Phosphoglucomutase of Green Gram (*Phaseolus radiatus*)

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Introduction

Phosphoglucomutase, the enzyme which converts glucose 1-phosphate (G 1-P) to glucose 6-phosphate (G 6-P) has been shown to occur in some plant materials. Hanes (1) detected the presence of the enzyme in pea extracts. Sisakyan and Kobyakova (2) demonstrated its activity in tomato leaf plastids. Recently Cardini (3) extracted the enzyme from jack bean seeds and studied some of its properties. Onodera (4) also reported the occurence of the enzyme in broad bean extracts.

In a previous communication from this laboratory, Sri Ram and Giri (5) showed the presence of two starch-synthesizing enzymes, viz., phosphorylase and Q-enzyme, in the extracts of green gram (*Phaseolus radiatus*). In view of the existence of the Embden-Myerhof glycolytic pathway in higher plants, it was of interest to investigate the occurence of other enzymes like phosphohexose isomerase and phosphoglucomutase in this source.

The present communication relates to the separation and characterization of phosphoglucomutase in green gram.

Experimental

The substrate G 1-P (potassium salt) was prepared enzymatically by the method of Hanes (6) and was recrystallized thrice from alcohol (50%).

Estimation of the Enzyme Activity

The enzyme activity was determined by estimating the unconverted G 1-P. This was done by measuring the inorganic phosphorus liberated on hydrolysis with *N* H_2SO_4 in a boiling water bath for 7 min. G 6-P is not hydrolyzed under these conditions. The decrease in inorganic phosphorus is an index of the G 6-P formed.

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Inorganic phosphorus was determined by the method of Fiske and SubbaRow (7).

A 2.5-ml. reaction mixture was always used, composed of 1 ml. of the enzyme preparation, and 1.5 ml. of veronal buffer of pH 7.5 in which are dissolved the substrate and magnesium sulfate, the final concentrations of the substrate, Mg^{++}, and the buffer being respectively, 10 mM, 2 mM, and 0.02 M. The reaction was carried out at 36° for 15 min. and was stopped by adding 5 ml. of 5% trichloroacetic acid. The precipitated proteins were filtered off and an aliquot of the filtrate was analyzed for G 1-P.

The activity of the enzyme was always adjusted such that 15–20% of G 1-P was converted to G 6-P under the conditions of the experiment.

Preparation of the Enzyme

The resting seeds of green gram were finely powdered and the powder was extracted with five times its weight of water under a layer of toluene for 12 hr. at 0°. The extract was centrifuged and the supernatant was dialyzed against running distilled water overnight and centrifuged again. This clear extract exhibited qualitatively similar properties as the partially purified product, obtained as follows.

The crude extract was adjusted to pH 5.0 with acetic acid and was heated to 45° with stirring. It was kept at this temperature for 10 min. and was rapidly cooled to 0° and the precipitate removed by centrifugation. To 100 ml. of the centrifugate, 30 g. of solid ammonium sulfate was added and the precipitate discarded. To the supernatant was added 15 g. of the salt, and the precipitate was collected by centrifugation. This was dissolved in a convenient volume of water and dialyzed as before, free of ammonium sulfate which interferes with the estimation of enzyme activity. The first precipitate could be refractionated as described to yield more material. By this procedure, a fourfold purification was achieved on the basis of milligrams of phosphorus converted into acid-stable form per milligram of nitrogen. Attempts at further fractionation resulted in much loss of the activity. The preparation was devoid of phosphorylase activity.

Results and Discussion

Phosphoglucomutase of green gram was found to be active over a wide range of pH with an optimum around pH 7.5 (Table I). The optimum temperature of the enzyme was 45° (Table II). The enzyme was completely inactive at 60° due to possible denaturation.

The crude extract was active without the addition of any activators. On prolonged dialysis much of the activity was lost indicating that some activators are present in the crude extract. The activity could be regained by the addition of Mg^{++} ions. Optimum activation was obtained at a concentration of 2 mM of Mg^{++} ions (Table III). Mn^{++} and Co^{++} also showed a similar activation at 1 mM concentration. Ni^{++} inhibited the enzyme activity. Higher concentration of Mg^{++} and Co^{++} inhibited

TABLE I

Effect of pH on Enzyme Activity

The reaction mixture contained 1.0 ml. of enzyme solution, 1.0 ml of substrate solution with magnesium sulfate, and 0.5 ml. of veronal buffer at various pH's. The final concentrations of G 1-P, Mg^{++}, and veronal were 10 mM, 2 mM, and 0.02 M, respectively. Other conditions were the same as specified in the text for the determination of the enzyme activity.

pH	Per cent conversion of G 1-P
6.8	10.6
7.2	12.6
7.5	16.0
7.8	12.6
8.2	7.4

TABLE II

Effect of Temperature on Enzyme activity

The experimental conditions were the same as specified in the text except that the temperature was varied.

Temperature °C.	Per cent conversion of G 1-P
16	6.8
25	9.7
35	22.6
45	45.2
50	40.0
55	15.3
60	Nil

the enzyme due to unspecific saline action as observed by previous workers (8, 9).

Sodium sulfite inhibited the enzyme in the presence of veronal buffer but activated in its absence. The optimum concentration of sodium sulfite for activation was between 10 and 20 mM (Table IV) which is in conformity with the finding of Jagannathan and Luck (9).

The effect of substrate concentration on the enzyme activity is shown in Table V. There is a large increase in activity with the substrate concentration in the range 3–10 mM. A slight inhibition is noted at higher concentrations of the substrate.

The activity was proportional to the time of incubation until about 60 % of the substrate was converted (Fig. 1). At equilibrium about 95 % of the substrate was found to be converted in agreement with the finding of previous workers (3, 8, 9).

TABLE III

Effect of Mg^{++} Concentration on Enzyme Activity

The experimental conditions were the same as specified in the text except that Mg^{++} concentration was varied.

Concentration of Mg^{++} m*M*	Per cent conversion of G 1-P
0.0	15.0
0.2	16.5
1	19.5
2	21.3
4	18.8
10	13.8
20	9.5
100	3.8

TABLE IV

Effect of Sodium Sulfite on Enzyme Activity

The experimental conditions were the same as specified in the text except that the veronal buffer was omitted as indicated. Sodium sulfite was added to make the required final concentration.

	Per cent conversion of G 1-P		
	No Buffer		0.02 *M* Buffer
Concentration of Na_2SO_3 m*M*	No Mg^{++}	2 m*M* Mg^{++}	2 m*M* Mg^{++}
0	10.0	18.8	13.0
2	11.6	19.2	6.0
5	13.2	28.4	8.0
10	18.4	29.6	7.0
20	19.6	27.6	6.0
40	12.8	16.0	3.2

The influence of various salts on the activity of the enzyme is presented in Table VI. The finding that Na ion is an inhibitor for phosphoglucomutase is in accord with that of Cori *et al.* (10), which, however, was not noticed by Jagannathan and Luck (9). Among the inhibitors tried, mercuric chloride was the most effective at a concentration of 0.05 m*M*.

Versene inhibited the enzyme completely at a concentration of 10 m*M*. The versene-treated and dialyzed enzyme could be reactivated by Mg^{++} and Ba^{++} at an optimum concentration of 2 m*M*. A similar observation was made by Sable and Calkins (11). Other metal-binding agents such as cysteine, histidine, and 8-hydroxyquinoline did not affect even the crude undialyzed extracts, while they produced slight inhibition of the dialyzed enzyme in the range of concentration of 5–50 m*M*.

TABLE V

Effect of Substrate Concentration on Enzyme Activity

The experimental conditions were the same as specified in the text except that the substrate concentration was varied.

Substrate concentration *mM/l.*	Amount of G 1-P converted *mM/l.*
3.0	0.135
6.5	0.767
10.0	2.200
13.0	1.755
16.0	1.632

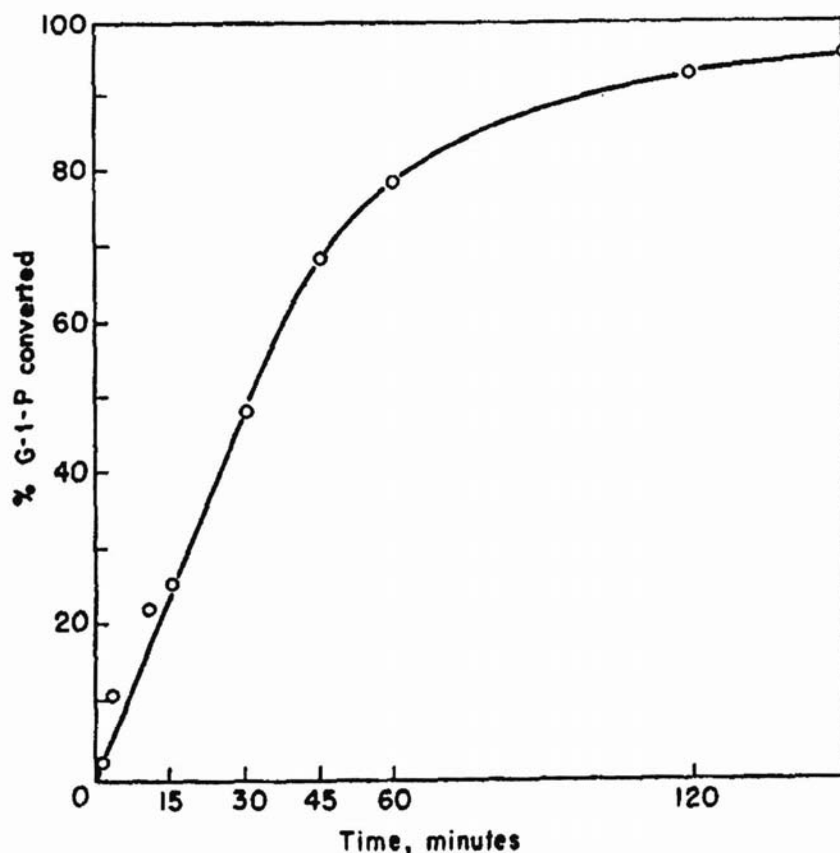

FIG. 1. Time course reaction of phosphoglucomutase activity and equilibrium between G 1-P and G 6-P. The experimental conditions were the same as specified in the text. Aliquots were withdrawn at various time intervals and were analyzed for G 1-P.

Phosphoglucomutase of green gram does not appear to depend on functional —SH groups for its activity, since iodoacetic acid at a concentration of 4 m*M* did not affect its activity when added immediately before the reaction. Preincubation of the enzyme with iodoacetic acid for 15 min., however, produced slight inhibition.

The foregoing studies were carried out without the addition of the known coenzyme, glucose 1,6-diphosphate, to the reaction mixture since

TABLE VI

Effect of Metallic Ions on Enzyme Activity

The experimental conditions were the same as specified in the text except for the additions.

Additions	Concentration *mM*	Per cent inhibition
NH_4Cl	50	18.7
NaCl	50	30.3
KCl	50	32.7
$CaCl_2$	25	100.0
$BaCl_2$	10	100.0
$ZnSO_4$	5	100.0
KCN	5	48.0
$PbNO_3$	1	70.0
$CuSO_4$	0.05	66.0
$AgNO_3$	0.05	73.0
$HgCl_2$	0.05	100.0

the needed catalytic amounts of the coenzyme were associated with the substrate. It was however found that the enzyme could be highly activated by further addition of the coenzyme. Using a coenzyme preparation of about 80% purity, it was found that maximum activation was produced by 1.6×10^{-5} to 1.6×10^{-4} M of it. At this concentration of the coenzyme, the reaction reached equilibrium (about 95% conversion) under the experimental conditions previously described, whereas in the absence of the coenzyme the percentage conversion of G 1–P was 25.3%.

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Summary

Phosphoglucomutase of green gram (*Phaseolus radiatus*) was partially purified by fractionation with ammonium sulfate following heat treatment.

The enzyme was activated by Mg^{++}, Mn^{++}, Co^{++}, and sodium sulfite. Among the several inhibitors for the enzyme are Na^{+}, Hg^{++}, Ag^{++}, Cu^{++}, KCN, and Ca^{++}. Metal-binding agents such as cysteine do not activate this enzyme.

The optimum pH of the enzyme was around pH 7.5. Temperature optimum was 45°. The enzymatic conversion of G 1-P to G 6-P attains equilibrium when about 95% of the substrate is converted.

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