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Domain versatility in plant AB-toxins: Evidence for a local, pH-dependent rearrangement in the 2γ lectin site of the mistletoe lectin by applying ligand derivatives and modelling

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Abstract **Mistletoe lectin is a potent biohazard. Lectin activity in the toxic dimer primarily originates from the 2γ-subdomain (Tyr-site) of the B-subunit. Crystallographic information on lectin–sugar complexes is available only at acidic pH, where lectin activity is low. Thus, we mapped ligand-binding properties including comparison to ricin's Tyr-site at neutral pH. Using these results and molecular dynamics simulations, a local conformational change was rendered likely. The obtained structural information is valuable for the design of potent inhibitors.**

Keywords: Agglutinin; Lectin; Mistletoe; Ribosome-inactivating protein; Ricin

1. Introduction

Ribosome-inactivating plant lectins are potent biohazards and also attractive models to study evolutionary routes of intra- and interprotein divergence [1,2]. AB-type toxins combine a toxic A-subunit with the lectin part (B-subunit). Starting from a primordial 40-residues peptide, a series of gene duplications is supposed to have led to the common structural arrangement of the B-chain. It consists of two tandemly arrayed domains (1 and 2) established by four subdomains each (α, β, γ, δ) [3]. Carbohydrate-binding activity is found in the 1α and 2γ subdomains. The potent toxin ricin, the role model of this class, harbours Trp37 in its low-affinity 1α site and Tyr248 in its high-affinity 2γ site [3]. In solution it is monomeric, in contrast to the weakly toxic dimeric agglutinin of the castor bean. The galactoside-specific agglutinin from *Viscum album* L. (VAA; also called viscumin or ML-I) uniquely combines the activities as toxin/agglutinin in one protein [4]. Binding to cells and multivalent glycoproteins is of nM affinity and can engage the 1α domain around Trp38 and the 2γ domain around Tyr249 [5–7]. Because dimer formation spatially restricts accessibility to the Trp-site, the Tyr-site is central for glycan binding and toxicity above submicrogram/ml-levels [7].

Of note for structural design of inhibitors, crystals of VAA-sugar complexes had been grown in acidic pH, i.e. pH 3.4 and 2.5 [8,9], a condition detrimental for lectin activity [10]. The concern for pH-dependent local conformational changes in the binding site arises. It is also valid in the case of ricin, which shares this sensitivity toward pH decrease [11]. To address this issue and also enable interprotein comparison, we here present mapping of the ligand-binding properties of VAA's Tyr-site and, assisted by molecular dynamics simulations, develop a structural model of this site at neutral pH.

2. Materials and methods

2.1. Saccharides

The panel of saccharides was established from sources as given in detail previously [12,13].

2.2. Isolation and labelling of VAA

Purification, labelling with a biotin derivative and quality/activity controls were performed as described previously [5,7]. Biotin-labelled VAA was subsequently acetylated with *N*-acetylimidazole, in the absence or presence of 0.1 M lactose, using a 2-fold excess (w/w) of the reagent [5]. Protein concentration was determined by the Lowry assay using concanavalin A (Sigma) as standard [14]. Radioiodination of VAA using IODO-GEN (Pierce Eurochemie) up to a typical specific activity of 15 ± 5 μCi/mg was carried out in the presence of 0.1 M lactose to protect the carbohydrate-binding sites from modification.

2.3. Quantitative binding studies

Plastic microwells were coated with 50 μl of asialofetuin solution for 16 h at 4 °C and binding of ^{125}I-VAA to the wells was assayed essentially as described [12], except that the buffer used was 5 mM sodium phosphate buffer, pH 7.2, 0.2 M NaCl. For binding assays with biotinylated VAA, precoated wells were incubated with 50 μl of lectin solution for 2 h at 20 °C, and the extent of bound lectin was monitored by measuring the amount of streptavidin associated to the wells after incubation for 1 h at 20 °C with 50 μl (15000 cpm) of ^{125}I-streptavidin solution in the same buffer. Streptavidin (Sigma) was iodinated using IODO-GEN.

The affinity of VAA for the tested saccharides was estimated by determining the amount of ^{125}I-lectin bound to wells coated with 50 μg/ml asialofetuin, after incubation at 20 °C with the ^{125}I-lectin solution in the absence or presence of different concentrations of the sugars.

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2.4. Molecular dynamics (MD) simulations

Crystallographic parameters of the B chain (pdb entry 1oql) were used as starting point to adapt the Tyr-site residues to neutral pH. Crystallographic water molecules were retained, *N*-acetyl-D-glucosamine groups were removed, and hydrogen atoms were added with Sybyl [15]. The Amber 03 force field was used to model the protein, the generalized amber force field for galactose [16]. Partial charges on galactose were calculated with the AM1-BCC method [17], using Antechamber in the AMBER 8 package [18]. Following addition of one chlorine ion, the system was solvated in an orthorhombic box of TIP3P water molecules ($83.1 \times 86.8 \times 68.6$ Å).

Simulations were carried out with the AMBER 8 suite of programs [18]. The particle mesh Ewald method [19] was used to treat long-range electrostatic interactions; a non-bonded cut-off of 10 Å was used for

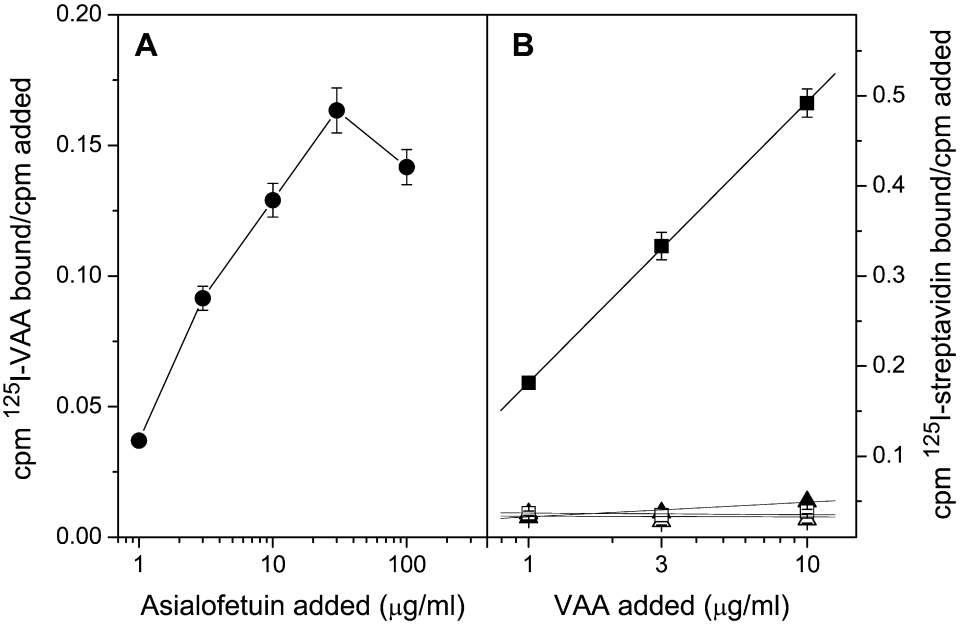

Fig. 1. Binding of VAA to asialofetuin-coated wells. (A) Binding of ^{125}I-VAA to wells coated with asialofetuin solutions of different concentrations was determined as described under Section 2. (B) Biotinylated VAA was treated with *N*-acetylimidazole in the absence (▲, Δ) or presence (■, □) of 0.1 M lactose. Microwell surfaces coated with asialofetuin (100 μg/ml) were then incubated, in the absence (filled symbols) or presence (open symbols) of 0.1 M lactose, with the acetylated biotinylated proteins at three different concentrations. The amount of bound lectin was finally assessed by measuring the binding of ^{125}I-streptavidin to the wells.

Table 1
Apparent association constants for the binding of methyl β-lactoside analogues to VAA

Unit derivatized	Compound	K_a (M^{-1} 10^{-3})		RelativeK_a	
		VAA	Ricin	VAA	Ricin
	Methyl β-lactoside	1.92 ± 0.02	17.8 ± 0.2	1	1
β-D-*Galactopyranose*					
C-2′	2′-Deoxy	0.23 ± 0.02	3.1 ± 0.1	0.12	0.17
	2′-*O*-Methyl	0.28 ± 0.01	13.0 ± 4.9	0.14	0.73
C-3′	3′-Deoxy-3′-fluoro	<0.1	<0.1	<0.05	<0.005
	3′-*O*-Methyl	<0.1	0.50 ± 0.01	<0.05	0.03
C-4′	4′-Deoxy	<0.1	0.33 ± 0.01	<0.05	0.02
	4′-Deoxy-4′-fluoro	<0.1	<0.1	<0.05	<0.005
	4′-*O*-Methyl	<0.1	0.61 ± 0.01	<0.05	0.03
C-6′	6′-Deoxy	0.78 ± 0.4	0.62 ± 0.01	0.4	0.03
	6′-Deoxy-6′-fluoro	0.53 ± 0.01	5.26 ± 0.8	0.3	0.29
	6′-*O*-Methyl	0.50 ± 0.05	10.3 ± 0.3	0.3	0.58
β-D-*Glucopyranose*					
C-1	1-Deoxy	1.50 ± 0.02	18.2 ± 1.0	0.78	1.0
C-2	2-Deoxy	1.15 ± 0.1	16.9 ± 2.6	0.59	0.95
	2-*O*-Methyl	1.1 ± 0.1	10.9 ± 0.7	0.57	0.61
C-3	3-Deoxy	1.8 ± 0.2	37.0 ± 12	0.93	2.1
	3-*O*-Methyl	0.4 ± 0.1	3.2 ± 0.03	0.20	0.18
	3-Deoxy-3-Methyl	0.17 ± 0.01	11.5 ± 0.4	0.1	0.64
	3-Epi	0.34 ± 0.02	2.6 ± 0.1	0.17	0.15
C-6	6-Deoxy	1.30 ± 0.05	8.8 ± 0.3	0.67	0.49
	6-*O*-Methyl	1.1 ± 0.1	7.7 ± 0.3	0.57	0.43

Relative K_a values were calculated taking K_a for methyl β-lactoside as unit. Values for ricin are from reference [11].