Preparation and preliminary X-ray studies of three acidic pH crystal forms of the anti-T lectin from peanut (*Arachis hypogaea*)

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1. INTRODUCTION

Lectins, by definition, are multivalent carbohydrate binding proteins. Their earlier generic name 'phytohaemagglutinins' arose from their ability to agglutinate erythrocytes and from the fact that, until recently, all the described lectins were from plant sources [1]. These proteins have been subsequently found in many animal species and probably they occur in all forms of life [2]. The interesting properties of lectins arise from their ability to bind specific cell-surface carbohydrates. Lectins have assumed considerable importance in recent years on account of their potential for use in studies on biological receptors and cell-surface phenomena.

Among the lectins studied so far, the agglutinin from peanut (*Arachis hypogaea*) is the only one with T-antigen specificity [3]. It is a tetrameric protein probably made up of identical subunits, with an M_r of 110000 [4,5]. We reported the crystallization of this protein at neutral pH and preliminary X-ray studies on the crystals [6]. These orthorhombic crystals contain a tetrameric molecule in the asymmetric unit and are suitable for high resolution X-ray studies. Our results were later confirmed by other workers [7]. In view of the probability of the subunits being identical, an exploration of the possibility of growing crystal forms with one or two subunits in the asymmetric unit was worthwhile. Also, it has been reported that the state of aggregation of the peanut lectin is influenced, as in the case of concanavalin A and wheat germ agglutinin, by the pH of the medium [8]. While the tetrameric state predominates above pH 4.75, the molecule dissociates into dimers at pH 4.75–3.0. Here, we report the preparation and preliminary X-ray studies of 3 new crystal forms grown at pH 4.6.

2. EXPERIMENTAL

A 4 mg protein/ml solution in phosphate buffer (pH 7.4), isolated and purified from the locally available peanut according to the procedure described in [9], was thoroughly dialysed against a 0.2 M NaCl, 0.05 M sodium acetate–acetic acid (pH 4.6) buffer containing 1.5 mM lactose. The protein solution at pH 4.6 thus obtained was used in crystallization experiments. A 40% (w/v) solution of polyethylene glycol (M_r 6000), obtained commercially from Sigma, in the same buffer was slowly added to the protein solution until a faint opalescence developed. The opalescence was cleared by the addition of small amounts of the protein solution. The solution, which contained 8–10% polyethylene glycol, was left standing at 20°C. Crystals of the type shown in fig.1, with

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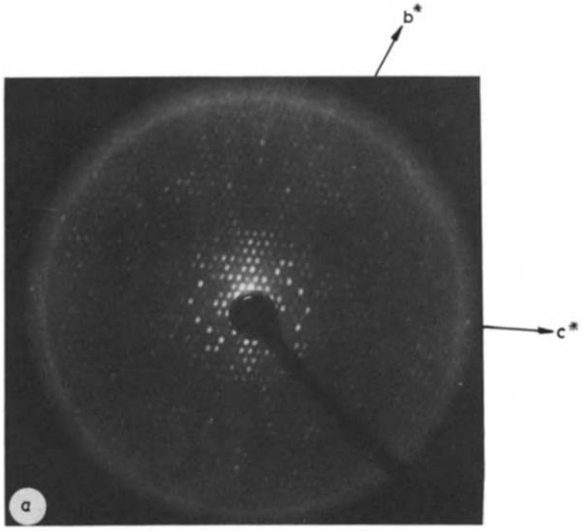
Fig.1. Crystals of peanut lectin grown at pH 4.6.

typical dimensions of 0.7 × 0.6 × 0.2 mm, grew in 2–3 weeks. Crystals were mounted in the usual way in thin-walled glass capillaries and X-ray precession photographs were recorded using nickel-filtered CuKα radiation. 15° precession photographs representing comparable zones from the 3 forms are shown in fig.2. X-ray photographs indicated the occurrence of 3 different crystal forms although all the crystals had nearly the same morphology.

3. RESULTS AND DISCUSSION

The space group and the unit cell dimensions of the 3 crystal forms are given in table 1. Two of the forms are monoclinic (monoclinic I and monoclinic II) while the third is triclinic. Table 1 also lists the volume of the solvent in each case, calculated using the method in [10]. In these calculations, the unit cell was assumed to contain two tetrameric molecules of M_r 110000, or 4



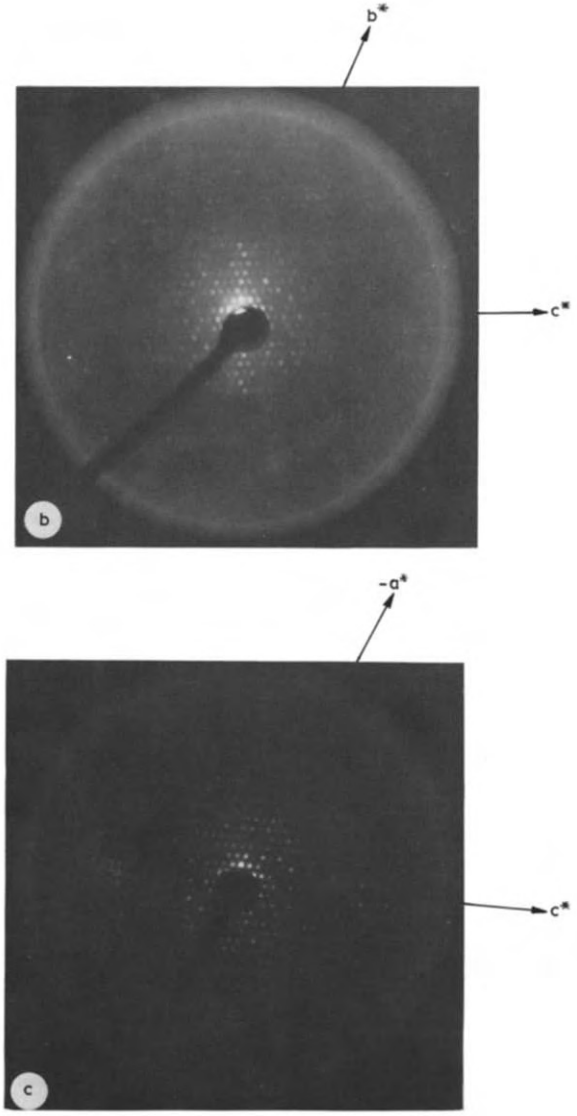


Fig.2. Comparable zero level 15° precession photographs from monoclinic form I (a), monoclinic form II (b) and the triclinic form (c).

Table 1

Space groups, unit cell dimensions (lengths in Å and angles in degrees) and the solvent content of the 3 crystal forms

	Space group	a	b	c	α	β	γ	Solvent volume (%)
Monoclinic I	$P2_1$	70.3	101.1	84.5	–	114.0	–	51
Monoclinic II	$P2_1$	70.5	107.3	85.0	–	114.0	–	54
Triclinic	P1	52.0	71.1	83.6	65.4	77.1	71.5	49

dimers with an M_r of 55000 each in the two monoclinic forms. The protein content of the unit cell in the triclinic form was assumed to be half of that in the monoclinic forms. The solvent content of each of the crystal forms grown at pH 4.6 is less than that (57%) of the crystals grown at neutral pH. The diffraction pattern extends to a resolution higher than 3 Å in all the 3 cases and the crystals are suitable for high resolution work.

The unit cell dimensions indicate the 3 crystal forms to be closely related. The two monoclinic crystal forms differ significantly only in the length of the unique axis. The intensities of the diffraction maxima from the crystals, however, differ markedly. The axial lengths a, b and c of the reduced cell [11] of the triclinic form is about equal to $B/2$, a and c of the monoclinic cells. Also the interaxial angle α of the triclinic form almost supplements β of the monoclinic forms. Thus the packing of molecules in the 3 crystal forms is likely to be similar.

The diffraction pattern of the monoclinic form I indicated the presence of an approximate non-crystallographic 2-fold symmetry axis perpendicular to the monoclinic unique axis. The approximate symmetry of diffraction spots in the h01 precession photograph (fig.2(a)) about the c^* axis and a direction perpendicular to it is a reflection of this non-crystallographic symmetry in the structure. Thus, the two halves of the tetrameric molecule (or two dimers, if the tetramers have dissociated into dimers) in the asymmetric unit are related by an approximate non-crystallographic symmetry element. This approximate symmetry is expected to be of some use in the contemplated structure analysis of the protein.

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