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A β-1,6-glucan glucanohydrolase involved in hydrolysis of cell-wall glucan in *Schizophyllum commune*

Regulation of a cell-wall glucan-degrading enzyme (R-glucanase) has been implicated in fruiting and sexual morphogenesis of the basidiomycete *Schizophyllum commune*[1,2]. The enzyme acts on the β-1,3, β-1,6-linked R-glucan that together with chitin constitutes the alkali-insoluble portion of the wall[3]. Another cell-wall glucan of *S. commune*, called S-glucan[3] and containing α-1,3 linkages[4], is not subject to degradation in the fungus. During morphogenesis, changes in R-glucanase activity of mycelial extracts are not paralleled by similar changes in enzymic activities as determined with laminarin (β-1,3-glucan) or pustulan (β-1,6-glucan) as substrates[2]. This suggests a peculiar specificity requirement for R-glucanase.

A crude external-enzyme preparation was prepared from the medium of fruiting cultures of *S. commune* K8 (refs. 1, 3) by acetone precipitation (2 vol., −20°). After dialysis against water, insoluble matter was removed and the enzyme solution was freeze-dried. External-enzyme preparation showed hydrolytic activity towards R-glucan (contaminated with approx. 15% chitin), laminarin, pustulan, yeast glucan (β-1,3, β-1,6-glucan), lichenan (β-1,3, β-1,4-glucan), *p*-nitrophenyl-β-D-glucoside, and glycogen (α-1,4, α-1,6-glucan). Virtually no activity was detected on acid-swollen cellulose (β-1,4-glucan), chitin (β-1,4-linked *N*-acetyl glucosamine), nigeran (α-1,3, α-1,4-glucan), and S-glucan.

External-enzyme preparation was fractionated on Sephadex G-100 (Fig. 1). Peak I, containing activities on all substrates tested, constitutes the excluded volume of the column. It should be remarked that enzyme activities are found exclusively in this volume when elution is performed with water instead of McIlvain buffer. This suggests that enzyme dissociation occurs during elution with buffer (*cf.* ref. 5). Estimation of reducing groups[6] and glucose (Glucostat reagents) in the laminarin hydrolysates indicated an exo-laminarinase (β-1,3-glucan glucohydrolase) in Peak II and an endo-laminarinase (β-1,3(4)-glucan glucanohydrolase, EC 3.2.1.6) in Peak III. The latter enzyme also acted on lichenan. Fig. 1 shows that these laminarinases do not hydrolyze R-glucan, but yeast glucan appears to be susceptible. R-glucanase and pustulanase activities peak together, although the ratios between these activities in Peaks I and IV are quite disparate.

Heat inactivation kinetics of the enzymes present in the first and second R-glucanase peaks were followed. R-glucanase and pustulanase from the second peak are inactivated concomitantly (Fig. 2B). The diphasic inactivation curve suggests the presence of two enzyme species, both with R-glucanase and pustulanase activities. The divergence of the curves for R-glucanase activity as determined by total carbohydrate analysis[7] may be interpreted as indicating that soluble products split off by the more heat-labile enzyme are larger than those produced by the more heat-stable enzyme. Fig. 2A indicates that the first peak contains a separate pustulanase in addition to R-glucanase. Thus, although enzymes with R-glucanase activity may act on pustulan, the external-enzyme preparation also contains an enzyme acting more specifically on pustulan.

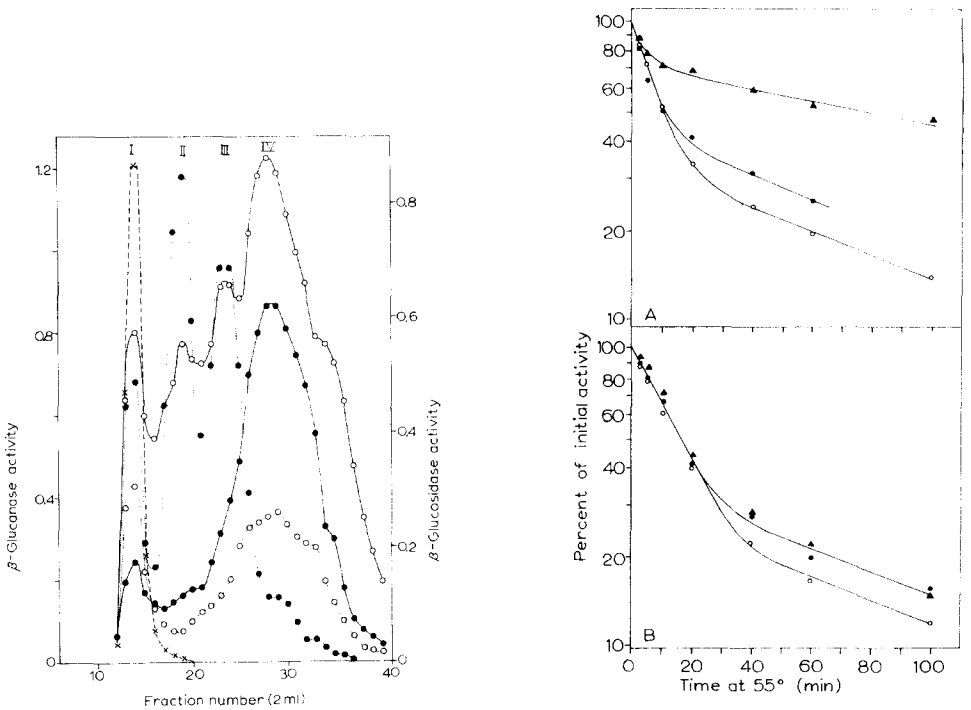

Fig. 1. Sephadex G-100 elution profile of external-enzyme preparation (4.5 mg). Eluant: 0.05 M McIlvain buffer (pH 5.5). Incubations were conducted in the same buffer. Hydrolysis of R-glucan (●—●) and yeast glucan (○—○): 0.25 ml substrate suspension (5 mg/ml) and 0.25 ml eluate were incubated at 30° for 2 h and the release of soluble carbohydrate was determined (anthrone). Hydrolysis of laminarin (●·····●) and pustulan (○·····○): 0.4 ml substrate solution (1.25 mg/ml) and 0.1 ml eluate were incubated at 30° for 1 h and the increase in reducing groups was determined. All glucanase activities are expressed as mg glucose equivalents liberated per ml eluate during the time indicated. β-Glucosidase activity (×– – – –×) was measured with *p*-nitrophenyl-β-D-glucoside as a substrate[2] and is expressed as units (μmoles/min) per ml eluate.

Fig. 2. Heat inactivation kinetics of enzymes after Sephadex G-100 fractionation (see Fig. 1). A. Enzymes in Peak I (Fractions 10–15). B. Enzymes in Peak IV (Fractions 24–33). ●, R-glucanase (reducing groups); ○, R-glucanase (total soluble carbohydrate); ▲, pustulanase (reducing groups). Enzyme solutions were incubated at 30° for 1 h.

Large soluble glucan fragments are the major reaction products of R-glucanase action. Fractions 28–35 (see Fig. 1) were pooled and incubated with R-glucan (30°, 2 h). The ratio of anthrone-positive material, reducing sugar, and glucose in the reaction products was 100:18:4. Thin-layer chromatography on cellulose (multiple development at 55° with *n*-propanol–water–ethyl acetate (7:2:1, v/v/v)) revealed glucose, gentiobiose and probably higher members of the β-1,6-linked series. However, most of the degradation products of R-glucan could be precipitated by adding 2 vol. ethanol. When the latter fraction was tested as a substrate for enzymes in the external-enzyme preparation, it was found to be susceptible to laminarinases. Similarly, when R-glucan was partially hydrolyzed with acid (2% HCl, 100°, 2 h)[8], both the ethanol-precipitable glucan in the hydrolysate and the residual glucan became susceptible to laminarinases in the external-enzyme preparation. The exo-laminarinase was the most active in this

respect. This indicates that the β-1,3 linkage in the intact R-glucan is inaccessible to enzymes which normally hydrolyze these bonds in laminarin.

Although no statements can be made concerning the linkages in R-glucan actually broken by R-glucanase (*cf.* ref. 9), the results suggest that enzymes with R-glucanase activity must be classified as β-1,6-glucan glucanohydrolases. However, since *S. commune* also manufactures an enzyme acting more specifically on the homopolymer pustulan, there appears to exist an as yet unknown specificity requirement in addition to the presence of 6-substituted glucosyl units. Therefore, R-glucanase activities in crude extracts cannot be assayed with pustulan as a substrate. Enzymes of the R-glucanase type may play important roles as initiators of softening or lysis of insoluble fungal cell-wall β-glucans.

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1 J. G. H. WESSELS, *Antonie van Leeuwenhoek J. Microbiol. Serol.*, 32 (1966) 341.
2 J. G. H. WESSELS AND D. J. NIEDERPRUEM, *J. Bacteriol.*, 94 (1967) 1594.
3 J. G. H. WESSELS, *Wentia*, 13 (1965) 1.
4 J. S. D. BACON, D. JONES, V. C. FARMER AND D. M. WEBLEY, *Biochim. Biophys. Acta*, 158 (1968) 313.
5 S. J. PILKIS, R. J. HANSEN AND M. E. KRAHL, *Biochim. Biophys. Acta*, 154 (1968) 250.
6 S. DYGERTS, L. H. LI, D. FLORIDA AND J. A. THOMA, *Anal. Biochem.*, 13 (1965) 367.
7 N. J. FAIRBAIRN, *Chem. Ind. London*, (1953) 86.
8 D. R. KREGER, *Biochim. Biophys. Acta*, 13 (1954) 1.
9 F. W. PARRISH AND A. S. PERLIN, *Nature*, 187 (1960) 1110.

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