ON THE MECHANISM OF DEXTRAN FORMATION
CHROMATOGRAPHIC STUDIES WITH C\textsuperscript{14}-LABELED SUGARS*

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Certain bacteria, e.g. Leuconostoc mesenteroides and related species, elaborate an enzyme, dextransucrase, which acts on sucrose to form dextrans, representing α-1,6-linked glucopyranose polymers, and free fructose. Hehre (1, 2) has demonstrated that this synthesis may also occur in cell-free systems. He postulated (3) that during the enzymatic reaction a glucosyl radical is split off from the substrate, sucrose, which can then attack the non-reducing end of a growing dextran chain. Although there is not yet direct evidence for such a “radical,” glucose in trace amounts has been detected in the reaction system (4).

The present experiments were undertaken in an attempt to obtain information on the mechanism of dextran synthesis with the aid of paper chromatography and C\textsuperscript{14}-labeled sugars.

EXPERIMENTAL

Materials and Methods

Substrate—Stock solutions of sucrose were made up at an initial concentration of 10 per cent in 0.05 M acetate buffer, pH 5.0. When radioactive sucrose was employed, stable sucrose was added to adjust the total concentration to 10 per cent. The radioactive sucrose was uniformly labeled with C\textsuperscript{14} and had a specific activity of 0.6 μc. per mg. For most experiments 10 mg. of C\textsuperscript{14}-sucrose were employed, since this activity was sufficient to give reproducible results both by means of direct counting and by autoradiography.

Dextransucrase—All enzyme preparations were obtained from culture filtrates of L. mesenteroides, strain NRRL B-512, grown on a yeast or corn steep medium (5, 6). The enzyme was purified by ethanol precipitation and subsequent adsorption on calcium phosphate gel. The most active enzyme solutions showed an activity of 500 units per mg. of dry weight.

* Some of the present data are taken from a thesis, to be submitted by one of the authors (A. G.) in partial fulfillment of the requirements for the degree of Doctor of Philosophy to the Polytechnic Institute of Brooklyn.
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1 dextransucrase unit is defined as the amount that will convert 1 mg. of sucrose per hour at 30° and pH 5.0 as determined by liberation of reducing sugar (fructose). Stock solutions containing 100 units per ml. in 0.05 M acetate buffer, pH 5.0, were employed in these experiments.

**Dextran Formation**—All experiments were performed at 30° and at pH 5.0. Equal volumes (0.1 to 1.0 ml. each) of the substrate and enzyme solutions were mixed rapidly and the time noted. From the enzyme concentration in the final reaction mixture, the time necessary for total conversion of the sucrose to dextran was estimated. The experiments were usually allowed to proceed somewhat longer to insure complete conversion. Aliquots of the reaction mixture were withdrawn by micro pipette for analysis by paper chromatography. In two of the experiments, the reaction was stopped by addition of an equal volume of hot 95 per cent ethanol. This precipitated the high molecular weight dextran which was separated by centrifugation in the angle head attachment of a refrigerated International centrifuge at 20,000 r.p.m. for 15 minutes. The dextran was washed several times with small portions of additional ethanol, and the wash fluids were added to the supernatant solution for concentration on a steam bath. The residue was then dissolved in 0.5 ml. of distilled water for chromatographic analysis.

**Chromatographic Technique**—The method of descending chromatography was employed. 60 cm. strips of Whatman No. 1 paper (10.5 cm. in width) were used. The samples to be studied were applied to the strips 10 cm. from one end in amounts of 10 μl. and irrigated with a solvent mixture (Solvent A) of n-butanol-pyridine-water (3:2:1 by volume) (7). In one case the irrigating solvent (B) was n-butanol-acetic acid-water (4:1:5 by volume) (8). Since the \( R_f \) values for the sugars are similar, viz. sucrose 0.28, glucose 0.37, fructose 0.40 for Solvent A and 0.14, 0.18, 0.23, respectively, for Solvent B, the resolution was increased by employing the multiple descent technique of Jeanes, Wise, and Dimler (7) or by serrating the lower edge of the paper and allowing the solvent to drip uniformly from the tips. This expedient increases the effective length of the chromatogram by increasing the distance of migration of the spots. In both methods the direct determination of the \( R_f \) value for an unknown component is very difficult, and it is therefore preferable to cut out the area containing the unknown component, elute it, and rerun it separately on another paper strip in the usual manner. The experiments were performed in duplicate and the chromatograms were developed (a) with aniline phthalate (9) for the detection of reducing sugars and (b) with urea-phosphoric acid\(^1\) for the detection of fructose-containing saccharides.

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\(^1\) Personal communication from Dr. H. M. Taushiya, Northern Regional Utilization Research Branch, United States Department of Agriculture, Peoria, Illinois.
Radioactivity Measurements—Autoradiograms were prepared by placing sections of the chromatographic strips in contact with Kodak "no screen" x-ray films for 2 to 4 weeks, depending upon the radioactivity of the sugars employed. The film was processed in the usual manner and the opacity measured with a Photovolt densitometer, model 525. For direct counting, the chromatographic strips were divided into narrow segments, and their activity was determined directly with a thin end window Geiger-Müller tube and an atomic scaler, model 1020A. From the counts obtained on the individual sections, curves were constructed relating relative activity to distance of migration.

\[
\begin{array}{|c|c|c|c|}
\hline
\text{STANDARDS} & \text{PANOSE} & \text{ISOMALTOSE} & \text{MALTOSE} \\
\text{SUCROSE} & & & \\
\text{+ENZYME} & & & \\
\text{+GLUCOSE} & & & \\
\text{+LOW MW DEXTRAN} & & & \\
\text{HYDROLYZED DEXTRAN} & & & \\
\text{STANDARDS} & & & \\
\hline
\end{array}
\]

Fig. 1. Schematic representation of descending chromatograms obtained with n-butanol-pyridine-water (3:2:1 by volume) as solvent mixture.

Action of Dextranucrase on Sucrose—10 mg. of C\textsuperscript{14} uniformly labeled sucrose in 0.1 ml. of the acetate buffer and 0.1 ml. of the enzyme solution were mixed as described above. 10 µl. aliquots were withdrawn at various intervals during the course of the reaction and applied directly to the chromatographic paper. The samples were rapidly dried with the aid of an infra-red lamp. A schematic chromatogram is shown in Fig. 1. After chromatographing, autoradiograms were prepared and the strips assayed by direct counting. Curves relating relative activity to distance of migration are presented in Figs. 2 and 3 for two different stages of the reaction. The most noteworthy feature of these autoradiograms is the presence of glucose and its increase with reaction time. This is further documented by Table I, which summarizes the results obtained on all samples withdrawn in the course of this experiment. It is seen that the glucose produced at 66 per cent conversion amounts to 2.17 per cent of the total activity.

The experiment was repeated with the addition of 10\textsuperscript{-4} M silver nitrate to
the reaction mixture. This concentration is sufficient to inhibit (10) the action of any invertase that may be present as a contaminant in the dextran-

![Chromatogram](image)

**FIG. 2**

**FIG. 3**

**TABLE I**

*Formation of Glucose during Conversion of Sucrose to Dextran*

<table>
<thead>
<tr>
<th>Time of reaction</th>
<th>Sucrose converted</th>
<th>Glucose formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>3.5</td>
<td>10.36</td>
<td>0.61</td>
</tr>
<tr>
<td>4.5</td>
<td>15.60</td>
<td>0.18</td>
</tr>
<tr>
<td>8.5</td>
<td>20.20</td>
<td>1.04</td>
</tr>
<tr>
<td>10.5</td>
<td>27.46</td>
<td>0.95</td>
</tr>
<tr>
<td>15.3</td>
<td>33.19</td>
<td>1.52</td>
</tr>
<tr>
<td>19.7</td>
<td>42.40</td>
<td>1.09</td>
</tr>
<tr>
<td>32.9</td>
<td>65.82</td>
<td>2.17</td>
</tr>
</tbody>
</table>

sucrase preparation. The results obtained were identical with those of the first experiment. A close examination of the chromatograms indicates the presence of trace amounts of oligosaccharides; the first in the series having an $R_f$ value identical with that of leucrose (11). The others could not be identified.
Effect of Low Molecular Weight Dextran on Reaction—10 mg. of C\textsuperscript{14} uniformly labeled sucrose plus 2 mg. of low molecular weight dextran \((\eta = 0.10, \text{molecular weight} = 10,000)\) were dissolved in 0.1 ml. of buffer, and 0.1 ml. of enzyme solution was added in the manner described above. Aliquots were withdrawn at various intervals during the reaction and chromatograms and autoradiograms were prepared as described above. In addition to the glucose, fructose, and sucrose spots, an oligosaccharide series was obtained with a spot coinciding with the \(R_F\) value of leucrose, its amount increasing with the time of reaction. The opacity of the autoradiograms was determined. By way of example, the curves corresponding to two different stages of the reaction (1 and 60 minutes) are reproduced in Fig. 4.

Effect of Glucose on Reaction—10 mg. of sucrose plus 10 mg. of C\textsuperscript{14} uniformly labeled glucose were dissolved in 0.1 ml. of the acetate buffer. To this was added 0.1 ml. of the enzyme solution. After 180 minutes, a 10 \(\mu\)l. aliquot was withdrawn and applied to the chromatographic paper. The balance of the reaction mixture was precipitated with hot ethanol as de-
scribed above, centrifuged, and the dextran separated from the supernatant solution containing the low molecular weight products. The dextran and the supernatant fluid were also chromatographed. As an internal standard, some radioactive glucose was added to one of the spots of the reaction mixture applied to the paper. Unfortunately, the only preparation of C\textsuperscript{14}-glucose available to us was contaminated with a small amount of radioactive fructose. In addition to fructose and glucose spots, the autoradiogram shows distinctly an oligosaccharide series, in confirmation of previous observations on stable glucose made by Koepsell et al. (11).

Fig. 5 represents a photograph of the autoradiogram. The slight darkening at the point of origin where the dextran is retained is presumably due to traces of C\textsuperscript{14}-glucose or oligosaccharides which have been adsorbed on the dextran. Mere washing of the dextran precipitates was found to be insufficient to remove this adsorbed material. However, when the dextran was redissolved and reprecipitated several times, its radioactivity decreased to zero upon direct counting with an end window Geiger-Müller tube, thus indicating that no free glucose is incorporated into the dextran molecules.

Preparation and Hydrolysis of C\textsuperscript{14}-Labeled Dextran—10 mg. of C\textsuperscript{14} uniformly labeled sucrose plus 40 mg. of sucrose were dissolved in 0.5 ml. of acetate buffer (0.05 M, pH 5.0). To this was added 0.5 ml. of the dextran-sucrase solution. After 180 minutes a sample for chromatography was taken from the reaction mixture by micro pipette. The bulk of the solution was added to 2 ml. of hot ethanol to isolate the high molecular weight radioactive dextran formed. The precipitate was washed as described...
previously. 1 ml. of 2 N HCl was added to the dextran precipitate, and the solution was allowed to remain at room temperature for 2 hours. The solution was then adjusted to pH 7.0 with dilute NaOH and the residual dextran precipitated with 2 volumes of 95 per cent ethanol. After centrifugation the supernatant liquid was concentrated and chromatographed. The precipitate was dissolved again in 1 ml. of 2 N HCl and heated to 100° in a sealed tube overnight. After hydrolysis, the solution was neutralized and chromatograms and autoradiograms were prepared. The chromatograms and autoradiograms show that cold hydrolysis does not seem to af-

![](https://example.com/diagram.png)

**Fig. 6.** Unit step in the growth of a dextran molecule from sucrose, as catalyzed by dextranucrase (from Hehre (3)).

fect the dextran materially, since no radioactivity could be detected in the supernatant fluid. The chromatogram obtained after hot acid hydrol-
ysis shows that the major degradation product has the relative mobility of glucose (see Fig. 1).

**DISCUSSION**

The scheme for the enzymatic conversion of sucrose to dextran, as postu-
lated by Hehre (3), implies an initial splitting of the substrate into fructose and a glucosyl residue. The latter links up with a glucose moiety at the non-reducing end of a growing dextran chain (see Fig. 6).

This scheme makes no provision for the formation of *free* glucose, and, in fact, Hehre attributes the finding of glucose in the reaction mixture by
Forsyth and Webley (4) to a contamination of their enzyme preparation with invertase. The present experiments prove that glucose is a by-product of the enzymatic conversion of sucrose to dextran by dextransucrase. This is readily explained by assuming that "glucosyl radicals" are produced from sucrose under the influence of that enzyme. While most of these "radicals" will react with sucrose or the growing polymer chain to form dextran, a small number may be expected to react with water as the acceptor to form stable glucose. The fact that glucose, when added to the system, is not incorporated into the dextran molecules to any detectable extent is further evidence that an active form (radical) of glucose is an intermediate in the synthesis. The appearance of the oligosaccharide series in the autoradiograms confirms the findings of Koepsell et al. (11) that free glucose may act as a weak glucosyl acceptor.

It would seem that sucrose may be acted on by enzymes in at least three different ways: (1) it may be split by invertase to yield stable glucose and fructose; (2) dextransucrase action leads to stable fructose and dextran via intermediate "glucosyl radicals;" and (3) levansucrase, conversely, produces stable glucose and levan through the production of "fructosyl radicals." These "radicals" need not necessarily be released into the reaction medium, but they may remain attached to the enzyme until they are transferred to a suitable acceptor (water, sucrose, dextran, etc.) in a manner analogous to other transglucosidation reactions. The term "radical" is used here merely to denote reactive forms of the sugars concerned.

SUMMARY

The synthesis of dextran from sucrose as catalyzed by dextransucrase has been studied with the aid of C\(^{14}\)-labeled substrates. It was demonstrated by means of chromatography; followed by autoradiography, that glucose is formed in the course of the reaction in small but increasing amounts as a function of time and sucrose conversion.

The production of glucose as a by-product of dextran synthesis is explained on the basis of the intermediate formation of "glucosyl radicals" from sucrose under the influence of the enzyme system.

The ability of added glucose to act as a weak glucosyl acceptor has been confirmed.

This investigation forms part of a research program conducted under the auspices of The Dextran Corporation, Yonkers, New York. It benefited greatly from the interest and encouragement shown by the late Dr. H. S. Paine, Director of Research and Development of that organization. Miss H. M. Lynch of The Dextran Corporation Laboratories kindly furnished the crude enzyme preparations employed as starting material.
Thanks are also due to Mr. W. Schaechter for assistance in the radioactivity determinations, to Mr. J. Pescatore for a gift of C\textsuperscript{14}-labeled sucrose, and to Dr. B. A. Silard for the loan of a Photovolt densitometer.

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