Chemistry of Natural Compounds, Vol. 44, No. 2, 2008

XEROPHYTIC *Cereus pterogonus* XYLOSE ISOMERASE IS A THERMOSTABLE ENZYME

S. Ravikumar and K. Srikumar

UDC 577.15

A thermostable xylose isomerase (D-xylose ketol-isomerase; EC.5.3.1.5) was isolated from the cladodes of xerophytic Cereus pterogonus*. The enzyme was purified to homogeneity. SDS-PAGE analysis estimated the molecular mass of the enzyme protein at 66 kDa. The enzyme exhibited temperature optima at 60 and 80°C. Though the enzyme activity was optimum at pH 7.0, the enzyme was found to be stable in both acidic and basic pH ranges in the presence of divalent ions Mn^{2+}, Co^{2+}, and Mg^{2+}. This enzyme may find potential use in the food and beverage industry.*

Key words: xylose isomerase, *Cereus pterogonus*, thermophilic, pH dependence.

D-xylose isomerase (XI) (D-xylose ketol-isomerase; EC.5.3.1.5) is an enzyme involved in pentose sugar metabolism. D-Xylose is formed during the decomposition of hemicelluloses by extracellular xylanases [1]. After transport through the plant cell membrane, D-xylose is converted to D-xylulose by the enzyme D-xylose isomerase. D-Xylulose is then phosphorylated by xylulokinase to direct the product into the pentose phosphate shunt pathway. D-Xylose isomerase is an important enzyme for biotechnology applications due to its ability to convert D-glucose to D-fructose [2]. This enzyme activity is therefore used in industry for the production of high fructose corn syrup employing glucose as a substrate for use as an alternative sweetener in food and beverages [3]. In the past, XI′s had been isolated and characterized from a number of eubacterial sources as also from rice and barley [4]. We report the identification, isolation, purification, and partial characterization of a thermophilic xylose isomerase from the eukaryotic source *Cereus pterogonus,* capable of converting glucose to fructose at 60 and 80°C with greater than 50% efficiency. We predict that this enzyme activity can potentially substitute for the eubacterial sources for use in the food and beverages industry.

The existence of a thermophilic xylose isomerase in the eukaryote *Cereus pterogonus* was identified. The enzyme activity was purified 25.5 – fold to homogeneity with a yield of 17.9% (Table 1). The molecular mass of the purified enzyme under reducing condition was determined at 66 kDa when subjected to SDS/PAGE (Fig. 1). The purified enzyme preparation showed two temperature optima at 60°C and 80°C and pH optimum at 7.0. The enzyme was found to be stable in acidic as well as basic pH in the presence of divalent ions Mn^{2+}, Co^{2+}, and Mg^{2+} (Fig. 2). The enzyme also exhibited activity for an extended time period at higher temperature in the presence of these ions (data not shown). This enzyme was found capable of converting greater than 50% of glucose to fructose both at 60 and 80°C. It is convincing that the thermostability and the neutral optimum pH of this eukaryotic xylose isomerase would allow its use as an indigenous supplement for fructose production in the sweetener industry.

Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Pondicherry – 605014, India, tel.: (91413) 2654422, e-mail: kotteazeth_srikumar@excite.com. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 169-170, March-April, 2008. Original article submitted December 21, 2006.

0009-3130/08/4402-0213

TABLE 1. Purification of Xylose Isomerase from *Cereus pterogonus*

Purification step	Total protein, mg	Total activity, Units	Specific activity, U/mg	Overall purification, fold	Overall yield, %
Cladode homogenate	64.0	444.94	7.99	1.0	100
Ammonium sulfate ppt.	26.5	341.37	13.82	1.72	71.6
Dowex anion-exchange					
T_{60} isoform	0.9	90.89	135.43	16.94	23.82
T_{80} isoform	1.2	96.91	109.9	13.75	25.78
Sephadex G 100					
T_{60} isoform	0.51	146.53	164.56	20.58	16.4
T_{80} isoform	0.45	169.85	204.26	25.55	17.96

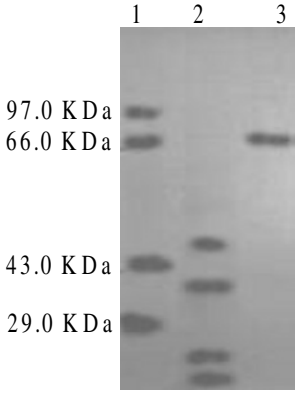

Fig. 1

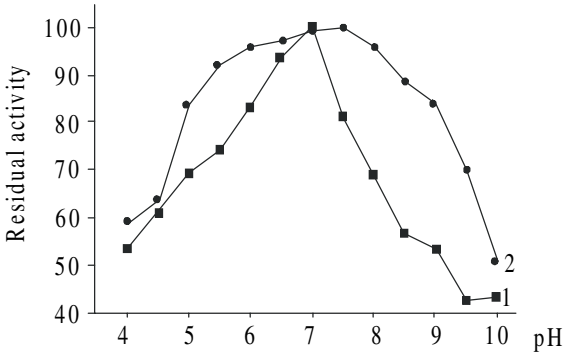

Fig. 2

Fig. 1. SDS –PAGE of xylose isomerase from *Cereus pterogonus*. 1 – Molecular weight standard; 2 – Crude homogenate; 3 – T_{80} isoform.

Fig. 2. Effect of pH on the xylose isomerase activity. Values are the means of three independent assays. 1 – activity; 2 – stability.

EXPERIMENTAL

All chemicals used were of analytical grade obtained from Sigma chemicals, USA, Bangalore genei, Himedia laboratories, Mumbai, India.

Cereus pterogonus cladode homogenate (20% w/v) was prepared in 0.05 M Tris-HCl buffer pH 7.0 containing 0.1 mM PMSF. The extract was filtered through cheese cloth and centrifuged for 15 min at 10.000×g in a refrigerated centrifuge employing a GSA rotor. The supernatant was used as the enzyme source. Protein content of the sample was also determined [5].

The enzyme activity was assayed employing 50 µL of a 1:10 diluted extract in a reaction mixture containing 70 mM xylose, 10 mM $MgCl_2$, 1 mM $CoCl_2$, and 50 mM Tris-HCl buffer (pH 7.5) (buffer A) and in a total reaction volume of 1 mL. Following incubation at 70°C for 30 min, 0.5 mL of the reaction volume was taken into 4.5 mL of perchloric acid (0.5 M) to stop the reaction. The quantity of fructose generated was measured using the cysteine-carbazole-sulfuric acid method [6]. One unit of isomerase activity was defined as the amount of enzyme required to produce 1 µmol of product per minute under the assay conditions.

The enzyme extract was precipitated using solid ammonium sulfate at 80% saturation. The preciptate was resuspended in 10 mL of buffer A and applied onto a column of Dowex-1 (1.2 cm × 30 cm) that had been pre-equilibrated with the same buffer. Following the column wash with buffer A (3 column volumes), the adsorbed proteins were eluted using a linear gradient of salt (0-1 M NaCl) in the same buffer. Fractions of 3 mL were collected and assayed for the enzyme activity and protein

content. Active fractions were pooled and taken for gel filtration on a Sephadex G-100 column (0.6 × 10 cm) that had been pre-swollen and pre-equilibrated with buffer A.

Molecular weight determination of the purified gel filtered enzyme active fraction was carried out on SDS-PAGE (12% w/v gel) as per the method of Laemmli [7].

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