Phytochemistry, Vol. 28, No. 2, pp. 355–357, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00
Pergamon Press plc.

LEGUMINOUS GLYCOSIDASES: HYDROPHOBIC AND LECTIN BINDING PROPERTIES

WOLFGANG EINHOFF and HAROLD RÜDIGER

Institut für Pharmazie und Lebensmittelchemie der Universität Würzburg, Am Hubland, D-8700 Würzburg, F.R.G.

(*Received* 25 *July* 1988)

Key Word Index—*Canavalia ensiformis*; *Glycine max*; *Lens culinaris*; *Pisum sativum*; *Phaseolus vulgaris*; *Sophora japonica*; *Wisteria floribunda*; Leguminosae; glycosidases; hydrophobic binding; lectin binding.

Abstract—The interactions of leguminous seed glycosidases with lectins from the same source, with concanavalin A (ConA) and with a hydrophobic adsorbent, phenyl-Sepharose, were studied. Only mannose/glucose specific lectins bind glycosidases from the same plant by means of the sugar-binding site, lectins with different sugar specificities either bind the glycosidases only at low ionic strength or not at all. ConA interacts with most glycosidases by its sugar binding site. All glycosidases interact with the hydrophobic adsorbent, they differ, however, in binding strength. The α-mannosidase from *Canavalia ensiformis* is exceptional. Though a glycoprotein, it interacts with ConA, the lectin from the same plant, by ionic forces. Binding to the hydrophobic adsorbent is much more tightly than with other glycosidases.

INTRODUCTION

In the seeds of Leguminosae, lectins, storage proteins and some glycosidases are located in the protein bodies. Interactions between these components may therefore be physiologically important [1, 2].

Recently we found that the α-mannosidase from *Canavalia ensiformis* specifically interacts with the lectin (ConA) from the same plant. We also found that the enzyme binds very effectively to a hydrophobic adsorbent, phenyl-Sepharose [3, 4]. In the present paper, we extend these studies to other species and to other glycosidases.

RESULTS AND DISCUSSION

Interaction with lectins

As seen from Table 1 (columns A), many glycosidases bind to the lectin from the source plant. Desorption is achieved either by the lectin-specific carbohydrate or by raising the ionic strength. Desorption by a carbohydrate is found only with mannose/glucose-specific lectins (glycosidases from *Lens culinaris* and *Pisum sativum* and the α-galactosidase from *Canavalia ensiformis*), in other species, the lectin-specific sugar (Gal) is ineffective. Ion strength dependent interactions are found between two *Canavalia ensiformis* enzymes (the α-mannosidase and the β-*N*-acetylglucosaminidase) and ConA, and between the α-mannosidases from *Glycine max* and *Phaseolus vulgaris* and the α-galactosidase from *G. max* with the respective lectin. Some glycosidases do not interact with the lectin from the same plant under the conditions used.

In contrast, most glycosidases interact with ConA (columns B). This applies even to those glycosidases that do not bind to the lectin from the same plant. Remarkably, all enzymes (with the exception of the *P. vulgaris* α-galactosidase) interact through the sugar binding site, even where the 'own' lectin interacts with the glycosidase by ionic binding. Not a single β-galactosidase binds to the lectin from the same plant. Nevertheless, all of them appear to be glycoproteins since they interact with the sugar-binding site of ConA.

α-Mannosidases, α-galactosidases and β-*N*-acetylglucosaminidases have been shown to reside in the protein bodies whereas β-galactosidases are outside [5]. The ability of the former three enzymes to interact with the lectins from the same plants and the failure of the β-galactosidases to do so correlate well with their intracellular locations.

Hydrophobic interaction

In contrast to ionic interaction, binding of proteins to hydrophobic adsorbents is generally strengthened by raising and weakened by lowering the ionic strength [6]. Therefore the salt concentration necessary for desorption provides a measure for the strength of interaction. Hydrophobic interactions are stronger at high than at low temperatures [6]. This was also observed for the enzymes studied here. Because of the instability of many enzymes and poor recoveries at room temperature, all chromatographies were performed at 4°.

The results of the chromatographies of glycosidases on phenyl-Sepharose are given in Scheme 1. At high ionic strengths, all glycosidases bind to this adsorbent. The α-mannosidase from *C. ensiformis* binds strongest and for

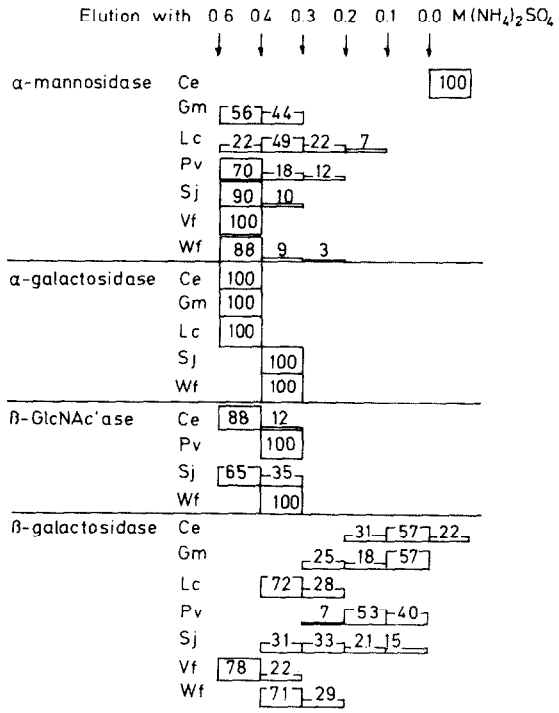
Enzymes: α-Galactosidase, α-D-galactoside galactohydrolase (EC 3.2.1.22); β-galactosidase, β-D-galactoside galactohydrolase (EC 3.2.1.23); α-mannosidase, α-D-mannoside mannohydrolase (EC 3.2.1.24); β-*N*-acetylglucosaminidase, β-*N*-acetyl-D-glucosaminide *N*-acetylglucosaminohydrolase (EC 3.2.1.30).

Abbreviations—ConA: concanavalin A, the lectin from *Canavalia ensiformis*.

Table 1. Interaction of glycosidases with immobilized lectins from the same plant (A) and with ConA (B)

	α-Mannosidase		α-Galactosidase		β-Galactosidase		β-*N*-Acetylglucosaminidase	
Plant	A	B	A	B	A	B	A	B
Canavalia ensiformis	++i		+c		—		+i	
Glycine max	+i	++c	++i	+c	—	+c	—	n.d.
Lens culinaris	++c	++c	++c	++c	—	++c	n.d.	n.d.
Pisum sativum	+c	+c	++c	++c	—	++c	n.d.	n.d.
Phaseolus vulgaris	+i	++c	—	+i	—	+c	—	n.d.
Sophora japonica	—	+c	—	—	—	++c	—	—
Wisteria floribunda	—	++c	—	++c	—	++c	—	+c

++, high capacity (>0.1 mg/ml gel); +, low capacity (<0.1 mg/ml gel); –, no binding; n.d., not determined; c, desorption by carbohydrate (0.1 M α-methylmannoside. Mannose, glucose and maltose are also effective); i, desorption at high ionic strength (0.1 M NaCl).



Scheme 1. Hydrophobic interaction of glycosidases with phenyl-Sepharose. Figures in the bars represent percentage recoveries at the ammonium sulphate concentrations indicated. Ce: *Canavalia ensiformis*; Gm: *Glycine max*; Lc: *Lens culinaris*; Ps: *Pisum sativum*; Pv: *Phaseolus vulgaris*; Sj: *Sophora japonica*; Wf: *Wisteria floribunda*.

desorption it requires a buffer free from ammonium sulphate. Other α-mannosidases bind more weakly and are desorbed at medium ammonium sulphate concentrations.

For the other three glycosidase activities investigated, no conspicuous differences could be detected between the enzymes from *C. ensiformis* and the other Leguminosae. α-Galactosidases and β-*N*-acetylglucosaminidases are desorbed at high salt concentrations in relatively narrow ionic strength ranges. β-Galactosidases, on the other hand, generally bind more tightly and appear at lower ammonium sulphate concentrations in broad ranges. This phenomenon may be due to the presence of β-galactosidase isoenzymes which differ in hydrophobicities. Recently, Biswas found that several β-galactosidases are present in the legume *Vigna sinensis* [7].

It has been proposed that it is possible to calculate hydrophobicities of proteins from amino acid compositions [8]. Of two α-mannosidases the amino acid compositions are known. The *P. vulgaris* enzyme has been analysed by Paus [9] and the *C. ensiformis* enzyme by us [10]. We calculated the hydrophobicities of both enzymes according to ref. [8]. The values obtained were 908 and 952 cal/mol for the enzymes from *C. ensiformis* and *P. vulgaris*, respectively, the experiment gives an inverse order of hydrophobicities (Scheme 1).

Apparently, hydrophobicity calculations only from amino acid analyses do not lead to meaningful predictions [11]. They do not differentiate between acidic amino acids and disregard differences in conformation and in degree of glycosylation. The *P. vulgaris* α-mannosidase is a glycoprotein (Table 1). This applies to the *Canavalia* enzyme as well [10, 12, 13], but in contrast to the *Phaseolus* enzyme, the *Canavalia* α-mannosidase contains a carbohydrate moiety that is buried and not accessible to either ConA [10] to endoglucosaminidase H [12].

As our data show, glycosidases from Leguminosae bind to lectins from the same plant and from other sources. They also show hydrophobic binding. The legume *C. ensiformis* is an exception in several respects. Its α-mannosidase is abundant by more than one order of magnitude as compared with other glycosidases in the same plant and with glycosidases in other Leguminosae [1]. Secondly, though it is a glycoprotein, it binds to its

'own' lectin by ionic forces. Thirdly it is much more hydrophobic than other leguminous glycosidases.

EXPERIMENTAL

Phenyl-Sepharose was from Pharmacia, *p*-nitrophenylglycosides from Sigma, seeds were from the suppliers mentioned in ref. [14].

Lectins were isolated from seeds by affinity chromatography on immobilized hog gastric mucin or ovomucoid as described in ref. [14], except for the lectins from *Canavalia ensiformis*, *Pisum sativum* and *Lens culinaris* for which Sephadex G-50 was used as an affinity adsorbent. Lectins were immobilized to Sepharose 4B by the cyanogen bromide procedure of ref. [15]. Protein concentrations of gels were determined by derivative spectrophotometry [16], usually 10–15 mg protein were immobilized per ml gel. Glycosidase activities were assayed with *p*-nitrophenylglycosides as substrates using the method of ref. [17] as modified in ref. [3].

Extracts were prepared from ground seeds with 0.05 M Tris–HCl buffer, pH 8.0. By acid pptn (pH 5.0), storage proteins could be removed nearly completely without serious losses (<20%) of glycosidases. For hydrophobic and affinity chromatographies this prepurified material (seed albumin) was applied to columns (0.7 × 4 cm) which were developed at a flow of 12 ml/hr in the cold (4°). In hydrophobic chromatography, 100–200 mg of albumin protein in 0.05 M Tris–HCl, pH 8.0–1 M $(NH_4)_2SO_4$, was loaded on phenyl-Sepharose which had been equilibrated with the same buffer. Enzymes were desorbed by applying a gradient of decreasing $(NH_4)_2SO_4$ concns in the pH 8 buffer.

For affinity chromatography, 20–30 mg of albumin in 0.05 M NaOAc buffer, pH 5.0–1 mM $ZnCl_2$, was loaded on the immobilized lectin in the pH 5 buffer. Elution was started with NaOAc, desorption was tried first with NaCl, then with the lectin specific sugars (α-methylmannoside or galactose) at 0.1 M concns.

REFERENCES

1. Einhoff, W., Fleischmann, G., Freier, T., Kummer, H. and Rüdiger, H. (1986) *Biol. Chem. Hoppe-Seyler* **367**, 15.
2. Freier, T. and Rüdiger, H. (1987) *Biol. Chem. Hoppe-Seyler* **368**, 1215.
3. Einhoff, W. and Rüdiger, H. (1986) *Biol. Chem. Hoppe-Seyler* **367**, 313.
4. Einhoff, W. and Rüdiger, H. (1986) *Biol. Chem. Hoppe-Seyler* **367**, 943.
5. Dey, P. M. (1984) *Phytochemistry* **23**, 257.
6. Hjertén, S. (1973) *J. Chromatogr.* **87**, 325.
7. Biswas, T. K. (1987) *Phytochemistry* **26**, 359.8.
8. Bigelow, C. C. and Channon, M. (1976) in *Handbook of Biochemistry and Molecular Biology* (Sassmann, D., ed.) pp. 210–243. *Proteins* Vol. 1. CRC Press, Cleveland, Ohio.
9. Paus, E. (1977) *Eur. J. Biochem.* **73**, 155.
10. Einhoff, W. and Rüdiger, H. (1988) *Biol. Chem. Hoppe-Seyler* **369**, 165.
11. Rose, G. D. (1987) *Proteins* **2**, 79.
12. Bowles, D. J., Chaplin, M. F. and Marcus, S. E. (1983) *Eur. J. Biochem.* **130**, 613.
13. Shepherd, V. and Montgomery, R. (1976) *Biochim. Biophys. Acta* **429**, 884.
14. Freier, T., Fleischmann, G. and Rüdiger, H. (1985) *Biol. Chem. Hoppe-Seyler* **366**, 1023.
15. Kohn, J. and Wilchek, M. (1982) *Biochem. Biophys. Res. Commun.* **107**, 878.
16. Schurz, H. and Rüdiger, H. (1982) *Anal. Biochem.* **123**, 174.
17. Li, Y. T. (1967) *J. Biol. Chem.* **242**, 5474.