Pak. J. Bot., 4 (2): 111—116, 1972.

COMPARATIVE STUDY OF PYRUVIC KINASE AND PHOSPHATASE IN GLYCOPHYTIC AND HALOPHYTIC SPECIES OF BEET ROOT

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Abstract

Effect of sodium ion concentration on potassium dependent pyruvic kinase reaction was investigated in glycophytic (*Beta vulgaris*) and halophytic (*Beta maritima*) plants. It was found that synthesis of pyruvic acid is mainly through phosphatase in *B. vulgaris*. Where as this was partly undertaken by kinase in *B. maritima*. These readjustments were attributed as biochemical adaptation in physiology of salt tolerance.

Introduction

Several enzymes like pyruvic kinase, acetothiokinase and those responsible for incorporation of amino acids in protein require specific cations for their actions. Presence of higher concentrations of sodium in the plants growing on saline soils is expected to show antagonism towards the other cations present in the system. This might cause inhibition of cation dependent specific enzymatic reactions. Since halophytes grow luxuriantly on saline substrate, it appears that either presence of greater concentration of sodium does not hinder the cation dependent enzymatic reactions or this interference is overcome by channelization of other non-specific enzymes. Present work is an attempt to find out the proper explanation for the same.

Glycophytic and halophytic species of a genus are often found growing in the nature, eg., *Beta vulgaris* is cultivated on non saline soils whereas *Beta maritima* grows in salt marshes near sea shore. Effect of sodium on potassium dependent pyruvic kinase reaction was investigated in B. *vulgaris* and *B. maritima*. Activity of phosphatase was also taken into consideration.

Materials and Methods

Acetone powders of *Beta vulgaris*, grown in the green house and *Beta maritima* growing in salt marshes were prepared. Fresh leaves were immediately frozen in solid carbon dioxide and thoroughly ground in a prechilled wareing blender in acetone cooled to 30°C. The supernatant was discarded and blending was continued by adding fresh amount of cool acetone. The slurry was filtered with suction and powder was again washed with cool acetone on buchner funnel. Acetone powder was dried in vacuo and kept in deep freezer.

One gm of acetone powder was shaken vigourously for 15 minutes in 20 ml of .05 M. HCl Tris buffer adjusted at pH 7.5. Extract was centrifuged at 20,000 g at 0°C.

Supernatant was dialysed against two fresh volumes of above mentioned buffer for eight hours. Enzymatic protein was determined using Folin Phenol reagent.

Activity of enzyme pyruvic kinase was determined by estimating the amount of pyruvate formed after enzymatic action on phosphoenole pyruvate (PEP) according the following equation:-

$$\text{i)}\quad PEP + ADP \xrightarrow[Mg^{++},\ K^{+}]{\textit{Pyruvic kinase}} \text{pyruvate} + ATP$$

$$\text{ii)}\quad PEP + H_2O \xrightarrow{\textit{Phosphatase}} \text{pyruvate} + Pi$$

Reaction mixture without ADP served as negative control (ii) and the amount of pyruvate formed was substracted from (i) to calculate pyruvate formed due to the activity of pyruvic kinase only. The reaction mixture contained the following:-

Stock solution:

Tris buffer (pH 7.5)	1.00M 1.25 ml
PEP	
(Tricyclohexylamine salt)	0.01M 3.75 ,,
ADP	0.01M 6.25 ,,
$MgSO_4$	0.10M 2.50 ,,
KCI	1.00M 0.05 ,,

made upto 25 ml by distilled water

1 ml of reaction mixture contains

Tris	50 μ mol
PEP	1.5 μ mol
ADP	2.5 μ mol
$MgSo_4$	10 μ mol
KCI	50 μ mol

Reaction mixture without ADP gave the amount of pyruvate formed due to the activity of phosphatase only. Pyruvic acid was estimated by the method of Kachmar & Boyer (1953). Enzyme extract containing about .6 mg protein was added to 1 ml reaction mixture and incubated at 37°C for ten minutes. Reaction was stopped by adding 3 ml cold 10% TCA. The volume of reaction mixture was adjusted upto 10 ml

by distilled water and centrifuged to remove the precipitate, 1 ml of .025 × dinintrophenyle hydrozine (made in 2N HCI) was added while shaking. Later, 6 ml of .5 N NaOH was added and tubes were allowed to stand for 10 minutes to develop colour. Optical density was taken at 510 n.m. and the amount of pyruvic acid was calculated from a standard curve.

Observations and Results

Data presented in Table 1 shows that .6 mg protein of enzyme complex is optimum concentration for the activity of pyruvic kinase, whereas activity of phosphatase kept on increasing with the increase of enzymatic protein during the period of experiment. Contents of Table 2 bring in light some interesting effects of sodium chloride on the activities of above mentioned enzymes in glycophytic and halophytic plants.

Table 1. Effect of enzyme complex concentration of *Beta vulgaris* on the activities of pyruvic kinase and phosphatases.

Concentration of enzymatic protein (mg)	Pyruvic acid formed due to phosphatases (μ mol)	Pyruvic acid formed due to kinase (μ mol)
.1	.12	.02
.2	.26	.06
.3	.42	.06
.4	.50	.06
.5	.52	.08
.6	.54	.12
.7	.64	.11
.8	.66	.10
.9	.70	.06
1.0	.76	Nil

Table 2. Effect of Sodium chloride on the activities of pyruvic kinase and phosphatases.

Concentration of NaCl.	*Beta vulgaris*		*Beta maritima*	
(μ mol)	Pyruvic Acid formed due to kinase (μ mol)	Pyruvic Acid formed due to phosphatase (μ mol)	Pyruvic Acid formed due to kinase μ mol	Pyruvic Acid formed due to phosphatases (μ mol)
Control (without NaCl)	.02	.76	.26	.32
20	.00	.76	.23	.26
40	.06	.72	.23	.26
60	.08	.70	.24	.25
80	.11	.70	.27	.25
100	.09	.66	.28	.21
120	.07	.55	.29	.20
140	.07	.55	.26	.20
160	.05	.55	.22	.20
180	.03	.55	.22	.18
200	.02	.53	.13	.18

Following conclusions could be drawn after critical analysis of the same.

(i) Whereas both the enzymes are equally responsible for the formation of pyruvate in the leaves of beet root growing in the salt marshes. Conversion of phenole pyruvic acid into pyruvic acid is mostly the result of the activity of phosphatase in the leaves of *Beta vulgaris* and pyruvic kinase has very little to do with it.

(ii) Activity of pyruvic kinase shows a slight rise with the initial increase of salt concentration. It reaches an optimum and then starts falling gradually to a minimum value. This effect is same on the enzymes extracted from both the type of plants.

(iii) Phosphatase activity shows a gradual decrease with increase in salt concentration. However, this inhibitory effect is more pronounced in the enzyme extracted from glycophytic species. The activity of phosphatase is much lower in halophytic in comparison with glycophytic species.

Discussion

Potassium is reported to be responsible for increasing the activity of pyruvic kinase in many plants (McCollum *et al.* 1959; Harold & Evans, 1963). Horovitz & Waisel (1970) noticed that addition of NaCl in growth medium resulted in stimulation of soluble ATPase activity in bean and corn but induced inhibition in its activity in *Suaeda* and *Atriplex* roots. Ahmad & Hewitt (1971) found an increase in the activity of PEP-phosphatase and a decrease of acid phosphatase in *Suaeda fructicosa* grown in saline culture.

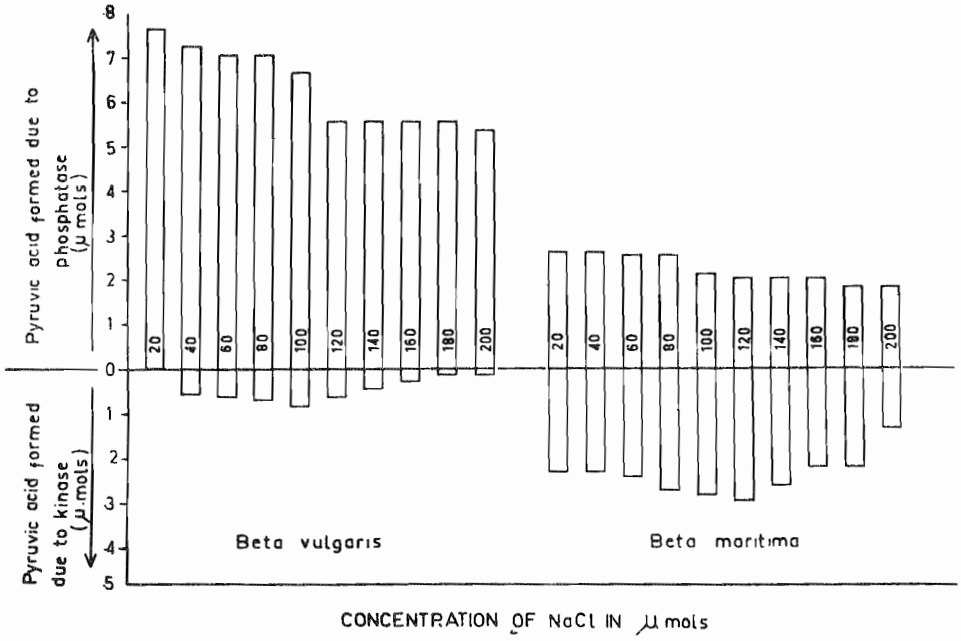

Fig. 1. Effect of sodium chloride on the pyruvic kinase and phosphatase in glycophytic and halophytic beet root.

In the present investigation NaCl was found to show almost similar effects on the activities of kinase and phosphatase irrespective of glycophytic or halophytic species. Activity of pyruvic kinase rises to an optimum with the increase of salt concentration and then gradually declines in both the kinds of plants, whereas, phosphatase activity decreases with the increasing salt concentration in reaction mixture. There seems to be a readjustment in the activities of various enzymes in halophytes. Synthesis of pyruvic acid, which is mainly through phosphatases in *Beta vulgaris*, is partly taken over by kinase in *Beta maritima* (Fig. 1). Diversions in metabolic pathways enable

the halophytes to overcome the inhibitory effects of cation dependent enzymatic reactions and thus growth and development is not affected.

Acknowledgements

The author wishes to express his gratitude to Dr. E.J. Hewitt for his encouragement and providing necessary facilities during this work at Long Ashton Research Station, University of Bristol. The receipt of bursary from Nuffield Foundation and grant of sabbatical leave from the University of Karachi are also gratefully acknowledged.

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