SUBSTRATE INHIBITION OF MAIZE ENDOSPERM SUCROSE SYNTHASE BY FRUCTOSE AND ITS INTERACTION WITH GLUCOSE INHIBITION*

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Sucrose synthase (EC 2.4.1.13) was purified to homogeneity from developing maize (Zea mays L.) endosperm. Substrate saturation and inhibitor kinetics were examined for the sucrose synthase reaction. The $K_m$-values for fructose and uridine diphosphate glucose (UDPGlc) were estimated to be 7.8 mM and 76 $\mu$M, respectively. Fructose concentrations over 20 mM inhibited sucrose synthase in an uncompetitive manner with respect to UDPGlc. Glucose was also found to be an uncompetitive inhibitor with respect to both fructose and UDPGlc. At inhibitory concentrations of fructose, the apparent $K_i$ for glucose increased linearly with increasing fructose concentration. The results suggest an ordered kinetic mechanism for sucrose synthase where UDPGlc binds first and UDP dissociates last. Fructose and glucose both inhibit by binding to the enzyme-UDP complex. Fructose and glucose, which are present in maize endosperm as the products of invertase, could inhibit sucrose synthase, especially in basal regions of the kernel where hexoses may accumulate.

Key words: Zea Mays L.; sucrose synthase; sucrose; fructose; glucose

Introduction

Growth of a developing maize kernel is dependent on a supply of sucrose from the phloem. Sucrose arriving at the kernel may be hydrolyzed by invertase ($\beta$-fructofuranoside fructohydrolase EC 3.2.1.26) to form glucose and fructose, or sucrose may be metabolized in the presence of UDP by sucrose synthase (uridine diphosphate glucose: D-fructose 2-glucosyltransferase, EC 2.4.1.13) to form uridine diphosphateglucose (UDPGlc) and fructose. Whereas invertase activity is highest in basal portions of the kernel, sucrose synthase activity is highest in the upper endosperm, where invertase activity is absent [1]. Both invertase and sucrose synthase activity are found in the lower endosperm. Glucose and fructose have been reported to accumulate in basal regions of the maize kernel to various extents as a consequence of sucrose hydrolysis by invertase [2,3]. Glucose has been reported to be an inhibitor of sucrose synthase [4—6], but details of the inhibition have not been described. Fructose has also been reported as a substrate inhibitor of sucrose synthase [6—8] and as a product inhibitor [9], but only the product inhibition has been described in detail.

Sucrose synthase has been purified and kinetically characterized from several sources [8—12], but many aspects of its regulation remain unclear. Although product inhibition studies have suggested an ordered Bi Bi kinetic mechanism [9], other mechanisms have been proposed [13,14]. In this study, substrate saturation kinetics of sucrose synthase in the direction of sucrose synthesis was examined. Inhibition of sucrose synthase activity by fructose and glucose were characterized. An
interaction between fructose and glucose inhibition was observed, and a mechanism by which fructose and glucose may inhibit sucrose synthase is suggested.

Materials and methods

Maize (Zea mays L.) was field grown and hand pollinated. Kernels were harvested 20 days after pollination, stripped from the cob and stored at -90°C until used. Endosperms were dissected and homogenized in extraction buffer containing 50 mM Hepes—NaOH (pH 7.5), and 1 mM dithiothreitol, at a ratio of 0.1 g fresh wt. tissue/ml of extraction buffer. The homogenate was centrifuged at 24000 × g for 15 min. Ammonium sulfate was added to the supernatant and the precipitate formed between 30 and 50% of saturation was collected. The precipitate was resuspended in extraction buffer and applied to a 2 × 120-cm Ultrogel AcA 34 column, equilibrated and eluted with 10 mM Hepes—NaOH (pH 7.5), and 1 mM dithiothreitol. The sucrose synthase activity that was eluted from the column was centrifuged at 24 000 × g for 15 min. Ammonium sulfate was added to the supernatant and the precipitate formed between 30 and 50% of saturation was collected. The precipitate was resuspended in extraction buffer and applied to a 2 × 120-cm Ultrogel AcA 34 column, equilibrated and eluted with 10 mM Hepes—NaOH (pH 7.5), and 1 mM dithiothreitol. The sucrose synthase activity that was eluted from the column was pooled and subjected to anion exchange chromatography using a Fast Protein Liquid Chromatography system (Pharmacia, Piscataway NJ, U.S.A.). The sample was applied to a Mono-Q anion exchange column equilibrated with a solution containing 10 mM Mes—NaOH (pH 6.0), and 1 mM dithiothreitol and was eluted with a linear gradient 0—300 mM NaCl in the same buffer. The sucrose synthase preparation obtained was homogeneous by SDS-polyacrylamide gel electrophoresis on 10% acrylamide slab gels, run at 30 mA for 3 h [15], where a single protein band with $M_r = 89 000$ was found, corresponding to the reported $M_r$ of sucrose synthase subunits [10].

Sucrose synthase assays contained 50 mM Bicine—NaOH (pH 8.0), 10 mM MgCl$_2$, 20 mM KCl, 0.4 mM phosphoenolpyruvate, 0.3 mM NADH, 1 IU/ml lactate dehydrogenase, 2 IU/ml pyruvate kinase and 3.8 μg/ml sucrose synthase protein in a final volume of 1 ml. Fructose, UDPGlc and glucose concentrations were varied as indicated in figures. Enzyme activity was measured by monitoring the decrease in absorbance at 340 nm at 30°C. One IU of activity is defined as the activity necessary to produce 1 μmol of product in 1 min. at 30°C. Protein was measured by BioRad (Bradford) protein reagent with ovalbumin as a standard.

Kinetic constants were determined by mean-square linear regressions of Hanes-Woolf type replots ($s/v$ against $s$), or Dixon plots ($1/V_{max}$ against $I$), as described by Dixon and Webb [16]. All values are means of three determinations.

Results

The substrate saturation response of sucrose synthase was hyperbolic for UDPGlc concentrations of 20—1000 μM and up to 15 mM fructose (data not shown). $K_m$-values for fructose and UDPGlc were 7.8 ± 1.0 mM and 76 ± 9 μM, respectively. $V_{max}$ was 15.1 ± 1.4 IU/mg protein. Fructose inhibited sucrose synthase activity at concentrations over 20 mM (data not shown). Hanes-Woolf ($s/v$ against $s$) replots of these data are shown in Fig. 1. In a Hanes-Woolf replot of kinetic data, the $X$ intercept is equal to $-K_m$, the slope is equal to $1/V_{max}$ and the $Y$ intercept is equal to $K_mV_{max}$ [16]. The data in Fig. 1 show that both the $K_m$-value for UDPGlc and $V_{max}$ decrease with increasing fructose, whereas $K_mV_{max}$ remains constant, indicating that fructose is uncompetitive with respect to UDPGlc [16]. A $K_i$-value for fructose of 27 ± 4 mM was calculated from a Dixon plot (Fig. 1 inset).

Glucose was an uncompetitive inhibitor of sucrose synthase activity with respect to fructose (Fig. 2) with a $K_i$-value of 43 ± 7 mM (Fig. 2 inset). Glucose was also an uncompetitive inhibitor with respect to UDPGlc (Fig. 3) with a $K_i$-value of 53 ± 7 mM (Fig. 4 inset).

At high (inhibitory) fructose concentrations, the $K_i$-value for glucose increased linearly with increasing fructose concentration (Fig. 4). Increasing glucose concentration also
Fig. 1. Hanes-Woolf (s/v against s) plots, showing uncompetitive substrate inhibition of sucrose synthase by fructose with respect to UDPGlc. The X intercept is equal to $-K_m$, the slope is $1/V_{max}$, and Y intercept is $K_m/V_{max}$. s, substrate concentration (mM fructose); v, enzyme reaction velocity (IU/mg protein). Inset: Secondary Dixon ($1/V_{max}$ against $I$) replot of the data. $V_{max}$, maximal enzyme velocity (IU/mg protein); $I$, inhibitor concentration (mM fructose).

Fig. 2. Hanes-Woolf (s/v against s) plot, showing uncompetitive inhibition of sucrose synthase activity by glucose with respect to fructose. UDPGlc concentration was 1.0 mM. s, substrate concentration (mM fructose); v, enzyme reaction velocity (IU/mg protein). Inset: Secondary Dixon ($1/V_{max}$ against $I$) replot of data. $V_{max}$, maximal enzyme velocity (IU/mg protein); $I$, inhibitor concentration (mM glucose).
Fig. 3. Hanes-Woolf ($s/v$ against $s$) plot, showing uncompetitive inhibition of sucrose synthase activity by glucose with respect to UDPGlc. Fructose concentration was 15 mM. $s$, substrate concentration (mM UDPGlc); $v$, enzyme reaction velocity (IU/mg protein). Inset: Secondary Dixon ($1/V_{max}$ against $I$) replot of data. $V_{max}$, maximal enzyme velocity (IU/mg protein); $I$, inhibitor concentration (mM glucose).

Fig. 4. Dixon ($1/v$ against $I$) primary plots of glucose inhibition of sucrose synthase activity at inhibitory fructose concentrations, showing an interaction between glucose inhibition and fructose inhibition. UDPGlc concentration was 1.0 mM. $v$, enzyme reaction velocity (IU/mg protein); $I$, inhibitor concentration (mM glucose).
increased the $K_i$-value for fructose linearly (data not shown).

Discussion

Uncompetitive substrate inhibition of sucrose synthase activity by fructose suggests an ordered kinetic mechanism [17], which is consistent with the product inhibition studies of Wolusik and Pontis [9]. Apparently UDPGlc binds first, followed by fructose. After catalysis, sucrose dissociates first, followed by UDP. Fructose would cause uncompetitive inhibition by binding to the enzyme-UDP complex, possibly at the site vacated by sucrose. Glucose would also cause uncompetitive inhibition of sucrose synthase activity by binding to the same enzyme-UDP complex. The binding of either fructose or glucose to the enzyme-UDP complex apparently prevents the binding of the other hexose, resulting in a competitive interaction between these two inhibitors (Fig. 4).

Fructose and glucose may accumulate to as much as 20% of the dry weight in some parts of the basal region of the developing maize kernel as a consequence of sucrose hydrolysis catalyzed by invertase [2,3], especially at early stages of development [3]. Although the results here deal with sucrose synthesis, a mechanism is provided by which glucose and fructose, products of invertase activity, could inhibit sucrose degradation by sucrose synthase. Inhibition of sucrose synthase activity by the products of invertase would be of greatest physiological significance in the lower endosperm and at early (0—15 days after pollination) stages of development when hexoses accumulate to the greatest extent.

References