STUDIES ON SUCROSE PHOSPHATE SYNTHETASE: REVERSAL OF UDP INHIBITION BY DIVALENT IONS

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1. Introduction

The enzyme sucrose phosphate synthetase (UDP-glucose: D-fructose-6-phosphate-2-glucosyltransferase, EC 2.4.1.14) catalyzing the reaction:

UDP-glucose + fructose-6-P → UDP + sucrose-6-P

is widespread in plant tissues [1–9]. It has been purified and its kinetic properties studied from wheat germ [2], broad bean cotyledons [10], rice scutellum [11], and potato tubers [9]. The effect of a number of cations on enzyme activity has been investigated in several preparations of sucrose phosphate synthetase [9,11,12]. Mg^{2+} and Mn^{2+} have been shown to produce a stimulative effect. Particularly, Preiss and Greenberg [13] found that Mg^{2+} decreases the concentration of UDP-glucose needed for half maximal activity. It has also been demonstrated, first by Mendicino [2], that UDP is a strong inhibitor of sucrose phosphate synthesis. However, no connection has been indicated to exist between Mg^{2+} or Mn^{2+} stimulation and UDP inhibition. We wish to report here that this inhibition can be reversed by Mg^{2+} or Mn^{2+}. No difference could be observed when the effect was studied at different pH.

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2. Materials and methods

All chemicals were purchased from Sigma Chemical Company, St. Louis, MO. Divalent cations were used as chloride salts.

Unless stated otherwise, all procedures were carried out in the cold.

Wheat germ (300 g) was suspended in 5 mM Tris-HCl buffer, pH 8, containing 5 mM mercaptoethanol and 10 mM KCN (900 ml). The suspension was occasionally stirred and after 1 h it was filtered through four layers of cheese-cloth. The filtrate was centrifuged at 27 000 *g* for 30 min and the supernatant dialyzed against three changes of 8 liters 20 mM Tris-HCl buffer, pH 8, containing 5 mM mercaptoethanol and 5 mM EDTA. The dialyzate was brought to 40% saturation with solid ammonium sulphate. The precipitate formed was removed by centrifugation and discarded.

The ammonium sulphate concentration of the supernatant was increased to 45% saturation and the precipitate collected by centrifugation at 27 000 *g* for 20 min. The precipitate was suspended in the minimum volume of 20 mM Tris-HCl buffer pH 7.5 containing 5 mM mercaptoethanol and 5 mM EDTA. The suspension was then dialyzed against a 40% saturated ammonium sulphate, pH 7, solution overnight. The equilibrated 40% ammonium sulphate suspension was centrifuged at 40 000 *g* for 30 min. The supernatant was separated and the precipitate was extracted with 1 ml of 40% ammonium sulphate, separating the supernatant by centrifugation at 40 000 *g* for 20 min.

This procedure was repeated twice. After deter-

mining enzyme activity, the supernatants richest in sucrose phosphate synthetase activity were pooled. This step allows the separation of sucrose phosphate synthetase and sucrose synthetase.

The resultant solution was dialyzed against 20 mM Tris-HCl, pH 7.5, containing 5 mM EDTA and 5 mM mercaptoethanol. The dialyzate was chromatographed in a DEAE column 2 X 20 cm equilibrated with the same buffer. After passing the sample, a linear sodium chloride gradient 0.1 to 0.8 M made up in the equilibration buffer, was started. The fractions with enzyme activity were pooled and concentrated in an Amicon ultrafiltration cell with a PM-10 membrane. The concentrate was used for the studies without further purification. The preparation catalyzed the formation of 0.2 μmol of sucrose-6-P per mg of protein per min, and it was free of sucrose synthetase and phosphatases activities.

Sucrose phosphate synthetase was assayed incubating in a total volume of 0.05 ml, 0.25 μmol UDP-glucose, 0.5 μmol Fru-6-P, 1 μmole NaF, 5 μmol HEPES buffer pH 6.7, enzyme, and compounds to be tested. After incubation at 30°C the sucrose phosphate formed was measured following the procedure already indicated by Cardini et al. [14] but measuring the colour developed with the method of Percheron [15]. Activity measurements were carried out under conditions where velocity was linear with both time and enzyme.

3. Results and discussion

It has already been shown for sucrose phosphate synthetase from other sources that divalent cations Mg^{2+} and Mn^{2+} activate the synthesis of sucrose phosphate [11–13,16]. It has also been shown before by Mendicino [2] for the wheat germ enzyme, that UDP is a strong competitive inhibitor. The effect was confirmed by Slabnik et al. [9] as well as by Nomura and Akazawa [11].

Preiss and Greenberg [13] on the other hand, working with the same wheat germ enzyme as Mendicino, reported no inhibition by UDP at 1 mM. Moreover, Fekete [10], speculating on these results, suggested that the enzyme used by Preiss and Greenberg was devoid of an activator, which would make the enzyme sensible to UDP inhibition. However,

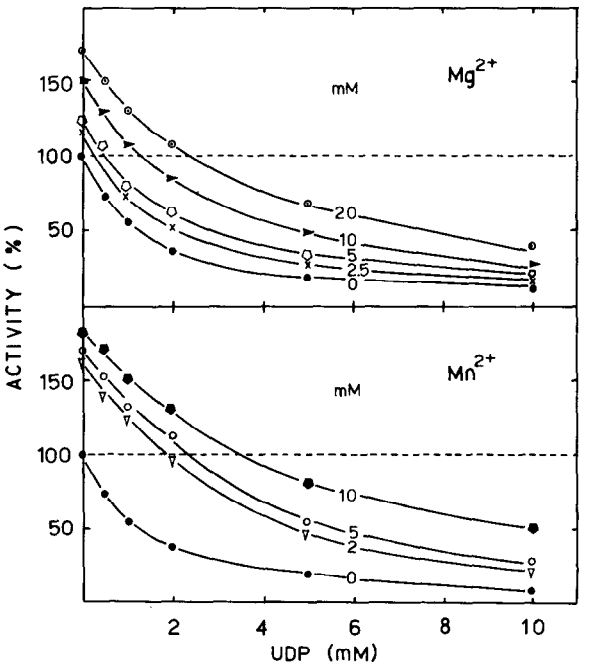

Fig.1. Reversal of UDP inhibition of modifying Mg^{2+} and Mn^{2+} concentration. The activity in the absence of UDP or divalent ions is taken as 100% (dotted line).

Preiss and Greenberg did include 22 mM Mg^{2+} in their reaction mixture. As can be seen in fig.1, UDP does indeed inhibit the sucrose phosphate synthetase reaction in the absence of Mg^{2+} or Mn^{2+}. Activity is reduced 80–90% of its value in the presence of UDP between 5–10 mM. In contrast, addition of Mg^{2+} or Mn^{2+} resulted in regaining activity to control values depending on the initial concentration of UDP present and the final divalent ion concentration attained. Thus, 5 mM Mn^{2+} fully reversed the inhibition caused by 2 mM UDP, while a higher Mg^{2+} concentration, 10 mM, was needed to obtain the same reversal.

The inhibition of sucrose phosphate synthetase by UDP is slightly altered by modifying the pH. The results presented in fig.2 indicate that 2 mM UDP produce about 40–60% inhibition between pH 5.5–7.5, with a plateau around pH optimum. The same figure shows that addition of 20 mM Mg^{2+} removes the inhibition and even more produces a slight activation above control values. For reference the activation effect produced in the reaction when Mg^{2+}

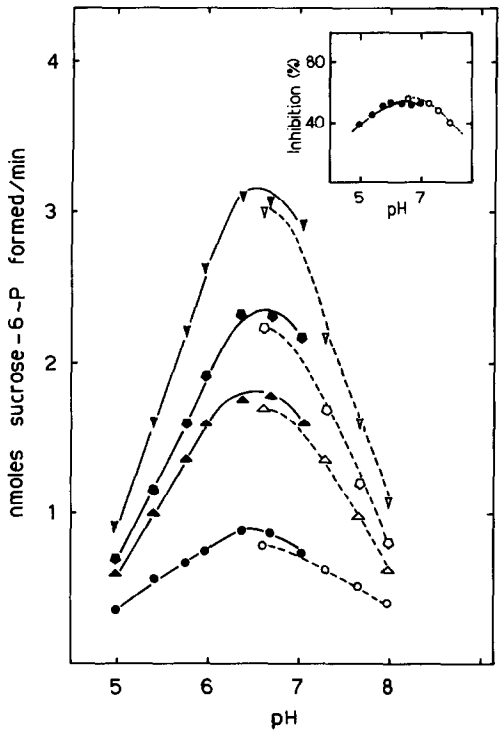

Fig.2. Effect of pH on sucrose phosphate synthetase activity. Closed symbols with MES buffer, open symbols with HEPES buffer. (▲–△) Control, no addition; (●–○) UDP 2 mM; (◔–◕) UDP 2 mM, Mg^{2+} 20 mM; (▼–▽) Mg^{2+} 20 mM. The inset illustrates the variation of the per cent inhibition by 2 mM UDP with pH.

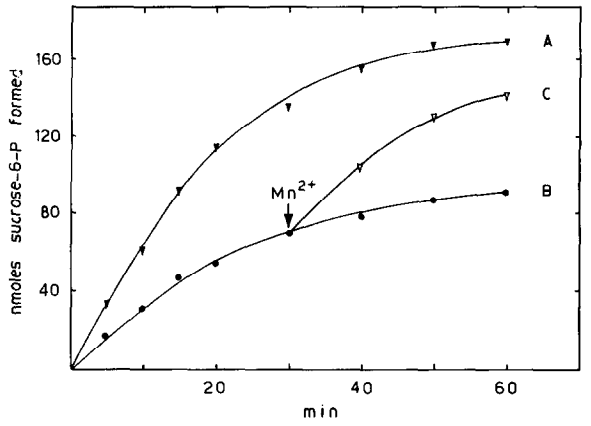

Fig.3. Effect of adding Mn^{2+} in the time curve. (A) Enzyme with 5 mM Mn^{2+}. (B) Control without addition of divalent ion. (C) At the time indicated by the arrow Mn^{2+} was added so the final concentration was the same as in curve A.

is added alone can also be seen. The presence of Mg^{2+} or Mn^{2+} could then regulate to a certain extent the activity of sucrose phosphate synthetase. The experiments presented in fig.3 illustrate the decrease of the rate of reaction with time as UDP accumulates.

When Mn^{2+} at 5 mM was present from the start of the incubation, the reaction was linear for at least 20 min (curve A). On the other hand, when the enzyme was incubated in the absence of divalent ion, the rate of the reaction slowed down earlier. On addition of Mn^{2+} to the same concentration as curve A, the inhibition produced by the UDP accumulated is removed allowing the reaction to proceed almost linearly again (curve C).

It should be mentioned that although the results with the same batch of the enzyme were reproducible, there was a certain variability from one preparation to another, especially in the extent of the activation by the divalent ions. The UDP inhibition was always reproducible.

The experiments described here indicate that Mg^{2+} or Mn^{2+} may exert a regulatory action on the activity of sucrose phosphate synthetase. It should be pointed out that a similar effect has been demonstrated for sucrose synthetase. In this latter case, the action is perhaps more relevant as divalent ions activate sucrose synthesis while inhibiting its cleavage. The fact that the two enzymes capable of synthetizing sucrose are affected by Mg^{2+} or Mn^{2+} and that in both cases the divalent ion can revert product inhibition, is significant. Furthermore, divalent ions play a role also on the activity of sucrose phosphate phosphatase [17]. However, it is difficult to asses its physiological significance. The present results, as has already been pointed out for sucrose synthetase [18], could be related with those of Gustafson and Gander [19]. These authors have suggested that the soybean plant might regulate the direction of catalysis by UDP-glucose pyrophosphorylase, if it could regulate the intracellular or intracompartment concentrations of free magnesium ion. Thus, an increase in Mg^{2+} or Mn^{2+} concentration would result in the enhancement of UDP-glucose synthesis. As a consequence, sucrose phosphate formation will also increase not only

because the substrate level (UDP-glucose) has risen, but also because the increase in magnesium ion concentration would reverse the inhibition by the UDP produced.

Acknowledgements

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