Pak. j. sci. ind. res., vol. 32, no. 1, January 1989

INVESTGATION OF INVERTASE ACTIVITY FROM *OXYSTELMA ESCULANTUM* R. BR. PLANT

A.N. Memon, M. Umer Dahot and A.R. Memon

Department of Biochemistry, Institute of Chemistry, Univresity of Sind, Jamshoro

(Received November 10, 1988, revised January 22, 1989)

Invertase activity was determined in crude exract of *Oxystelma esculantum* R. Br. plant by the use of sucrose as a substrate. The optimum pH and temperature was found to be 5.5 and 30° respectively. Vitamin C, potassium cyanide and cobalt chloride stimulated 15.0, 25 and 25% activity whereas marked decrease in invertase activity occured with $CuSO_4$, urea, calcium chloride and EDTA (87.5, 75, 75 and 62.5%) respec - tively.

Keywords: Oxystelma esculantum R. Br., Root tuber, Invertase.

INTRODUCTION

Sucrose is important in higher plants both as a storage compound and as a translocation form of photosynthetic products. Inspite of its importance the degradation of sucrose is not well understood. Among the degradative enzymes of sucrose, acid invertases are wide spread in plants. There are reports of a good correlation between invertase increment and plant growth [1-4], but the function of plant invertases is poorly understood so far.

Now a days glucose and fructose syrup is commercially produced by enzymatic process by degradation of sucrose with invertase enzyme [5]. Invertases can be classified into insoluble (bound to cell wall) or soluble (present in the cell) according to their association with cellular components. Soluble acid invertases are thought to be vacudar enzyme, as shown in the case of beet root [6], carrot, potato and red beet [7], cultured tobaco cells [8], *Ricinus communis* leaves [9] and date fruit [10].

Invertases can also be calssified, on the basis of their optimum pH, like acid invertases, neutral invertases and alkaline invertases. Glasziou [11] has reported that sugar cane contains acidic (pH 5.0 - 5.5) and neutral (pH 7.0) invertases. On other hand acidic and alkaline invertases were reported to be present in soybean nodules [12] and root nodules of *Lupinus angustifoltus* [13].

Oxystelma esculantum (Asclepiadaccae family), a wild growing plant, commonly grows in sandy hill slopes in deserted areas of Pakistan, India, Sri Lanka and Java. Tuber part of this plant is commonly used by the people of Tharparkar as a fruit and vegetable.

However, a general survey on chemical analysis and enzymes activities in seeds, fruits and tuber of plants of arid origin have been reported earlier from this laboratory [14-17]. *Oxystelma esculantum* plant tuber which is available in large quantity locally during the monsoon season, was selected for undertaking detail investigation on the characterisation of its invertase activity.

MATERIALS AND METHODS

Chemicals. All reagents were of analytical grade and were used without further purification. Sucrose was the product of BDH chemicals, 3, 5-dinitrosalicylic acid was obtained from Sigma chemicals, bovine serum albumin was obtained from E. Merck chemicals.

Plant material. Root tuber of *Oxystelma esculantum* (Kondhir) were collected in the months of June-September after monsoon rain from diplo District Tharparkar, Sind, (Pakistan).

Preparation of crude enzyme extract. The enzyme extract was prepared according to the previous report [18]. 10 grams of root tubers were peeled, cut into thin slices and crushed with glass powder in 30 ml ice cold distilled water and centrifuged at 4000 g for 10 minutes. the supernatant was transferred in 100 ml of volumetric flask and this procedure was repeated twicely and total volume of 100 ml was made up with ice cold distilled water to get soluble crude enzyme extract.

Determination of protein. Protein content of crude enzyme extract was determined by the method of Lowary *et al.* [19] with crystalline bovine serum albumin as standard and was found to be 2.3 mg/g root tuber.

Determination of invertase activity. Invertase activity was determined in 2 ml containing 1mM sucrose 20mM acetate buffer pH 4.6, 0.1ml of enzyme sample incubated at 37°. After 30 minutes aliquots of 0.2 ml assay mixture were withdrawn and 0.5 ml of 3,5 dinitrosalicylic acid was added. The solutions were boiled for 10 minutes in a boiling water bath. The colour developed was measured at 540 nm according to Bernfeld method [20].

A unit of invertase is defined as the amount of enzyme which catalyzes the liberation of one mg of reducing sugar per 30 minutes under the condition of the assay.

Characteristics of invertase. The effect of pH on invertase activity was determined through pH 3-8.0 with 0.2Macetate buffer and 0.2M phosphate buffer.

The effect of temperature on invertase activity was investigated by incubating the assay material of invertase at different temperatures ranging from 20-45° at pH 5.5 for 30 minutes.

The heat treatment experiments were carried out by pre-incubating a crude enzyme preparation of *Oxystelma esculantum* for ten minutes at various temperatures ranging from 50-80°. The invertase activities estimated as reported above.

RESULTS AND DISCUSSION

The effect of time and enzyme concentration on the rate of enzymatic reaction was studied. The rate of reaction increased linearly with increase in time and enzyme concentration as shown in Fig. 1 and 2. Thus in subsequent experiments time of incubation was kept for 30 minutes.

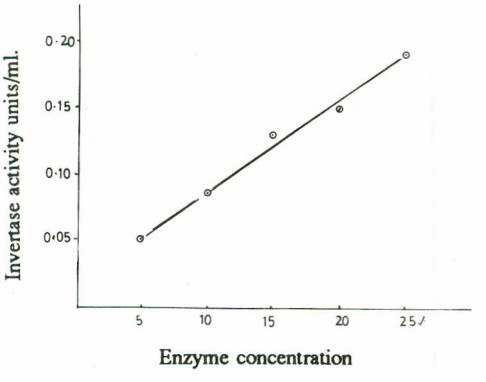

Fig. 1. Effect of enzyme concentration incubated with 1mM sucrose substrate concentration at 37° for 30 min.

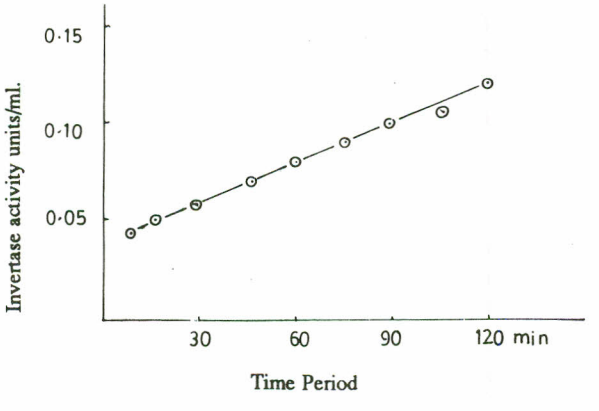

Fig. 2. Time course of invertase activity. Reaction mixture contained 0.1 ml of enzyme solution, 1mM. Sucrose were incubated at 37° for 30 min.

Figure 3 shows the pH curves obtained by using the crude extract. Crude enzyme preparation was found to exhibit optimum activity at pH 5.5.

The variation of invertase activity with temperature is shown in Fig. 4. The temperature profile indicates that the temperature optimum for 30 minutes reaction period is 30°, for crude enzyme preparation.

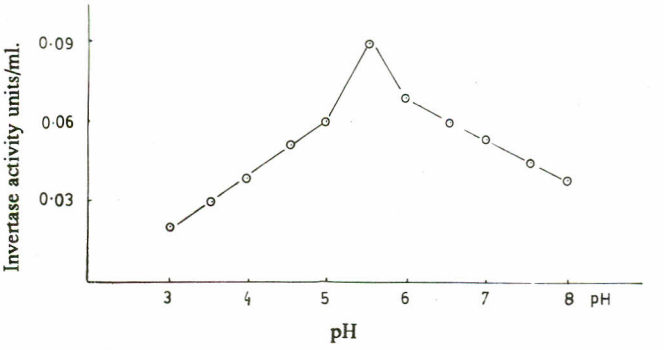

Fig. 3. Effect of pH on invertase activity.

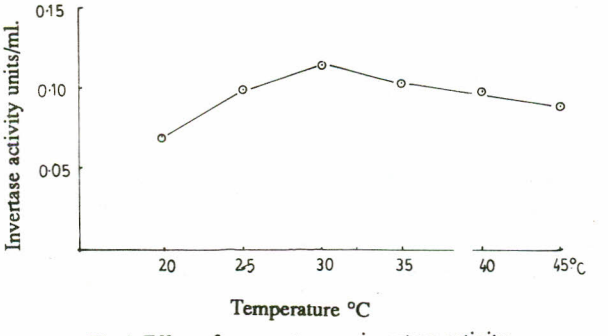

Fig.4. Effect of temperature on invertase activity.

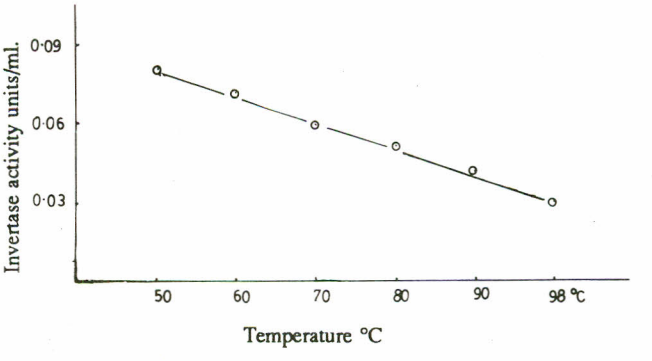

Fig. 5. Effect of heat treatment on invertase activity.

The effect of heat treatment was used to determine the thermosensitivity of invertase activity. Invertase activity of crude enzyme preparation was found to be heat stable even at 98° which retained 26% of its activity as shown in Fig. 5.

The influence of various reagents on the enzymatic activity of invertase was studied and is tabulated in Table 1. It was observed that the invertase activity of *Oxystelma esculantum* was significantly inhibited in the presence of $CuSO_4$, urea, $CaCl_2$ and EDTA.

The invertase activity was inhibited (75%) in the presence of 5mM $CaCl_2$, may suggest that the concentration of Ca^{2+} present in the *Oxystelma esculantum* is already optimal because enzyme action in presence of high concentration of Ca^{2+} is reported to be inhibitory whilst in low concentration of Ca^{2+} enzyme activity is markedly activated. The invertase of

Table 1. Effect of various reagents on invertase activity of *Oxystelma esculantum.*

Reagent added	Amount added	Activity	Relative activity%	Activation inhibition%
Control	-	0.08	100	-
$CuSO_4$	5mM	0.01	12.5	87.5 (I)
Vitamin-C	5mM	0.2	250	150 (A)
KCN	5mM	0.1	125	25 (A)
$CaCl_2$	5mM	0.02	25	75 (I)
EDTA	10mM	0.03	37.5	62.5 (I)
$CoCl_2$	1mM	0.1	125	25 (A)
Urea	2mM	0.02	25	75 (I)

Oxystelma esculantum was inhibited by 10mM EDTA to the extent of 62.5% suggesting that it may be a metalloenzyme [21,22]. Vitamin C, potassium cyanide and cobalt chloride activated the rate of invertase reaction 150%, 25% and 25% respectively. The activation effect of vitamin C, KCN and $CoCl_2$ was also reported by other workers [23]. Purification work on invertase activity from *Oxystelma esculantum* is under progress.

REFERENCES

1. R.L. Lyne and T.ap. Rees, Phytochemistry, **10,** 2593 (1971).
2. R.R. Gayler and T. Glasziouk, Plant Physiol., **27**, 25 (1972).
3. B.G. Combe, Ann. Rev, Plant. Physiol., **27**, 507 (1976).
4. R. Pressay and J.K. Avants, Plant Physiol., **65**, 136 (1980).
5. M. Umar Dahot, J. Pure and Appl. Sci., **5**, 23 (1986).
6. R. A. Leigh, T.ap. Rees, W.A. Fuller and J. Bandfield, Biochem. J., **178**, 539 (1979).
7. D. Vaughan and I.R. MacDonald, Plant Physiol., **42**, 456 (1967).
8. T. Boller and H. Kende, Plant Physiol., **63**, 1123 (1979).
9. M.A. Vattuone, O.L. Fleischmacher, F.E. Prado, A. Lopez Vinals and A.R. Sampietro, Phytochemistry, **22**, 1361 (1983).
10. B.A. Marouf and L. Zeki, J. Fd. Sci., **47**, 678 (1982).
11. K.T. Glasziou, Nature, **193**, 1100 (1962).
12. M. Morell and L. Copeland, Plant Physiol., **74**, 1030 (1984).
13. J.G. Robertson and M.P. Taylor, Planta, **112**, 1 (1973).
14. A.R. Memon, K.M. Bhatti and A.N. Memon, J. Chem. Soc. Pakistan, **6**, 71 (1984).
15. A.N. Memon, A.R. Memon and M. Umer Dahot, J. Pure and Appl. Sci., **6**, 5 (1987).
16. A.N. Memon, A.R. Memon and M. Umer Dahot, Pakistan J. Biochemistry (Submitted).
17. M. Umar Dahot and M.S. Memon, Tehqique, **4**, 9 (1986).
18. A.R. Memon and M. Umar Dahot, J. Chem. Soc. Pakistan, **7**, 7 (1985).
19. O.H. Lowary, N. J. Rose Brough, A.L. Farr and R. J. Roandell, J. Biol. Chem., **193**, 265 (1951).
20. P. Bernfeld, *Methods in Enzymology*, S.P. Colowick and N. O. Kaplan (Academic Press, Inc., New York, 1955), Vol. I, pp. 49.
21. P.D. Boyer, H. Lardy and K. Myrback ed., *The enzymes*(Academic Press, New York, 1960), 2nd ed., Vol.IV, pp. 389.
22. K. Balasubramaniam and P.N. Kannangara, J. Natn. Sci. Coun. Srilanka, **10**, 169 (1982).
23. J.A. Sacher, M.D. Hateh and K.T. Glaszious, Plant Physiol., **16**, 836 (1963).