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OCCURRENCE OF GLYCOPROTEIN GLYCOSIDASES IN MATURE SEEDS OF MUNG BEAN (*VIGNA RADIATA*)

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Key Word Index—*Vigna radiata*; mung bean; Leguminosae; glycoproteins; glycosidases; multiple forms; mung beans; protein bodies.

Abstract—The presence of nine different glycosidases was demonstrated in the crude extract of mature mung bean seeds. *N*-Acetyl β-D-glucosaminidase, α-D-galactosidase and β-D-glucosidase were each resolved into two respective active forms by gel filtration. The other glycosidases showed single forms only. The apparent MWs of the glycosidases were determined. The glycosidases were absorbed to Con A-Sepharose column, with the exception of a small percentage of α-galactosidase and α-mannosidase which were eluted unretarded. The bound enzymes displayed varying affinities for the immobilized lectin, indicating differences in glycosylation. With the exception of β-galactosidase and invertase, all the glycosidase activities were detected in the protein bodies isolated from the seeds.

INTRODUCTION

A wide variety of legume seeds are known to contain glycoproteins which may be lectins and reserve proteins [1]. Several plant glycosidases are also known to be glycoprotein in nature [2–5]. Protein bodies have been demonstrated to be the site for many of these glycoproteins [6–8]. During seed ripening many of the synthesized proteins migrate from their sites of synthesis on the endoplasmic reticulum [9] to the protein bodies [10]. A number of hydrolytic enzymes, including glucosidases, were found in the vacuoles formed from 'empty' protein bodies [11] and indeed they were also found in the intact protein bodies [8].

The function of the carbohydrate moieties of plant glycoproteins is somewhat unclear. A class of glycoproteins, termed extensin are related to cell extension [12]. The carbohydrate parts of glycoproteins may also be involved in recognition phenomena such as interaction between cells and in targeting the protein to specific sites [13–16]. In this respect the targeting of mammalian lysosomal glycoprotein enzymes is well studied [17]. In the present study attempts have been made to examine a number of glycosidases from mung bean for their possible glycoprotein nature. The presence of glycosidase activities in isolated protein bodies has also been investigated.

RESULTS AND DISCUSSION

Separation of glycosidases

Dry resting seeds were milled to a fine powder and extracted with acetate buffer, pH 5. Use of citrate–phosphate buffer was avoided as citrate ions could cause possible interconversion between enzyme multiple forms [18]. The presence of zinc chloride (1 mM) in the extracting buffer was important for the display of full activity of α-mannosidase. This zinc-dependent enzyme [19] was unstable when EDTA (10 mM) was present in the buffer. Difficulties were encountered in assaying glycosidase activities because of turbidity produced by zinc on addition of sodium carbonate (0.1 M) for stopping the enzyme-catalysed hydrolysis of *p*-nitrophenyl glycosides. It was therefore necessary to centrifuge the final assay solution before measuring the absorbance of the yellow colour of released *p*-nitrophenol. The glycosidase activities detected in the crude extract are shown in Table 1. While all activities were assayed using 0.1 M acetate buffer, pH 5, invertase was measured using 0.1 M phosphate buffer, pH 7.5. This enzyme displayed no activity at pH 5. This is in accordance with the general observation that acid invertases are found in the plant organs where rapid growth occurs, whereas the alkaline invertases are present in storage organs [20, 21]. The pH optima of all other enzymes were determined separately using the crude extract and the respective *p*-nitrophenyl substrate. The optima were found to lie in the pH range of 4.5–5.5. The use of acetate buffer at pH 5 for assays gave almost 90% of maximal activities of the enzymes.

The crude extract was fractionated by using solid ammonium sulphate and the 35–75% fraction dissolved in the enzyme extraction buffer displayed *ca* 70% of the starting activities. A sample of this fraction was then loaded on a Sephacryl S-200 column. The elution profiles of the enzymic activities are shown in Fig. 1. A single enzyme form was observed for α-L-arbinofuranosidase, α-L-fucosidase, β-D-galactosidase, invertase, α-mannosidase and β-xylosidase; however, for *N*-acetyl β-D-glucosaminidase and α-D-galactosidase, two active forms were resolved. Whereas invertase was eluted in the void volume, other enzymes lay within the resolving limit of the Sephacryl column. The apparent MWs of these enzymes were assessed by calibrating the column with proteins of known MWs. The results presented in Table 1 indicate that the two forms of β-glucosidase and *N*-acetyl β-D-glucosaminidase may have monomer/dimer relationship whereas α-D-galactosidase have monomer/tetramer relationship. The latter enzyme commonly occurs in these

Table 1. Glycosidase activities in the crude extract of mung bean seeds

Enzymes	Activity (m units/g dry wt. of seeds)	Apparent MW
1. *N*-Acetyl β-D-glucosaminidase (EC 3.2.1.30)	75	90000 and 45000
2. α-L-Arabinofuranosidase (EC 3.2.1.55)	53	162000
3. α-L-Fucosidase (EC 3.2.1.51)	81	90000
4. α-D-galactosidase (EC 3.2.1.22)	625	162000 and 42000
5. β-D-galactosidase (EC 3.2.1.23)	160	162000
6. β-D-Glucosidase (EC 3.2.1.21)	890	170000 and
7. Invertase (alkaline) (EC 3.2.1.26)	515	> 250000
8. α-D-Mannosidase (EC 3.2.1.24)	430	79000
9. β-D-Xylosidase (EC 3.2.1.27)	217	42000

forms in legume seeds [22–24].

In order to demonstrate possible affinities of the glycosidases for Concanavalin A, a dialysed sample of the ammonium sulphate fraction was passed through a column prepared from the immobilized lectin (Con A-Sepharose). The protein elution pattern shown in Fig. 2 demonstrates that a major fraction was unadsorbed (peak A) while four peaks (B–E) were resolved by a linear gradient of methyl α-D-glucoside. Thus, peaks B–E represent glycoproteins displaying increasing binding intensities with the lactin. The lack of binding of peak A is not due to overloading of the column, since when this peak was re-run on a larger column, it was also eluted unretarded. The material in peak A is therefore either not a glycoprotein or contains carbohydrate units of a composition and/or structure unreactive with Concanavalin A. The following enzymic activities were observed in peaks A–E (the percentage activities recovered as compared to those applied are shown in brackets): A—α-D-galactosidase (25%), α-D-mannosidase (32%); B—α-D-galactosidase (70%), α-L-fucosidase (95%), β-D-xylosidase (90%); C—*N*-acetyl β-D-glucosaminidase (95%), α-D-mannosidase (65%), β-D-glucosidase (92%); D—α-L-arabinosidase (96%), β-D-galactosidase (95%); E—invertase (96%). These results suggest that the glycosidases present in mung bean differ in their glycosylation. The two glycosidases in peak A may be precursors of the respective glycosylated enzymes present in peaks B and C. The ratio of the low and high MW forms of α-galactosidase in peak A was the same as in peak B, showing that if this enzyme were unglycosylated, its monomeric active form was able to aggregate to yield the active tetramer. Finally, it can be commented that lectin affinity columns may prove as important tools for resolving glycoproteins with varying extent of glycosylation by eluting with a concentration gradient of the specific sugar molecule. Immobilized lectins with differing ligand-binding affinities may also be used for this purpose.

Glycosidase activities in protein body

The protein body fraction was isolated by the glycerol method as described earlier [25, 26]. The fraction was solubilized [26] and assayed for the glycosidase activities. The results presented in Table 2 show that various proportions of seven glycosidase activities were present in the protein body fraction. The low recovery of activities may be due to losses during the isolation and extraction procedures. However the yields of *N*-acetyl β-D-glucosaminidase, α-D-galactosidase and α-D-mannosidase were higher than that of the other enzymes. The β-galactosidase and invertase activities were entirely absent from the protein body preparation.

The glycoprotein nature of the protein body glycosidases was examined by passing the solubilized fraction through a Con A-Sepharose column. The elution pattern of the protein peaks and the distribution of glycosidase activities were essentially the same as shown in Fig. 2

Table 2. Distribution of glucosidase activities in mung bean protein body fraction

Enzymes	Activity in protein body* fraction (%)
N-Acetyl β-D-glucosaminidase	62
α-L-Arabinofuranosidase	45
α-L-Fucosidase	33
α-D-Galactosidase	58
β-D-Glucosidase	41
α-D-Mannosidase	61
α-D-Xylosidase	26

*This was calculated based upon the enzyme units present/g dry wt of seeds and that found in protein body fraction obtained from 1 g dry wt of seeds.

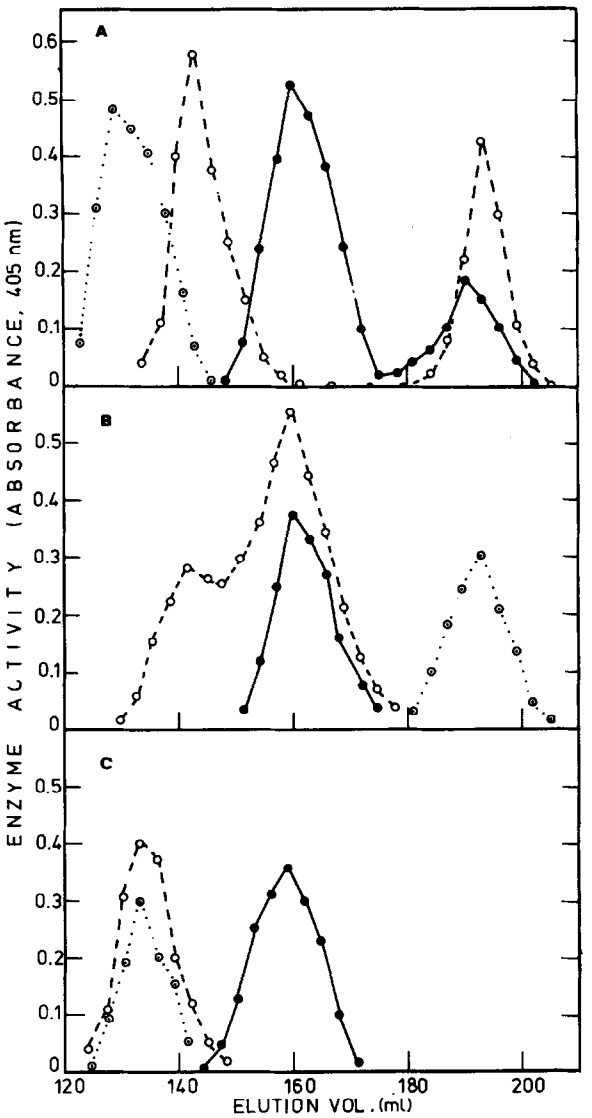

Fig. 1. Gel-filtration on Sephacryl S-200 column of a sample of $(NH_4)_2SO_4$ fraction of mung bean extract. Glycosidase activities were determined in each fraction (volume used in assay is shown in brackets): (A) ●——● *N*-acetyl β-D-glucosaminidase (0.4 ml), O----O α-D-galactosidase (0.05 ml), O · · · · O invertase 0.05 ml); (B) ●——● α-L-fucosidase (0.2 ml), O----O β-D-glucosidase (0.04 ml), O · · · · O β-D-xylosidase (0.05 ml); (c) ●——● α-D-mannosidase (0.04 ml), O----O β-D-galactosidase (0.3 ml), O · · · · O α-L-arabinofuranosidase (0.2 ml). Absorbance at 405 nm are plotted; other conditions are described in the Experimental section.

except that there was no glycosidase activity in peak A and E and no β-galactosidase activity in peak D. Peaks B–D were further examined by their passage through the Sephacryl S-200 column. Two forms of activities were resolved for *N*-acetyl β-D-glucosaminidase, α-D-galactosidase and β-D-glucosidase. The apparent MWs of these forms and the other glycosidases determined from the respective elution volumes were comparable to those shown in Table 1.

The absence of alkaline invertase from the protein bodies is not surprising. These cellular compartments seem to be the sites of acid hydrolases [8, 27], similar to those of mammalian lysosomes. The site of acid β-galactosidase, on the other hand, may be the cell wall [28, 29]. Protein bodies are regarded as protein-filled vacuoles [8, 30–32]. Thus the hydrolases present in protein bodies may also be expected in the vacuoles. It was recently shown that in bean cotyledons α-mannosidase activity was present in endoplasmic reticulum, protein bodies and cell wall fractions [27]. It is tempting to speculate that glycosylation of the newly synthesized protein may be one important factor in targeting and transport of the glycosidases to the protein body site. If comparison of vacuoles and protein bodies is made with mammalian lysosomes, it is interesting to note that most acid hydrolases present in the latter are glycoprotein in nature [33, 34] with affinity for Con A.

EXPERIMENTAL

Enzyme isolation and assay. Mature mung bean seeds (*Vigna radiata*) purchased locally were milled in a mechanical grinder and the powder (100 g) suspended in 0.1 M NaOAc buffer, pH 5 containing 1 mM $ZnCl_2$ (200 ml). The slurry was continually stirred at 4° for 1 hr and then centrifuged for 30 min at 20000 *g*. The supernatant was used as the crude enzyme extract. This extract was fractionated with $(NH_4)_2SO_4$ and the 35–75% fraction was dissolved in the extraction buffer (10 ml) and dialysed against the same buffer. The enzyme preparation so obtained was employed in gel-filtration experiments and affinity chromatography using Con A-Sepharose column. Glycosidase activities were assayed by using *p*-nitrophenyl glycosides as substrates and the conditions were the same as those of α-galactosidase assay [4]. 0.1 M NaOAc buffer, pH 5 containing 1 mM $ZnCl_2$ was used. Unit of enzyme activity is expressed as the amount required to convert 1 μmol of the substrate under the given conditions.

Chromatographic methods. The gel-filtration experiments were carried out at 4° using Sephacryl S-200 column (2 cm × 100 cm) equilibrated with the enzyme extraction buffer. Enzyme sample (3 ml) was applied and the column was eluted with a downward flow (30 ml/hr); 3 ml fractions were collected. Affinity chromatography was performed using a Con A-Sepharose column (1 cm × 5 cm) equilibrated with 0.1 M NaOAc buffer, pH 5 containing 1 mM each of $MnCl_2$, $MgCl_2$, $CaCl_2$ and 1% NaCl. Enzyme sample (1 ml) was applied and the column eluted with the equilibrating buffer; 2 ml fractions were collected. When no more proteins were eluted, a gradient of 0–0.3 M methyl α-D-glucoside made in the equilibration buffer was applied for eluting the adsorbed proteins.

Isolation of protein bodies. This was carried out by following the previously described method [25, 26].

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REFERENCES

1. Lis, H. and Sharon, N. (1973) *Annu. Rev. Biochem.* **42**, 541.
2. Dey, P. M., Pridham, J. B. and Sumar, N. (1982) *Phytochemistry* **21**, 2195.
3. Dey, P. M., Naik, S. and Pridham, J. B. (1982) *FEBS Letters* **150**, 233.

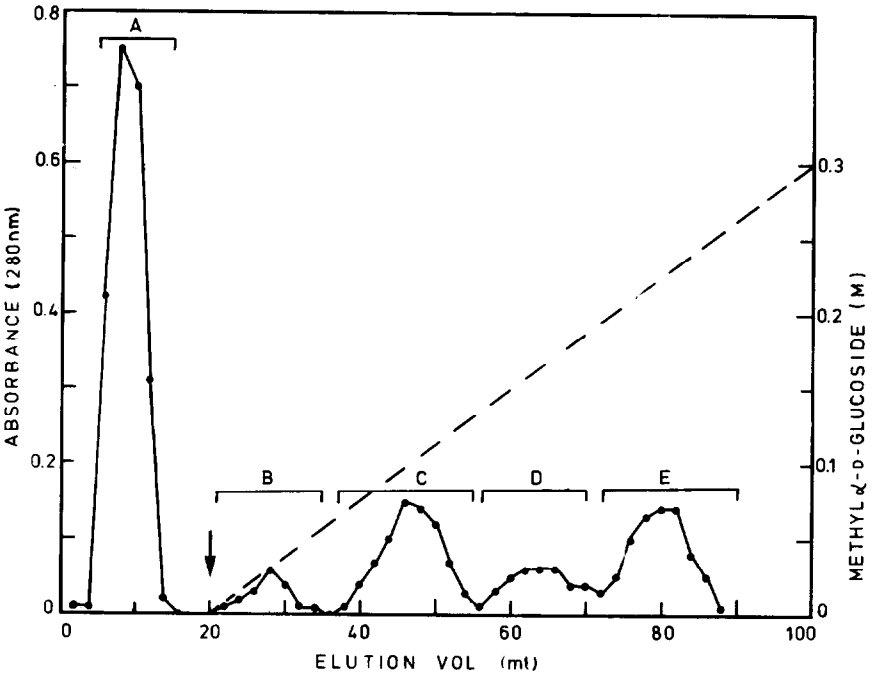

Fig. 2. Affinity chromatography on Con A-Sepharose column of a sample of $(NH_4)_2SO_4$ fraction of mung bean extract. Protein peaks marked A–E were assayed for glycosidase activities. Arrow indicates the start of the methyl α-D-glucoside gradient. Other conditions are described in the Experimental section.

4. Dey, P. M., Del Campillo, E. and Pont Lezica, R. (1983) *J. Biol. Chem.* **258**, 923.
5. Dey, P. M. and Del Campillo, E. (1983) *Adv. Enzymol.* **56**, 141.
6. Pernollet, J. C. (1978) *Phytochemistry* **17**, 1473.
7. Matile, P. (1975) *The Lytic Compartment of Plant Cells*, Vol. 1. Springer, New York.
8. Van der Wilden, W., Herman, E. M. and Chrispeels, M. J. (1980) *Proc. Natl. Acad. Sci., U.S.A.* **77**, 428.
9. Bollini, R. and Chrispeels, M. J. (1979) *Planta* **146**, 487.
10. Millerd, A. (1975) *Annu. Rev. Plant Physiol.* **26**, 53.
11. Nishimura, M. and Beevers, H. (1978) *Plant Physiol.* **62**, 44.
12. Lamport, D. T. A. and Catt, J. W. (1981) in *Encyclopedia of Plant Physiology, New series* (Prison, A. and Zimmermann, M. H., eds) Vol. 13B, pp. 133–165. Springer, New York.
13. Brown, R. G. and Kimmins, W. C. (1977) in *International Review of Biochemistry* (Northcote, D. H. ed.) Vol. 13, pp. 183–209. University Park Press, Baltimore.
14. Olden, K., Parent, J. B. and White, S. L. (1982) *Biochim. Biophys. Acta* **650**, 209.
15. Bowles, D. J. (1979) *FEBS Letters* **102**, 1.
16. Bowles, D. J. and Marcus, S. (1981) *FEBS Letters* **129**, 135.
17. Neufeld, E. F. and Ashwell, G. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, W. J., ed.) pp. 241–266. Plenum Press, New York.
18. Dey, P. M., Hustler, M. J., Pridham, J. B. and Sumar, N. (1982) *Phytochemistry* **21**, 1557.
19. Snaith, S. M. and Levvy, G. A. (1973) *Adv. Carbohydr. Chem. Biochem.* **28**, 401.
20. Lyne, R. L. and ap Rees, T. (1971) *Phytochemistry* **10**, 2593.
21. Gaylor, K. R. and Glaszion, K. T. (1972) *Plant Physiol* **27**, 25.
22. Hankins, C. and Shannon, L. M. (1978) *J. Biol. Chem.* **253**, 7791.
23. Del Campillo, E., Shannon, L. M. and Hankins, C. (1981) *J. Biol. Chem.* **256**, 7177.
24. Dey, P. M. and Pridham, J. B. (1972) *Adv. Enzymol.* **36**, 91.
25. Yatsu, L. Y. and Jacks, T. J. (1968) *Arch. Biochem. Biophys.* **124**, 466.
26. Plant, A. R. and Moore, K. G. (1982) *Phytochemistry* **21**, 985.
27. Van der Wilden, W. and Chrispeels, M. J. (1983) *Plant Physiol.* **71**, 82.
28. Asamizu, T., Inone, Y. and Nishi, A. (1981) *Plant Cell Physiol.* **22**, 469.
29. Klis, F. M., Dalhuizen, R. and Sol, K. (1974) *Phytochemistry* **13**, 55.
30. Craig, S., Goodchild, D. J. and Miller, C. (1980) *Aust. J. Plant Physiol.* **7**, 329.
31. Baumgartner, B., Tokuyasu, K. T. and Chrispeels, M. J. (1978) *J. Cell Biol.* **79**, 10.
32. Boller, T. and Kende, H. (1979) *Plant Physiol.* **63**, 1123.
33. Beutler, E., Guinto, E. and Kuhl, W. (1975) *J. Lab. Clin. Med.* **85**, 672.
34. Kencht, D. A. and Diamond, R. L. (1981) *J. Biol. Chem.* **256**, 3564.