Inhibition of Sucrose Phosphatase by Sucrose

By J. S. HAWKER

*David North Plant Research Centre, Colonial Sugar Refining Co. Ltd.,
Indooroopilly, Queensland, Australia*

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1. Partially purified sucrose phosphatase from immature stem tissue of sugar-cane is inhibited by sucrose. The enzyme was also inhibited by maltose, melezitose and 6-kestose but not by eight other sugars, including glucose and fructose. 2. The relative effectiveness of sucrose, maltose and melezitose as inhibitors is different for sucrose phosphatase from different plants. 3. The inhibition of the sugar-cane enzyme by sucrose was shown to be partially competitive. The K_i for sucrose is about 10 mM. 4. Melezitose is also a partially competitive inhibitor of the enzyme but the inhibition by maltose is probably mixed. 5. The possibility that sucrose controls both the rate of accumulation of sucrose in stems of sugar-cane and sucrose synthesis in leaves by inhibiting sucrose phosphatase is discussed.

Sucrose inhibits the specific sucrose phosphate phosphohydrolase (sucrose phosphatase) from sugar-cane stem mitochondria (Hawker & Hatch, 1966). Subsequently, sucrose phosphatase has been found in soluble extracts of all plant tissues examined (Hawker, 1966). The present paper describes the inhibition by sucrose and other sugars of sucrose phosphatase from sugar-cane and other plants.

MATERIALS

Preparations of sucrose phosphatase from several plants were used (Hawker & Hatch, 1966; Hawker, 1966). Yeast invertase was obtained from Difco Laboratories, Detroit, Mich., U.S.A. Sucrose phosphate, phosphorylated at the 6-position of fructose and containing radioactivity only in the fructose moiety, was prepared enzymically as described by Hatch (1964). 6-Kestose (6^P-β-fructosylsucrose) and 1-kestose (1^P-β-fructosylsucrose) were supplied by Dr J. Gagolski, Colonial Sugar Refining Co. Ltd., Sydney, New South Wales, Australia.

METHODS

Sucrose phosphatase from tobacco leaves. Tobacco-leaf preparations were made by grinding leaves in the medium described by Hawker & Hatch (1966). The homogenate was filtered through muslin and frozen and thawed.

Measurement of enzyme activity. The rates of hydrolysis of [fructosyl-¹⁴C]sucrose phosphate were determined at 22° as described by Hawker & Hatch (1966) except that the tris-maleate buffer was adjusted to pH 6.7.

Reversal of sucrose inhibition. Partially purified sucrose phosphatase from sugar-cane stem (Hawker, 1966) was incubated with and without 50 mM-sucrose in standard reaction mixtures minus sucrose phosphate for 10 min.

One of the reaction mixtures containing sucrose was treated for a further 15 min. with yeast invertase (5 μl. of a solution containing 25 mg./ml.). Radioactive sucrose phosphate was then added to all tubes and the reactions were stopped at the end of 15 min. by heating at 100° for 2 min. The mixtures not already containing invertase were treated for 5 min. with invertase and then heated as before. After paper chromatography the percentage hydrolysis of sucrose phosphate was determined by counting the sucrose phosphate and fructose spots (Hatch, 1964).

RESULTS

Effect of sugars on the activity of sucrose phosphatase from sugar-cane. The effect of several sugars on the activity of partially purified sucrose phosphatase from immature stem tissue of sugar-cane (Hawker, 1966) is shown in Table 1. Of the sugars studied, only four markedly inhibited the activity of the enzyme. After storage for 80 days the enzyme preparation exhibited only 35% of its original activity but still showed the same degree of inhibition by sucrose. The presence of sucrose (100 mM), added after boiling the reaction mixtures, did not interfere with the assay procedure.

Inhibition by sugars of sucrose phosphatase from different plants. Table 2 shows the effect of some of the sugars, found to be inhibitors of sugar-cane stem sucrose phosphatase, on sucrose phosphatase from other tissues of sugar-cane and from other plants. In separate experiments, similar results were obtained for partially purified enzymes and crude preparations. Comparison of the inhibitions of the activity of the enzymes from different plants by sucrose, maltose and melezitose at a

concentration of 100mm reveals four distinct patterns of inhibition. The enzymes from immature stem tissue, mitochondria and leaves of sugar-cane (Pindar) all show the same pattern of inhibition, which is different from the inhibition shown by preparations from two other species of sugar-cane, *Saccharum spontaneum* L. and *S. robustum* Brandes and Jeswiet ex Grassl. Examination of the paper chromatograms showed that there was no significant breakdown of any of the sugars during the assays.

Reversibility of sucrose inhibition. Since glucose and fructose do not inhibit sucrose phosphatase a rapid method could be used to determine the reversibility of the inhibition by sucrose. The rate of hydrolysis of sucrose phosphate by partially

purified enzyme from immature stem tissue of sugar-cane was inhibited 63% by 50mm-sucrose. When sucrose phosphate was added to similar reaction mixtures that had been treated with invertase, its rate of hydrolysis was the same as in controls with no added sucrose.

Table 1. *Effect of sugars on the activity of partially purified sucrose phosphatase from immature stem tissue of sugar-cane*

The reaction mixtures contained 71 μ M-[fructosyl-¹⁴C]-sucrose phosphate and sugars at a concentration of 100mm. Other details are given in the Methods section.

Sugar	Inhibition (%)	Sugar	Inhibition (%)
Sucrose	76	Fructose	0
Maltose	45	Lactose	0
Melezitose	86	Cellobiose	0
6-Kestose	34	Trehalose	0
1-Kestose	0	Gentiobiose	0
Turanose	7	Raffinose	0
Glucose	0

Fig. 1. Inhibition of sugar-cane sucrose phosphatase by sucrose. Double-reciprocal plots are shown of data obtained in the presence of the following concentrations of sucrose: ●, none; ○, 10mm; ▲, 100mm; △, 250mm; ■, 500mm. The rate of hydrolysis of sucrose phosphate by partially purified sugar-cane sucrose phosphatase was determined as described in the Methods section. The units of *v* are μ -moles of sucrose phosphate hydrolysed/reaction mixture/30 min.

Table 2. *Effect of sugars on sucrose phosphate hydrolysis by enzyme preparations from several tissues*

Values are percentage inhibitions by sugars at a concentration of 100mm of the hydrolysis of 71 μ M-[fructosyl-¹⁴C]sucrose phosphate. Partially purified enzyme from sugar-cane stem tissue and enzymes from other tissues were prepared as described by Hawker (1966) except partially purified enzymes from immature-stem mitochondria and carrot-root mitochondria (Hawker & Hatch, 1966) and tobacco-leaf enzyme. The preparation of the tobacco-leaf enzyme and other details are given in the Methods section.

Enzyme source	Type of preparation	Inhibition (%)			
		Sucrose	Maltose	Melezitose	Turanose
Sugar-cane (Pindar)					
Immature stem	Partially purified	74	45	86	7
Immature-stem mitochondria	Partially purified	70	50	78	—
Leaf laminae	Crude	70	39	80	—
<i>S. robustum</i>					
Immature stem	Crude	20	50	40	—
<i>S. spontaneum</i>					
Immature stem	Crude	15	44	25	—
Carrot root					
Supernatant	Crude	86	82	36	36
Precipitate	Crude	86	84	36	44
Mitochondria	Partially purified	90	87	44	57
Tobacco leaf	Crude	33	72	28	—

Fig. 2. Inhibition of sugar-cane sucrose phosphatase by sucrose at the following concentrations of sucrose phosphate: ●, 36 μ M; ○, 280 μ M. Data from Fig. 1 and additional data are plotted.

Fig. 3. Data of Fig. 2 plotted as $1/v$ against sucrose concentration.

Sucrose as a partially competitive inhibitor of sucrose phosphatase. The rate of hydrolysis of sucrose phosphate by sugar-cane enzyme was linear with time for at least 30 min. The inhibition by 100 mM-sucrose was the same when measured after 10, 15 or 30 min.

Double-reciprocal plots (Lineweaver & Burk, 1934) showed a series of straight lines that intercepted at the $1/v$ axis (Fig. 1). However, the percentage inhibition did not reach 100% (Fig. 2). Also, when the above data and other data were

plotted as $1/v$ against the concentration of sucrose (inhibitor) at constant sucrose phosphate concentrations, the lines were curved instead of being straight (Fig. 3). Thus the data are consistent with the inhibition by sucrose being partially competitive (Webb, 1963; Dixon & Webb, 1964).

For partially competitive inhibition:

$$R = \frac{\alpha K_i + \alpha [I]}{\alpha K_i + [I]}$$

Where R is the ratio of the slopes of the inhibited and uninhibited curves in a double-reciprocal plot, K_i is the inhibitor dissociation constant, $[I]$ is the inhibitor concentration, and α represents the change in affinity of substrate for enzyme-inhibitor complex and also the change in affinity of the inhibitor for the enzyme-substrate complex (Webb, 1963).

Rearranging the above equation:

$$K_i = \left(\frac{[I]}{R-1} \right) \left(1 - \frac{R}{\alpha} \right)$$

Now:

$$K'_i = \frac{[I]}{R-1} \quad (1)$$

where K'_i is the calculated K_i (Webb, 1963). Substituting in eqn. (1):

$$K_i = K'_i \left(1 - \frac{R}{\alpha} \right) \quad (2)$$

Rearranging:

$$\frac{1}{K'_i} = \frac{1}{K_i} - \frac{R}{\alpha K_i}$$

Since K_i and α are constants, a plot of $1/K'_i$ against R will give a straight line that cuts the $1/K'_i$ axis at $1/K_i$ and the R axis at α . The suggestion by Webb (1963) that plotting K'_i against R results in a straight line is not true since it is based on incorrect substitution in eqn. (1) above to give:

$$K'_i = K_i \left(1 - \frac{R}{\alpha} \right)$$

instead of the correct eqn. (2). When two sets of data were plotted a value of K_i of about 10 mM and a value for α of about 11 was obtained (Fig. 4).

When data for the inhibition of carrot sucrose phosphatase were plotted as $1/v$ against sucrose concentration, curved lines were obtained (Fig. 5). The inhibition by lower concentrations of sucrose was consistently higher for carrot enzyme than for the sugar-cane enzyme. The K_i for the carrot sucrose phosphatase was found to be less than half the K_i for the sugar-cane sucrose phosphatase, but the values of α were similar for both enzymes.

Inhibition of sucrose phosphatase by melezitose

Fig. 4. Plot of R against $1/K_i$ calculated from the data of Fig. 2 (O) and additional data (●). The definitions of R , K_i and the relevant equations are given in the Results section.

Fig. 5. Inhibition of carrot sucrose phosphatase by sucrose at the following concentrations of sucrose phosphate: ●, $36 \mu\text{M}$; ○, $140 \mu\text{M}$. The rates of hydrolysis (v) of sucrose phosphate by carrot sucrose phosphatase were determined as described in the Methods section and are expressed as $\mu\text{moles/reaction mixture/30 min}$.

and maltose. The inhibition of partially purified sucrose phosphatase from immature stem tissue of sugar-cane by melezitose was qualitatively similar to the inhibition of the enzyme by sucrose. The K_i for melezitose was about 7 mm and the value of

Fig. 6. Inhibition of tobacco sucrose phosphatase by maltose at the following concentrations of sucrose phosphate: ●, $36 \mu\text{M}$; ○, $280 \mu\text{M}$. The lines intersect at -23 mm . The methods were similar to those described in the legend to Fig. 5.

Fig. 7. Inhibition of tobacco sucrose phosphatase by maltose. Double-reciprocal plots are shown of data obtained in the presence of the following concentrations of maltose: ●, none; ○, 50 mm; ▲, 100 mm. The methods were similar to those described in the legend to Fig. 5.

α was 16. However, the lines obtained by plotting $1/v$ against maltose concentration were not curved and intercepted at -145 mm .

Since sucrose phosphatase from tobacco leaf is inhibited more by maltose than is the sugar-cane enzyme it was used for more detailed study. A plot of $1/v$ against maltose concentration for the tobacco enzyme also produced straight lines (Fig. 6). Double-reciprocal plots (Lineweaver & Burk, 1934) showed a series of straight lines that intercepted at neither axis (Fig. 7). The results show that the inhibition of sucrose phosphatase by

maltose is mixed inhibition (Webb, 1963) and the K_i for maltose is about 23 mM.

DISCUSSION

The similarity of the results for inhibition of partially purified and crude preparations of sucrose phosphatase by sugars further supports the claim (Hawker, 1966) that the amount of sucrose phosphatase present in tissue homogenates can be determined without further purification.

The inhibition of the activity of the enzyme from immature tissue of sugar-cane by sugars is reasonably specific. All the sugars found to be inhibitors contain an α -linked glucose moiety and in each case, except maltose, this is linked to a fructose moiety. Substitution at the 6-position of the glucose moiety (raffinose), substitution at the 1-position of the fructose moiety (1-kestose) or replacement of the α -anomer by a β -linked glucose moiety (e.g. cellobiose) results in complete loss of activity of the sugar as an inhibitor. However, substitution at the 3-position (melezitose) or 6-position (6-kestose) of the fructose moiety modifies but does not destroy the activity of the sugar. Since glucose and fructose have no effect on the activity of the enzyme it is likely that all monosaccharides would be ineffective as inhibitors.

The partially purified enzymes from immature stem tissue of sugar-cane and from carrot have many properties in common (Hawker & Hatch, 1966). However, the quantitative differences observed in the effect of sugars on the two enzymes show that the enzymes are not identical. There appears to be only one form of the enzyme in the sugar-cane variety Pindar, because as the partially purified sucrose phosphatase loses activity the percentage inhibition by sucrose of the remaining activity remains the same. Also, the inhibition by sugars of the enzyme from both supernatant and mitochondria is similar to that of the enzyme from sugar-cane leaves (Table 2). The existence of a slightly different sucrose phosphatase in other species of *Saccharum* could prove useful in tracing the evolution of the *Saccharum* complex.

Sucrose phosphatase does not have the properties of an allosteric enzyme as described by Monod, Wyman & Changeux (1965). The substrate did not exhibit co-operative homotropic interactions either in the presence or absence of inhibitor, and the inhibitor itself did not show any co-operative effects (Figs. 1, 2 and 3). The kinetics of the inhibition of sucrose phosphatase by sucrose are, however, those of a system of partially competitive inhibition. With this type of inhibition the enzyme is never completely inhibited (Dixon & Webb, 1964). Thus sucrose phosphatase in sugar-cane tissue would still be partially active even if the

enzyme is situated in the vacuole, where the concentration of sucrose can be higher than 600 mM (Glasziou, Bull, Hatch & Whiteman, 1965).

Sucrose also acts as a partially competitive inhibitor of sucrose phosphatase from carrot (Fig. 5). Under the same experimental conditions sucrose is a more effective inhibitor of carrot sucrose phosphatase than sugar-cane sucrose phosphatase. This fact is due to the different affinities shown by the two enzymes for sucrose and not to a difference in the affinity of substrate for the inhibitor-enzyme complex, i.e. α is the same for each. In contrast, both a decrease in K_i and an increase in the α value result in melezitose being a more effective inhibitor than sucrose of sugar-cane sucrose phosphatase. Again in contrast, the affinity of maltose for the sugar-cane enzyme is much less than that of sucrose or melezitose, but the inhibition by maltose is probably mixed. Even though under similar conditions the inhibition by sucrose of sugar-cane sucrose phosphatase is nearly the same as the inhibition by maltose of the tobacco enzyme (Table 2) there is more than a twofold difference in the values of K_i for the two inhibitors. This difference is balanced by the difference in the type of inhibition exerted by the two inhibitors.

Since sucrose has the characteristics of a partially competitive inhibitor of sucrose phosphatase, sucrose and sucrose phosphate probably combine with different groups on the enzyme (Dixon & Webb, 1964). Melezitose probably combines with the enzyme at the sucrose site. It seems likely that maltose also combines with the sucrose-binding site on the enzyme, but in contrast with sucrose and melezitose prevents the breakdown of enzyme-substrate complex to yield products.

The involvement of sucrose phosphatase in sugar accumulation in sugar-cane tissues has been suggested and its possible location discussed (Hawker, 1966). The inhibition of sucrose phosphatase by sucrose could act as a control mechanism limiting the concentration of sucrose in the vacuole.

If sucrose phosphatase is also involved in sucrose synthesis during photosynthesis (Hawker, 1966), the inhibition of sucrose phosphatase by sucrose could act as a control on the rate of photosynthesis. Inhibition of sucrose phosphatase could be expected to result in a build-up of sucrose phosphate. Sucrose phosphate does not inhibit UDP-glucose-fructose 6-phosphate glucosyltransferase from wheat germ (Mendicino, 1960), but it is not known whether sucrose phosphate affects the activity of the enzyme from sugar-cane. Whether or not there is any effect, the possibility that sucrose phosphate inhibits an enzyme or enzymes further back in the pathways of both sucrose synthesis during photosynthesis and sucrose accumulation cannot be overlooked.

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