Phytochemistry 1974 Vol 13 pp 55 to 57 Pergamon Press Printed in England

WALL-BOUND ENZYMES IN CALLUS OF *CONVOLVULUS ARVENSIS*

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(*Received* 23 *May* 1973 *Accepted* 8 *August* 1973)

Key Word Index—*Convolvulus arvensis*, Convolvulaceae, morning glory, glycosidases, phosphatases, cell wall

Abstract—Isolated cell walls of *Convolvulus* callus contain α- and β-galactosidase, α- and β-glucosidase, α- and β-mannosidase, acid invertase and acid phosphatase activities No neutral invertase or alkaline phosphatase activities could be detected Acid invertase activity per mg cell wall increased considerably during incubation of callus fragments in nutrient solution, as opposed to the activities of the other enzymes mentioned

INTRODUCTION

THE ACTIVITY of acid invertase in the cell wall of *Convolvulus* callus increases considerably upon subculturing Addition of inhibitors of RNA or protein synthesis does not prevent this rise but, on the contrary, results in an even higher invertase activity [1] Wall-bound glycosidases are common among higher plants,[2–7] so it seemed worthwhile to search for other wall-bound enzymes in *Convolvulus* callus, and to study the effect of subculturing on these enzymes

RESULTS AND DISCUSSION

Table 1 shows that cell walls contain a number of glycosidases (E C 3 2 1 20–3 2 1 26) and an acid phosphatase (E C 3 1 3 2) No evidence for the presence of alkaline phosphatase or neutral invertase in the cell wall was obtained These wall-bound enzymes could not be solubilized by non-ionic detergents, demonstrating[8] that they were not artefacts due to the formation of wall-tannin-protein complexes during homogenization In general their pH optima agreed closely with the values reported for the corresponding cytoplasmic enzymes in higher plants [9–15] Apparently, the binding to the cell wall did not influence the location of the pH optima

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TABLE 1 WALL-BOUND ENZYMES IN *Convolvulus* CALLUS

Enzyme	Optimal pH	Enzyme* activity at optimal pH	Enzyme† activity after incubation	Enzyme activity (%) released by isolated cell walls in H_2O	Triton X100‡ 0·4%
α-Galactosidase I	5·2–5·6	128	83	1	3
II	6·0–6·4	107	116	1	4
β-Galactosidase	4·2 4·6	68	–	-	-
α-Glucosidase	4·6–5·0	13	99	0	0
β-Glucosidase	5·2–5·6	32	94		-
Acid invertase	4·5–4·8	18	700	0	0
α-Mannosidase	4·4 5·0	132	52	0	0
β-Mannosidase	4·7–6·2	16	-		-
Acid phosphatase	5·0–5·4	740	102	1	1

* nmol of substrate hydrolyzed per hr and per mg dry wt of cell wall material

† Enzyme activity expressed as percentage of the initial activity Incubation took place in nutrient solution and lasted 48 hr

‡ Two other non-ionic detergents were tested namely Carbowax 1540 (12%) and Tween 60 (2%) The results were similar to those obtained with Triton X100

As distinct from wall-bound invertase activity the activities of the other cell wall enzymes did not increase upon subculturing callus fragments into nutrient solution Addition of actinomycin D or cycloheximide to the nutrient solution further stimulated the invertase development in the cell walls The α-galactosidase I and α-glucosidase activities were however, inhibited for *ca* 50 and 13% respectively (Table 2) Though only these two cell wall enzymes of those not stimulated by subculturing were tested, it seems questionable if the other ones will behave quite differently

TABLE 2 EFFECT OF ACTINOMYCIN D AND CYCLOHEXIMIDE ON WALL-BOUND INVERTASE α-GALACTOSIDASE AND α-GLUCOSIDASE ACTIVITIES

Treatment		Invertase* activity	α-Galactosidase I activity	α-Glucosidase activity
Before incubation		10	131	10·9
After incubation†		150	155	12·2
+ Act D mg/l	5	150	153	—
	15	213	114	-
	70	256	99	10·4
	100	220	79	11·0
+ CHI mg l	0·1	230	115	
	1	256	81	-
	10	382	76	11·2
	100	369	76	10·3

Enzyme activities have been expressed as nmol of substrate hydrolyzed per hr and per mg dry wt of cell wall material All values are means of determinations on three samples

* These data are shown here for comparison

† Incubation lasted 48 hr

EXPERIMENTAL

Material After lyophilization, the tissue was pulverized and the powder suspended in a solution of cysteine (6 mg ml pH 7·0 2·5 ml g callus fr wt) Cytoplasmic constituents were washed out with H_2O by centrifuging ×5 at 270 *g* The residue was finally suspended in two vol of H_2O All operations were carried out at 0·4

Measurement of enzyme activities The invertase activity was determined according to Klis and Hak [1] The reaction mixtures for the assay of the other glycosidase activities respectively contained sodium phthalate buffer or in the case of β-galactosidase, α- and β-mannosidase activities sodium acetate buffer (60 mM, optimal pH for each enzyme separately, see Table 1), suitable substrate (*p*-nitrophenyl-glycoside or *o*-nitrophenyl-β-D-galactopyranoside in case of β-galactosidase activity, 0 4 mM) and cell walls, together making up a final volume of 2 5 ml After incubation in a rotary shaker at 30° and 300 rpm the reaction was stopped with 2 5 ml 0 2 M Na_2CO_3 The mixture was subsequently centrifuged and the amount of *p*-nitrophenol formed determined at 400 nm Phosphatase activity was measured with *p*-nitrophenyl phosphate as a substrate using phthalic buffer for acid phosphatase and Tris-HCl for alkaline phosphatase The reaction was stopped by adding 2 5 ml 1 M Tris-HCl containing 0 4 M sodium phosphate and the enzyme activities were determined as above All enzyme activities were expressed as nmoles of substrate hydrolyzed hr^{-1} mg^{-1} (dry wt) of cell wall material

Incubation of callus tissue The incubation soln was prepared according to Earle and Torrey,[16] but contained glucose instead of sucrose carbon source Incubation was under sterile conditions in flasks containing about 1 g callus in 15 ml soln The flasks were rotated (45 rpm) in the dark at 25° for 48 hr

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