Crystallization and preliminary X-ray data for peanut agglutinin

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Peanut agglutinin *X-ray structure* *Affinity chromatography*

1. INTRODUCTION

Lectins are polyvalent carbohydrate-binding proteins that can agglutinate cells having the appropriate saccharides on their surfaces. Although they were discovered in plants, lectins are found in all types of living organisms. Their biological functions are not completely understood, but they tend to be involved in interactions with components of the cell surface or the extracellular environment [1]. There are several hypotheses regarding their functions. In plants, lectins may participate in binding of nitrogen-fixing bacteria to the roots of legumes [2,3] and in defense against infection [4]. In animals, lectins may also serve protective function [5], as well as roles in receptor-mediated pinocytosis of partially degraded glycoproteins [6] and in intercellular recognition during differentiation [7]. Because of the specificity of their interactions with the carbohydrate moieties of glycolipids and glycoproteins, lectins have played an increasingly important role in studies of membranes from normal and cancerous cells [8], blood typing and the study of blood group substances [8], the purification of glycoproteins and polysaccharides [8] and the triggering of lymphocytes to undergo blastogenesis [9].

Peanut agglutinin (PNA) is a lectin that is specific for terminal D-galactosyl residues [10,11] with an additional preference for *N*-acetylgalactosamine in the penultimate position [10]. This specificity allows the use of PNA to detect the T-antigen on erythrocyte surfaces [12], to study T lymphocyte subpopulations [13], and to separate mature and immature thymocytes [14]. The physical properties of PNA have been examined by circular dichroism, gel chromatography and sedimentation techniques [15]. It is a tetramer of M_r 98 000. The circular dichroism spectrum of PNA changes in the near ultraviolet upon binding lactose; whereas, the far ultraviolet spectrum remains unchanged [15]. The thermodynamic parameters, which have been determined by ultraviolet difference spectroscopy, are similar to those of other plant lectins [16]. The first forty residues have been sequenced and are homologous to the sequences of lectins from ten other leguminous plants [17]. The homology to concanavalin A (Con A) is particularly interesting since the amino terminal residue of PNA would be aligned with residue 123 of Con A. There are additional similarities between these two proteins in their metal binding properties [16] and CD spectra [15].

In this paper we are reporting conditions for the crystallization of PNA in the presence and absence of lactose. The crystals of the PNA–lactose complex are suitable for structural analysis, and their space group has been determined.

2. EXPERIMENTAL

2.1. *Materials*

The peanuts were purchased from a local grocery store and were of the variety Florigiant. The epoxy-activated Sepharose 6B was obtained from Pharmacia Fine Chemicals; the lactose, from Sigma; and the polyethylene glycol (carbowax 6000), from Fisher.

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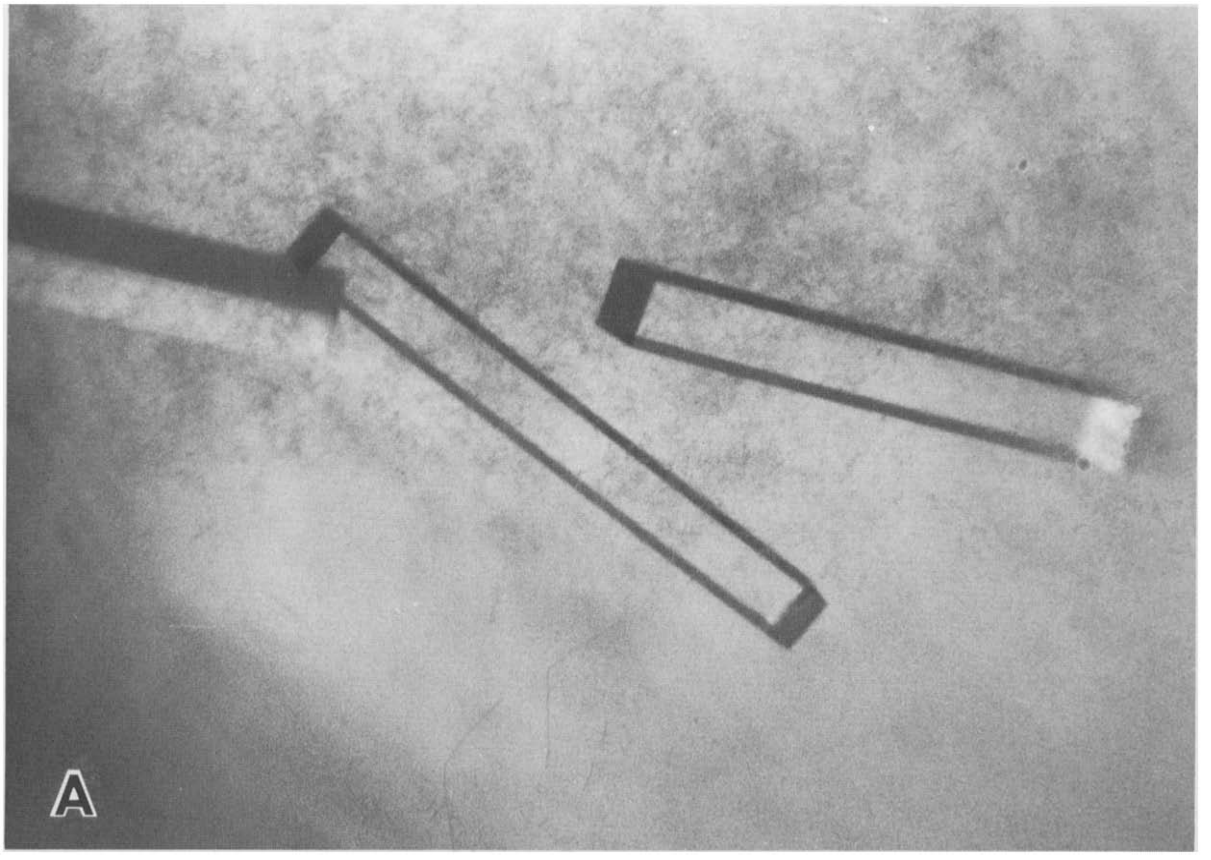

Fig.1A. Crystals of peanut agglutinin grown in the presence of lactose.

2.2. *Synthesis of lactosyl–Sepharose*

Three grams of epoxy-activated Sepharose 6B were swollen and washed in water. The moist, activated Sepharose was suspended in 20 ml of 0.1 N NaOH containing 0.72 g of lactose and mixed by end-over-end tumbling overnight. The lactosyl–Sepharose was washed in a scintered glass funnel with 100 ml of 0.5 M NaCl and then with 100 ml of 0.1 M NaCl, 0.05 M Tris–HCl (pH 7.5) at 5°C.

2.3. *Preparation of peanut agglutinin*

Green peanuts (6000 g) were extracted by homogenizing 200 g batches in 500 ml of 0.9% NaCl in a Waring blender at top speed for one minute and then in a Polytron homogenizer for one minute at top speed. The homogenate was centrifuged at 16 000 × *g* for 20 min, and the lipid layer was removed by filtering through glass wool. The pellet was re-extracted with 1 litre of 0.9% NaCl, and the two supernatants were combined. The proteins in the combined supernatant that precipitated between 40% and 60% saturation with $(NH_4)_2SO_4$ were isolated, dialyzed against 0.1 M NaCl, 0.05 M Tris–HCl (pH 7.5) buffer and applied to a lactosyl–Sepharose column. After washing the column with the NaCl–Tris buffer, the PNA was eluted by adding 0.1 M lactose to the eluting buffer.

2.4. *Crystallization of PNA*

Crystals of a PNA–lactose complex were grown in vials from solutions of polyethylene glycol (PEG). The best conditions were 6.5 mg/ml PNA, 10 mM sodium phosphate (pH 7.0), 5 mM lactose, 0.02% NaN_3 and 6.8% (v/v) PEG. The conditions giving crystals ranged from pH 5.5 to pH 8.0 and from 6.0 to 7.5% PEG.

Crystals of the lectin without lactose were also grown in vials from PEG solutions. Suitable conditions ranged from pH 5.5 to 6.0, from 8% to 9.5% PEG and from 2.9 to 7.3 mg/ml of PNA.

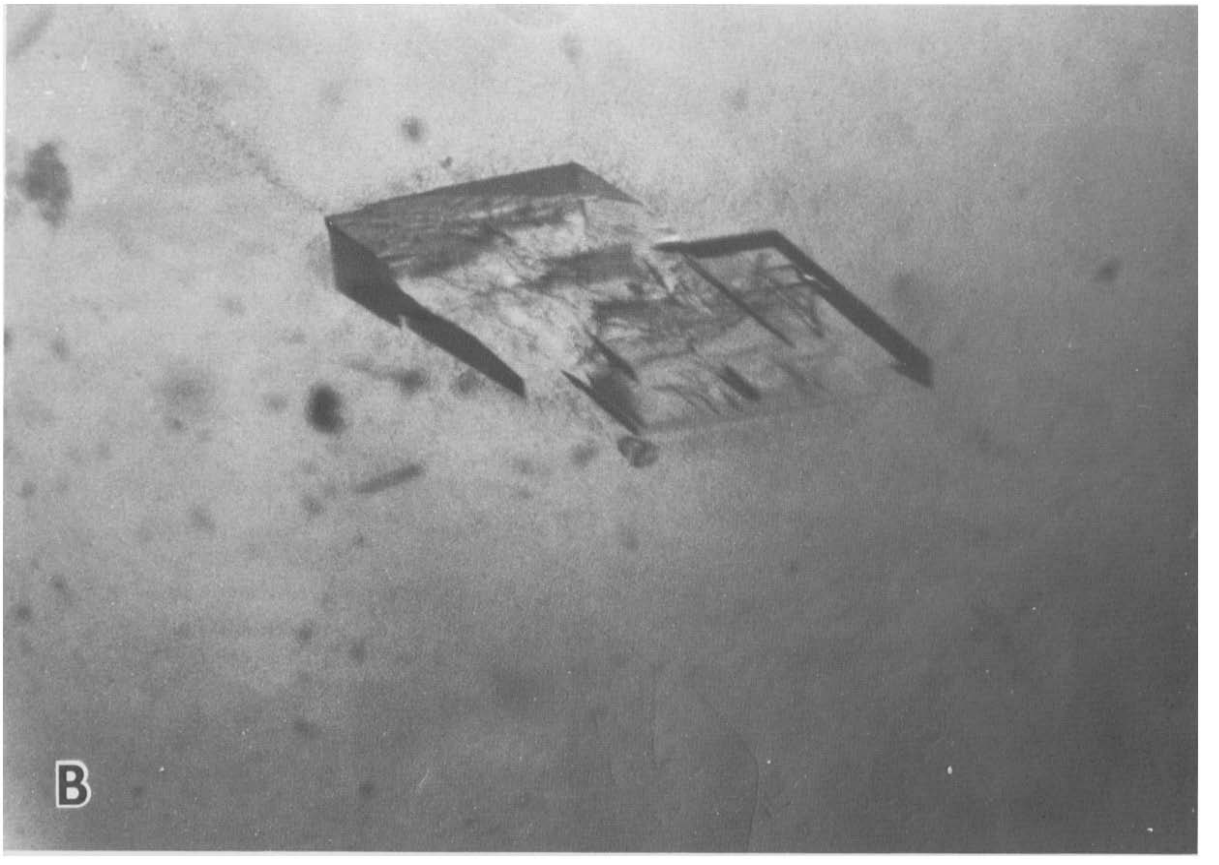

Fig.1B. Crystals of peanut agglutinin grown in the absence of lactose.

The crystals were mounted in quartz capillaries for X-ray studies. Precession photographs were taken at a precession angle of 16° with Ni-filtered CuK_α radiation produced by a Picker X-ray generator equipped with a Philips fine focus tube.

3. RESULTS

Figure 1 shows the PNA crystals grown in the presence and absence of lactose. The crystal morphologies are obviously different. The crystals of the PNA–lactose complex (fig.1A) are perfectly formed rectangular prisms, while the ones grown in the absence of the disaccharide (fig.1B) are twinned rhombohedrons. Preparations of peanut agglutinin may contain as many as eight isolectins, as detected by isoelectric focusing and isolated by chromofocusing [18]. When single crystals of PNA, grown either in the presence or absence of lactose, are dissolved and the protein subjected to isoelectric focusing in polyacrylamide gels, all of the isolectins appear to be present. Only the lactose-containing crystals are suitable for diffraction studies. The *Okl* plane of reciprocal lattice is shown in fig.2, which demonstrates that high resolution data can be collected. The space group is $P2_12_12$ with unit cell dimensions of $a = 130$ Å, $b = 127$ Å and $c = 78$ Å. The corresponding unit cell volume is 1.3×10^6 Å^3, with four asymmetric units per unit cell. If there is one tetramer per asymmetric unit, the value for the ratio of the unit cell volume to protein molecular weight (V_m) is 3.3 Å^3/dalton. While this is higher than the average value for protein crystals (2.4 Å^3/dalton), it is within the range of previously observed values [18]. Although the space group has not been determined for the twinned crystals grown in the absence of lactose, it is definitely not the same as those of the PNA–lac-

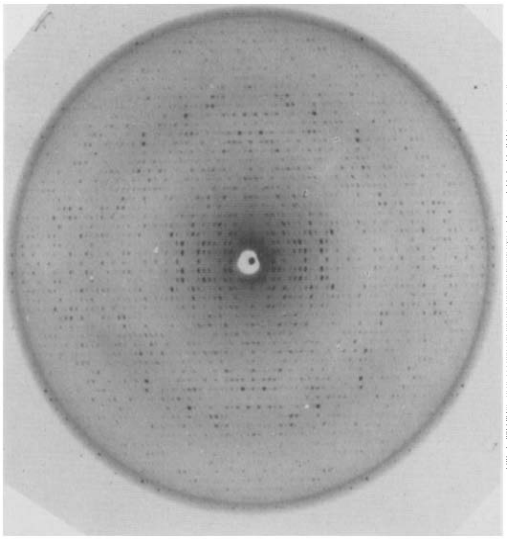

Fig.2. X-ray precession photograph of the *Okl* reciprocal lattice sections of a crystal of the PNA–lactose complex. The precession angle was 16° and the data extends past 2.8 Å resolution.

tose complex. It is an orthogonal space group, but the unit cell dimensions and symmetry elements are different.

4. DISCUSSION

Crystals of peanut agglutinin grown in the presence of lactose are suitable for high resolution X-ray diffraction studies. They can become quite large (4 mm in the longest direction), but the usual dimensions are 1.0 × 0.3 × 0.1 mm. The crystals contain eight isolectins, as observed by isoelectric focusing. The chemical nature of the differences among these isolectins is not known; however, it must be slight since the diffraction pattern extends past 2.8 Å resolution. Wheat germ agglutinin was also crystallized in a form that contained more than one isolectin, and these crystals were used to determine its structure [20].

The differences in the crystal morphology and space group between PNA crystallized in the presence and absence of lactose are quite striking. The obvious explanation is that a conformational change occurs when the disaccharide binds to the protein. This interpretation is consistent with the CD changes upon binding lactose [15]. Other explanations for the two crystal forms are also possible. For example, adjacent molecules in a crystal might interact via bound carbohydrate or via the carbohydrate binding sites. Since our work was completed, Salunke et al. have reported the crystallization of PNA in the absence of lactose [21]. Their crystals seem to be very similar to the ones we grew in the presence of the sugar. These data might imply that there is no structural transition upon disaccharide binding or that the crystals of Salunke et al. contain lactose, which had been used to remove the protein from an affinity column.

Further study of the PNA crystals is underway with the eventual aim of obtaining its three-dimensional structure at high resolutions. A search for heavy atom derivatives is in progress. Since the asymmetric unit contains the entire tetramer, the non-crystallographic symmetry may be useful in determining the positions of the heavy atoms. Work is also in progress to obtain suitable crystals of PNA in the absence of lactose.

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