INDUCTION OF α-AMYLASE OF BACILLUS STEAROTHERMOPHILUS
BY MALTODEXTRINS

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ABSTRACT


—Technical-grade maltose contained 3.5% glucose, 0.5% maltotriose, and 2.5% of the higher molecular weight maltodextrins. The first five homologies (maltose being the first in the series) of the maltodextrin series were isolated and purified. Each member of the series was found to be chromatographically pure. The physical and chemical properties were determined. It was shown that the contaminating maltodextrins found in technical grade maltose were linear (1–4 linked) polymers of glucose, ranging from maltotriose to maltohexaose. The addition of maltose, maltotriose, maltohexaose, maltopentaose, and maltotetraose (all at 10−4 M) to cultures growing in a chemically defined medium resulted in a stimulation in the differential rate of α-amylose synthesis by 1.2, 1.6, 1.9, 2.3, and 3.0 times that of the sucrose control, while glucose had no effect. The induction data indicate that Bacillus stearothermophilus 1503–4 is a partial constitutive strain with respect to α-amylose synthesis.

Markovitz and Klein (1955) reported that growing cultures or washed-cell suspensions of Pseudomonas saccharophila could be induced to form α-amylose in the presence of starch or maltose. Tomomura et al. (1961) found that amylose, kojibiose, maltose, isomaltose, and panose stimulated α-amylose formation in washed mycelial suspensions of Aspergillus oryzae. A transglucosylase was also found in this mold which produced isomaltose and panose from maltose. They concluded that the natural inducer of α-amylose in this organism was isomaltose, which was produced from maltose.

Nomura, Maruo, and Akabori (1956) concluded that the α-amylose of Bacillus subtilis was not inducible, since it was produced only during the stationary phase of growth. The commercial production of B. subtilis α-amylose is accomplished by use of high concentrations of starch (8 to 12%); therefore, it is possible that α-amylose synthesis, in this organism, is induced by starch or the lower molecular weight oligosaccharides resulting from α-amylose hydrolysis.

In most cases where α-amylose has been shown to be induced by either maltose or starch, the actual inducer(s) has not been isolated and characterized. In these instances, kinetic studies are difficult to interpret.

In the preceding paper (Welker and Campbell, 1963), it was shown that maltose (or the contaminating maltodextrins found in technical-grade maltose) increased the rate of synthesis of α-amylose, when added to growing cultures of B. stearothermophilus. The same maltodextrins were also found in supernatant fluids of cultures utilizing starch as a carbon source. The present paper is concerned with the isolation and characterization of the maltodextrins, and the determination of their efficiency as inducers of the α-amylose of B. stearothermophilus.

MATERIALS AND METHODS

Isolation of sugars and oligosaccharides found in technical-grade maltose. The starting material was technical-grade maltose (Difco or Pfanstiehl Chemical Corp., Waukegan, Ill.). A 50% solution of the sugar was autoclaved for 20 min at 121°C and filtered to remove the precipitate.

The initial purification procedure was that of Whistler and Durso (1950), modified as follows.
The adsorbant, composed of equal parts of charcoal (Darco-60; Atlas Powder Co., Wilmington, Del.) and Celite (Johns-Manville, New York, N.Y.; no. 535), was washed exhaustively with water to remove the fines. The water was decanted, leaving a thick slurry.

A chromatographic column (4.5 cm diameter) was filled to a height of 40 cm with the charcoal-Celite mixture. The column was then washed with 2 liters of water, followed by 2 liters of 0.1 N HCl to remove basic ash (Whistler, 1954). The acid was removed from the column by washing exhaustively with distilled water.

A total of 100 g of sugar (200 ml of a 50% solution) was placed on the column. Desorption of the sugars and oligosaccharides was accomplished by passing 2 liters each of water, 5, 15, 30, and 95% ethanol through the column. The effluent was collected in 100-ml volumes. The reducing sugars in each 100-ml fraction were determined by the reductometric method with 3,5-dinitrosalicylic acid. Qualitative determinations of the sugars present in each 100-ml fraction were accomplished by multiple ascending chromatography as described by Welker and Campbell (1963).

The water fractions contained glucose and varying amounts of maltose and maltotriose. The 5, 15, 30, and 95% ethanol fractions contained: maltose; maltotriose; a mixture of maltotriose, -tetraose, and -pentaose; and a mixture of maltohexaose and high molecular weight oligosaccharides, respectively. None of the fractions contained these sugars in the pure form. The respective effluent fractions were combined and concentrated to 1/50 their original volume under reduced pressure.

The sugars and oligosaccharides were further purified by large-scale multiple ascending paper chromatography. The samples were placed in a narrow continuous streak, 4 cm from one edge, on Whatman 3-MM filter paper (46 by 46 cm). The filter paper was stapled in the form of a cylinder and placed in a Chromatocab (model A-300; Research Specialties Co., Richmond, Calif.). The Chromatocab was made airtight and placed in the dark at ambient room temperature. The developing solvent and the silver-dip method of detecting reducing sugars were described in the accompanying paper (Welker and Campbell, 1963). Four ascents of 30 cm each were found to be sufficient to separate the first six members of the homologous series of maltodextrins.

Vertical strips from the chromatogram were developed and used as markers for sectioning the remaining portions of the chromatogram. The individual maltodextrins were extracted from the paper with boiling water. The sugar solution was treated with charcoal (Darco-60), filtered, and concentrated in vacuo. The syrupy material was dried with acetone and washed with a small amount of n-butanol. The syrup was taken up in 50 ml of water and lyophilized in a freeze-drying apparatus (Virtis Co., Inc., Gardiner, N.Y.). The dried amorphous sugars and maltodextrins were stored in a vacuum desiccator over NaOH pellets.

**Production of maltodextrins from amylose.** The method of Pazur and Budovich (1956) was used to obtain the maltodextrins in greater quantity, identical to those found in technical-grade maltose. Amylose (20.0 g) was refluxed in 400 ml of 0.1 N HCl for 1 hr. The cooled solution was neutralized with solid Na2CO3 and filtered. The filtered solution was deionized by the addition of Amberlite MB-3 (mixture of Amberlite IR-120 and IRA-410). The resin was removed by centrifugation, and the solution was concentrated to approximately 5.0 ml under reduced pressure. The individual maltodextrins were isolated by multiple ascending paper chromatography and purified as described above.

**Identification of maltodextrins found in technical-grade maltose.** The identity of the sugars and maltodextrins can be determined by plotting the logarithm of a partition function (α') against molecular size as described by French and Wild (1953). The partition function was calculated from the multiple ascent chromatograms.

**Determinations of chain length of maltodextrins.** The determination of the chain length of the maltodextrins was accomplished by periodate oxidation (Hassid and Abraham, 1957). The chain length of the maltodextrins, in terms of anhydroglucose units, was obtained by calculating the g of maltodextrin per 3 moles of formic acid formed, and dividing the result by 162 (molecular weight of anhydroglucose).

**Quantitation and determination of molecular weights of maltodextrins.** Quantitative determination of the maltodextrins was performed by the method of Pazur (1953). Molecular weights were determined by the procedure of Whelan, Bailey, and Roberts (1953).

**Determination of optical rotation of maltodextrins.** The optical rotation of the maltodextrins...
was measured in a double-field polarimeter (O. C. Rudolph & Sons, Inc., Caldwell, N.J.; model no. 301) equipped with a sodium lamp. The sugar samples were dissolved in 2.0 ml of water (0.2 to 0.5% solutions), and 0.01 ml of 0.01 N HCl was added to hasten equilibrium. These experiments were carried out at 24 C. The specific rotation was calculated for each sample and compared with the literature value.

The optical rotations of the maltodextrins were also plotted, by the Freudenberg and Blomquist (1935) relationship in which $A/n$ is plotted against $n - 1/n$, where $A$ is the molecular rotation and $n$ the number of glucose residues per molecule.

Measurement of inducing efficiency of maltodextrins. The media, conditions for growth studies, and the measurement of $\alpha$-amylase formation were described by Welker and Campbell (1963).

Results

Isolation and purification of maltodextrins found in technical-grade maltose. Technical-grade maltose contained 3.5% glucose, 0.5% maltotriose, and 2.5% higher molecular weight oligosaccharides. The first five homologues in the maltodextrin series (maltose being the first) were isolated and purified. Examination of Fig. 1 reveals that each member of the series gave only a single spot on paper chromatograms. The mobilities of the maltodextrins, prepared by acid hydrolysis of amylose, were identical to those isolated from technical-grade maltose.

Degree of polymerization. Figure 2 shows that a linear relationship exists between the log of the partition coefficient ($a'$) and the assumed degree of polymerization. The degree of polymerization, determined by periodate oxidation of each of the maltodextrins (Table 1) was in agreement with the theoretical values for 1–4 linked glucose residues.

Physical properties of maltodextrins. The values obtained for the molecular weight and optical rotation of the maltodextrins are shown in Table 1.

Molecular weights of the individual members of the homologous series of maltodextrins were in close agreement with the calculated values. The specific rotations for glucose and the maltodextrins were similar to those reported by Whelan et al. (1953).

The specific rotations of glucose and the maltodextrins were used in the Freudenberg-Blomquist (1935) relationship (Fig. 3), which shows the correlation between optical rotation and molecular structure.

Molecular weights and specific rotations of the homologous series of maltodextrins obtained by acid hydrolysis of amylose agreed with those found for the maltodextrins present in technical-grade maltose.
Induction of \( \alpha \)-amylase by maltodextrins. Glucose and the maltodextrins (\( 10^{-4}\) M) were each added to cultures which had just entered the logarithmic phase of growth on sucrose (0.5%). The effect of glucose and the maltodextrins on

**TABLE 1. Physical properties of maltodextrins isolated from technical-grade maltose**

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Molecular weight</th>
<th>Optical rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found</td>
<td>Calculated</td>
<td>Found in [lit]</td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Glucose........</td>
<td>180</td>
<td>51.6</td>
</tr>
<tr>
<td>Maltose.........</td>
<td>342</td>
<td>132.0</td>
</tr>
<tr>
<td>Maltotriose.....</td>
<td>504</td>
<td>155.0</td>
</tr>
<tr>
<td>Maltotetraose.</td>
<td>664.8</td>
<td>176.0</td>
</tr>
<tr>
<td>Maltopentaose.</td>
<td>800.1</td>
<td>181.1</td>
</tr>
<tr>
<td>Maltohexaose.</td>
<td>980.2</td>
<td>185.0</td>
</tr>
</tbody>
</table>

* Whelan et al. (1953).

† Determined by periodate oxidation.

**TABLE 2. Effect of maltodextrins on differential rate (K) of \( \alpha \)-amylase formation by Bacillus stearothermophilus**

<table>
<thead>
<tr>
<th>Sugar</th>
<th>K</th>
<th>Fold increase in K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose........</td>
<td>73</td>
<td>--</td>
</tr>
<tr>
<td>Glucose........</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>Maltose.........</td>
<td>85</td>
<td>1.2</td>
</tr>
<tr>
<td>Maltotriose.....</td>
<td>116</td>
<td>1.6</td>
</tr>
<tr>
<td>Maltotetraose.</td>
<td>220</td>
<td>3.0</td>
</tr>
<tr>
<td>Maltopentaose.</td>
<td>170</td>
<td>2.3</td>
</tr>
<tr>
<td>Maltohexaose.</td>
<td>143</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Conditions for growth and induction of \( \alpha \)-amylase are described in Materials and Methods. The growth rate constant (\( k \)) was 0.42 for all sugars.

\( \alpha \)-amylase formation is shown in Fig. 4. The growth rates of the cultures were not changed by the addition of glucose or the maltodextrins. Table 2 shows that, at a concentration of \( 10^{-4}\) M, maltotetraose gave the highest increase in the differential rate of \( \alpha \)-amylase synthesis (\( K \)), while glucose had no effect.
The addition of the purified sugars and the maltodextrins to purified α-amylase had no effect on the enzymatic activity.

**DISCUSSION**

Technical-grade maltose was shown to contain contaminating maltodextrins which were linear (1-4 linked) polymers of glucose, ranging from maltotriose to maltohexaose. Maltoheptaose and maltooctaose were present in small amounts, but no attempt was made to isolate them. Although not definitely established, it seems likely that the linkages of the maltodextrins are alpha (1-4), since the maltodextrins obtained from amylose (which contains only alpha 1-4 linkages) had the same physical and biological properties as those obtained from technical-grade maltose.

MacQuillan, Winderman, and Halvorson (1960), working with a yeast hybrid which produces large amounts of β-glucosidase constitutively, reported that the addition of inducers to the culture medium doubled the differential rate of enzyme synthesis. They called this yeast "semi-constitutive" with respect to β-glucosidase. Mutants of *Escherichia coli*, constitutive for tryptophanase, were called "partial constitutives" by Ng and Gartner (1963) because the enzyme levels were increased by growth in the presence of inducer. The constant rate of α-amylase formation in the absence of maltose or the maltodextrins and the increased rate of formation in their presence show that *B. stearothermophilus* 1503-4 exhibits both constitutive and inducible characteristics. We have therefore called *B. stearothermophilus* 1503-4 a partial constitutive strain with respect to α-amylase synthesis.

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**LITERATURE CITED**


