The Purification and Properties of Sucrose Synthetase from Etiolated Phaseolus aureus Seedlings*

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SUMMARY

UDP-glucose:f-ructose 2-glucosyltransferase (EC 2.4.1.14), commonly called sucrose synthetase, has been purified from etiolated Phaseolus aureus seedlings to homogeneity by electrophoretic, immunological, and ultracentrifugal criteria. Molecular weight determinations by low speed equilibrium centrifugation yields a value of 375,000 ± 12,000. A value of 405,000 ± 20,000 was obtained by gel filtration. Other physical determinations for the enzyme yielded the following data: 

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Since the discovery of sucrose synthetase (UDP-glucose:f-ructose 2-glucosyltransferase EC 2.4.1.14) and sucrose phosphate synthetase (UDP-glucose:f-ructose 6 phosphate 2-glucosyltransferase EC 2.4.1.13) by Cardini, Leloir, and Chiriboga (1) and Leloir and Cardini (2) in 1955, much work has been done to study the physiological role these enzymes play in the biosynthesis, or metabolism of sucrose, or both, in higher plants. Present evidence strongly indicates that sucrose phosphate synthetase

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Amino Acid Analysis—The sample was dialyzed exhaustively against 0.05 M sodium phosphate, pH 7.5, 0.1 M NaCl, containing 0.1% SDS, 0.02% thioglycolate, and 0.0015 M EDTA. Electrophoresis was performed against this buffer. A concentration of 0.01 M NaCl gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreitol and EDTA (ρ = 1.001). Similar runs in the same buffer containing 0.1 M NaCl (ρ = 1.004) gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreitol and EDTA (ρ = 1.001). Similar runs in the same buffer containing 0.1 M NaCl (ρ = 1.004) gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreitol and EDTA (ρ = 1.001). Similar runs in the same buffer containing 0.1 M NaCl (ρ = 1.004) gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreitol and EDTA (ρ = 1.001). Similar runs in the same buffer containing 0.1 M NaCl (ρ = 1.004) gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreitol and EDTA (ρ = 1.001). Similar runs in the same buffer containing 0.1 M NaCl (ρ = 1.004) gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreitol and EDTA (ρ = 1.001). Similar runs in the same buffer containing 0.1 M NaCl (ρ = 1.004) gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreitol and EDTA (ρ = 1.001). Similar runs in the same buffer containing 0.1 M NaCl (ρ = 1.004) gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreitol and EDTA (ρ = 1.001). Similar runs in the same buffer containing 0.1 M NaCl (ρ = 1.004) gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreitol and EDTA (ρ = 1.001). Similar runs in the same buffer containing 0.1 M NaCl (ρ = 1.004) gave comparable results. A 1% of 0.733 was used which was calculated from amino acid composition data. For subunit analyses, the enzyme was dialyzed 24 hours at 4°C against 0.05 M Tris, pH 7.5, containing 0.1 mM dithiothreito|
lyophilized to complete dryness, resuspended in H₂O, and re-
lyophilized. The sample was resuspended in constant boiling
HCl and hydrolyzed under vacuum at 110° for 24 hours. Analy-
yses were performed by standard procedures on the short and long
column of the Beckman-Spinco amino acid analyzer model 1205.
Tryptophan content was determined in a similar manner except
that the protein was hydrolyzed in saturated barium hydroxide.
Cysteine was oxidized with performic acid to cysteic acid prior
to analysis.

**Enzyme Assays—** Sucrose synthetase was assayed in the direc-
tion of sucrose synthesis by the method of Grimes et al. (14)
(labeled Assay A in Table III). Several different assays have
been employed in the reverse direction. For the Kₘ determina-
tions of nucleoside diphosphates illustrated here, the enzyme was
assayed by incubation at 25° in Pasteur pipettes with drawn tips
(Assay B). The reaction contained 0.1 mM [³H]sucrose (8 × 10⁶
cpm), nucleoside diphosphate, and buffered enzyme in appropri-
ate concentrations in a final volume of 20 μl. Reactions were
terminated by the addition of 5 μl of chromatography solvent
and spotting on Whatman No. 1 paper. Nucleoside diphosphate
glycolates were separated from [³H]sucrose and fructose by
descending chromatography in 1 M ammonium acetate (pH 3.8)-
95% ethanol (3:7). The nucleoside diphosphate glycolate formed
was cut out and counted in toluene scintillation fluid containing
42 ml of Spectrofluor (American-Searle) per liter of toluene.
The determination shown here for sucrose Kₘ was made by as-
saying fructose formed with the Nelson-Somogyi reducing sugar assay (29) (Assay C). In this assay, nucleoside diphosphate,
Tris buffer, sucrose, and enzyme were incubated 25° in a final
volume of 0.5 ml. Reactions were terminated by the addition of
Nelson-Somogyi Solution III and further analyzed for fructose
as described in the reference. This assay was also employed for
some of the equilibrium constant determinations. A different
assay (Assay D) was used for the equilibrium constant deter-
mination. The assay was similar in all respects to Assay B but
the reaction was terminated by spotting on discs of Whatman
DE-81 paper. The discs were then washed free of [³H]sucrose
with 20 ml of distilled H₂O, dried, and the adsorbed nucleoside
diphosphate glycolate was counted in the previously described
scintillation fluid.

**Table I**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Fold purification</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialyzed crude...</td>
<td>1630</td>
<td>(37.4)</td>
<td>(0.023)</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>40 to 52% ammonium sulfate cut</td>
<td>907</td>
<td>37.4</td>
<td>0.364</td>
<td>11.5</td>
<td>100</td>
</tr>
<tr>
<td>DEAE-cellulose...</td>
<td>7.74</td>
<td>18.7</td>
<td>2.42</td>
<td>105.2</td>
<td>50</td>
</tr>
<tr>
<td>Bio-Gel A-15M...</td>
<td>2.14</td>
<td>12.4</td>
<td>5.80</td>
<td>252.0</td>
<td>33</td>
</tr>
</tbody>
</table>
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**RESULTS**

**Purification of Enzyme**—Table I shows the scheme of purifica-
tion for *P. aureus* sucrose synthetase. Because the specific ac-
tivity of crude extracts changes rapidly during the growth of
etiolated seedlings, reaching a maximum between 3 and 4 days
after purification, the final-fold purification has varied from 148
to 580 depending upon the exact age of the beans at time of
harvest. Such a low-fold of purification to obtain purity seemed
unusual at first, until it was realized just how abundant this pro-
tein is in crude extracts of seedlings. Fig. 1 gives an indication
of this. The gel on the left is of the purified enzyme; the one on
the right is of the 40 to 52% ammonium sulfate cut, the first step
of purification. It is quite evident that the sucrose synthetase
band is by far the most abundant protein in this fraction. That
the band representing the purified enzyme is indeed sucrose syn-
thetase has been confirmed by slicing the gels, extracting in buffer
and assaying for activity. Fig. 1 also shows another criterion for
purity of the enzyme. Antibody was prepared against the puri-
fied enzyme and reacted against a 40 to 52% ammonium sulfate
fraction in an Ouchterlony double diffusion experiment. As
shown in Fig. 1, a single band is obtained in such an experiment.

**Structural Properties of Purified Enzyme. Partial Specific Vol-
ume—** Grimes et al. (14) reported a ϑ of 0.83 (measured in sucrose
gradients) and 0.78 (in CsCl₂) for the partially purified enzyme and
on this basis suggested that the enzyme might be a lipopro-
tein. Similar redeterminations with the purified enzyme yield
the following values for Vm = 0.826 (in 30 to 60% sucrose gra-
derients) and 0.760 (in CsCl₂). However, certain considerations lead
to the conclusion that these values for Vm may be inaccurate due
to solvent interactions, particularly in the case of the sucrose
gradients. For example, when purified *Escherichia coli* β-galac-
tosidase (ϑ of 0.70 from amino acid composition) was centrifuged
to equilibrium in sucrose under identical conditions, a Vm of 0.80
was measured. Furthermore, in CsCl₂, catalase (ϑ of 0.73)
banding nearly coincident with sucrose synthetase at ϑ = 0.775.
Finally, analyses (see below) for nonprotein components support
the conclusion that *P. aureus* sucrose synthetase contains no sig-
nificant amounts of nonprotein components. Therefore, for
molecular weight determinations, we have used Vm = 0.733 calcul-
ated from the amino acid composition of the enzyme.

**Nonprotein Components**—Analytical, after mild hydrolysis,
showed no detectable carbohydrate (by the indole method (30)
or by a reducing sugar test (31)) or phosphorus (by the Ames
procedure (32)) or phosphorus (by the indole method (30)) or phosphorus (by the indole method (30)) or phosphorus (by the indole method (30)) or phosphorus (by the indole method (30)) or phosphorus (by the indole method (30)) or phosphorus (by the indole method (30)) or phosphorus (by the indole method (30)) or phosphorus (by the indole method (30)). The organic phase of preparations obtained by
extraction of the enzyme with organic solvents. Such results
tend to rule out the presence of phospholipid or goliploid.
These organic extracts also showed no detectable light absorp-
tion or fluorescence in the range of 200 to 750 nm. Analyses for
sterols were negative. Analyses for fatty acids by gas chroma-
tography before or after acid hydrolysis of the enzyme yielded
values of <1%. Significant, but very low levels (<3%) of car-
bohydrate and phosphorus were detectable upon analysis of the
nonextracted native enzyme. An ultraviolet and visible absorp-
tion spectrum of the enzyme in 0.03 M Tris buffer, pH 7.5, was
typical of normal proteins showing absorption maxima at 215
and 276 nm. The A₂₅₀/A₂₆₀ absorption ratio was 1.45.

**Amino Acid Analyses**—Table II shows the results of an amino
acid analysis of the purified enzyme. The results show an av-
average distribution of the common amino acids. The average
percent (five determinations) of recovery by weight was 71% with values ranging from 62 to 84%. No real explanation can be offered for the low recoveries; the most probable cause lies in the uncertainty of the determination of the $A_{420}$ (see "Experimental Procedure").

Molecular Weight by Equilibrium Sedimentation—Plots of log

\[ \Delta \rho / \rho \text{ versus } log M \] for the native enzyme taken from data from either low or high speed equilibrium runs at all speeds and concentrations of enzyme were linear, indicating homogeneity and ideality of the enzyme under the conditions run. A representative plot (6400 rpm and 1.5 mg per ml) yielded a line with a slope of 0.400 ± 0.005. Speeds of 4800, 5600, and 6400 rpm were used for low speed centrifugation and three different protein concentrations (1.0, 1.5, and 2.0 mg per ml) were used at each speed. A mean molecular weight of $375,000$ with a standard deviation of ±12,000 was calculated from the slopes of the low speed runs. A meniscus depletion run at 11,000 rpm and 1.0 mg per ml yielded a similar molecular weight of 382,000.

Sedimentation Velocity—With catalase (11.3 S) as a marker, purified sucrose synthetase was found to have an $s_{20,w}$ of 12.4 × $10^{-13}$ sec$^{-1}$ by the method of Martin and Ames (33).

Diffusion Constant and Frictional Ratio—With molecular weight $= 375,000$, $\bar{V} = 0.733$, and $s_{20,w} = 12.4$, a diffusion constant $(D_{20,w})$ of $3.63 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ was calculated by use of the Svedberg equation. The corresponding $f_{20,w}$ was calculated to be 1.49.

Molecular Weight by Gel Filtration—Fig. 2 shows a plot of $K_{av}$ versus log $M$ for sucrose synthetase and various marker proteins. Based on its $K_{av}$, sucrose synthetase has an estimated molecular weight of 405,000 ± approximately 20,000 by this technique.

Subunit Analyses: SDS Gels—Purified enzyme was reacted with [3H]dimethylsulfate to label the enzyme, dialyzed against SDS (0.1%), and run on SDS gels as described under "Experimental Procedure." [3H]Labeled influenza virus subunits were run simultaneously as molecular weight markers. The gels were fractionated on a gel slicer and the fractions counted for 3H and tritium. Under these conditions, it has been shown (34) that proteins are separated into individual subunits which migrate as a function only of molecular weight. Fig. 3 shows the results of such an experiment. Arrows indicate the peaks of the marker

### Table II

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues/94,000 mol wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>44.9</td>
</tr>
<tr>
<td>Histidine</td>
<td>26.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>49.9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>16.4</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>70.6</td>
</tr>
<tr>
<td>Threonine</td>
<td>50.7</td>
</tr>
<tr>
<td>Serine</td>
<td>40.6</td>
</tr>
<tr>
<td>Glutamine acid</td>
<td>100.8</td>
</tr>
<tr>
<td>Proline</td>
<td>34.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>59.1</td>
</tr>
<tr>
<td>Alanine</td>
<td>56.2</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>3.1</td>
</tr>
<tr>
<td>Valine</td>
<td>53.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>10.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>45.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>93.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>29.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>40.5</td>
</tr>
</tbody>
</table>

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Fig. 1. Analytical acrylamide gels and Ouchterlony double diffusion slides of sucrose synthetase preparations. Details of procedures are under "Experimental Procedure." Gel on left is of purified sucrose synthetase, gel on right is of 40 to 52% ammonium sulfate fraction from the first step of purification. Center well of Ouchterlony slides contained undiluted antiserum prepared against purified sucrose synthetase. Adjacent side wells contained a 1:3 dilution of the 40 to 52% ammonium sulfate fraction.
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A single species of subunit of molecular weight 90,000 is observed, indicating the enzyme is a multimer of identical protein subunits. With the high degree of sensitivity resulting from the radioactive labeling techniques, the sharpness of the single peak is a further criterion of purity of the enzyme. That the enzyme contains only one species of subunit is further supported by NH2-terminal analyses by dansylation of the purified enzyme which yielded only one conclusive NH2-terminal amino acid, threonine. Furthermore, low speed equilibrium sedimentation of the enzyme in 6 M guanidine-HCl yielded linear plots for log $A_q / q$ which yielded an average molecular weight of 93,800 ± 6,800, in good agreement with the results of SDS gels. Runs were performed at 11,000 and 13,000 rpm with three different protein concentrations at each speed (1.2, 2.4, and 3.6 mg per ml).

### Equilibrium Constant of Reaction

Reports of the equilibrium constant for the sucrose synthetase reaction in the direction of sucrose synthesis have varied between 1.6 and 8 (1, 10, 12). Table III shows the results of five separate determinations at 7.5 and 25°. A mean value for the equilibrium constant of 0.149 in the direction of UDP-glucose synthesis was obtained.

$K_m$ Values for Substrates—Grimes et al. (14) reported $K_m$ values for the purified enzyme for all of the possible substrates in the direction of sucrose synthesis. Where repeated with the purified enzyme, our results agree with their figures. Table IV shows $K_m$ values for sucrose determined from Lineweaver-Burk plots with the purified enzyme and assayed in the presence of saturating UDP or ADP. For comparison, the $K_m$ for P. aureus invertase performed at its pH optimum of 5.5 is shown. The invertase determination was made with a partially purified preparation obtained from the excluded fraction of the DEAE-cellulose fractionation of sucrose synthetase. With sucrose synthetase, a $K_m$ for sucrose of 17 mM with UDP and 29 mM with ADP was obtained. $K_m$ of 32 mM for invertase was calculated.

### Table III

<table>
<thead>
<tr>
<th>Assay used</th>
<th>Sucrose</th>
<th>UDP</th>
<th>UDP-glucose</th>
<th>Fructose</th>
<th>$K_{eq}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$7.0 \times 10^{-4}$</td>
<td>$7.0 \times 10^{-4}$</td>
<td>$3.0 \times 10^{-4}$</td>
<td>$3.0 \times 10^{-4}$</td>
<td>0.183</td>
</tr>
<tr>
<td>B</td>
<td>$7.1 \times 10^{-4}$</td>
<td>$7.1 \times 10^{-4}$</td>
<td>$2.9 \times 10^{-4}$</td>
<td>$2.9 \times 10^{-4}$</td>
<td>0.166</td>
</tr>
<tr>
<td>C</td>
<td>$7.5 \times 10^{-4}$</td>
<td>$7.5 \times 10^{-4}$</td>
<td>$2.5 \times 10^{-4}$</td>
<td>$2.5 \times 10^{-4}$</td>
<td>0.111</td>
</tr>
<tr>
<td>D</td>
<td>$1.45 \times 10^{-2}$</td>
<td>$1.45 \times 10^{-2}$</td>
<td>$5.5 \times 10^{-4}$</td>
<td>$5.5 \times 10^{-4}$</td>
<td>0.143</td>
</tr>
<tr>
<td>Mean</td>
<td>$1.38 \times 10^{-2}$</td>
<td>$1.38 \times 10^{-2}$</td>
<td>$1.6 \times 10^{-2}$</td>
<td>$1.6 \times 10^{-2}$</td>
<td>0.149</td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.024</td>
</tr>
</tbody>
</table>

**Fig. 3.** SDS-acrylamide gel of purified sucrose synthetase. Details are given under "Experimental Procedure." Arrows indicate the positions of 14C-labeled marker proteins (influenza virus subunits).
Table IV

<table>
<thead>
<tr>
<th>Substrate varied</th>
<th>Second substrate in saturating levels</th>
<th>$K_m$ of varied substrate</th>
<th>$V_{max}$ (relative numbers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>UDP</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>ADP</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>None</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>UDP</td>
<td>Sucrose</td>
<td>0.19</td>
<td>100.0</td>
</tr>
<tr>
<td>ADP</td>
<td>Sucrose</td>
<td>0.19</td>
<td>28.6</td>
</tr>
<tr>
<td>TDP</td>
<td>Sucrose</td>
<td>0.30</td>
<td>5.8</td>
</tr>
<tr>
<td>CDP</td>
<td>Sucrose</td>
<td>0.44</td>
<td>3.4</td>
</tr>
<tr>
<td>GDP</td>
<td>Sucrose</td>
<td>0.17</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* Invertase substituted for sucrose synthetase.

From the data presented, *P. aureus* sucrose synthetase appears to be a protein with a molecular weight of approximately 375,000. This is considerably smaller than the value estimated by Grimes et al. (14) by gel filtration, but in this case only a void volume marker was run. Subunit analyses with SDS gels and equilibrium sedimentation in 6 M guanidine-HCl indicate that the protein contains identical subunits of approximate molecular weight 94,000. The molecular weight data lead to the conclusion that the enzyme is most likely a tetramer of identical 94,000 molecular weight subunits.

In terms of its kinetic properties, it is interesting that, although the equilibrium constant of the enzyme favors sucrose synthesis, the kinetic parameters with regard to substrate affinities are so arranged as to make the ratio of $V_f/V_b$ approximately unity. As pointed out in an earlier paper (3), the finding that the levels of the enzyme in *P. aureus* are high only in nonphotosynthetic tissues suggests very strongly that its prime physiological role is that of the catalysis of synthesis of nucleoside diphosphate glucoses from the high levels of sucrose normally translocated to these tissues. In this regard, the relationship between sucrose synthetase and invertase becomes of interest. Although the sucrose synthetase reaction is far more efficient from an energetic standpoint, the high levels of invertase, as well as sucrose synthetase, present in these tissues indicate that both enzymes may function in sucrose metabolism. The determination of the $K_m$ for sucrose for these two potentially competitive enzymes was performed in an attempt to clarify this relationship. The finding of very similar $K_m$ values for sucrose for these two enzymes offers no opportunity for discrimination of roles in this regard. However, it should be noted that the pH optima of the two enzymes are quite different (5.5 for invertase versus 7 to 9 for sucrose synthetase), and information on intracellular localization of these enzymes might be crucial to understanding their relative effectiveness in sucrose degradation. Machlachlan et al. (17) have also studied the relationship between these enzymes in pea seedlings, and they have found a marked difference in distribution of these enzymes in various regions of the plant, suggesting another way in which control of the pattern of sucrose degradation could occur.

We find in etiolated seedlings of *P. aureus* that the levels of sucrose synthetase are highest in the region of elongation whereas the levels of invertase are highest in the region below the zone of elongation. The fact that sucrose synthetase has the potential capability of synthesizing all of the nucleoside diphosphate glucoses which serve as cell wall precursors, adds interest to this distribution pattern. Recent kinetic studies by this author (35)* indicate that sucrose synthetase has a number of regulatory effectors. These include NADP, NADPH, fructose-1-P, Mg$^{2+}$, pyrophosphate, and indoleacetic acid, the plant hormone which controls cell elongation. We hope that more information on how these compounds may act in combination to control the activity of this enzyme will shed more light on the physiological role of this enzyme which is so abundant in these tissues.

The nature of the specificity of the enzyme for the various nucleoside diphosphates is also relevant to the physiological role this enzyme plays in higher plants. The data presented here on those kinetic parameters imply very strongly that UDP-glucose would be the major nucleoside diphosphate glucose produced physiologically, although one cannot exclude the possibility that the others, particularly ADP-glucose, may be synthesized under the appropriate conditions. In the reverse direction, Grimes et al. (14) showed that UDP-glucose was a potent inhibitor of sucrose biosynthesis from ADP-glucose. It remains to be determined whether a similar phenomenon occurs in the direction of sucrose degradation. Such questions are particularly relevant to the possible and, as yet unresolved role sucrose synthetase

* D. P. Delmer. unpublished information.
plays in the conversion of sucrose to starch during seed ripening, although current views seem to favor the opinion that sucrose degradation by sucrose synthetase primarily occurs via a conversion to UDP-glucose rather than ADP-glucose, and that the ADP-glucose pyrophosphorylase is the primary enzyme responsible for ADP-glucose biosynthesis (11, 19, 20, 36, 37).

All reported relevant data on sucrose synthetase from various plants show that the enzyme prefers the uridine-containing substrate (UDP or TDP-glucose). Although this important similarity exists, clearly the enzyme is not identical in its kinetic parameters in all plants. For example, Milner and Avigad (38) report that the sucrose synthetase from the beet roots has a higher \( V_{\text{max}} \) with TDP as substrate than with ADP. The work of deFekete and Cardini (11) with sweet corn indicate that the "preference" of this sucrose synthetase for TDP over ADP is a result of a \( K_m \) difference (5 \( \times 10^{-4} \) M for TDP versus 2 \( \times 10^{-3} \) M for ADP), which is not the case for \( P. \) aureus sucrose synthetase.

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