound to the PA-cellulose, even after washing with 2% Triton X-100. Urea (6 M) could be used to remove additional enzyme from the PA-cellulose, but a large proportion remained bound even under these conditions.

The L-producing enzyme was then used for determining whether it could modify B-512F dextran to make it more resistant to dextranase hydrolysis. The experimental procedure was the same as previously outlined, except that B-742 L dextransucrase (PA-cellulose fraction I; Fig. 6) was substituted for the B-742 CSDC used in the previous experiment. As is shown in Table III, B-512F dextran was not significantly modified by B-742L dextransucrase.

Although the B-742 CSDC was shown to modify dextran by synthesizing α-D-(1→3) branch linkages, only α-D-(1→6) linkages formed in acceptor reactions with D-glucose, maltose, and isomaltose. This was shown by allowing B-742 CSDC to

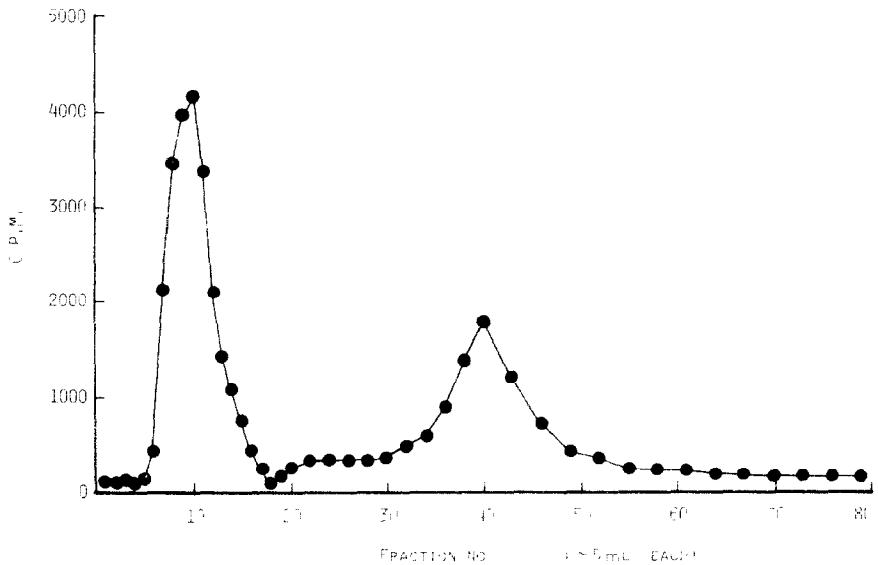


Fig. 6. (Phenoxyacetyl)cellulose chromatography of B-742 CSDC. CSDC (20 mL) was applied to a column (2.5 × 10 cm) of PA-cellulose, and first eluted with 20mM pyridine acetate buffer, pH 5.3 (fractions 1–17) and then with 1% Triton X-100 in buffer (fractions 18–80). Value in c.p.m. is amount of D-[^{14}C]glucose incorporated into methanol-insoluble D-glucan in the following assay: each fraction (20 μL) was incubated at 24° with 10 μL of 0.3M (U)-[^{14}C]sucrose (108 c.p.m./μg) in pH 5.3 buffer for 2.5 h. Each assay-mixture (20 μL) was adsorbed onto filter-paper squares (1.5 × 1.5-cm), which were then washed with methanol, dried, and counted for ^{14}C in toluene cocktails.

react with sucrose plus one of the three acceptors already named, in the same manner as described for B-1355 alternansucrase[2]. Thin-layer chromatography of the products showed that only α-D-(1→6)-linked products were formed, and that these were identical to those formed by B-512F dextransucrase[28,29]. The unusual saccharides characteristic of alternansucrase acceptor-reactions[2], compounds that contain α-D-(1→3), as well as α-D-(1→6) linkages, were not detected in the acceptor reactions catalyzed by B-742 CSDC.

DISCUSSION

The history of *Leuconostoc mesenteroides* NRRL strain B-742 can be traced back to 1930, when Hucker and Pederson[5] first isolated it from spoiled canned-tomatoes, and referred to it as *Leuconostoc mesenteroides*, strain 5. Shortly thereafter, Tarr and Hibbert[6] studied the conditions required for optimum production of dextran by this strain, which they referred to as culture 4. Early methylation studies[7,8] of the dextran produced by this strain showed the presence of (1→6) and (1→4) linkages, although it is not known whether the methods used would have distinguished the different types of branched D-glucose residues. It was not until the early 1950's that two separate dextran fractions were isolated from this

strain[10,11] and their structures analyzed by periodate oxidation. These early studies indicated 20–26% of α-D-(1→3) branches in fraction S dextran, as well as the 17–24% of α-D-(1→4) branches also found therein. Fraction L was consistently found to contain 18–20% of α-D-(1→4) branches, with no α-D-(1→3) linkages[9–13].

In 1954, in a survey of the dextrans produced by 96 strains of bacteria, Jeanes *et al.*[13] found differences in results obtained from analyses of the B-742 dextrans as compared with earlier studies, and attributed these differences to changes in the organisms over time, and to different conditions of storage and culture. They also described *Leuconostoc mesenteroides* strain B-1142, which had originated from an early isolate of B-742, but had changed with storage in a different culture-collection, so that it produced only an S dextran fraction[13]. More-detailed, structural analyses of the B-742 dextrans did not occur until 1979, when Seymour *et al.*[16] published the methylation data shown in Table I.

In light of this history of variability in the properties of the B-742 dextrans, then, it is not too surprising that the fraction S dextran produced by our enzyme preparation differs from that studied by others. This may be partially attributable to differences in culture conditions, but the conditions under which the dextran is synthesized also play an important role. This observation is not entirely without precedent. For example, it has been reported that calcium, which is required by B-512F dextransucrase, may play a role in branch formation by another dextransucrase[26,30]. It was found[31] that both the pH and the concentration of divalent metal ions influenced the degree of branching in a dextran produced by the dextransucrase from *Leuconostoc mesenteroides* IAM 1046.

Because branch formation by dextransucrase may occur by acceptor reactions with preformed dextran[3,28,32], anything that affects the relative rate of acceptor reactions with dextran should affect the degree of branching. It is for this reason that the dextrans formed in the dialysis-bag experiments are more highly branched. Under the usual circumstances, as dextran is formed from sucrose, D-fructose is released. Acceptor reactions with D-fructose will give rise to leucrose[33], whereas acceptor reactions with dextran give branched dextran[3]. Under normal circumstances, the concentrations of D-fructose and dextran both increase as the reaction proceeds. However, in the dialysis bag, the concentration of D-fructose, and, therefore, of leucrose, remains relatively low, because they are able to diffuse out of the bag as soon as they are formed. The concentration of dextran, on the other hand, increases within the bag, and acceptor reactions with this dextran, to give more highly branched dextran, are considerably favored. This is apparently the reason why the B-742 S dextran formed in the dialysis bag is more highly branched. The presence of α-D-(1→3) branches in the fraction L dextran formed in the dialysis bag can be explained in the same way, *i.e.*, B-742 L dextran, normally a poor acceptor for B-742 S dextransucrase, can act as an acceptor for α-D-(1→3) branch formation if conditions favor such a reaction with the S dextransucrase. The transfer of α-D-(1→3) branches to B-742 L dextran would also explain why some workers[15,16,34] have observed the presence of small proportions of α-D-(1→3) linkages in fraction L.

The results in Table III show that the dextransucrase that forms B-742 fraction S dextran is capable of transferring D-glucosyl groups from sucrose to an α-(1→6)-linked D-glucan, to form α-D-(1→3) branches. It is the presence of a high percentage of branched residues that makes branched dextrans more resistant to hydrolysis by endodextranase.

These results indicate that branch formation by dextransucrase can be attributed to acceptor reactions, even for such a highly branched dextran as B-742 S. It is possible, then, that the only major difference between a dextransucrase that forms a dextran having few branches, such as B-512F, and one that forms a highly branched dextran, such as B-742 S, is the size, or the stereochemistry, of the site to which acceptors bind, or the affinity of such a site for polymeric acceptors. An increased affinity for unbranched dextran and an increased rate of acceptor reactions with dextrans, relative to the rate of chain elongation by D-glucosyl insertion[35], would result in more, as well as shorter, branches. Thus, the more highly branched is a dextran, the shorter would be the average branch-length. For a dextran having a high degree of branching, such as B-742 S, all of the branches would be single D-glucosyl groups, whereas a dextran such as B-512F would have a considerable number of longer branch-chains.

The structure of B-742 fraction S dextran, then, is determined to some extent by the conditions of synthesis. It is probably a comb-like polymer, as suggested by Seymour *et al.*[16], but the "comb" may have "missing teeth", *viz.*, unbranched D-glucosyl residues within the main chain. The extent of branching may, thus, vary. The distribution of branches seems to be fairly random, as indicated by the distribution of hydrolysis products after dextranase treatment. This is what would be expected if the branching resulted from acceptor reactions.

The dextran fraction L, which we did not study in as much detail as fraction S, differs in some respects from the more commonly studied dextrans. These differences seem to arise from the presence of α-D-(1→4) branch linkages. It is possible that an α-D-(1→4) branch point imparts to the dextran chain a conformation different from that given by an α-D-(1→3) branch point; this was first suggested by Torii *et al.*[36] to explain the "anomalous" behavior of B-742 fraction L with certain carbohydrate-binding proteins[36,37]. They stated that inspection of molecular models showed that an α-D-(1→4) branch causes a perturbation in the conformation of the dextran chain compared to that of unbranched or α-D-(1→3) branched dextrans. This perturbation may be responsible for rendering a greater portion of the dextran molecule inaccessible to dextran-binding proteins, whether they be immunoglobulins or endodextranases. This appears to be the most likely explanation for the fact that B-742 L dextran, with only 12–14% of α-D-(1→4) branching, is hydrolyzed by endodextranase to the same extent as our CSDC-produced B-742 S dextran, having >30% of α-D-(1→3) branching.

The lower affinity of B-742 L dextransucrase for (phenoxyacetyl)cellulose, compared to that of the S dextransucrase, is similar to that in the B-1355 system[1], where the enzyme which was bound more tightly to the PA-cellulose was the one that formed the α-D-(1→6)-glucan having α-D-(1→3) branch linkages

We have observed that the structure of our CSDC-produced, B-742 fraction S dextran bears a number of similarities to the soluble D-glucan formed by an extracellular glucansucrase (GTF-S) from *Streptococcus mutans* 6715. Preliminary results suggest that branch formation by the streptococcal enzyme may proceed in a manner similar to that of the *Leuconostoc mesenteroides* B-742 S dextransucrase.

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