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SHORT COMMUNICATION

α-AMYLASE ACTIVITY IN SUGAR CANE (*SACCHARUM OFFICINARUM*) CHLOROPLASTS

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INTRODUCTION

THE PRIMARY photosynthetic products of tropical plants, including sugar cane, are C_4 dicarboxylic acids[1-4] and these plants possess two types of chloroplast. One of these (grana type) has a normal structure, stores little starch and is associated with the leaf mesophyll cells. The other type (non-grana) stores relatively large quantities of starch and is found in the bundle sheath cells.[5-7]

The following study, which was undertaken as part of a general investigation of carbohydrate metabolism in sugar cane chloroplasts, has revealed that most of the amylase activity of cane leaves is associated with these organelles and particularly with those of the non-grana type. Moreover, this activity is mainly attributable to α-amylase, but the possibility of traces of β-amylase has not been excluded.

RESULTS AND DISCUSSION

Initial studies with soluble protein from whole chloroplast fractions showed the presence of an enzyme which degraded starch with the formation of maltose, maltotriose and higher molecular weight oligosaccharides (DP 2-5; paper chromatography). The pH optimum of this activity was 6·9 when measured with whole chloroplast, grana and non-grana protein fractions. Whole chloroplast fractions produced the same hydrolytic products when incubated with starch at both pH 4·8 and 6·9 thus suggesting that the activity was due mainly to α-amylase and not β-amylase. The hydrolytic activity at pH 4·8 was only 20 per cent of that at pH 6·9.

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[5] W. M. LAETSCH, D. A. STETLER and A. J. VLITOS, *Z. Pflanzenphysiol.* **54**, 472 (1966).
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Table 1 shows that the amylase from whole chloroplast fractions is activated by chloride and by calcium ions and inclusion of both in the incubation medium increases the activity approximately 5-fold: incubation of the enzyme with NaCl (10 mM) and EDTA reduces the activity 4-fold compared with NaCl alone, which suggests that some calcium ion is bound to the crude protein fraction. These conclusions are substantiated by the ion-addition experiments in Table 2 where the protein fractions have been dialysed against EDTA prior to examination. Here again it is apparent that it is mainly α-amylase and not β which is present in the cane chloroplasts as only the former enzyme is normally activated in this way by calcium and chloride ions.

TABLE 1. THE EFFECT OF IONS ON α-AMYLASE ACTIVITY

Salt		Relative activity (%)* Tris-acetate buffer pH 6·8	Phosphate buffer pH 6·8
No addition		20·0	13·6
NaCl	5 mM	89·1	87·7
	10 mM	100·0	92·3
	20 mM	100·0	93·2
$Ca(NO_3)_2$	5 mM	41·3	—
	10 mM	45·0	—
	20 mM	41·3	—
$Ca(NO_3)_2$ 10 mM + NaCl 10 mM		93·5	—
EDTA 10 mM + NaCl 10 mM		25·5	—

* Fully activated enzyme (+ 10 mM NaCl) = 100.

TABLE 2. THE EFFECT OF DIALYSING α-AMYLASE AGAINST EDTA

Addition	Relative activities (%)
Original activity (NaCl, 10 mM)	100
Boiled enzyme	0
After dialysis against 0·1 M EDTA	
No addition	0
NaCl (10 mM)	13·6
$Ca(NO_3)_2$ (10 mM)	19·4
$Ca(NO_3)_2$ (10 mM) NaCl (10 mM)	65·2

Haapala[8] has claimed that *Stellaria media* chloroplasts contain β-amylase as judged from mercuric ion inhibition and the pattern of starch hydrolysis products. A recent survey by Gates and Simpson[9] has shown that α-amylase is ubiquitous in higher plants. These workers only concerned themselves with whole leaf preparations, however. An unspecified amylase has been shown to occur in whole leaf preparations of sugar cane.[10]

The levels of α-amylase in whole leaf and chloroplast protein fractions from cane (Table 3) show that the specific activity of the enzyme is considerably greater in the organelles than in

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the remainder of the leaf; indeed *in vivo* the amylase may well be completely organelle-bound. The other point of interest is that the non-grana type chloroplasts have an activity twice as high as that of the grana-type. This may be related to the large quantities of starch found in the former organelles, i.e. they possess a more efficient method for starch degradation than the grana-type.

TABLE 3. LEVELS OF α-AMYLASE IN CELL FRACTIONS

	α-Amylase (μg apparent maltose/hr/μg protein)
Leaf batch A (collected in June 1968)	
1000 g supernatant	1·9
Whole chloroplast fraction	44·0
Leaf batch B (collected in May 1969)	
Whole leaf protein	0·7
Grana type chloroplast	5·6
Non-grana type chloroplast	12·5

Variations in the levels of α-amylase, which are probably seasonal, have been noted on several occasions. Highest levels of enzyme appeared to be associated with periods of rapid growth.

Further studies are continuing on the levels of other starch-hydrolysing enzymes and of synthetases in cane chloroplasts.

EXPERIMENTAL

Plant Material

Sugar cane plants were grown in pots in greenhouses at the University of London Botanical Supply Unit under natural illumination. Mature leaves were harvested during the spring and summer months after 6–8 hr daylight.

Preparation of Chloroplasts

Whole chloroplast pellets were prepared by the method of Bucke, Walker and Baldry and washed once with the isolating medium.[11] Chloroplast fractionation into grana and non-grana types was effected by centrifugation through 50% sucrose and the pellets washed, as described by Baldry *et al.*[12]

Preparation of Soluble Protein

Pellets were suspended in 10 mM-Na_2HPO_4/KH_2PO_4 buffer (pH 6·9) and homogenized in a Potter–Elvehjem homogenizer. The suspension was twice frozen and thawed to facilitate disruption of the chloroplast membrane. The resulting brei was centrifuged at 40,000 *g* for 30 min to remove the membranes and the soluble protein precipitated by gradual addition, with stirring at 0°, of solid $(NH_4)_2SO_4$ to a final conc. of 80%. The protein was centrifuged at 10,000 *g* for 20 min, redissolved in 2–5 ml of 10 mM-phosphate buffer (pH 6·8) and finally dialysed against 1 l. of the same buffer at 5° for 12 hr.

Enzyme Assay

The enzyme was assayed by a modification of the method of Bernfeld[13] using a colorimetric procedure to estimate the liberated reducing groups.[14,15] This method has been recommended by Robyt and Whelan.[16]

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[12] C. W. BALDRY, J. COOMBS and D. GROSS, *Z. Pflanzenphysiol.* **60**, 78 (1968).
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The normal reaction mixture (total v. 1·0 ml) contained A.R. soluble starch (British Drug Houses Ltd.) in 0·1 M-Tris-acetate or phosphate (pH 6·9) buffer (10 mg/ml; 0·5 ml) and 0·1 M-NaCl (0·1 ml). The reaction was started by adding enzyme (0·1–0·4 ml) and digests were incubated at 35°. Alkaline copper reagent (1·0 ml) was added to stop the reaction and the mixture boiled for 20 min and then cooled. Nelson's arsenomolybdate reagent (1·0 ml) was added, the solution diluted with water to 25 ml and the absorbance measured at 520 nm. A linear relationship between reducing power (expressed in terms of μg maltose/hr/μg protein) and time up to 60 min was observed. Protein was determined by the method of Lowry *et al.*[17]

Paper Chromatography

Oligosaccharides were examined on paper chromatograms using two solvent systems; ethyl acetate: acetic acid: formic acid: water (18:3:1:4) and ethyl acetate: pyridine: water (10:4:3). The compounds were located with a silver nitrate/NaOH reagent.[18]

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