INTERACTIONS BETWEEN THE TWO LECTINS FROM *VICIA CRACCA*

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1. Introduction

Many lectins have been described from both plant and animal sources [1–3]. Usually one lectin of a peculiar specificity can be isolated from one species. Only a few plants contain lectins which differ in their sugar specificities. *Vicia cracca*, the common vetch, belongs to these plants. It contains a GalNAc (VcGalNAc) and a mannose/glucose-binding lectin (VcMan). Both have been isolated and characterized [4,5]. The VcGalNAc lectin (M_r 125 000) is composed of 4 subunits equal in molecular size (M_r 33 000) whereas the VcMan lectin contains pairs of 2 subunit types (α, M_r 5700; β, M_r 17 500) resulting in a tetramer of M_r 44 000. Though occurring in the same plant, both lectins are only distantly related in their amino acid sequences [5,6].

Lectins are frequently used to purify and to characterize glycoproteins, glycolipids and cells from alien sources. The interaction between lectins and these biological structures are, however, accidental and never appear in nature. In view of the biological role of plant lectins which is totally unknown, any interaction found between a lectin and a constituent of the same plant deserves attention. We have described the interaction of Leguminosae lectins with proteins of the same plant (lectin binders) [7]. This result has been confirmed in [8]. Here, we report that both lectins from *Vicia cracca* react with each other. This interaction was studied by two independent methods: affinity chromatography and nephelometry.

2. Materials and methods

Sephadex G-100, CH-Sepharose and Ficoll were from Deutsche Pharmacia (Freiburg), polyethylene glycol and *N*-hydroxysuccinimide from Serva (Heidelberg), lactate dehydrogenase from Boehringer (Mannheim) and bovine serum albumin from Behringwerke (Marburg). All other chemicals were bought from Sigma Chemie (München). The *Vicia cracca* lectins were isolated by affinity chromatography as in [4,5]. The VcGalNAc lectin is a glycoprotein. It contains ~7% of neutral sugar [9], predominantly mannose as shown by gas–liquid and thin-layer chromatography. In the VcMan lectin, only 0.7% neutral sugar was found, i.e., <1 sugar residue/subunit. No amino sugars could be detected [10].

Nephelometers equipped with a He–He laser from Hyland-Travenol (München) and Behringwerke (Marburg) were used for determining the relative light scattering (RLS) which results from protein–protein interaction. Readings were taken between 2–4 h after mixing. Affinity adsorbents were prepared by coupling lectins to CH-Sepharose 4B by the *N*-hydroxysuccinimide method [11] using the water soluble 1-ethyl-3(dimethylaminopropyl)-carbodiimide as a condensing agent. The amount of lectin bound to CH-Sepharose was estimated by a novel spectrophotometric method in which the concentration of an immobilized protein is assayed by its first derivative spectrum [12]. Unless otherwise indicated, all experiments were run in 0.05 M Tris–HCl (pH 8.0), 0.1 M in NaCl, at room temperature (20°C).

3. Results and discussion

In isolating the VcMan lectin, we observed that the VcGalNAc lectin had to be removed prior to affinity chromatography on Sephadex G-100 to optimize the capacity of the gel. Thus, we suspected that sugar residues of Sephadex and the VcGalNAc lectin compete with each other for the VcMan lectin. Isolation of the VcGalNAc lectin, on the other hand, was not

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impaired by the presence of the VcMan lectin. This may be explained by the fact that the association constants of the VcGalNAc lectin for GalNAc is ~20-times higher than the glucose–VcMan association constant [5].

To see whether an interaction between both lectins might be of biological significance, we examined the intracellular localization of the proteins. *Vicia cracca* seeds were processed and the soluble fraction and the crude protein bodies separated according to [13]. The protein bodies were further purified by centrifugation in a Ficoll gradient as in [14]. By these methods, both lectins were clearly shown to appear together in the soluble fraction.

3.1. *Affinity chromatography*

If one of both lectins is attached covalently to CH-Sepharose, it is able to retard the other one from a solution (fig.1). Control experiments confirmed that retardation by far exceeds gel-filtration effects arising from different M_r-values. By adding 0.1 M glucose to the eluant, retardation volumes are decreased by 50%. Apparently, the mannose/glucose binding site of the VcMan lectin takes part in the interaction. In order to quantify the interaction, we transformed the law of mass action:

$$A + B \rightleftarrows AB$$

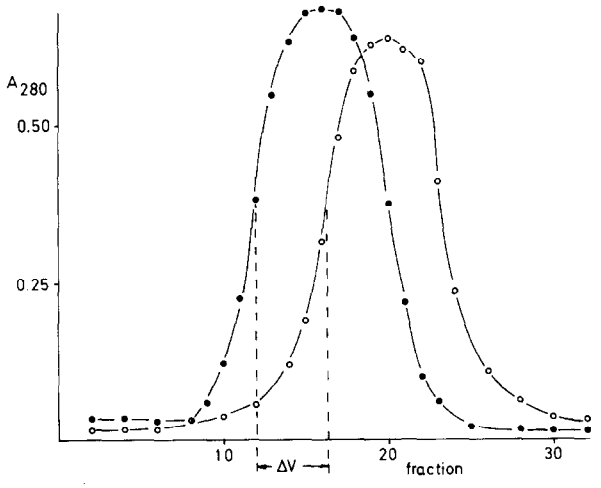

Fig.1. Gel-filtration of the VcMan-lectin (o——o) and bovine serum albumin (•——•) on VcGalNAc–CH-Sepharose 4B (immobilized VcGalNAc, 3 μM). Column size, 0.35 × 7.1 cm; fractions, 0.42 ml/9 min. The retardation volume is given by ΔV.

to yield:

$$ab = 1/2 \times (a_0 + b_0 + 1/K) \pm$$

$$1/2 \times \sqrt{(a_0 + b_0 + 1/K)^2 - 4a_0b_0} \qquad (1)$$

(a_0, b_0, ab, concentrations of the immobilized lectin A, of the lectin B in solution, and of the lectin–lectin complex at equilibrium, respectively; K, association constant) K and ab are unknown in eq. (1). From chromatographic runs, we obtained retardation volumes ΔV (fig.1) which represent the difference between the elution volumes of the lectin and an unretarded reference protein (bovine serum albumin, lactate dehydrogenase). We determined ΔV-values for different values of b_0 using a gel which contained 3 nmol immobilized lectin/ml ($a_0 = 3 \times 10^{-6}$ M). In a b_0 *vs* ΔV-diagram, a straight line is obtained. The intercept on the ΔV-axis represents the retardation volume V_0 at infinitely small b_0. Deviation in a set of experiments (3–5) was ~3%. As shown in [15], $(V_0 - \Delta V)$ is proportional to the complex concentration ab. By arbitrarily assuming K-values, we calculated complex concentrations from eq. (1). As ab values resulting from positive square roots exceeded a_0 and b_0, we took into consideration only values from negative roots. The ab values computed for different K were plotted against $(V_0 - \Delta V)$ taken from the experiment. A family of straight lines resulted the intercept of which on the $(V_0 - \Delta V)$-axis depended on the K-value used. Since ab is directly proportional to $(V_0 - \Delta V)$ [15], only that line is valid which runs through zero. This line was calculated from:

$$K = (6.7 \pm 1.0) \times 10^4 \text{ l/mol}$$

which thus represents the association constant.

3.2. *Nephelometry*

Laser nephelometry is an extremely sensitive method to visualize and to quantify antigen–antibody interactions. We studied the lectin–lectin interaction by this method. Fig.2 shows that on mixing both lectins, nearly no light-scattering (RLS) can be observed. Addition of PEG 6000, however, dramatically increases the measurable effect. This has also been observed in other lectin–glycoprotein interactions [16]. No PEG concentration optimum could be obtained. In routine experiments, 2–5% PEG were employed. Control experiments show that the

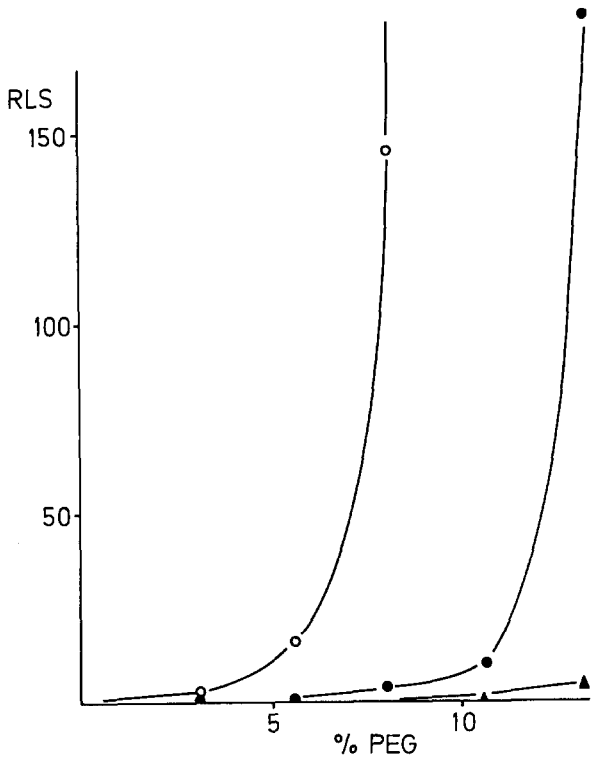

Fig.2. Dependence of relative light scattering (RLS) on the concentration of PEG 6000. Mixture of VcMan (1.8 μM) and VcGalNAc (0.8 μM) (o——o); controls, VcMan alone (▲——▲); VcGalNAc alone (●——●).

VcGalNAc lectin does not precipitate considerably at <10% PEG and that light scattering caused by the VcMan lectin is negligible at all PEG concentrations tested (fig.2).

For determining the pH dependence, we used the buffers proposed in [17] to minimize inconsistencies caused by ions of different properties. Interaction between both lectins is highly pH-dependent with a maximum about pH 6 (fig.3). Determinations below pH 5.5 were impossible because the VcGalNAc lectin itself precipitated at this pH. As seen from fig.4, the interaction is diminished at high ionic strength. This indicates that the interaction is partially ionic in character. At >2 M NaCl, the curve drops only very slowly. The influence of sugars is shown in fig.5. Glucose decreases the RLS considerably. At 0.4 mM glucose, the complex formation is inhibited by 50%. By using 2.5 mM glucose, the interaction is depressed to 15% of the original value. Apparently, the sugar binding site of the VcMan lectin participates since

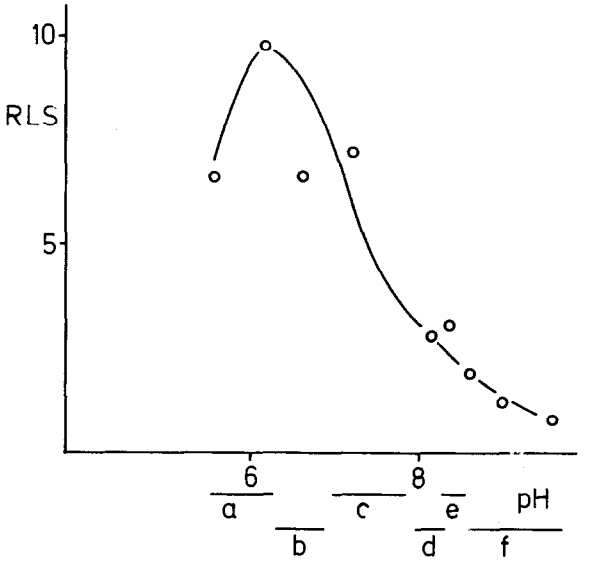

Fig.3. Dependence of relative light scattering (RLS) on pH: VcMan 2.5 μM; VcGalNAc 1.4 μM; NaCl 0.1 M; PEG 6000 2%; buffer 0.15 M; morpholinoethanesulfonic acid (a), morpholinopropanesulfonic acid (b), *N*-tris(hydroxymethyl)-methyl-2-aminopropane sulfonic acid (c), *N*-tris(hydroxymethyl)-methylglycine (d); *N,N*-bis(2-hydroxyethyl)glycine (e); *N*-tris(hydroxymethyl)methyl-3-aminopropane sulfonic acid (f).

the dissociation constant of the lectin–glucose complex is of the same order of magnitude [5]. Addition of GalNAc, however, does not result in a further decrease. On the other hand, the RLS is diminished by only 20% in presence of GalNAc. This effect is

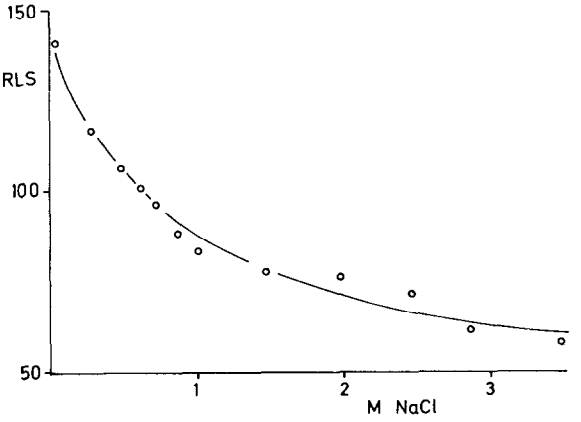

Fig.4. Dependence of relative light scattering (RLS) on the ionic strength: VcGalNAc 1.9 μM; VcMan 2.6 μM; PEG 6000 5%; Tris–HCl (pH 8.0) 0.02 M.

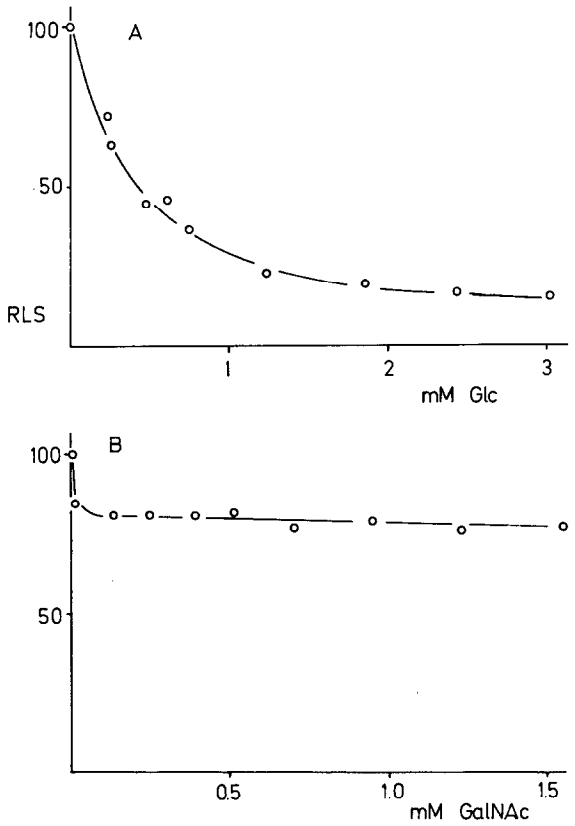

Fig.5. Dependence of relative light scattering (RLS) on the concentration of glucose (A) and GalNAc (B) added: VcGalNAc 1.9 μM; VcMan 2.6 μM; PEG 6000 5%.

achieved by <0.1 mM of this sugar. Higher amounts of GalNAc do not cause any further change. Presumably, this may reflect a conformational change of the VcGalNAc lectin which occurs in this concentration range [4]. Additional presence of glucose reduces the RSL from 80–15% of the initial value. The sugars have to be added prior to mixing the lectins. After precipitates have formed, they undergo secondary changes and cannot be reproducibly dissolved by sugars. If increasing amounts of the VcMan lectin are added to a fixed concentration of VcGalNAc, saturation curves are obtained (fig.6). Linearisation according to Hofstee [18] results in parallel straight lines from which $K = (8.3 \pm 1.0) \times 10^4$ l/mol was calculated. This value is in good agreement with the constant from affinity chromatography (see above).

These results suggest that both proteins, which not only occur in the same plant but also in the same cell compartment, may also be associated at their natural

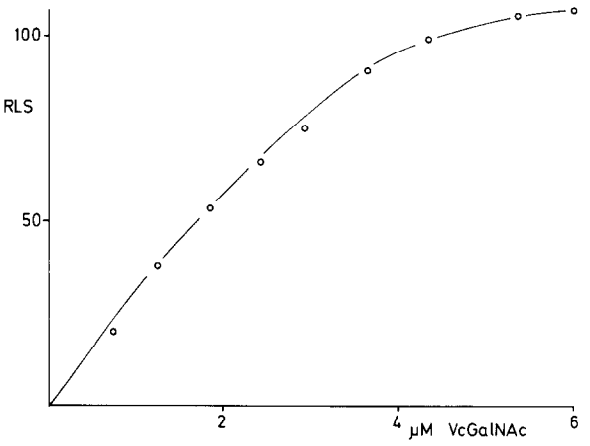

Fig.6. Dependence of relative light scattering (RLS) on the concentration of VcGalNAc added to a fixed concentration (14.6 μM) of VcMan; 2% PEG 6000, 3 h incubation.

site. This interaction is dependent on the environmental conditions as, e.g., pH, sugar or glycoside concentrations, and ionic strength. Within the cell, these parameters can be modulated rendering the interaction of both proteins reversible. Such a reversibility, however, has to be postulated for interactions of biological importance.

Acknowledgements

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