CRYSTALLOGRAPHIC STUDIES ON CONCANAVALIN B

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ABSTRACT

Concanavalin B has been grown as hexagonal needles and analyzed by x-ray diffraction and electron microscopy. The crystals are of space group $P6_1$ with $\underline{a}$ = 81Å and $\underline{c}$ = 101Å. There is one molecule of 30,000 molecular weight as the asymmetric unit of the crystal. Electron micrographs demonstrate that the crystals maintain considerable order after dehydration and exhibit large intersticial solvent regions.

INTRODUCTION

Concanavalin B is one of four proteins from the Jack Bean (Canavalis Ensiformis) which were crystallized by J. B. Sumner between 1918 and 1935 (1, 2, 3). The other three are urease, the only one of the four to be assigned a distinct enzymatic function, canavalin, and concanavalin A. The hexameric subunit composition and arrangement of canavalin have recently been established by x-ray diffraction analysis (4), and the entire three-dimensional structure of concanavalin A has been determined (5, 6). This latter protein exhibits a number of interesting physiological properties due to its ability to bind to cell surfaces (7, 8). The function of concanavalin B in the seed, like that of concanavalin A, is unknown, and very little research has been conducted on its biochemical properties to date. The molecular weight was estimated by Sumner in 1938 to be 45,000 by ultra-centrifugal analysis (9).

We have crystallized concanavalin B by a modification of Sumner's procedure (1) and studied it by x-ray diffraction analysis, electron microscopy and SDS-polyacrylamide gel electrophoresis.

METHODS

Crystallization - One hundred grams of Jack Bean meal purchased from Sigma Co. was extracted with 30% acetone and centrifuged to remove insoluble residue. The supernatant was brought to 50% acetone concentration, the precipitate collected and dissolved in minimal Tris-HCl buffer pH 8.0. The protein solution was dialyzed for 48 hours vs. distilled water at 4°C and a heavy yield of micro-crystalline needles collected by centrifugation. The crystals were dissolved in aqueous ammonia and dialyzed for several days against 15% 2-methyl-2, 4-pentanediol whereupon large hexagonal needles of concanavalin B were formed.

X-Ray Analysis and Electron Microscopy - For x-ray examination, crystals were sealed in quartz capillaries by the conventional means, and diffraction photographs recorded on a Supper precession camera using nickel filtered CuK_{α} radiation generated by an Elliot rotating anode source operated at 40 kV and 40 mA. For examination with the electron microscope, microcrystals suspended in a drop of water were placed on a carbon covered grid followed by a drop of 2% uranyl acetate. Micrographs were taken on a JOEL 100-B operating at 80kV and a magnification of 60,000.

Acrylamide Gel Electrophoresis - SDS polyacrylamide gel electrophoresis was performed according to Laemmli (10) using a discontinuous tris-glycine buffer system. Eight proteins of known molecular weight were run as standards on parallel gels and the molecular weight of concanavalin B calculated according to Weber and Osborn (11).

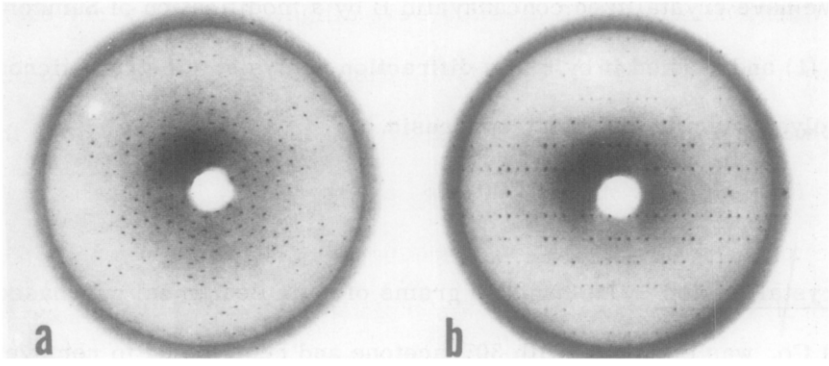

Figure 1 - An 8° precession photograph of (a) the hk0 zone and (b) the h0ℓ zone of the reciprocal lattice of hexagonal concanavalin B crystals.

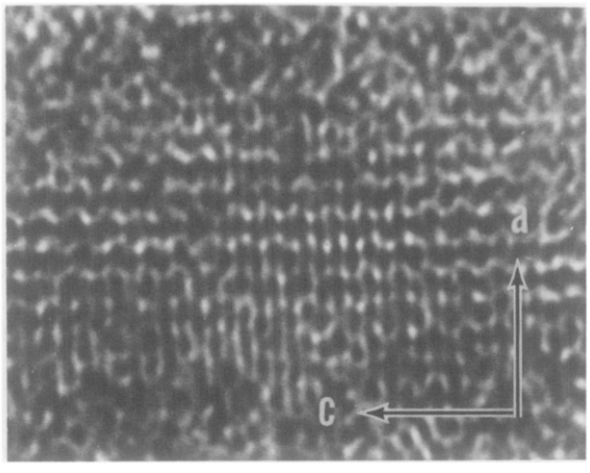

Figure 2 - An electron micrograph of a small section of a negatively stained microcrystal of concanavalin B. The light areas correspond to protein and the dark areas to stain. The magnification is x 750,000.

RESULTS

Electrophoresis of concanavalin B on SDS-polyacrylamide gels and comparison with known standards showed the protein to have a minimum molecular weight of 30,000±500, which is about 30% lower than Sumner's 1938 result.

When concanavalin B crystals were alligned with the x-ray beam parallel with the long axis of the crystal, photographs like that shown in figure 1a were recorded. When rotated by 90° about the spindle axis, the pattern in figure 1b was observed. The Laue symmetry is 6, and only those 00ℓ reflections for which $\ell = 6n$ are present. The space group is $P6_1$ (or its enantiomorph $P6_5$) and the unit cell dimensions are a = 81Å and c = 101Å.

Assumption of one molecule of 30,000 molecular weight as the asymmetric unit of the crystal implies a unit cell volume to protein mass of V_m=3.18. This value is within the range of crystalline proteins compiled by Matthews (12) although it is rather high, and indicates a large solvent volume in the crystals. According to the formula derived by Matthews the solvent volume for concanavalin B crystals would be 61%.

To verify that this was the case, microcrystals of concanavalin B were examined in the electron microscope by negative staining. Figure 2 is a small portion of the microcrystal. The light areas correspond to protein and dark areas to the stain which fills intersticies between molecules. It is immediately evident from the extensive distribution of stained areas in the crystal that there must be a large solvent regions in the fully hydrated state.

The long dimension of the crystal is the crystallographic c axis, the direction of the electron beam is approximately along the 110 direction, and the protein is seen projected onto the 100 plane. Regions of heavy staining join to form striking longitudinal lines running the length of the crystal as well as cross striations. The crystallographic period along c is the distance between alternate cross lines which is 68Å. Along a the crystallographic period is the distance between longitudinal lines, 62Å. These dimensions represent shrinkages of 32% and 23% along c and a respectively. The volume of the dehydrated unit

cell is $2.26 \times 10^5 \text{Å}^3$. This represents a shrinkage in unit cell volume after loss of solvent of 61%, exactly that predicted by the formula of Matthews. We suspect that the precise equivalence of the two values is partly coincidental, since some small volume in the dry state must be occupied by stain, but the agreement is sufficient evidence that the asymmetric unit is one molecule.

X-ray diffraction intensities were observed in still photographs that extended to less than 3.0Å resolution, and no appreciable degradation of the crystals with exposure time was evident for 60 hours. The crystals of concanavalin B, therefore, seem suitable for a three dimensional structure analysis.

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