Three-dimensional structure of lectin from pea (*Pisum sativum*) at 5 Å resolution

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The protein lectin from pea (M_r 49 000) crystallizes in space group $P2_12_12_1$ with cell dimensions a = 51.0 Å, b = 61.7 Å, c = 137.6 Å and z = 4. The three-dimensional structure of pea lectin at 5 Å resolution was determined by multiple isomorphous replacement method. The data were collected on an ARGUS multichannel diffractometer. The pea lectin molecule can be described as a dimer with approximate dimensions 85 × 55 × 40 Å. The borderline between the globules appears in the three-dimensional model as a shallow groove on its surface. Both globules have two dense layers. The molecule proved to be quite similar to the dimer of concanavalin A.

Lectin *Crystal* *X-ray diffraction* *Three-dimensional structure* *Multichannel diffractometer*

1. INTRODUCTION

The protein lectin from pea (*Pisum sativum*) belongs to the group of lectins: polyvalent membrane-active proteins that bind specifically to the carbon receptors on cell membranes. They are widespread in nature and have been found in bacteria, plants and animals. The lectins play an important part in intercellular interactions, promote the agglutination of cells, cause precipitation of glycoproteins, and stimulate mitosis. There is a considerable body of literature on lectins. Authors in [1] have published a monograph with a vast bibliography.

Lectin from pea has an M_r of about 49 000 and consists of 4 subunits ($2\alpha + 2\beta$), $M_{r\alpha} \simeq 6000$ and $M_{r\beta} \simeq 17\,500$. The molecule contains two Mn atoms and two Ca atoms without which it is biologically inactive. The pea lectin interacts specifically with D-mannose and D-glucose. The primary structure of the molecule (about 210 amino acid residues) has been only partially determined: the α-subunit has been sequenced in full (53 residues) [2], while in the β-subunit only the N-terminal 26-residue region is known [3]. The great similarity in the primary structures of favin, lentil lectin, concavalin A (Con A) and the sequence part of pea lectin suggests that the rest of the pea lectin molecule should also be homologous to these proteins.

We began work on the three-dimensional structure of this protein using X-ray diffraction in 1976 [4]. The cell parameters (a = 51.0 Å, b = 61.7 Å, c = 137.6 Å), the space group $P2_12_12$ and z = 4 were determined. The paper [5] published in 1982 presented data on the three-dimensional structure of pea lectin at 6 Å resolution. The structure factor phases were determined with the help of one heavy-atom derivative and anomalous scattering. The American authors mentioned the similarity between the dimensions, shape and some structural features of pea lectin molecule and the Con A dimer.

2. MATERIALS AND METHODS

2.1. *Isolation, crystallization and heavy-atom derivatives*

Lectin was isolated from commercial pea seeds

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as in [1]. Crystals were grown from a 1.8–3% solution of the protein in 0.01 M acetate buffer (pH 5.6), with 10–15% ethanol. After centrifugation the solution was kept at 3–6°C. After 3–10 days polymorphous crystals appeared attained dimensions of $0.4 \times 0.5 \times 1\ mm^3$. Crystallization in an ultracentrifuge [6] was also used. Native protein crystals were kept in a solution of 20% ethanol in 0.01 M acetate buffer (pH 5.6).

The heavy-atom derivatives were obtained by soaking protein crystals in solutions containing heavy atoms in a concentration of about 10^{-3} M for 1–2 weeks. The binding of heavy atoms was checked from diffraction patterns obtained by the screen precession method ($\mu = 22°$).

More than 20 different compounds with heavy atoms were used to obtain 3 isomorphous protein derivatives (table 1).

Dialysis of the lectin crystals against an EDTA solution and subsequent atomic absorption spectroscopy tests for Ca and Mn demonstrated the absence of Mn and the presence of Ca in the crystals. It seems that lectin, like Con A [14], has a higher binding coefficient for Ca than for Mn.

2.2. *Data collection*

Complete diffraction data sets with a resolution of 2.4 Å for the native protein and its heavy-atom derivatives were collected on an ARGUS multichannel diffractometer [7–9]. The diffractometer is based on a 16 000-channel position-sensitive X-ray detector controlled by an M600 computer. A schematic representation of the diffractometer system is given in fig.1. Table 2 lists some statistical characteristics of the $|F_{hkl}|^2$ data sets.

Diffraction data were collected by the rotation method. The use of the high-speed multichannel diffractometer considerably reduced the radiation damage of the sample, making it possible to collect a complete three-dimensional data set from one crystal.

Table 1

Preparation of pea lectin isomorphous derivatives

Compound	Concentration (mM)	Soaking time (days)
$K_3UO_2F_5$	1	5
$HgCl_2$	0.5	7
K_3IrCl_6	1	3

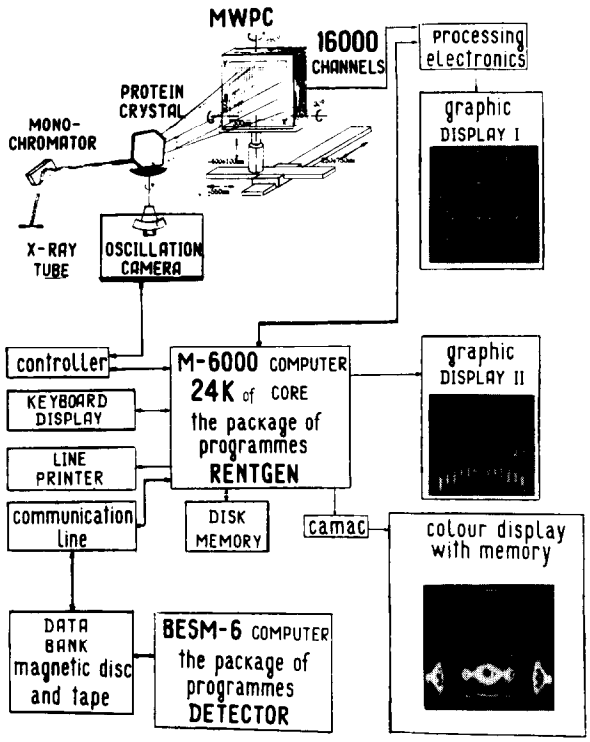

Fig.1. Schematic representation of the ARGUS multichannel diffractomer system. Displays I and II show diffraction patterns of rotating lectin crystals in real time and stored in computer memory, respectively.

Table 2

Data collection statistics

Number of the independent reflections at 2.4 Å resolution	~16 000
Proportion of $\|F_{hkl}\|^2 \geqslant 3\sigma$	75%
Maximum rate of measurements	1400 reflections per h
Average rate of measurements	500 reflections per h
R factor: native protein derivatives	5.5–9.5%

σ is the mean-square error in $|F_{hkl}|^2$. The R factor was calculated for all symmetry related reflection pairs in a set from the formula

$$R = \frac{\Sigma|F_{hkl}|_i^2 - |F_{hkl}|_j^2|}{\Sigma(|F_{hkl}|_i^2 + |F_{hkl}|_j^2)}$$

2.3. *Phase determination*

The positions of heavy atoms in the crystal were determined by difference Patterson syntheses with the coefficients $|F_{PH}| - |F_P|^2$, where $|F_P|$ and $|F_{PH}|$ are structure factor modules of the native protein and its derivative. Analysis of Harker sections enabled determination of the major sites of uranium and mercury atoms in their respective derivatives. The structure factor phases were computed according to the algorithm in [10]. The heavy atom parameters were refined by minimizing the sum $\Sigma m|F_{PH}| - |D_H|$, where m is the figure of merit representing the cosine of the phase angle error, $D_H = F_P + f_H$, and f_H is the estimated contribution of the heavy atoms. For location of the iridium sites as well as the minor sites of uranium and mercury, difference Fourier syntheses were calculated with the coefficients $m|F_{PH}| - |F_P|\exp(i\varphi_P)$, where φ_P is the structure factor phase of the protein. The results of heavy atom parameters refinement are listed in table 3.

3. RESULTS

The position of the molecule in the unit cell and its boundary were established from the 5 Å resolution electron density map. Fig. 2 shows 5 stacks of superimposed sections of the unit cell along the '*c*' axis with a 2 Å spacing, where the boundary of the molecule can be seen quite clearly. A three-dimensional model of the molecule constructed on the basis of such sections is shown in fig.3. The molecule proved to be quite similar to the Con A dimer [12,13]. The approximate dimensions of the pea lectin molecule are 85 × 55 × 40 Å; those of the Con A dimer are 84 × 40 × 39 Å. The lectin molecule can also be represented to consist of two symmetry related globules; the borderline between the globules appears in the three-dimensional model as a shallow groove on the surface (fig.3a) running from left to right and upward at an angle of 30° to the horizontal line (as in Con A). There are high electron density regions near the upper and lower ends of the molecule; these may be the sites of the Ca and Mn atoms, as in the Con A dimer. In pea lectin the distance between these sites is about 70 Å, as compared with 67 Å in the Con A.

Both globules of the lectin molecules have two dense layers (fig.4) whose size and orientation are very similar to the first and second sheets in the Con A subunits.

A more detailed discussion of the structure of lectin from pea and its similarity to Con A will have to wait until higher resolution data are obtained.

Table 3

Phase refinement statistics ($\langle m \rangle = 0.67$)

Heavy-atom compound	Number of atomic sites	R_D	R_K	R_{ST}	E	$\langle f_H \rangle$	N
$K_3UO_2F_5$	2	0.19	0.9	0.53	0.50	0.91	1682
$HgCl_2$	3	0.16	0.9	0.64	0.49	0.72	1694
K_3IrCl_6	4	0.13	0.8	0.70	0.45	0.52	1700

$$R_D = \frac{\Sigma_N|F_{PH}| - |F_P|}{\Sigma_N|F_P|}, \quad R_K = \frac{\Sigma_N|F_{PH}| - |D_H|}{\Sigma_N|F_{PH}|}, \quad [11]$$

$$R_{ST} = \frac{\Sigma_N|F_{PH}| - |D_H|}{\Sigma_N|F_{PH}| - |F_P|},$$

$$E = \left(\sum_N |F_{PH}| - |D_H|^2 \Big/ N\right)^{1/2},$$

$$\langle f_H \rangle = \left(\sum_N |f_H|^2 \Big/ N\right)^{1/2}$$

where $|f_H|$ and $|D_H|$ are the estimated structure factors of the heavy atoms and derivatives, $|F_P|$ and $|F_{PH}|$ are the experimental structure factor values of the protein and its derivatives, and N is the number of reflections involved in the calculations

Fig.2. Five stacks of superimposed $x - y$ sections of 5 Å resolution electron density map, z ranging from 0.686 to 0.743 with a spacing of 0.014 (d, in the range 20–5 Å).

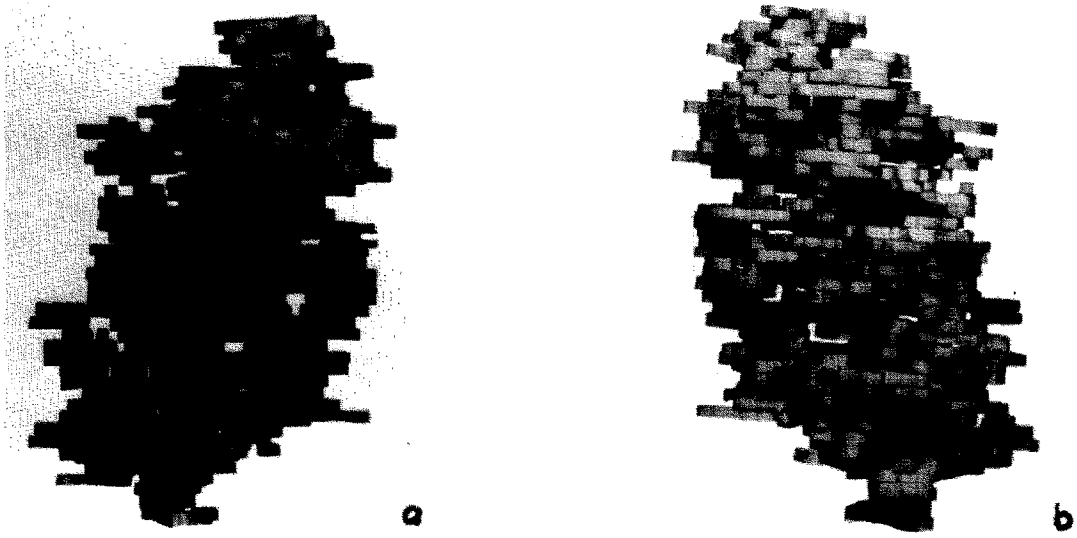

Fig. 3. Model of pea lectin molecule at 5 Å resolution. Views from front (a) and back (b) sides along '*b*' axis (layer thickness 2 Å; the *c* axis is vertical).

Fig.4. Stereoscopic image of a part of the pea lectin molecule viewed along the *c* axis, *z* ranging from 0.357 to 0.429 with a spacing of 0.014 (*d*, in the range 20–5 Å).

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