A Method for Converting Glucose to Fructose*

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The unavailability of singly labeled fructose has been one of the drawbacks in studies on the metabolism of this sugar. Since several types of single carbon labeled glucose are commercially available, a good method for converting glucose to fructose would be of considerable benefit. A commonly used method for effecting this conversion involves the phosphorylation of glucose to glucose 6-phosphate employing hexokinase, isomerization of glucose 6-phosphate to fructose 6-phosphate and crystallization of the latter as the barium salt. Following removal of barium, fructose 6-phosphate is hydrolyzed with a phosphatase, and the free sugar is then recovered and purified. The yields are low, and the procedure is rather difficult.

In the method to be described, glucose is quantitatively reduced to sorbitol, and this in turn is converted to fructose in one incubation with a coupled enzymatic reaction. The yield of fructose is 90% of theoretical in the enzymatic conversion step and 75% through the final purification.

The following are the pertinent reactions in outline form.

\[
\text{Glucose} + \text{NaBH}_4 \rightarrow \text{sorbitol} + \text{borohydride oxidation products}
\]

\[
\text{Sorbitol} + \text{DPN}^+ \rightarrow \text{fructose} + \text{DPNH} + \text{H}^+
\]

\[
\text{Pyruvate} + \text{DPNH} + \text{H}^+ \rightarrow \text{lactate} + \text{DPN}^+
\]

Net: Glucose + pyruvate → fructose + lactate

**EXPERIMENTAL**

**Materials**

Sodium pyruvate and DPN (95% based on 4 H₂O) were purchased from the Sigma Chemical Company. Crystalline lactic dehydrogenase as purchased from the Sigma Chemical Company contained 25,200 units per mg of protein. A 0.5-ml sample of the suspension containing 11 mg of protein was centrifuged, and the crystals were dissolved with 2 ml of 0.1 M potassium phosphate buffer, pH 8. The activity of this enzyme solution was checked by a spectrophotometric assay (1).

Sorbitol dehydrogenase, renamed ketose reductase by Williams-Ashman and Banks (2), was prepared from six rat livers by making preparation B as described by Blakley (3). The supernatant fluid from the chloroform-ethanol treatment was dialyzed three times successively against 2 liters of distilled water, 30 minutes each time. It was then fractionated in the cold with ammonium sulfate solution saturated at 4°C and adjusted to pH 7 with ammonia. Protein fractions precipitating between 0 to 45%, 45 to 60%, and 60 to 70% saturation were collected by centrifugation. These were dissolved in 0.01 M phosphate, pH 7.8 to give a final volume of approximately 15 ml, and each was dialyzed against one liter 0.001 M phosphate, pH 7.8, for 1.5 hours. Each fraction was tested for its sorbitol dehydrogenase activity according to Wolf (4). Williams-Ashman and Banks (2) used the fraction precipitating between 40 and 60% saturation with ammonium sulfate for further purification, but we found that most of the activity was present in the 60 to 70% saturation fraction. This fraction (150 units per mg of protein) had a 3-fold enrichment of activity per unit of protein over the original supernatant fluid. It was used without further purification and can be stored frozen for at least 9 months without loss of activity.

Amberlite IR-120, 20 to 50 mesh, Rohm and Haas Company, was converted to the hydrogen form with 2 N H₂SO₄ and then washed with distilled water until sulfate free. Duolite A-4, Chemical Process Company, Redwood City, California, was converted to the hydroxide form with 2 N NaOH and washed with distilled water until the effluent was nearly neutral. Sodium borohydride, purity 98%, was obtained from Metal Hydrides Inc., Beverly, Massachusetts. Protein precipitating reagents were 0.3 N Ba(OH)₂ and ZnSO₄ (44.5 g of ZnSO₄·7H₂O per 500 ml of solution).

**Method**

The method has been developed for the conversion of 36 mg of glucose. This amount dissolved in 2 ml was placed in a 100-ml round bottom flask adapted for use on the Rinco rotating vacuum-type evaporator. It was treated with 36 mg sodium borohydride and allowed to stand 20 minutes at room temperature. Excess borohydride was then destroyed by adding 1.5 ml of 1 N HCl and heating the flask for 1 minute in a boiling bath to insure complete destruction. After cooling, a drop of brom cresol green indicator was added and the mixture adjusted to pH 4 with 1 N NaOH. The mixture was taken to dryness on the Rinco, and the salt treated four times with 10 ml of absolute methanol, each time evaporated to dryness, to remove borate as the methyl borate azeotrope.

The remaining salts were dissolved with 4 ml of H₂O and the solution desalted by passing it through a double ion exchange column consisting of 4.5 cm² IR-120 layered over 6.5 cm² A-4 in a 1-cm diameter column. The flow rate was 1 ml per minute. Three successive washings, each with 4 ml of H₂O, served to wash all the sorbitol from the column. The combined eluents were taken to dryness in the 100-ml Rinco flask, and the enzymatic conversion carried out in the same flask.

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To the flask containing the sorbitol were added: 5.4 ml of H₂O, 1 ml of phosphate buffer (0.1 M, pH 8), 1 ml of DPN (0.005 M, pH 7), 1.1 ml of sodium pyruvate (0.2 M), 0.5 ml lactate dehydrogenase, 1 ml of sorbitol dehydrogenase (approximately 2,000 units). This mixture was incubated for 150 minutes at 37°. It was deproteinized by adding 4 ml of Ba(OH)₂ followed by 2.2 ml of ZnSO₄, the balanced proportions of these reagents. After centrifuging sharply and collecting the supernatant fluid, the precipitate was washed by resuspending it in an additional 5 ml of H₂O, followed by 1 ml of Ba(OH)₂ and 0.55 ml of ZnSO₄. This was centrifuged again, and the supernatant fluid added to the first. Fructose determinations were made on aliquots of the fluid (5) to ascertain the extent of the conversion.

The solution was passed through a triple ion exchange column, 4 cm³ of IR-120 over 6 cm³ of A-4 over 2 cm³ of IR-120. If some cation exchanger is not put in the bottom of the column, subsequent paper chromatography of the sugars is erratic and often unsatisfactory. The column was washed with water as before to regain all sugar, and the combined effluents were taken to dryness. We did this in two stages; first in a 100-ml round bottom flask and then transfer of the residue with 5 ml of H₂O to a pear-shaped flask. This in turn was concentrated to a drop of syrup in the tip of the flask.

The syrup was dissolved with 0.15 ml of H₂O and 0.01-ml aliquots were spotted on Whatman 3 MM paper sheets to separate the fructose from the unreacted sorbitol.1 It was necessary to spot the syrup; if it was streaked on the paper, the components did not separate well. Descending chromatography was carried out at 37° using the upper layer of an ethanol 5, n-butanol 45, H₂O 50 mixture, equilibrated at 30° and separated from the aqueous layer.2 Adequate separation was achieved in 36 to 48 hours as compared to 3.6 mm for sorbitol. The sheets were allowed to dry air dry until no trace of butanol remained, and the components were located by cutting strips from each side of the sheet and spraying them with the indicator of Bradfield and Flood (6).1 The corresponding area on the main sheet was cut out, set up as a strip. It was concentrated to the desired volume and used as such in various experiments.

As an alternative to the paper chromatographic separation of fructose and sorbitol, they may also be separated by ion exchange chromatography of their borate complexes, according to certain general procedures (7, 8). However, we found the methods to be highly empirical, and the conditions necessary to achieve a separation may have to be redetermined for each batch of resin. A mixture consisting of 40 μmoles sorbitol and 160 μmoles fructose in 10 ml of 0.008 M K₂B₄O₇ was put on 2-ml of Dowex 1 resin (200 to 400 mesh, borate form). The column was then washed with 50 ml of 0.005 M K₂B₄O₇ and elution carried out with 0.012 M K₂B₄O₇ at a flow rate of 1 ml per minute. Fructose was eluted in a broad band between 170 and 800 ml with the peak at 480 ml. Sorbitol appeared in the fraction between 630 and 1100 ml with its peak at 850 ml. Approximately 150 μmoles of fructose were eluted free of sorbitol. It may be possible to achieve more quantitative separation of fructose and sorbitol by increasing the amount of resin. Fructose was recovered from the eluate by

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1 It is permissible to have as much as 15 μmoles of sugar or polyol per 1-cm spot. Such amounts migrated at the same rate and with no more spreading than did smaller samples.

2 If the solvent mixture is saturated with water at 37° and the temperature should fall slightly during chromatography, some water may separate from the mixture in the flask and cause streaking of the spots.

3 Although the polyols usually stand out as yellow spots on a light blue background, the contrast can be sharpened by holding the papers momentarily in an atmosphere containing some ammonia.

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**Table I.** Characterization of product of sorbitol dehydrogenase reaction and comparison with authentic sugars

<table>
<thead>
<tr>
<th>Sorbitol dehydrogenase product</th>
<th>d-Fructose</th>
<th>L-Sorbose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating time for appearance of osazone at 100°</td>
<td>5 min</td>
<td>5 min</td>
</tr>
<tr>
<td>Crystallization of osazone in ice bath</td>
<td>yellow crystals</td>
<td>yellow crystals</td>
</tr>
<tr>
<td>Melting point of osazone (uncorrected)</td>
<td>203 to 204°</td>
<td>203 to 204°</td>
</tr>
<tr>
<td>Fermentability with bakers' yeast</td>
<td>complete</td>
<td>complete</td>
</tr>
<tr>
<td>Oxidation in 1 N KOH in oxygen</td>
<td>K arabonate, formed</td>
<td>K arabonate, formed</td>
</tr>
</tbody>
</table>

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**FIG. 1.** The enzymatic conversion of the borohydride reduction product to fructose. The incubation mixture was the same as that described under “Method.” Aliquots (0.25 ml) were removed at the time intervals shown, deproteinized by adding 1.0 ml of Ba(OH)₂ followed by 0.55 ml of ZnSO₄, making the volume to 10 ml, and analyzing 2-ml aliquots for fructose (5).
passing the latter through Amberlite 120 (H-form), taking the effluent mixture of fructose and boronic acid to dryness and then removing the boronic acid by repeated distillations with methanol.

RESULTS AND DISCUSSION

It was first ascertained that the borohydride reduction of glucose to sorbitol was complete and in agreement with Abdel-Akher et al. (9) no reducing sugar could be detected after 15 minutes reaction time. Since the maximum yield of fructose in the subsequent enzymatic reaction was around 90% of theoretical, there was a possibility that some sorbitol was retained on the resin column during the desalting treatment. However, carbon determinations (10) on aliquots of the effluent showed that the recovery of sorbitol was complete.

A number of experiments have shown that the conversion of sorbitol to fructose is not quite complete. A typical experiment is shown in Fig. 1. Attempts to increase the fructose yield by increasing the pyruvate/sorbitol ratio were unsuccessful, in fact slightly lower yields were obtained when the ratio exceeded 2:1.

It was necessary to establish that the ketose formed in this reaction was solely fructose. If the sorbitol dehydrogenase attacked indiscriminately either end of the sorbitol molecule, L-sorbose as well as D-fructose would be formed. Cummins et al. (11) have shown that an extract prepared from Acetobacter suboxydans catalyzed the conversion of sorbitol to fructose when DPN was added, however sorbose was formed when TPN was added.

We have characterized the product of the sorbitol dehydrogenase reaction by formation and characterization of the osazone, testing its fermentability by yeast and demonstrating that arabinose is formed by alkaline oxidation (12). The results of these tests are summarized in Table I. They are in agreement with those obtained by Blakley (3), and as he had shown, although the sole product of sorbitol oxidation is fructose, the enzyme will catalyze the reduction of L-sorbose in the presence of DPNH.

In this case the product is L-iditol.

It was also necessary to establish that in the conversion of carbon-labeled glucose to fructose no change in the position of the label occurs. This was done by employing the alkaline oxidation procedure (12) on single-labeled fructose prepared from single-labeled glucose. Potassium arabinate derived from fructose-C\(^\text{14}\) had the same specific activity as the fructose from which it was made. This would exclude randomization between C-6 and C-1 since in the oxidation to arabinose, C-1 is oxidized to formate, and any shift of label from C-6 to C-1 in the course of fructose formation would diminish the specific activity of C-6.

Application of this same oxidation to fructose-C\(^\text{14}\) gave potassium arabinate with virtually no radioactivity. After the second recrystallisation the arabinonate had 0.8% of the specific activity of the parent fructose and a constant specific activity had not yet been achieved. This means that in preparing fructose-C\(^\text{14}\) from glucose-C\(^\text{14}\) there is no significant randomization of label to positions 2, 3, 4, 5, and 6.

SUMMARY

A method for converting glucose to fructose is described. It involves chemical reduction of glucose to sorbitol and oxidation of the sorbitol to fructose in a coupled enzyme reaction. Fructose is formed in 90% yield in the enzymatic reaction. In converting single-carbon labeled glucose to the corresponding fructose no significant randomization of label occurs.

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REFERENCES

A Method for Converting Glucose to Fructose
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