CRYSTALLOGRAPHIC STUDIES OF A MITOGENIC LECTIN FROM PEA SEEDS

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Received May 13,1977

Summary: A new crystal form of a mitogenic lectin from pea seeds (Pisum sativum) has been obtained which is suitable for high resolution structural work. The crystals are orthorhombic, space group $P2_12_12_1$, with unit cell dimensions: a = 64.2Å, b = 72.7Å, c = 108.3Å. The asymmetric unit contains one protein molecule.

Lectins are an interesting class of proteins which have the unusual property of agglutinating erythrocytes. Most of them are obtained from plant seeds and they appear to have a high binding specificity for particular carbohydrates. In addition, some lectins are designated mitogenic because they are known to stimulate cell division in lymphocytes. Considerable interest has been associated with lectins recently as some are known to preferentially agglutinate malignant cells (1). The molecular basis for their specificity can only be understood through a knowledge of their three-dimensional structure which is normally obtained by an X-ray diffraction crystal structure analysis. Here we report the preparation of crystals of a mitogenic pea lectin which have suitable properties for a three-dimensional X-ray diffraction analysis.

Two closely related lectins are present in pea seeds (Pisum sativum). They have been purified and studied in several laboratories (1-3). These lectins have been shown to bind D-mannose as well as methyl-α-D-glucoside,

ISSN 0006-291X

and they have mitogenic activity (2, 3). The proteins have a total molecular weight of 49,000 with an $\alpha_2\beta_2$ subunit structure where the smaller subunit α has a molecular weight of 7,000 and β is 17,000.

The pea lectins were purified according to the method of Trowbridge (2). Two elution peaks obtained by DEAE-cellulose (Whatman DE52) chromatography were labelled fraction A (isoelectic point pI = 4.1) and fraction B (pI = 6.5). Both fractions were dialyzed extensively against distilled water to remove all sugars and were then lyophilyzed. Each fraction was crystallized at 4°C from a solution which contained 10 mM sodium cacodylate (pH = 5.5), 15-20 mg protein/ml, and 10% (w/v) polyethylene glycol 4000 or 6000 (J. T. Baker & Co.). Both fractions A and B as well as a mixture of them produced similar crystals. All subsequent work was performed on crystals obtained from fraction B.

The crystals could be grown until their edges were 2-3 mm in length (Figure 1). The crystals were chunky and had variable shapes with prismatic features. X-ray studies indicated that the crystals are of orthorhombic symmetry with unit cell dimensions $\underline{a}$ = 64.2Å, $\underline{b}$ = 72.7Å and $\underline{c}$ = 108.3Å. The observed systematic absences ($\underline{h}$ = 2n + 1 for $\underline{h}00$, $\underline{k}$ = 2n + 1 for $0\underline{k}0$, $\underline{l}$ = 2n + 1 for $00\underline{l}$) clearly showed that the space group is $P2_12_12_1$ (Figure 2). Assuming that there are four molecules in the unit cell and one molecule of the protein (MW = 49,000) in the asymmetric unit, the volume per dalton, $\underline{V}m$, is 2.58Å^3/dalton, which is close to the average value for proteins found by Matthews (4).

The crystals diffract to at least 1.9Å resolution as judged from a 2° oscillation photograph. There is no appreciable intensity change in the X-ray diffraction patterns after exposure to an X-ray beam from an Elliott

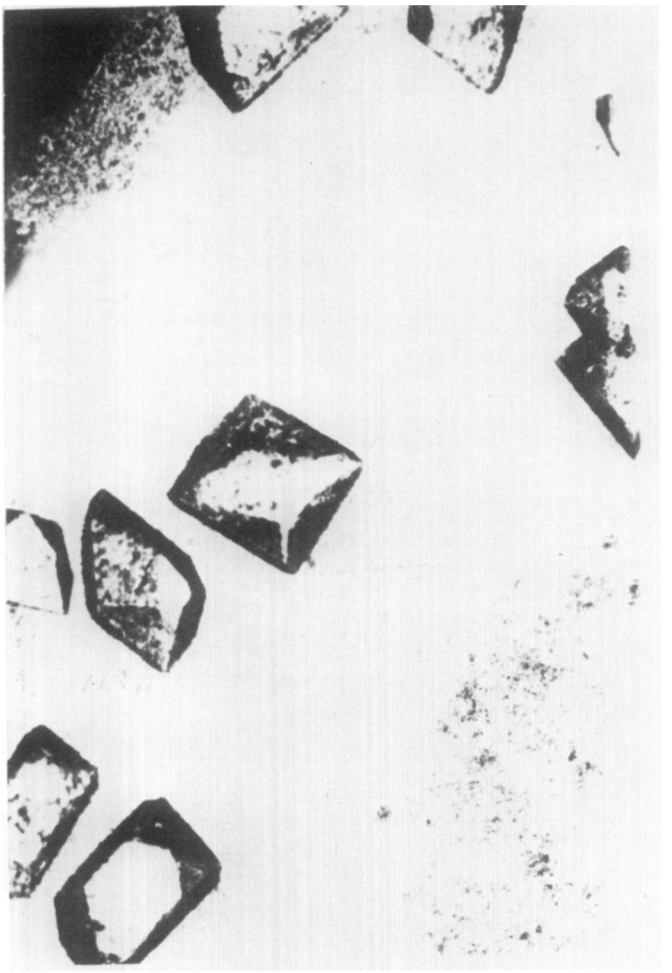
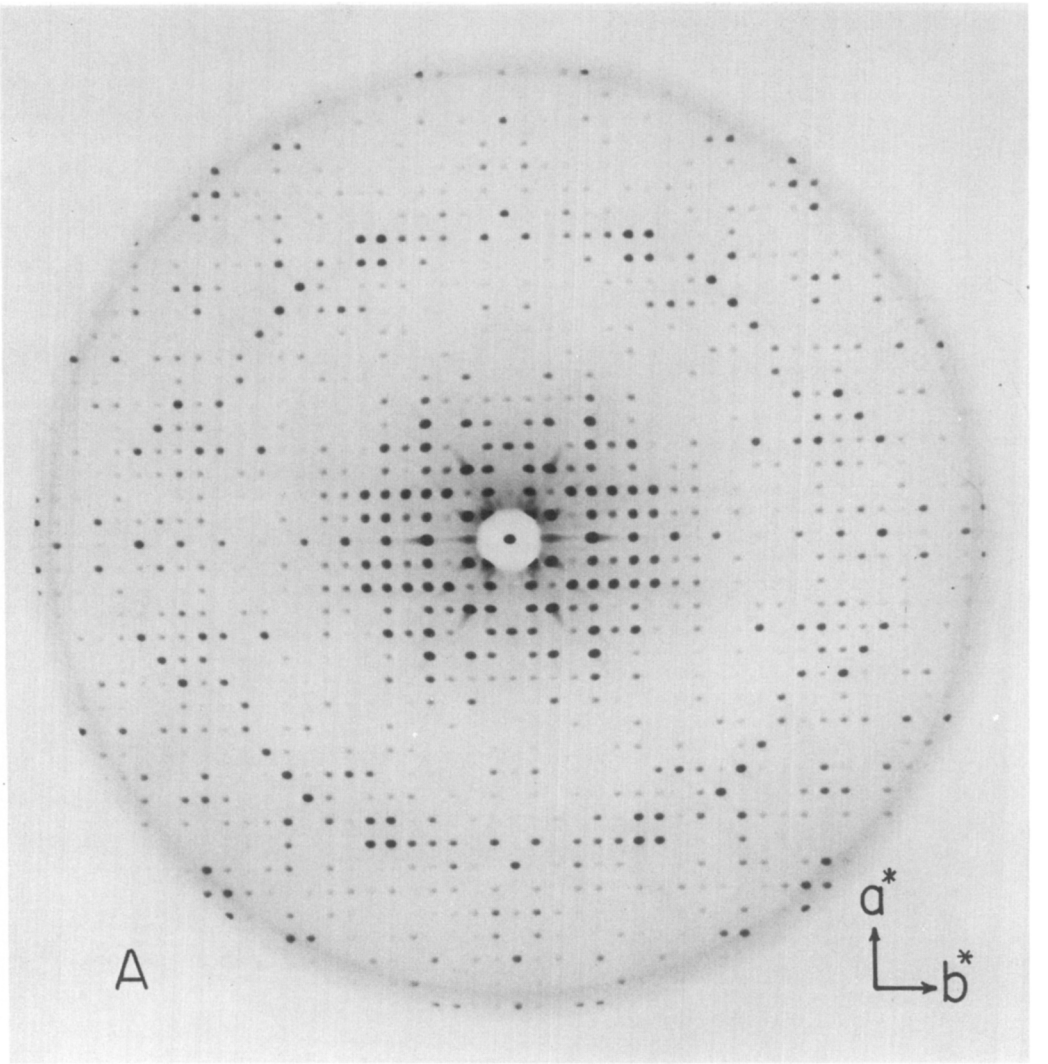


Figure 1. Photomicrograph of pea lectin crystals. The average length of the crystals is approximately 1.0 mm.

rotating anode for over 100 hours. Thus, it appears that this crystal form is suitable for high resolution structural work.

We have also crystallized the protein in the presence of a 10 to 40-fold mole ratio excess of D-mannose, which is 5-20 times the concentration necessary to give half occupancy in solution (K_a = 1.4 x 10^3 l/mole) (2). The crystals appear to have slightly different morphology although no change of the diffraction patterns was detected by visual inspection. This suggests



that the sugar binding sites of the pea lectin molecules are not involved in crystal lattice packing interactions if they are present in the crystal.

A different crystal form of pea lectin has been obtained by V. A. Bryzgunov et al. (3) from an ethanolic solution. Those crystals are in the same space group ($P2_12_12_1$) but have different cell dimensions (a' = 51.0Å, b' = 61.7Å, c' = 137.6Å). It is interesting to note that occasionally we have obtained the Bryzgunov crystal form under the same

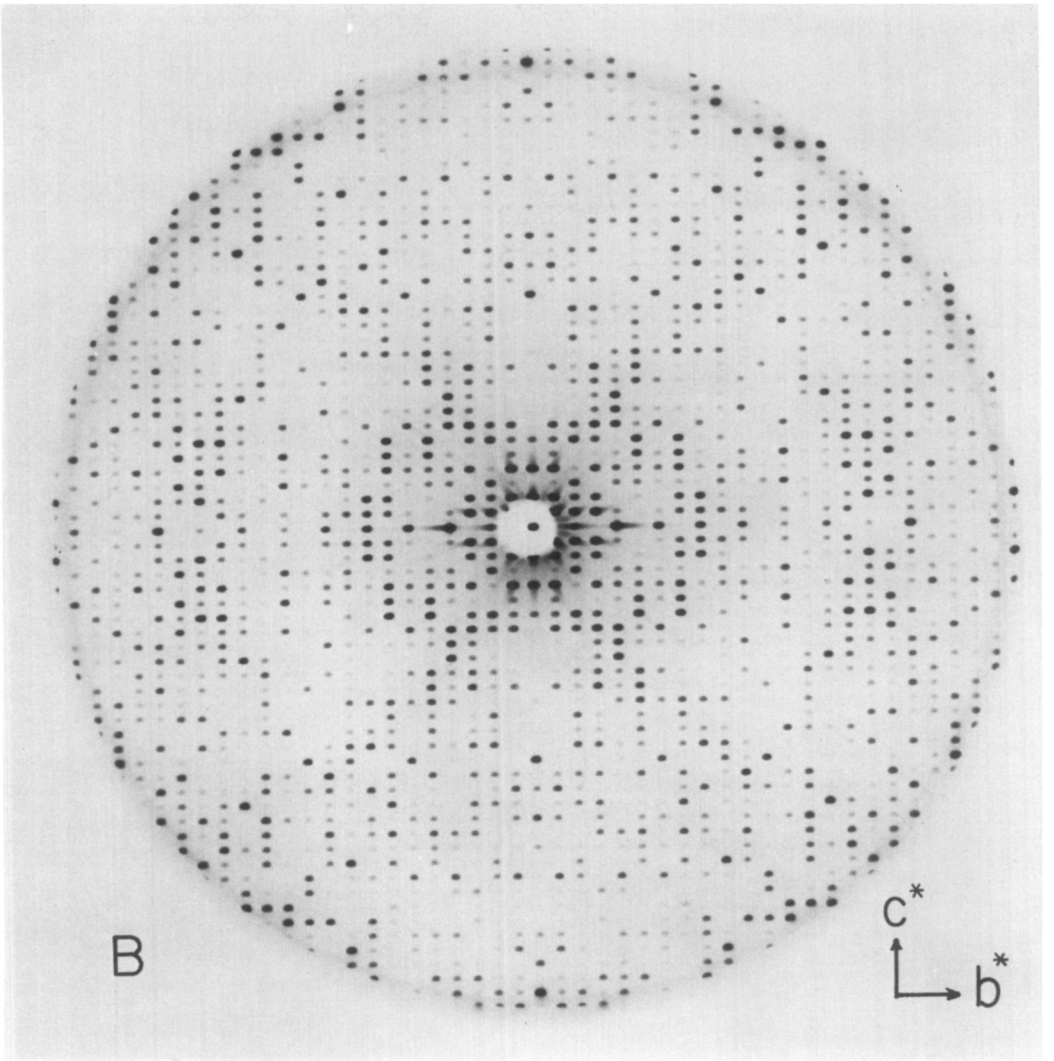


Figure 2. X-ray diffraction precession photographs (μ = 14°) of pea lectin crystals. a) hk0 zone b) 0kl zone. The X-ray beam source was an Elliott rotating anode emitting CuKα radiation. Exposure time approximately 20 hrs. The crystals with mother liquor were mounted in sealed glass capillaries.

conditions described above for the preparation of our new crystal form. We do not know what circumstances promote this.

On comparing the two crystal forms of pea lectins, we see that their cell dimensions are somewhat related since $\underline{a}(64.2) \approx \underline{b}'(61.7)$; $\underline{b}(72.7) \approx \underline{c}'(137.6$

$c(108.3) \approx 2a'(51.0)$ where a', b', c' are the cell dimensions of the crystals reported by Bryzgunov et al. (3). This suggests that some of the same protein-protein interactions may be preserved in the two crystal forms.

We have collected a native data set of reflections to 3.5Å resolution using a Picker FACS-I diffractometer. We are currently using this data set to compute a rotation function search in order to locate a non-crystallographic two-fold axis which could be useful in the interpretation of heavy-atom derivatives.

Acknowledgments: We thank Emil Shen and Dr. Martha Kimball for their assistance in this project. The research was supported by grants from the NIH, NSF, NASA and the American Cancer Society. A. H.-J. Wang was partially supported by the Center for Cancer Research at M. I. T.

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