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Crystallization of *Helix pomatia* agglutinin (HPA), a protein from the edible snail

John N. Lisgarten,[a] James E. Pitts,[a] Rex A. Palmer,[a]* Colin D. Reynolds,[b] Minh Hoa Dao-Thi,[c] Edilbert Van Driessche[d] and Sonia Beeckmans[d]

[a]Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, England, [b]School of Biomolecular Sciences, Liverpool John Moores University, Max Perutz Building, Byrom Street, Liverpool L3 3AF, England, [c]Laboratory of Ultrastructure, Institute for Molecular Biology and Biotechnology, Vrije Universiteit Brussel, Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium, and [d]Laboratory of Protein Chemistry, Institute for Molecular Biology and Biotechnology, Vrije Universiteit Brussel, Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium

Correspondence e-mail: r.a.palmer@mv3b.cryst.bbk.ac.uk

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Crystals of *Helix pomatia* agglutinin (HPA) have been grown by the hanging-drop technique using polyethylene glycol as the precipitant at 293 K. Over a period of one to two weeks the crystals grew to maximum dimensions of 0.10 × 0.05 × 0.02 mm. The crystals belong to space group $P6_322$, with unit-cell dimensions $a = b = 63.3$, $c = 105.2$ Å and $Z = 12$ identical monomers of $M_r = 13$ kDa, aggregating into two 78 kDa hexameric protein molecules per unit cell, each with symmetry 32 (D_3). The diffraction pattern extends to 3.6 Å at 293 K.

1. Introduction

Proteins known today as lectins were first described to be present in the seeds of *Ricinus communis* by Herman Stillmark in 1888 at the University of Dorpat. *Ricinus* seed extracts were found to display haemagglutinating activity and the protein responsible for this activity, ricin, was called a phytohaemagglutinin. Today, it is known that proteins which are able to cause agglutination of erythocytes (and other cells) are not only found in the seeds of a wide variety of plants, but are also often present in vegetative parts (stems, leaves, roots, rhizomes) of plants. Tissues of vertebrates and invertebrates, bacteria and viruses also contain haemagglutinins, and these proteins are now generally referred to as lectins, *i.e.* proteins of non-immune origin which reversibly bind oligosaccharides or glycoconjugates (glycoproteins or glycolipids) or polysaccharides without modifying their covalent structure (Kocourek & Horejsi, 1981; Barondes, 1988). Lectins can often be inhibited by simple sugars and consequently they are often classified on the basis of their sugar-binding properties.

Lectins are employed in a number of important applications over a wide variety of immunological and biochemical fields. Moreover, they can be regarded as model systems for studying the molecular basis of protein–carbohydrate interactions, as they occur in cell–cell recognition events (for reviews, see Lis & Sharon, 1986, 1998). The structures of several plant lectins, including some lectin–carbohydrate complexes, are known at high resolution (see Loris *et al.*, 1998 for a review). The X-ray structures of a number of animal lectins have also recently been reported (see Rini, 1995 and Gabius, 1997 for reviews).

However, the specific mechanisms by which lectins can discriminate between different but closely related oligosaccharides are not well understood. In order to gain a detailed understanding of the mechanisms which govern their exquisite carbohydrate-binding specifity, further knowledge of their high-resolution three-dimensional structures and conformations is required.

Although lectins are synthesized by virtually all living organisms, to date it is plant lectins which have attracted the most attention. This is not surprising, as the lectins which occur in plants are abundant and easy to extract and purify, and are furthermore stable proteins which can be derivatized without their sugar-binding properties being affected. Nevertheless, it is now widely recognized that invertebrates are also rich sources of lectin proteins (Vasta, 1992). The edible snail *Helix pomatia*, for example, has been shown to contain large amounts of a lectin which specifically agglutinates human type A erythrocytes (Uhlenbruck & Prokop, 1966). It has been proposed (Vasta, 1992) that this lectin, found to be localized in the snail albumin gland (Prokop *et al.*, 1968), plays an important role in the protection of eggs and developing embryos against bacterial and fungal infections.

Detailed screening of a number of breast cancers against lectins of different specificities (Leathem & Brooks, 1987) demonstrated the interesting histochemical staining patterns of *H. pomatia* agglutinin (HPA). It was discovered that while HPA binding is minimal in healthy breast tissue, it is more marked in cancerous breast tissue, and particularly when the axillary lymph nodes are affected. HPA binding is associated with poor prognosis and is a good predictor of long-term patient outcome (Brooks & Leathem, 1991; Schumacher *et al.*, 1996). By using different HPA-binding oligosaccharides which bind to HPA extracted from breast tumours, it will be possible to model

Table 1
Data-collection statistics.

Resolution range (Å)	R_{merge} (%)	$I/\sigma(I)$	Completeness (%)	N_{hkl} unique	N_{hkl} collected	M
20.00–6.52	3.6	20.9	76.8	221	1935	11.4
6.52–5.29	8.4	11.1	89.2	206	1874	10.2
5.29–4.64	7.6	9.9	90.4	208	1918	10.2
4.64–4.23	9.2	8.6	91.3	204	1769	9.5
4.23–3.94	12.9	6.4	91.5	199	1493	8.2
3.94–3.72	19.2	4.4	87.1	176	935	6.1
3.72–3.58	29.8	2.9	87.0	169	794	5.4

changes in the three-dimensional structure of this lectin when it binds to different ligands. An understanding of the distribution of the HPA-binding ligands *in vivo* and the mechanisms of binding to the lectin's carbohydrate-binding sites will facilitate the development of novel mimetic compounds for imaging and targetting micro-metastases prior to their clinic manifestation. Such a diagnostic tool would be invaluable in the early detection of this widespread disease.

Although HPA is widely used as a tool in several biochemical and clinical protocols, its structure and physicochemical properties have surprisingly received little attention to date. Previous studies on HPA (Hammerström *et al.*, 1972) have indicated that it is a 78 kDa protein consisting of six identical (or very similar) subunits of 13 kDa, each containing one intra-chain disulfide bond and a single saccharide-binding site. The subunits are covalently linked in pairs by a single intra-chain disulfide bond to form dimers, and the three dimers aggregate to form a hexamer which is held together by non-covalent interactions. Isoelectric focusing experiments indicate the presence of at least nine bands in HPA preparations, having isoelectric points between pH 6 and 8 (Kühnemund & Köhler, 1975). Hammerström & Kabat (1969) have shown HPA to be a glycoprotein containing approximately 7%(*w*/*w*) of bound carbohydrate, consisting mainly of galactose and mannose. HPA has also been shown to bind to polysaccharides containing terminal non-reducing *N*-acetyl-D-galactosamine or *N*-acetyl-D-glucosamine (Hammerström & Kabat, 1969, 1971).

2. Methods and results

HPA was purified from the albumin glands of *Helix pomatia*, as will be described in detail elsewhere (Bourjolly *et al.*, in preparation). In short, the procedure consists of the following steps: dissection of the albumin glands from wild *H. pomatia* snails, homogenization of the glands and extraction in phosphate-buffered saline, precipitation of the lectin with ammonium sulfate and affinity chromatography. Purity of the lectin was assessed by SDS–PAGE and FPLC analysis using a Superose-12 gel-filtration column.

Single crystals of purified HPA were obtained by the hanging-drop vapour-diffusion method. Crystallization droplets of 10 µl initial volume were prepared on siliconized glass cover slips and suspended over 1.0 ml reservoirs containing 100 m*M* HEPES pH 7.5 and 10% PEG 8000. The crystallization droplets consisted of 5 µl (5 mg ml^{-1}) protein solution and 5 µl well solution. Crystallization was carried out at 293 K. Small crystals appeared after a few days and grew to approximately 0.10 × 0.05 × 0.02 mm after about two weeks.

Fig. 1 shows crystals of *H. pomatia* agglutinin crystals obtained by the present method. X-ray diffraction data of the crystals mounted in a quartz capillary were collected on the beamline at CLRC Daresbury Station 9.6. Data-collection statistics are given in Table 1. The wavelength was set to 0.87 Å and the data were measured at 293 K and collected with a MAR Research image plate using the rotation method with 2.0° oscillations. Determination of unit-cell parameters, space group, integration of reflection intensities and scaling were performed using *DENZO* and *SCALEPACK* (Otwinowski, 1993). The autoindexing procedure of *DENZO* indicated that the crystals belong to the hexagonal system, with unit-cell dimensions $a = b = 63.3$, c = 105.2 Å and V_c = 3.65 × 10^5 Å^3. Systematic absences for a 6_3 screw axis parallel to c and Laue symmetry 6/*mmm* are consistent with space group $P6_322$. A unit-cell content of Z = 12 monomers of M_r 13 kDa per unit cell requires approximately 41% solvent by weight, which is within the range normally found in protein crystals (Matthews, 1968). These results are also consistent with the earlier findings of Hammerström *et al.* (1972) that HPA is a hexameric protein of M_r 78 kDa comprising a trimer of dimers (Fig. 2). The required symmetry for the hexamer is thus 32 in space group $P6_322$, with two hexamers per unit cell. Fresh crystals diffracted to approximately 3.4 Å resolution. An 87.6% complete data set to 3.6 Å was collected with an R_{merge} of 11.4%.

A search for suitable heavy-atom derivative crystals is in progress.

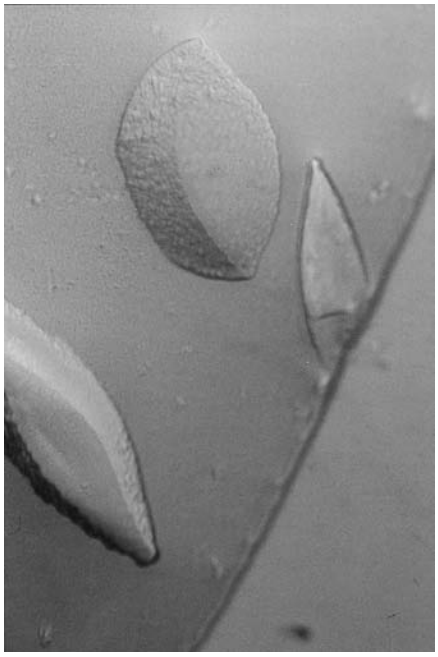

Figure 1
Crystals of the lectin *H. pomatia* agglutinin. The largest dimension is 0.1 mm.

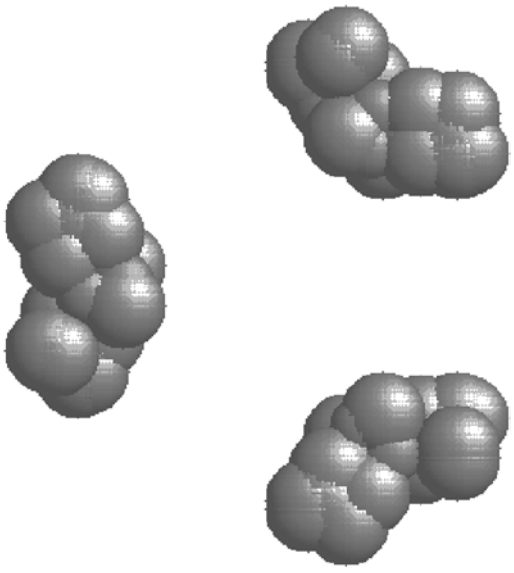

Figure 2
HPA proposed trimer of dimers, simulated structure. Crystallographically, the required symmetry of the hexamer is 32 (D_3) in space group $P6_322$.

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