XXVIII Polish Biochemical Society Meeting, Łódź, 1992

Acta Biochimica Polonica
Vol. 40 No. 1 1993

GalNAc/Gal specific lectin from German iris (*Iris germanica* L.) rootstock

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Most of the plant lectins characterised so far have been isolated from tissues of Dicotyledones. Among the lectins of Monocotyledones that from wheat germs (WGA) was the first known. During the last decade the *Gramineae* family was well characterised with respect to the occurrence of lectins and their structural and immunological relationships [1 - 3]. Less is known about lectin activity in other representatives of Monocotyledones, although during the last few years the presence of lectins in bulbs of tulip, snowdrop and daffodil was reported [4 - 7].

We have found the hemagglutination activity in the saline extracts from rootstocks of the German iris (*Iris germanica* L.). The plant samples for the experiments were rootstocks and leaves of iris cv. Black Taffeta, obtained from the Wrocław University Botanical Garden collection. The extracts agglutinated human erythrocytes of A1, A2, and 0 groups, but they showed very weak activity towards the B group of red cells. Rabbit erythrocytes were agglutinated quite well, while the sheep and hen ones were not agglutinated at all.

Table 1

Inhibition of the hemagglutination activity of iris rootstock lectin in the presence of mono- and disaccharides

Saccharide	Concentration causing 50% inhibition of the hemagglutination activity (mM)
Glucose	200
Mannose	–*
GlcNAc	100
GalNAc	1.5
Galactose	6.25
Lactose	3.12
Arabinose	100
Xylose	–*
Fucose	200

* – No inhibitory effect at 200 mM concentration. The rootstock extracts after $(NH_4)_2SO_4$ precipitation were samples for these experiments

To determine the iris rootstock lectin specificity we have examined the inhibition of lectin activity by typical saccharides (Table 1). Among the eight monosaccharides and one disaccharide tested, GalNac, Lac, and Gal were found to be the most potent inhibitors, as they caused a 50% decrease of lectin activity at concentrations as low as 1.5 mM, 3.12 mM and 6.25 mM, respectively. This permits to classify this lectin as a typical GalNac/Gal specific plant lectin; however experiments on a limited number of saccharides examined might, when extended, point to a complex pattern of lectin specificity. The hemagglutination activity in iris rootstocks varied over the wide range during the vegetative period (Fig. 1). In summer and autumn the amount of lectin is very low, not exceeding 10 agglutinating units per gram of tissue fresh weight. In the spring time lectin activity increased rapidly, reaching 550 agglutinating units per gram of tissue fresh weight. In the leaves of iris we have also found a remarkable hemagglutination activity, appearing by the end of April and increasing in the later period. It is worth noting that the high lectin activity in leaves, reaching 400 - 500 units/g of fresh weight, persists for at least four months, even after flowering, while in the rootstock the

high level of lectin was found to occur only during 3 - 4 weeks preceding flowering. Such a picture of hemagglutination activity is rather atypical. In the so far published studies lectin was reported to accumulate in the seeds (*Gramineae, Leguminosae* [3, 8]) or storage vegetative tissues of plants [4 - 6]. The amounts of lectins found in green parts of plants are usually very low. Van Damme *et al.* [9, 10] have reported a high level of lectin in dry, resting bulbs of tulip and daffodil followed by a rapid decrease of lectin content during the sprout development. These results led those authors to the conclusion that lectins might serve as the storage material utilized in the early stages of development. The unusual changes of the lectin activity observed in iris rootstock and leaves are inconsistent with such a hypothesis. In our opinion the involvement of lectins in the plant metabolism or their regulatory functions might be more complex than that believed so far.

For the purpose of further molecular characteristics of iris lectin we worked out the procedure of its isolation, including homogenization of tissue in 0.9% NaCl, protein precipitation at 80% $(NH_4)_2SO_4$ saturation, and the ion exchange chromatography on DEAE-Sephadex. The active fractions were eluted from the column with 0.25 M phosphate buffer, pH 7.2. The capability of lectins for specific and reversible binding of sugars enables isolation of these proteins by the affinity chromatography on immobilized sugars. Since the iris lectin specificity has been determined as GalNac/Gal, we have chosen 0.2 M HCl treated Sepharose 6B as the chromatography matrix [11]. After washing out of the unbound proteins, the lectin fraction was released from the column using 0.2 M lactose in 0.9% saline, 0.1 M acetate buffer, pH 5.1. The material obtained after this step was highly active; even the amount as low as 0.3 µg of protein showed the hemagglutination activity. On SDS-PAGE [12] we found two predominant fractions of about 35 kDa and 30 kDa, although trace protein contaminations were also present.

The iris lectins need further examination. The first question is whether the rootstock lectin is of dimeric structure, or isolectins are present in this tissue. An other problem is whether the leaf lectin is the same protein as the rootstock one, or are they completely different organ-specific proteins exhibiting lectin activity.

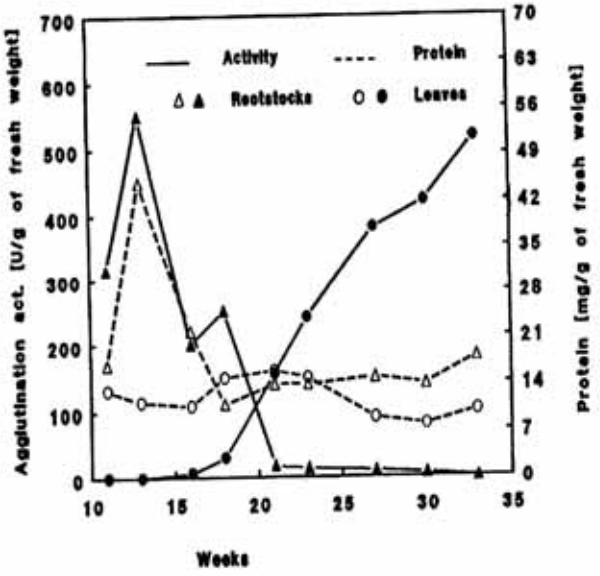

Fig. 1. Changes in hemagglutination activity in the rootstocks and leaves of the german iris during the vegetative period.
The time is expressed as weeks counting from Jan. 1st 1992

REFERENCES

1. Peumans, W.J., Stinissen, H.M. & Carlier, A.R. (1983) *Biochem. Physiol. Pflanzen* **178**, 423 - 431.
2. Stinissen, H.M., Peumans, W.J. & Carlier, A.R. (1983) *Planta* **159**, 105 - 111.
3. Peumans, W.J. & Stinissen, H.M. (1983) in *Chemical Taxonomy, Molecular Biology and Function of Plant Lectins* (Goldstein, I.J., ed.) pp. 98 - 116, A.R. Liss Inc. N.Y.
4. Oda, Y. & Minami, K. (1986) *Eur. J. Biochem.* **159**, 239 - 245.
5. Cammue, B.P.A., Peeters, B. & Peumans, W.J. (1986) *Planta* **169**, 583 - 588.
6. Van Damme, E.J.M. & Peumans, W.J. (1987) *FEBS Lett.* **215**, 140 - 144.
7. Kaku, H., Van Damme, E.J.M., Peumans, W.J. & Goldstein, I.J. (1990) *Arch. Biochem. Biophys.* **279**, 298 - 304.
8. Van Driessche, E. (1983) in *Advances in Lectin Research* (Franz, H., ed.) vol. 1, pp. 73 - 134, VEB Verlag Volk & Gesundheit.
9. Van Damme, E.J.M. & Peumans, W.J. (1989) *Planta* **178**, 10 - 18.
10. Van Damme, E.J.M. & Peumans, W.J. (1990) *Planta* **182**, 605 - 609.
11. Ersson, B., Asperg, K. & Porath, J. (1973) *Biochim. Biophys. Acta* **310**, 446 - 452.
12. Laemmli, U.K. (1970) *Nature* **227**, 680 - 585.