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EFFECT OF A STRONG ENZYME DENATURANT (UREA) ON THE STABILITY OF SOLUBLE ACID INVERTASES FROM SUGARCANE

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Abstract

An investigation was performed to determine the effect of urea, a strong enzyme denaturant on the stability of invertases from two cultivars of sugarcane. The stability of native soluble acid invertases (SAI) of COJ-84 (local cultivar of sugarcane) to urea showed that they were more stable than those of CP-77-400 (local cultivar of sugarcane). The stability of SAI of CP-77-400 to urea gave biphasic trend at different salinity levels, and the stability at these levels decreased significantly as compared to control. The stability of 50 mM salinity level SAI was also decreased, while at 150 mM salinity level remained unchanged. The SAI of COJ-84 secreted under saline growth media presented extreme stability to unfolding by urea. The SAI at 150 and 200 mM salinity levels gave an activation trend and did not show denaturation.

Introduction

Sugarcane storage tissues accumulate sugar against a gradient, probably using energy provided by respiration, and this is accompanied by continuous cleavage and synthesis of sucrose during accumulation of sucrose in storage tissues (Batta & Singh, 1986). Invertases have been suggested as key regulators for the accumulation of sucrose in sugarcane parenchyma, a stem storage tissue (Gayler & Glasziou, 1972; Batta *et al.*, 2002; Sachedeva *et al.*, 2003). Neutral invertases occur in the cytoplasm and other compartments of the cell (Sturm, 1999; Jin *et al.*, 2009). SAI activities are usually high in storage tissues that are rapidly growing during internode growth and development (Walker *et al.*, 1997; Lontom *et al.*, 2008). The *Saccharum* group that retains high levels of SAI activity in mature stem storage tissue usually do not store high level of sucrose (Zrenner *et al*., 1996; Pan *et al*., 2009), but sometimes the reverse is true. Apparently, SAI must be low before sucrose can accumulate and this high sucrose accumulation can be the result of other factors as well (Gayler & Glasziou, 1972; Pan *et al.*, 2009). To acquire knowledge of these factors both in terms of stability and catalysis is a pre-requisite for their physiological applications. Sugarcane is the prime source of sugar production in Pakistan, but its growth is considerably reduced due to high levels of salts present in most soils (Wiegand *et al.*, 1996; Hussain *et al*., 2004; Gomathi & Thandapani, 2005). Thus, it is important to investigate physiological and biochemical basis of yield reduction under saline conditions. Keeping in view this, an attempt was made to isolate, purify and characterize invertases from sugarcane cultivars grown under saline conditions to evaluate the possible effect of salt stress on various characteristics of these enzymes. In this study, we investigated the effect of urea on invertase stability in two sugarcane

cultivars differing in salt tolerance so as to determine how far invertase stability is related to salt tolerance potential of the varieties.

Materials and Methods

Cultivation of sugarcane: Local sugarcane cultivars CP-77-400 and COJ-84 were grown in under field conditions the details of which have been described elsewhere (Hussain et al., 2009). The soil salt level was 25 mM.

Harvesting of canes: After six month growth of the crop in saline medium, the sugarcanes were cut from the soil surface and the trash removed. The canes were washed well with tap water and stored at 4° C.

Isolation and purification of invertases: The crude invertases were isolated and purified to homogeneity by a combination of ammonium sulphate, Hiload Q-Sepharose, Mono-Q ion exchange, hydrophobic interaction, and gel filtration chromatography on a FPLC system following Siddiqui *et al.,* (1997). The cane juice dialyzed against distilled water at 4°C for the removal of salts and soluble sugars and concentrated by freeze-drying. Total proteins and invertase activity were appraised.

All the methodology for enzyme assay after purification by different techniques and characterization have been described elsewhere (Niaz *et al.,* 2004) and Siddiqui *et al.* (1997). Effect of urea on invertases was determined following the method of Rangarajan *et al*. (1992). The freezedried powder of cane invertase was dissolved in 10 mM Tris/HCI, pH 7.0 which contained 8.0 M urea and it was incubated at 20°C. Different timecourse aliquots were taken and spontaneously assayed for enzyme activity. The rate of denaturation was determined by applying pseudo-first order plots (Rashid & Siddiqui *et al*. (1999).

Table 1. Kinetics of stability to 8 M urea of soluble acid invertases at 20º C from sugarcane cultivars CP-77-400 and COJ-84 grown under salinity.

Invertases of different salinity level	CP-77-400		COJ-84	
	K_d (min^{-1})	t ½ (min)	K_d (min^{-1})	t½ (min)
Native	0.0094	74	0.0018	6 t½
50 mM	0.0127	55	0.0006	19 t½
100 mM*	0.0091*	8*	0.0023	5 t½
	0.005*	139*		
150 mM	0.0089	78	0.0007	17 t_d
200 mM**	0.148**	5**	0.00065	18 t_d
	0.0055**	126**		

The values against 100mM* &200mM** salinity levels in CP-77-400 indicate biphasic trend of enzyme stability; **t½** = half life, **t_d** = doubling time, calculated using the relation: In2/K_d, where K_d is the denaturation rate obtained from Fig. 1.

Results and discussion

The stability of native SAIs of COJ-84 to 8 M urea at 20° C showed that they were more stable than those of CP-77-400, and their half-lives were 6 h and 1.23 h (74 min),

respectively. The pseudo-first order plots for stability of SAI of CP-77-400 to urea gave biphasic trend at 100 and 200 mM salinity levels, and the stability at these levels decreased significantly as compared to control because 50 % activity of these invertases was lost only after 8 and 5 min, respectively, as compared to 74 min in the case of control. The stability of SAI at 50 mM salinity level was also decreased, while at 150 mM the level remained unchanged (Fig. 1, Table 1).

The SAI of COJ-84 secreted under saline growth media gave an interesting behaviour, and surprisingly all SAI under salinity presented extreme stability to unfolding by urea, with the exception of 100 mM, which showed some decrease in half-life. The SAI at 150 and 200 mM salinity levels gave an activation trend and did not show denaturation, and their doubling time was equal to 17 and 18 h, respectively (Table 1). The stability of native SAI in CP-77-400 to proteolytic degradation by chymotrypsin was very high (t½=315 min) as compared to that in COJ-84 (t½=73 min).

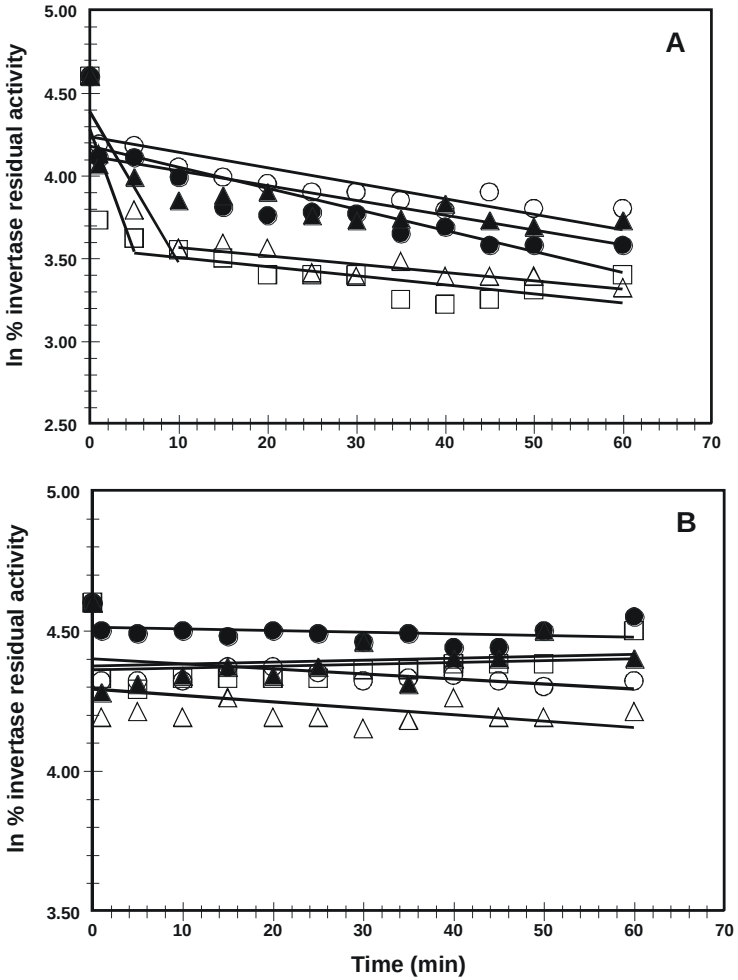

Fig. 1. Pseudo-first-order plots for the effect of saline growth media on the stability to 8 M urea, at 20°C, of soluble acid invertases of sugarcane cultivars: plot-A (CP-77-400) and plot-B (COJ-84): native (O), 50 mM (●), 100 mM (□), 150 mM (▲) and 200 mM. (△)

The rate of an enzyme catalyzed reaction may be reduced by a variety of substances. Of these substances, urea has been reported as one of the potential non-specific protein denaturants. It is generally believed that the thermostable enzymes are mostly stable to other denaturants like urea. Similarly, in the present case stability of native SAI of COJ-84 to 8 M urea, at 20 °C, showed that they were more stable than those of CP-77-400. The SAI of COJ-84 secreted under saline growth medium gave very interesting behaviour, and surprisingly all SAI under salinity showed extreme stability to unfolding by urea. The activation against urea of COJ-84 SAI, secreted under salinity, elucidated that the surface of the enzyme might be more hydrophobic in nature. The effect of urea on stability strengthened the belief that the expression of invertases might be influenced by salinity (Ben-Hayyim & Rana, 1990).

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