ENZYMATIC SYNTHESIS OF DEXTRAN
ACCEPTOR SPECIFICITY AND CHAIN INITIATION

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Dextran, a glucose polymer composed predominantly of α-1,6-glucopyranosidic linkages, is produced from sucrose by *Leuconostoc mesenteroides* and related organisms and from dextrins by other bacteria (see the review by Hehre (1)). Conversion of sucrose to dextran is catalyzed by an enzyme, dextransucrase, elaborated by the organisms. Dextransucrase preparations as culture filtrates (2, 3) or as dry purified concentrates are prepared readily and some of the properties of the enzyme have been described (2–5).

A study of the course of enzymatic dextran synthesis indicated that dextran having an average molecular weight of several millions is produced from the beginning of reaction. In recent experiments it has been found that the presence of certain sugars, notably maltose and isomaltose, as auxiliary sugars in dextran-synthesizing reaction mixtures alters the normal course of reaction so that the usual opalescent, viscous type of solution, indicative of extensive high polymer production, is not obtained. Such reaction mixtures contain instead oligosaccharides as major products. It appeared that the auxiliary sugars acted as glucosyl acceptors, alternate with the normal acceptor, and also initiated chain formation.1 A study of acceptor specificity in dextran synthesis was therefore undertaken with the object of elucidating the polymerization mechanism. It was hoped that clues leading to the direct enzymatic synthesis of “clinical” dextran with an average molecular weight in the range of 50,000 to 100,000 would be found. The results are described here.

Methods

Enzyme Preparations—*Leuconostoc mesenteroides* NRRL B-5122 from the Culture Collection of this Laboratory was used as source of dextran-

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1 Such auxiliary sugars will hereafter be referred to as alternate glucosyl acceptors.
2 This strain of *L. mesenteroides* was isolated in 1943 by Dr. R. G. Benedict, Fermentation Division, Northern Regional Research Laboratory.

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sucrase. This strain produces a relatively linear, water-soluble dextran (6).

The organisms were grown on a large scale in a medium containing su-
crose, corn steep liquor, and salts with continuous pH regulation, as has
been described (3). Purified dextran-sucrase, as a lyophilized dry powder,
was prepared from the culture filtrates by alcohol and ammonium sulfate
precipitation. A preparation containing 100 enzyme units per mg. was
used. 1 dextran-sucrase unit is defined as the amount of enzyme which
will convert 1 mg. of sucrose to dextran in 1 hour at 30° and pH 5.0, as
determined by increase in reducing power, calculated as fructose (2, 3).

Dextran Synthesis—Reaction mixtures with or without alternate ac-
ceptors were held at pH 5.0 and 30°. Dextran synthesis at 0.125 M sucrose
in the absence of alternate glucosyl acceptors proceeds by the appearance
of opalescence and increase in viscosity. Such changes, indicating pro-
duction of dextran of very high molecular weight, were looked for. The
extent of reaction was determined by measuring the increase in reducing
power, calculated as fructose, by the method of Somogyi (7), corrected as
necessary for the presence of the auxiliary reducing sugar originally added.

The yield of high molecular weight dextran, referred to as native dex-
tran, was determined by alcohol precipitation. An equal volume of ab-
solute ethanol was added to a 2 ml. aliquot of reaction mixture. The
precipitated gum was dissolved in 2 ml. of water, reprecipitated, and re-
dissolved at suitable strength for polarimetric determination of dextran.
Formamide was used when necessary to dispel opalescence. The results
were calculated on the basis that B-512 dextran has an [α]D of +200° in
water and of +215° in formamide at the dextran concentrations read.

Detection of Oligosaccharides—The alcoholic supernatant liquids from
the first precipitation of dextran were spotted on triplicate sheets of filter
paper. The papers were irrigated by the solvent descent technique with
a solvent containing 3 volumes of n-butanol, 2 volumes of pyridine, and 1
volume of water, as recommended by Jeanes, Wise, and Dimler (8). After
being dried, individual papers were sprayed with (a) ammoniacal silver
nitrate or (b) alkaline 3,5-dinitrosalicylic acid (8) to detect reducing sugars,
or (c) with dilute urea-phosphoric acid solution2 to detect fructose-con-
taining sugars. Spots indicating the location of sugars appeared on heat-
ing. Standard solutions of known sugars were spotted to provide ref-
erence spots.

EXPERIMENTAL

Survey of Sugars and Sugar Derivatives As Alternate Glycosyl Acceptors—
Deposition of glucosyl radicals on acceptors other than the normal ac-

2 Dimler, R. J., private communication.
ceptor was manifested to varying degrees in three ways: (a) the rate of production of reducing power, calculated as fructose, was increased or decreased; (b) the yield of native dextran was decreased; and (c) the production of oligosaccharides, difficult to detect in the presence of sucrose only, was readily detectable in the presence of a new acceptor. All three effects were examined in a survey of sugars and sugar derivatives for possible alternate glucosyl acceptors.

Test reaction mixtures (10 ml.), which were 0.125 M in sucrose, 0.125 M in auxiliary sugar or sugar derivative as alternate acceptor, 0.02 M in acetate buffer at pH 5.0 containing 25 units of enzyme per ml., were set up and incubated at 30°. Purified enzyme was used in these experiments. In one type of control the auxiliary sugar was omitted; in a second type the sucrose was omitted. The reaction rate was determined as reducing power liberated in 30 minutes, and the final reducing power and yield of high molecular weight dextran were measured. Oligosaccharide formation was examined on paper chromatograms at the end of reaction. The individual reaction mixtures were spotted at uniform original sucrose concentration to permit comparison of spot intensities.

The paper chromatograms indicated the formation in all sucrose-containing reaction mixtures of small amounts of a new reducing disaccharide. This sugar, to which the name "leucrose" has been given (9), is a fructo-glucoside. It appeared at a position half way between isomaltose and maltose. Instead of the greenish blue spot characteristic of fructose saccharides when sprayed with urea-phosphoric acid reagent, it gave a light pink spot after prolonged heating. Approximately 3 per cent of the sucrose utilized was converted to leucrose, as estimated by comparing the spot formed with those given by standard solutions of pure leucrose.

A second new reducing sugar, as yet unidentified, appeared in all sucrose reaction mixtures as a much fainter spot, approximately coinciding with the maltose guide spot. Presumably this sugar is a disaccharide, judging from its mobility. It gave a blue color with urea-phosphoric acid reagent and was assumed to contain fructose in its structure.

None of the auxiliary sugars or sugar derivatives tested as glucosyl acceptors was attacked by the enzyme in the absence of sucrose. Xylose, d- and L-arabinose, ribose, rhamnose, mannose, sorbose, cellobiose, trehalose, lactose, melezitose, raffinose, inulin, inositol, mannitol, sorbitol, gluconate, 2-ketogluconate, 5-ketogluconate, and glycerol were inert in the dextran synthesis in the presence of sucrose. Specimens of β-amylase limit dextrin, soluble starch, amylpectin, native B-512 dextran, and a commercial preparation of low (about 80,000) molecular weight clinical dextran did not affect reaction rate or oligosaccharide production appre-
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Ciably when present at 0.125 M on an anhydroglucose unit basis. Interference by the latter substances with alcohol precipitation prevented examination of their effect on dextran yield. Isomaltose, maltose, α-methyl glucoside, glucose, fructose, leucrose, melibiose, and galactose affected reaction rate, as well as the reducing power and native dextran accumulated by the end of the experiment, as shown in Table I. The effects on oligosaccharide production are shown in part in Fig. 1.

Isomaltose had the greatest effect on the reaction and appeared to be the best alternate glucosyl acceptor. Its presence approximately doubled reaction rate. The reaction mixture remained essentially clear and nonviscous, and the yield of normal dextran was very low. The chromatograms showed very heavy production of a series of oligosaccharides whose sugars coincided with those obtained in the hydrolysis of dextran. If therefore appeared that the oligosaccharide series produced was the ascending α-1,6-glucopyranosidic series, and that normal dextran synthesis had been diverted to major formation of these oligosaccharides. Decrease in the intensity of isomaltose spots before and after reaction indicated that isomaltose had been used in the reaction, presumably by initiating chain formation.

Maltose was the second best alternate glucosyl acceptor. It sharply increased reaction rate and depressed dextran yield. No appreciable opalescence or increase in viscosity occurred. Intensity of the maltose spot

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**Table I**

*Effects of Auxiliary Sugars on Dextran Synthesis*

<table>
<thead>
<tr>
<th>Auxiliary sugar</th>
<th>Reducing power*</th>
<th>Dextran yield†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min.</td>
<td>Final</td>
</tr>
<tr>
<td></td>
<td>mg. per ml.</td>
<td>mg. per ml.</td>
</tr>
<tr>
<td>None</td>
<td>6.5</td>
<td>22.8</td>
</tr>
<tr>
<td>Isomaltose</td>
<td>12.8</td>
<td>22.8</td>
</tr>
<tr>
<td>Maltose</td>
<td>10.8</td>
<td>21.4</td>
</tr>
<tr>
<td>α-Methyl glucoside</td>
<td>8.6</td>
<td>21.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>7.1</td>
<td>24.5</td>
</tr>
<tr>
<td>Fructose</td>
<td>5.4</td>
<td>22.2</td>
</tr>
<tr>
<td>Leucrose</td>
<td>5.0</td>
<td>19.6</td>
</tr>
<tr>
<td>Melibiose</td>
<td>5.6</td>
<td>21.2</td>
</tr>
<tr>
<td>Galactose</td>
<td>6.2</td>
<td>23.8</td>
</tr>
</tbody>
</table>

* Corrected for auxiliary sugar. The theoretical final value is 22.8 mg.
† Theoretical yield, 20 mg.

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4 We are indebted to Dr. A. Jeanes and Dr. R. J. Dimler for providing the specimen of dextran hydrolysate.
decreased as a result of the reaction, and a series of oligosaccharides was again produced as a major product. The series differed, however, from that formed in the sucrose-isomaltose reaction mixture. The trisaccharide spot coincided with panose, a glucose trisaccharide whose constitution is 4-α-isomaltopyranosyl-β-glucose (10, 11). This sugar would be the expected product if a glucosyl radical were linked through an α-1,6-

![Diagram](image)

**Fig. 1.** Oligosaccharides produced in dextran synthesis in the presence of auxiliary sugars. A tracing of a paper chromatogram sprayed with ammoniacal silver nitrate is shown. The paper was irrigated five times by the multiple descent technique (8). Spots indicating the auxiliary sugar supplied are indicated by X.

glucopyranosidic bond to the non-reducing glucose moiety of maltose. Successive deposition of additional radicals would result in a series of oligosaccharides related to that found in the dextran hydrolysate, but different in that the disaccharide moiety at the reducing end would be maltose instead of isomaltose. Presumptive evidence for such a series of oligosaccharides was the relative positions of individual members of the two series. For example, the trisaccharide (coinciding with panose) formed in the reaction had a somewhat greater mobility than isomaltotriose, and a comparable difference was found between other equivalent members. It was concluded, therefore, that maltose not only had acted as a glucosyl acceptor but had initiated formation of new dextran chains as well.
Glucose accelerated the reaction, but the reducing power of 7.1 mg. per ml. found at 30 minutes for glucose was only 0.6 greater than the value in the control experiment. It is felt from examination of the experimental deviations that a difference of 0.6 indicates that the rate has been definitely altered, whereas a difference of 0.3 is only suggestive. Glucose had little effect on dextran yield. An oligosaccharide series was again readily detectable, but the individual sugars were present in smaller amounts. The series coincided with the dextran hydrolysate series, as would be expected if glucose were an alternate glucosyl acceptor and also initiated chain formation. It appeared that glucose was a less effective acceptor than either maltose or isomaltose.

α-Methyl glucoside increased the reaction rate and decreased dextran yield. A different series of spots was evident on papers sprayed with silver nitrate, but not on those sprayed with dinitrosalicylic acid. It appeared that α-methyl glucoside initiated formation of a series of non-reducing oligosaccharide derivatives having the obvious relationship to the dextran series of sugars.

Fructose as auxiliary sugar differed from the four previous acceptors in decreasing, rather than increasing, the reaction rate. There was little effect on dextran yield. The paper chromatograms indicated that there had been considerably greater formation of leucrose and also of the fructose-containing disaccharide coinciding with maltose. There was only a slight increase in the amounts of higher oligosaccharides. Leucrose itself decreased reaction rate slightly and did not affect dextran yield, but had a definite though minor effect on oligosaccharide production. The latter sugars appeared to be a still different series.

Melibiose as auxiliary sugar caused a decrease in reaction rate, without major effect on dextran yield. The chromatograms, however, indicated moderate production of a reducing sugar appearing below melibiose and presumed to be a trisaccharide. Other oligosaccharides were present in only small amounts. Only oligosaccharide production was affected to any degree by galactose, and the effect was small.

Raffinose contains the labile sucrose linkage and might be expected to act as glycosyl donor. Provision of maltose as acceptor in a maltose-raffinose reaction mixture did not lead to detectable reaction.

**Leucrose Formation**—The genesis of leucrose was examined. Leucrose might be expected to be formed by either (a) deposition of a glucosyl radical on free fructose or (b) deposition of a glucosyl radical on the fructose moiety of sucrose, followed by splitting of the labile sucrose linkage. An experiment was performed in which fructose at levels between 0 and 10 per cent was added to reaction mixtures containing 5 per cent sucrose and 50 units of purified dextranaseurase per ml. The amount of leucrose ap-
pearing on the chromatograms was proportional to fructose concentration, and fructose seemed to behave as the glucosyl acceptor.

DISCUSSION

It has been shown that certain sugars, especially isomaltose, maltose, α-methyl glucoside, and glucose, when present during dextran synthesis, can act as glucosyl acceptors alternate with the natural acceptor. They can also initiate chain formation. In many respects these sugars perform a function in dextran synthesis analogous to priming in starch synthesis. Chain initiation by abnormal acceptors, such as maltose, α-methyl glucoside, melibiose, fructose, and leucrose, as observed on paper chromatograms, leads to formation of new series of oligosaccharides related to dextran oligosaccharides but differing in the nature of the saccharide moiety at the reducing end. Extended growth of the maltose-derived series of oligosaccharides to a dextran analogue has recently been demonstrated and will be reported elsewhere. It is to be expected that similar analogues can be synthesized from other alternate acceptors as primers. For convenience it is proposed to name such new dextran analogues after the initial acceptor, as, for example, “maltodextran” from maltose and “α-methylglucosidodextran” from α-methyl glucoside. In this nomenclature the type of dextran normally produced would be called “isomalto-dextran” and would be derived by deposition of glucosyl radicals on glucose, isomaltose, or the natural acceptor, whatever its identity.

Provision of alternate glucosyl acceptors would be expected to result in competition for glucosyl radicals between the natural and alternate acceptors. The effectiveness of alternate acceptors in modifying the normal synthetic process would depend on (a) the acceptor’s intrinsic reactivities as well as on (b) their concentrations relative to that of the natural acceptor. Variation in the first factor was evident in the present work. Provision of an efficient alternate acceptor, such as maltose or isomaltose, appears to divert normal dextran synthesis, in which high polymer is produced throughout the course of reaction and in which no appreciable quantities of oligosaccharides are found (1), to an abnormal process in which many chains are built up simultaneously. It would seem that extension of the second type of process should lead to control over size of the product, and as such would be valuable as a means for obtaining directly the type of small dextran polymers desired as a blood plasma substitute.

The increase in reaction rate resulting from the presence of maltose, isomaltose, or α-methyl glucoside suggests that the nature and amount of the acceptor are rate-controlling factors in normal dextran synthesis. The
observed increase in reaction rate, taken with the near absence of detectable oligosaccharides at all stages of normal dextran synthesis, arouses speculation as to the nature of the natural chain initiator and the acceptors derived from it. Consideration of the numbers of sucrose molecules converted, in relation to the molecular weight of the dextran produced, indicates that chain initiation must occur during the course of extensive dextran synthesis. One possibility is that chain initiation occurs infrequently and that the initiator and growing polymer are highly active as acceptors; consequently, the few growing chains rapidly outgrow the oligosaccharide range. Studies on the kinetics of polymerization have been undertaken and may disclose the character of normal dextran formation.

The effect of fructose and melibiose in decreasing reaction rate sets these sugars apart from other major acceptors and is not explainable from obvious considerations. One possible explanation is that deposition of glucosyl radicals on these sugars withdraws enzyme temporarily from its normal sucrose-splitting function. Fructose and melibiose appear to be acceptors of low reactivity, inasmuch as oligosaccharides are not abundant. However, low oligosaccharide concentration may indicate a higher reactivity of the oligosaccharide than of the parent sugar in the manner discussed above for conventional dextran synthesis.

Although results of the survey indicate that saccharides containing terminal glucose units, such as occur in maltose, isomaltose, and α-methyl glucoside, constitute three of the major alternate acceptors found, yet rigid and simple correlation between sugar structure and acceptor suitability is not yet permissible since other saccharides, such as trehalose, share this attribute and were inert.

We wish to acknowledge our indebtedness to Dr. Edward J. Hehre, Cornell University Medical College, for stimulating discussions of enzymatic dextran synthesis, especially as to the nature and effects of primers.

SUMMARY

1. A survey of a large number of sugars and sugar derivatives indicated that isomaltose, maltose, α-methyl glucoside, and glucose can act as efficient alternate glucosyl acceptors and can also initiate chain formation when present during enzymatic conversion of sucrose to dextran. These sugars increased reaction rate. In contrast to normal dextran synthesis, the process in the presence of efficient alternate acceptors led to extensive oligosaccharide formation and simultaneous growth of many dextran chains.
2. Fructose, leucrose, melibiose, and galactose also appeared to act as glucosyl acceptors, but were much less efficient.

3. The oligosaccharides produced appeared to comprise the series to be expected from successive addition of glucose by α-1,6-glucopyranosidic linkage on the alternate acceptor.

4. Formation of leucrose and a second fructose-containing disaccharide was enhanced by the presence of fructose as auxiliary sugar.

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