STUDIES ON SUCROSE PHOSPHATE SYNTHETASE

The inhibitory action of sucrose

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1. Introduction

Plant biochemists are confronted with two pathways leading to sucrose, both of which involve UDP-glucose as glucosyl donor. Sucrose synthetase [1] (UDP-glucose: D-fructose-2-glucosyltransferase, EC 2.4.2.13) and sucrose phosphate synthetase [2] (UDP-glucose: D-fructose-6-phosphate-2-glucosyltransferase, EC 2.4.2.14) with its associate sucrose phosphate phosphatase [3,4] (sucrose-6-phosphate phosphohydrolase, EC 3.1.3.00) catalyze the following reactions:

$$\text{UDP-glucose} + \text{fructose} \rightleftharpoons \text{UDP} + \text{sucrose}$$

$$\text{UDP-glucose} + \text{fructose-6-P} \rightleftharpoons \text{UDP} + \text{sucrose-6-P}$$

$$\text{sucrose-6-P} \longrightarrow \text{sucrose} + P_i$$

The existence of these two separate mechanisms for the synthesis of sucrose raises the question of their respective roles in vivo [5]. Present views tend to stress that the physiological role of sucrose synthetase would be sucrose cleavage, while the role of sucrose phosphate synthetase coupled to sucrose phosphate phosphatase would correspondingly be that of sucrose synthesis [6]. Thus, sucrose synthesis would be the result of two consecutive, practically irreversible reactions. Since it is generally considered that irreversible reactions provide the most sensitive loci for exertion of metabolic control [7], a search for compounds acting as inhibitors or activators was carried out.

This paper reports the inhibitory action of sucrose on the activity of sucrose phosphate synthetase. Its kinetic analysis indicates the probable existence of two enzyme forms with markedly different affinities for sucrose. On the other hand, the data may also fit with an enzyme which exhibits negative cooperativity towards sucrose binding.

2. Materials and methods

All chemicals were purchased from Sigma Chemical Company, St Louis, MO. UDP-[^{14}C]glucose was obtained from the Instituto de Investigaciones Bioquímicas, Fundación Campomar, Buenos Aires. Sucrose phosphate synthetase from wheat germ was obtained as in [8].

Sucrose phosphate synthetase activity is generally estimated by the thiobarbituric acid method [9], but this method was not suitable in the presence of oligosaccharides. In this case the activity was determined by three alternative methods.

2.1. *Enzyme assay a*

Measurement of the formation of UDP using pyruvate kinase, by quantifying the pyruvate formed as 2,4-dinitrophenylhydrazone as in [10] with slight modifications*. Sucrose phosphate synthetase was

* It was reported [5] that sucrose interferes with pyruvate kinase. It was later found that this does not occur when the kinase is in excess

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assayed incubating in total vol. 0.05 ml, 0.5 μmol UDP-glucose, 0.5 μmol fructose-6-P, 5 μmol Hepes-K buffer, pH 6.5, enzyme and compounds to be tested. Incubation was carried out at 30°C and stopped by heating at 100°C for 1 min. Blanks were run with fructose-6-P added at the end of incubation. After cooling, 0.06 ml solution containing in μmol: Tris–HCl buffer pH 8, 10; PEP, 0.25; KCl, 10; $MgSO_4$, 2.5, and pyruvate kinase 15 μg (465 units/mg protein) was added. After 15 min at 37°C, the reaction was stopped by addition of 0.15 ml 2,4-dinitrophenylhydrazine 0.1% in 2 M HCl. After 5 min at room temperature, 0.2 ml 10 M NaOH and 0.5 ml ethanol were added and the tubes were shaken. The A_{520} was measured.

2.2 *Enzyme assay b*

Measurement of the formation of UDP previously separated from the other components of the reaction mixture by paper chromatography. The incubation was carried out as for the thiobarbituric acid method, but the reaction was stopped by heating at 100°C for 1 min. Blanks were run with fructose-6-P added at the end of incubation. Aliquots (40 μl) were spotted on Whatman No. 1 chromatographic paper and chromatographed in 1 M ammonium acetate (pH 7.5): 95% ethanol (3:7) by descending chromatography. The UDP areas were cut and eluted with HCl 0.1 M. UDP was determined by A_{260}.

2.3. *Enzyme assay c*

Measurement of labelled sucrose after submitting the sucrose phosphate formed to the action of alkaline phosphatase. The experimental procedure was as in [11].

One unit of sucrose phosphate synthetase activity catalyzes the formation of 1 μmol product/min. Activity measurements were carried out under conditions where velocity was linear with time and enzyme.

3. Results and discussion

The results presented in table 1 illustrate the effect of various sugars on the activity of sucrose phosphate synthetase. It can be seen that only sucrose produces a significant inhibition. Moreover, the sucrose inhibitory effect seems to be rather specific. This is supported by the fact that turanose (which has a similar structure) or raffinose and melezitose (trisaccharides where sucrose is part of their molecules) were not inhibitory.

Table 1
Effect of oligosaccharides

Oligosaccharide (50 mM)	UDP formed (nmol)	Activity (%)
None	19.5	100
Sucrose	10	52
Raffinose	20	102
Melezitose	20	102
Stachyose	21	107
Maltose	20	102
Cellobiose	19.5	100
Trehalose	19.6	100
Turanose	20	102
Melibiose	20	102

Activity was measured by assay a

The extent of sucrose inhibition varied with the enzyme batch from 18–60%. Furthermore, in some enzyme preparations the inhibition seemed to decrease with their age. This variability could explain the contradictory results [12,13] of no sucrose inhibition in enzyme preparations, and [14] 10% inhibition at 10 mM sucrose. Nevertheless, in order to ascertain that sucrose inhibition was not caused by the analytical procedures, sucrose phosphate synthetase activity was measured in the presence of sucrose using three different methods (assays a, b and c). All methods gave similar results confirming the sucrose inhibition.

Having ascertained the inhibitory effect of sucrose, it was studied kinetically varying the concentration of fructose-6-P and UDP-glucose, at fixed levels of sucrose. Figure 1 shows a double-reciprocal plot of initial velocity with varying concentrations of fructose-6-P in the presence of different concentrations of sucrose. This plot indicates that sucrose is a non-competitive inhibitor with respect to fructose-6-P. However, in the secondary plot, straight lines are not obtained when the slopes and intercepts of the primary plot are replotted as functions of sucrose concentration. Both replots are hyperbolic, indicating that the inhibition is a partial noncompetitive one. This inhibition occurs when the inhibitor causes the

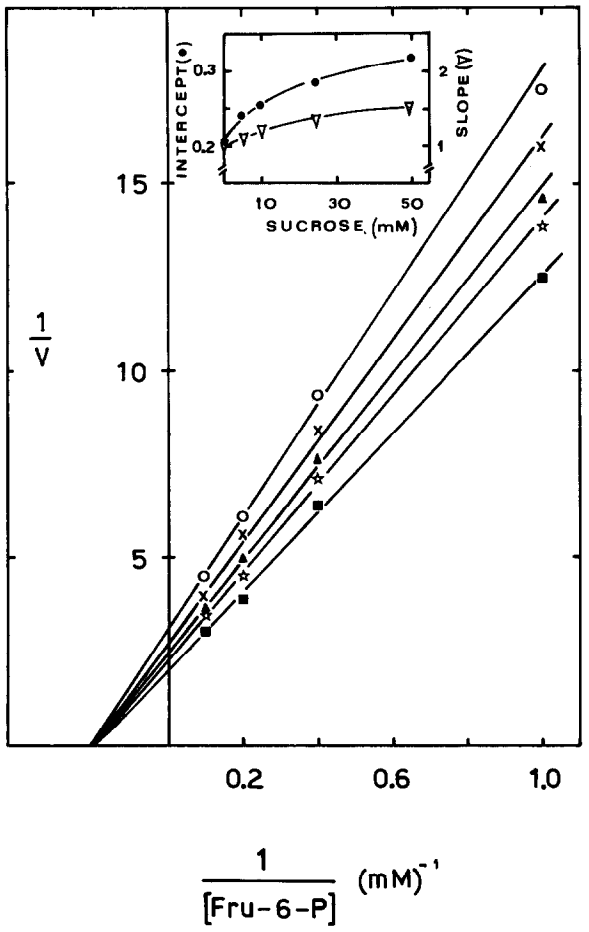

Fig.1. Effect of variable concentrations of fructose-6-P on initial velocity at different levels of sucrose: (☆) 5 mM; (▲) 10 mM; (X) 25 mM; (○) 50 mM; (■) no addition of sucrose. UDP-glucose concentration was fixed (10 mM). Activity was measured by assay c. Velocity is in μmol sucrose-6-P/min/ml. In the secondary plot slopes and intercepts of the primary plot are replotted as a function of sucrose concentration.

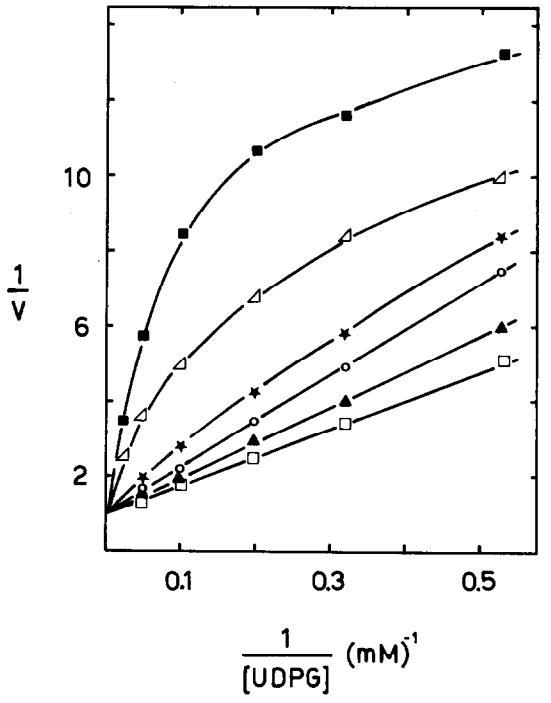

Fig.2. Effect of variable concentrations of UDP-glucose on initial velocity at different levels of sucrose: (▲) 5 mM; (○) 20 mM; (★) 50 mM; (◿) 100 mM; (■) 200 mM; (□) no addition of sucrose. Fructose-6-P concentration was fixed (10 mM). Activity was measured by assay a. Velocity is in μmol/UDP formed/min/ml.

reaction flux to be diverted to an alternate pathway that is slower than the normal one [15].

The inhibitory effect of sucrose when UDP-glucose is the variable substrate and fructose-6-P the fixed one, is shown in fig.2. Here it can be seen that the plots are actually curved. At high substrate concentrations and high inhibitor concentrations the curvature of the reciprocal plots in the region close to the $1/v$ axis is more obvious. For a unireactant enzyme this type of kinetic can be observed, according to [16], for multiple forms of the same enzyme that catalyze the same reaction but are unequally sensitive to a given inhibitor. (If the system is multireactant, the kinetic will be similar, except that the kinetic constants are apparent constants for the given concentration of co-substrate which is held constant).

However, when the data presented in fig.1,2 were plotted according to the Hill equation, the apparent order of reaction n for UDP-glucose was 0.5 at 100 mM sucrose, while for fructose-6-P it was 0.8 at 50 mM sucrose. This would suggest that sucrose phosphate synthetase exhibits negative cooperativity towards sucrose binding [17].

The similarity in structure between sucrose and sucrose phosphate would suggest that the former may occupy the site of the latter on the enzyme. However, it should be remembered that sucrose phosphate does not inhibit the reaction [3]. This was confirmed [8].

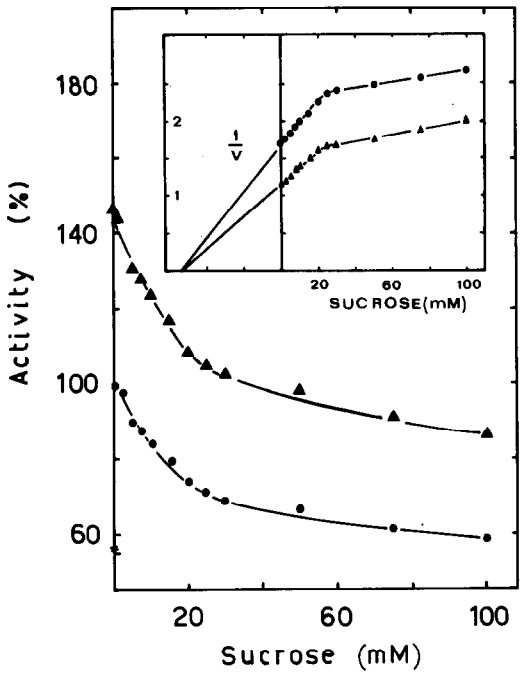

Fig.3. Effect of sucrose concentration: (•) activity in the absence of Mg^{2+} and (▲) in the presence of Mg^{2+} 20 mM. Activity was measured by assay a. The inset shows the data plotted according to [18].

Thus, it seems reasonable to assume that sucrose and sucrose phosphate occupy different sites on the enzyme.

The inhibitory action of sucrose was also studied in the presence of Mg^{2+}, an anion that produces a stimulative effect on sucrose phosphate synthetase activity. The results presented in fig.3 indicate, firstly, that sucrose inhibition takes place at disaccharide levels normally found in plant tissue and, secondly, that the inhibition approaches a plateau when higher concentrations of sucrose are attained. Activity is reduced to 60% of its value in the presence of sucrose 60–100 mM. In contrast, addition of 20 mM Mg^{2+} resulted in regaining activity to control values, depending on the initial concentration of sucrose present. The inset of fig.3 also shows that when the data are plotted according to [18], linear plots are obtained which seems to have two distinct regions that can be fitted with different straight lines. This plot again suggest the presence of two enzyme forms with different reactivity towards sucrose: one with K_i 50 mM, while the other apparently would be one order of magnitude higher. Mg^{2+} addition does not change the pattern neither influence K_i values.

The inhibition of sucrose phosphate synthetase by sucrose could act as a control mechanism of its activity. The results presented here do not, however, permit to decide the type of mechanism. The data seem to indicate that the enzyme may exist in two forms: one very sensitive to sucrose inhibition, while the other one not. If this were the case, it is tempting to speculate that the regulation of sucrose phosphate synthetase may take place by appearance and disappearance of the enzyme form susceptible to sucrose inhibition. The detection of isoenzymes of sucrose phosphate synthetase [19] as well as the change of sucrose inhibition with age, may point in this direction.

The existence of isoenzymes will also produce the appearance of negative cooperativity in terms of Hill coefficients of less than one [17]. Nevertheless, the possibility that sucrose phosphate synthetase may be a negatively cooperative enzyme should not be ruled out yet. An enzyme with this characteristic would also be conveniently built to deal with the high concentration of sucrose existing in plant tissue.

It should be remembered that sucrose also inhibits sucrose phosphate phosphatase [20]. However, the inhibition found for sucrose phosphate synthetase seems to be more useful for the cell economy, as it would allow the use of UDP-glucose for other reactions.

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