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SHORT COMMUNICATION

β-(1→3) GLUCANASES FROM PLANT CALLUS CULTURES*

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Abstract—Enzymes capable of hydrolyzing β-(1→3) glucan (laminarin) have been found in callus cultures of bean (*Phaseolus*), lettuce (*Lactuca*), carrot (*Daucus*) and pepper (*Capsicum*). Laminaribiose is the major product. Lichenin and oat glucan were not attacked by these laminarinases.

INTRODUCTION

β-(1→3) GLUCANASES have been reported from molluscs;[1-3] bacteria;[4] fungi;[5,6] algae;[7,8] and higher plants.[7-11] The β-(1→3) glucan substrates are found in algae[8,12-15] as laminarin, leucosin, or paramylon; in fungi[16-19] as pachyman; and in sieve tubes and germinating pollen grains of higher plants[20-22] as callose. Recently we have found enzymes capable of hydrolyzing laminarin in a number of callus cultures from higher plants, but we have not found therein any substrate for these enzymes.

EXPERIMENTAL AND RESULTS

Undifferentiated callus cultures of bean, lettuce, carrot and pepper were initiated and carried for at least ten transfers on the defined medium of Murashige and Skoog[23] with 3·0%

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sucrose as carbon source and with 0·05 mg/l. of 2,4-dichlorophenoxyacetic acid as the auxin. Cells grown on solid medium (0·6% agar) were suspended in 0·05 M phosphate buffer pH 6·0 containing 0·01 mg cysteine per ml, using 10 ml of buffer per gram fresh weight of cells, homogenized for 5 min in a Virtis homogenizer, and then centrifuged (600 *g* for 10 min) to remove solids. The homogenate was dialyzed (24 hr, 5°, buffer), or the proteins precipitated with 66% acetone. Acetone precipitates were gummy and gave low enzyme activities. The greatest activities were obtained from dialyzed homogenates adjusted to pH 5·0.

Enzyme activities were measured by adding 0·5 ml of homogenate to 0·5 ml of substrate (5 mg/ml in 0·05 M citrate buffer pH 5·0) and incubating for 1–4 hr at 40° or 50°. Reducing sugars produced were measured by a dinitrosalicylic acid method[24] and corrected for appropriate enzyme and substrate blanks. A value of 0·50 mg as glucose in this test run at 50° for 1 hr equals one unit per ml of the enzyme preparation.[25]

The following substrates gave negative (less than 0·10 mg as glucose) results in all tests: methyl α-D-mannopyranoside, methyl β-D-mannopyranoside, salicin, xylan [β-(1→4)], carob gum [β-(1→4) mannan], ivory mannan [β-(1→4)], konjac mannan [β-(1→4)], pectic acid, chitin, mycodextran [α-(1→3) and α-(1→4) glucan], dextran [α-(1→6)], lichenin [β-(1→3) and β-(1→4) glucan], oat glucan [β-(1→3) and β-(1→4) glucan], carboxymethylcellulose, and pustulan [β-(1→6) glucan].

Sucrose and starch are the usual reserve carbohydrates of higher plants and enzymes hydrolyzing one or the other of these substrates were found in all cultures tested. Enzymes hydrolyzing laminarin were always present (Table 1).

TABLE 1. ENZYMES FROM PLANT CELL CULTURES ON SOLID MEDIA

Culture	Enzyme units/g fresh weight of cells		
	Sucrose	Starch	Laminarin
Pepper stem	42	142	88
Carrot root	13	0	2
Lettuce leaf	3	0	8
Bean leaf	0	84	9

These plant β-(1→3) glucanases have a pH optimum of from 5 to 6 and digestion is most rapid at 40° to 50°. The hydrolysis products from laminarin consist of a series of oligosaccharides with little glucose, (Fig. 1). Pachyman, the β-(1→3) glucan from *Poria cocos*, is also hydrolyzed by the pepper enzyme giving a series of similar oligosaccharide products (Fig. 1). The higher oligosaccharides appear early in the digestion suggesting a random acting endoenzyme. Laminaribiose is a major product. Similar results were obtained with preparations from bean, carrot, and lettuce. Lichenin and oat glucan were not hydrolyzed even at enzyme concentrations ten times those showing strong digestion of laminarin.

The action of pepper enzyme (1 g fresh wt of callus homogenized in 20 ml of buffer and dialyzed) was compared to related endo-β-glucanases: a random laminarinase from *Rhizopus arrhizus* QM 1032 (0·1 mg/ml), a random cellulase from *Streptomyces* QM B814 (0·1 mg/ml), and a specific lichenase in Novo Amylase a commercial preparation from *Bacillus subtilis* (1 mg/ml),[25] all acting on β-(1→3) and β(1→4) glucans (Table 2). Oat glucan and lichenin are similar glucose polymers containing β-(1→4) and β-(1→3) bonds in the approximate ratio of 2:1. They are readily hydrolyzed by random cellulases or laminarinases to give well characterized products.[26] Novo Amylase contains a specific lichenase that apparently binds to the trimer product.[27] This enzyme does not act on either cellulose or laminarin. A similar enzyme found in germinated barley,[28] acts on barley glucan (mixed β-(1→3) and β-(1→4) linkages) but not on cellulose or laminarin. The pepper enzyme may be a specific β-(1→3) glucanase. It acts only on β-(1→3) glucans producing much laminaribiose. It appears to be an endoenzyme, but the possibility that it may be an exoenzyme removing laminaribiose units, analagous to the action of β amylase on starch, cannot be ruled out. Further studies will be required to elucidate the action of this enzyme.

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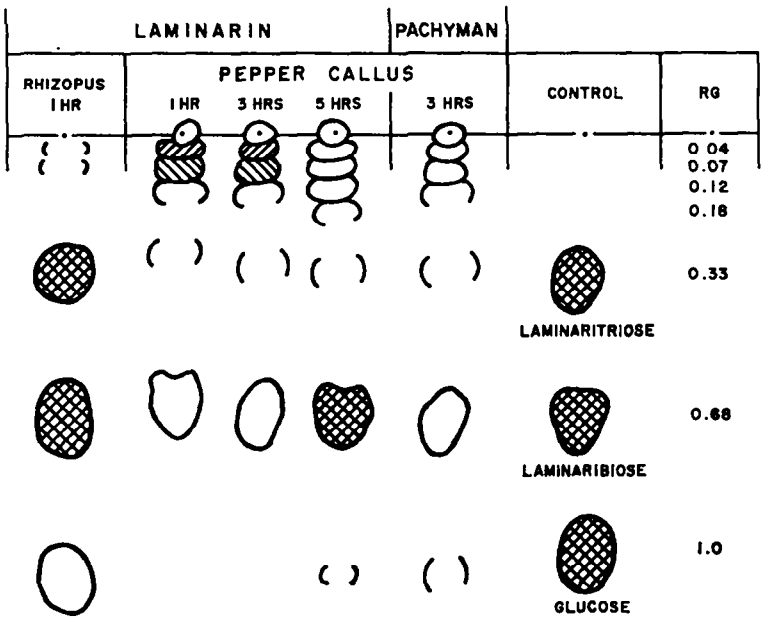

FIG. 1. CHROMATOGRAM OF THE DIGESTS OF LAMINARIN AND PACHYMAN BY THE β-(1→3) GLUCANASES OF PEPPER AND *Rhizopus arrhizus*.

Spotted on Whatman No. 1 paper, developed in isopropanol:glacial acetic acid:water (27:4:9) then sprayed with benzidine. There were no spots at 0 time. R_G = distance component moved/distance glucose moved.

TABLE 2. PRODUCTS OF ENZYME ACTION ON LAMINARIN, LICHENIN, OAT GLUCAN, AND CELLULOSE*

	Enzyme			
Substrate	Pepper Specific laminarinase	Novo Amylase Specific lichenase	*Rhizopus* Random laminarinase	*Streptomyces* Random cellulase
Laminarin	Laminaribiose (laminaritriose) higher oligosaccharides	No action	Laminaribiose laminaritriose higher oligosaccharides	No action
Lichenin	No action	Trimer *a*	Laminaribiose Trimer *a* Tetramer *a*	Cellobiose Trimer *b* (tetramer *b*)
Oat glucan	No action	Trimer *a* Tetramer *a*	Laminaribiose Trimer *a* Tetramer *a*	Trimer *b* Tetramer b_1, b_2
Cellulose	No action	No action	No action	Cellobiose Cellotriose

Products by chromatography†: trimer $a = 4^2$-O-β-glucosyl laminaribiose; trimer $b = 3^2$-O-β-glucosyl cellobiose; tetramer $a = 4^2$-O-β-cellobiosyl laminaribiose; tetramer $b_1 = 3^2$-O-β-cellobiosyl cellobiose; tetramer $b_2 = 4^2$-O-β-laminaribiosyl cellobiose.

* 1 ml enzyme plus 1 ml substrate (5 mg) at pH 5·0 and 50° incubated for 2 and 4 hr.

† Solvents on Whatman No. 1: Isopropanol:acetic acid:water (27:4:9) and ethyl acetate:acetic acid:water (9:2:2). Spray: benzidine or $AgNO_3$.

A search made for the substrate of the β-(1→3) glucanase in the callus cultures was unsuccessful. Polysaccharides containing glucose (acid hydrolysis) could be extracted from callus tissues, but neither the extracts nor the insoluble residues were acted on by the pepper enzyme.

The yields of plant laminarinase are equivalent to about 40–800 enzyme units per gram dry weight of tissue. These are much lower than the yield of about 12,000 units/g dry weight of mycelium for the random laminarinase of *Rhizopus arrhizus*. Both enzymes produce laminaribiose as a major product of action on laminarin, but the Rhizopus enzyme produces much more laminaritriose and the pepper relatively more of the higher oligosaccharides. The *Rhizopus* enzyme is also active on glucans of mixed β-(1→3) and β-(1→4) linkages, substrates which are resistant to the pepper enzyme. Both enzymes may prove useful in studies of polyglucan structure.