Pakistan J. Bot. 2 (2) : 9—12

STUDY OF INVERTASES FROM *TARAXACUM* ROOTS *

M. I. KHAN

Department of Botany, University of Karachi, Karachi-32.

Abstract

pH specificities of invertases from the roots of *Taraxacum officinale* have been investigated. Two acidic and one neutral invertases were found to be present in this root. The acid invertases were more effective in hydrolyzing sucrose than the neutral one. Paper chromatographic separation of these enzymes have shown that all three have different rf values.

Introduction

Enzyme invertases occur in almost every plant. Its main function is to hydrolyze sucrose into glucose and fructose. Cabib (1951), while studying the paper chromatographic separation of some proteins in brewer's yeast, found two types of invertases different in their rf values and having two different pH optimas. Bealing (1952) however, failed to show the activity of two invertases in brewer's yeast. It was, therefore, felt desirable to investigate the sucrose hydrolyzing enzymes in *Taraxacum* roots.

Material and Methods

Roots of *Taraxacum officinale* W. were used throughout this study. Roots were collected from the open ground in the vicinity of Sheffield University (UK) and brought to the laboratory in polythene bags. After washing with tap water, whole roots were used for the extraction of the enzymes.

Extraction of the enzymes

The roots (20 g fresh weight) were crushed with acid-washed sand and 10 ml of 0.2 M citrate phosphate buffer, pH 7.4 at 5 C. The slurry so obtained was filtered through eight layers of muslin cloth and the filtrate centrifuged at 4000 × g for 20 mins at 5 C. The supernatant solution containing the crude enzyme extract, was used for the estimation of pH optimas of invertases.

For paper chromatographic separation of invertases, the crude enzyme extract was partially purified by dialyzing for 18 hrs in four litres of 0.002 M citrate phosphate buffer pH 7.4 at 5 C and centrifuged at 4000 × g for 10 mins at 5 C. The protein in the supernatant solution was precipitated by adding solid ammonium sulphate to 50% saturation (Dixon and Webb 1964), and was removed by centrifugation at 6000 × g for 10 mins at 5 C. The precipitate was washed twice with 50% ammonium sulphate solution and then dissolved in 2 ml of 0.02 M phosphate buffer pH 7.4.

* This work was carried out at the Department of Botany, University of Sheffield and is based on part of a thesis accepted for the degree of Ph. D. The author wishes to thank Dr. A. Booth for taking keen interest in this work.

Enzyme assay

Invertase was estimated by measuring the increase in free reducing sugars after enzymatic hydrolysis of sucrose by Somogyi's method as modified by Nelson (1944).

0.5 ml of the crude enzyme extract was mixed with 0.5 ml of 0.2 M sucrose, 0.5 ml of citrate phosphate buffer of pH ranging from 4.8 to 8.0 and 0.5 ml of chloroform as an antiseptic. In the control only sucrose was omitted. The tubes were incubated for an hour at 30 C after which the reaction was stopped by plunging the tubes into boiling water for 3 mins. Free hexoses released due to enzymatic hydrolysis were measured.

In the crude enzyme extract, protein was measured by the method of Lowry *et al.* (1951).

Paper chromatographic separation of invertases

The separation of invertases were achieved by ascending paper chromatography in the manner described by Cabib (1951). About one cm wide band of the enzyme solution was loaded on to a 8 cm wide Whatman paper No. 4 at 15 C. Care was taken to maintain the paper in wet condition, to avoid the risk of protein denaturation by drying. The solvent used was a solution of equal volumes of 0.05 M phosphate buffer pH 7.2, and 25% ethanol. The temperature of the tank was maintained at 5 C during the development of the chromatogram. When the solvent front reached 25 to 30 cm, the paper was removed from the tank and suspended in the room at a temperature of 15 C for two hrs for partial drying. Then the paper, still moist, was cut into four 2 cm wide longitudinal strips. One of the strips was used as an enzyme control and was not sprayed while the others were sprayed with 0.02 M sucrose solution in 0.02 M phosphate buffer at pH 5.4, 6.5 and 7.2 respectively. The strips, after spraying, were hung at room temperature (25 C) overnight for drying.

The sucrose hydrolysed by the enzyme invertase was detected by employing 2, 3, 5-trimethyl tetrazolium chloride spray (White 1952), which gave red colour with reducing sugars only. The enzyme control strip was also sprayed with the reagent for the detection of any reducing sugars present in the enzyme extract.

Results and Discussion

Three distinct pH optimas of invertases were found in *Taraxacum* roots (Fig. 1). The two acid invertases were found to be more effective in hydrolyzing the sucrose than the neutral one. The question whether these three invertases are also different in some other properties was further investigated by their paper

chromatographic behaviour. It is evident from the result presented in Fig. 2(a) that, the enzymes more active at three pH's are different in their rf values too. The rf values of enzymes were 0.23-0.45, 0.40-0.65 and 0.25-0.55 at pH's 5.4, 6.5 and 7.2 respectively.

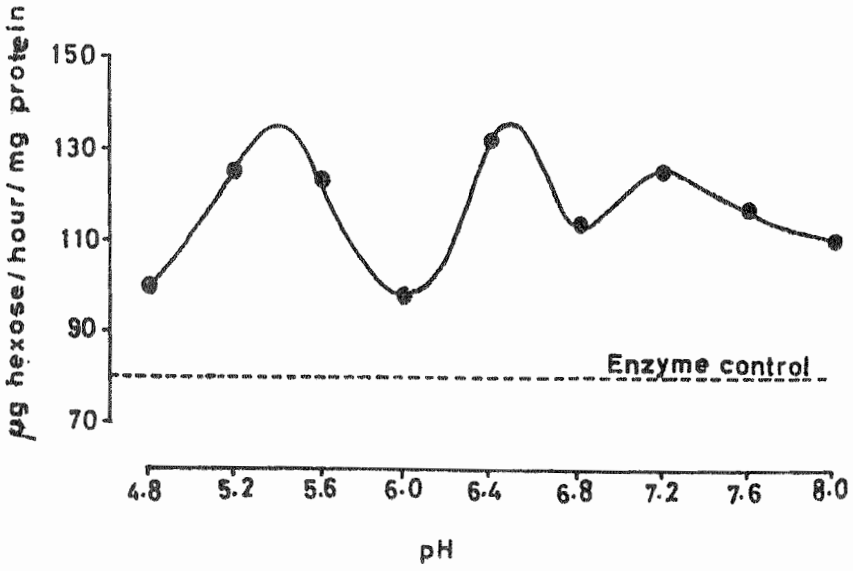

Fig.1 pH optima of invertase

It was thought that the spots of the hydrolyzed sucrose might diffuse through the paper while the paper is wet. This was investigated in another experiment of similar nature. In this case the preliminary procedure was similar to the one described in chromatographic method. The two mm strips of the deve-

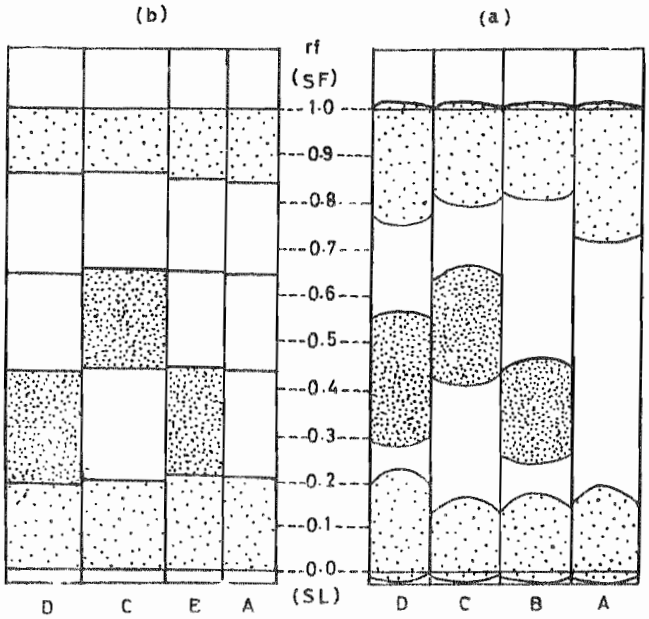

Fig. 2. Three distinct pH optimas of invertases

loped chromatogram were transversly divided into five equal segments (Fig. 2-b), and these segments were then sprayed with sucrose in appropriate pH's. The results of this experiment is presented in Fig. 2(b). It is clear from this result that the relative positions of the hydrolyzed sucrose is the same in both the experiments, and there appears to be no diffusion of sucrose during its hydrolysis.

The three pH optimas and rf values of invertases indicate the presence of three different types of the enzyme in the roots of *Taraxacum officinale*. Similar results were obtained by Cabib (1951) who demonstrated the presence of two different invertases in Brever's yeast. Hatch and Glasziou (1963) while studying the sugar accumulation cycle in sugar-cane found two types of invertases. One was active at pH 5.4 while the other at 7.1. In these studies, the invertases were found in different tissues, the acid being present in the young while the neutral in mature tissues. Hatch *et al.* (1963) concluded that these enzymes regulate the movement of sucrose from vascular to storage tissue in the internode of mature sugar-cane plant.

References

Bealing, F. J. 1952. The transfructosidase activity of mould invertase preparation Ph.D. Thesis, Univ. of Sheffield.

Cabib, E. 1951. Paper chromatography of some enzymes and the plasma proteins. Biochem. Biophy. Acta. 7 : 604-606.

Dixon, M. and E. C. Webb. 1964. Enzymes. Longmans Green and Co. Ltd.. London, W.C.1.

Hatch, M. D. and K. T. Glasziou. 1963. Sugar accumulation cycle in sugar-cane. II. Relationship of invertase activity to sugar content and growth rate in storage tissue of plants grown in controlled growth environments. Pl. Physiol. **38** : 344-348.

————J. A. Sacher and K. T. Glasziou. 1963. The sugar accumulation cycle in sugar-cane. I. Studies on the enzymes of the cycle. Pl. Physiol. **38** : 338-343.

Lowry, O. H., N. J. Rosebrough, A. L. Afarr and R. J. Randav. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. **193** : 265-273.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153** : 375-380.

White, J. W. 1952. The action of invertase preparations. Arch. Biochem. Biophy. **39** : 238.