Phytochemistry, 1978, Vol. 17, pp. 1015–1016 © Pergamon Press Ltd. Printed in England. 0031-9422/78/0601-1015 $02.00/0

SUBUNIT STRUCTURE OF CANAVALIN

RIMONA MARGALIT and EZRA DANIEL
Department of Biochemistry, George S. Wise Center for Life Sciences, Tel-Aviv University, Ramat-Aviv, Israel

(*Received* 9 *September* 1977)

Key Word Index—*Canavalia ensiformis*; Leguminosae; jack beans; canavalin; subunit structure; proteins.

Abstract—The subunit structure of canavalin was studied. The native protein has a sedimentation coefficient of 6.4 S and a MW of 91 000. SDS gel electrophoresis of the fully dissociated protein gave a single band, corresponding to a polypeptide chain with a MW of 22 700. A minimal MW of 20 700 was estimated from the amino acid composition. On a MW basis the native molecule consists of 4 chains. Support for the tetrameric structure of canavalin is provided by the electrophoretic pattern of partially dissociated protein.

INTRODUCTION

Canavalin is a globular protein from jack beans, first isolated by Sumner [1] in 1919, together with urease, concanavalin A and concanavalin B. The last 3 crystallized upon precipitation while canavalin did not. Canavalin did, however, crystallize after incubation with trypsin under mild conditions [2]. Sumner [1, 2] referred to both the native protein and the trypsin-treated protein as canavalin.

For the trypsin-treated canavalin, Sumner [3] estimated a sedimentation coefficient of 6.4 S (for a 1% protein solution) and a MW, from sedimentation and diffusion, of 113000. The corresponding values for canavalin have not previously been reported.

Since the biological function of canavalin is unknown, it has not attracted much attention in the past. Recently, however, there has been renewed interest in the structure of the protein. McPherson and Rich [4] and McPherson and Spencer [5] crystallized the trypsin-treated canavalin and investigated its subunit structure using X-ray diffraction and electron microscopy. In the work presented here, we have studied the subunit structure of native canavalin.

RESULTS

Sedimentation

In sedimentation velocity experiments native canavalin gave a single symmetrical boundary, $s^0_{20} = 6.42$ S. The sedimentation pattern was independent of pH in the range 3–11. The MW of native canavalin was determined by sedimentation equilibrium. A linear relation was found on plotting log c vs r^2. From the slope, a MW of 91 000 ± 1700 was calculated, using a value of 0.73 ml/g for the partial specific volume [3].

Electrophoresis

The banding pattern of SDS gel electrophoresis of canavalin depends on the conditions under which the protein is incubated. If preincubation is carried out in strong dissociating conditions (1% SDS and 1% mercaptoethanol for 5 min at 100°) all the protein migrates as a single band with a relative mobility of 0.69 corresponding to a MW of 22 700 ± 900. If preincubation is carried out under mild conditions (0.1% SDS and 0.1% mercaptoethanol for 15 min at room temperature) only a trace of the fast moving band can be detected and virually all the protein migrates as a slow band with a relative mobility of 0.27 corresponding to a MW of 89 000 ± 1200. When a concentrated solution of protein was incubated under the strong dissociation conditions three faint bands were seen to follow the main fast-moving band.

Amino acid composition

The amino acid composition of canavalin is presented in Table 1. The data show that the protein does not contain tryptophane, as was first noted by Sumner [1]. The minimal MW of canavalin calculated per one cystine residue is 20 300 ± 2000. From the amino acid composition a value of $\bar{v} = 0.739$ ml/g was calculated for the partial specific volume of the protein.

DISCUSSION

Homogeneity of the canavalin preparation used in this study was inferred from the observation of a single peak in sedimentation velocity, and from the linearity of the log c vs r^2 plot obtained in sedimentation equilibrium experiments (MW 91 000). Canavalin shows a remarkable stability towards dissociation. Neither acid pH, down to 3, nor alkaline pH, up to 11, effects any appreciable change in the sedimentation coefficient. Complete dissociation could only be achieved in strong dissociating medium—1% SDS and 1% mercaptoethanol.

The MW (22 700) was then consistent with the minimal MW determined from the amino acid composition. The corresponding value of 19 500 reported by McPherson and Rich [4] is somewhat lower; the difference between their value and ours might be due to the fact that their determination was performed on trypsin-treated canavalin. Taking into account the MW's of the undissociated molecule and that of the constituent polypeptide chain, we can conclude that canavalin contains 4 polypeptide chains per molecule.

Support for the tetrametric structure of the molecule was afforded by the electrophoretic pattern of partially

Table 1. Amino acid composition of canavalin

Residue	% by wt	Moles residues* per mole cystine	Colorimetric determination† % by wt	Moles residues per mole cystine
Asp	11.9 ± 0.11	21 ± 2		
Thr	2.5 ± 0.05	5 ± 1		
Ser	3.1 ± 0.04	7 ± 1		
Glu	16.9 ± 0.13	27 ± 2		
Pro	3.9 ± 0.05	8 ± 1		
Gly	3.2 ± 0.03	10 ± 1		
Ala	4.6 ± 0.08	12 ± 2		
Val	7.5 ± 0.10	15 ± 2		
Met	2.5 ± 0.08	4 ± 1		
Ile	5.0 ± 0.06	9 ± 1		
Leu	11.7 ± 0.11	21 ± 2		
Tyr	4.6 ± 0	6 ± 0	5.5	6.4
Phe	5.6 ± 0.07	8 ± 1		
Trp	0	0	0.24	0.25
Lys	5.6 ± 0.06	9 ± 1		
Hys	2.0 ± 0	3 ± 0		
Arg	8.2 ± 0	11 ± 1		
Cysteine			0	0
Cystine‡	1.0 ± 0	1 ± 0	1.0	0.9

* Determined by this work.
† Determined by Sumner [8].
‡ Determined as cysteic and residues.

dissociated canavalin. Four bands are obtained in this case: a fast-moving band, corresponding to fully dissociated polypeptide chain; a slow-moving band, corresponding to the undissociated protein; and two additional bands, corresponding to 2- and 3-chain intermediates. The 4 polypeptide chains are probably linked together through interchain S—S bonds, since in a reduced concentration of mercaptoethanol—0.1% instead of 1%—little or no dissociation takes place.

The 4 subunit structure found by us for canavalin is different from the 6-subunit structure reported by McPherson and Rich [4] and McPherson and Spencer [5] for trypsin-treated canavalin from X-ray crystallography.

While any explanation for the discrepancy must, for the present, be only tentative, the findings may reflect a genuine difference in the mode of association of individual polypeptide chains from intact canavalin and from trypsin-treated canavalin. In fact, the difference between the two proteins has been suggested as the reason for the different morphology of their crystals [4, 9]. A crystallographic study of intact canavalin similar to the one carried out on the trypsin-treated protein is needed to provide a definite answer to this problem.

EXPERIMENTAL

Jack bean meal was from Sigma Chemical Co. (St. Louis, No. 63178). Acrylamide was from Merck (Munich, Germany). Bis acrylamide was from Fluka, AG (Switzerland).

Preparation of the protein. The procedure of ref. [2] for purifying canavalin from jack bean meal includes a final step in which trypsin is added to the aq. soln of canavalin to initiate crystallization. The canavalin used in this investigation was prepared according to this method with the omission of the trypsin-addition step. The purified canavalin was lyophilized and the resultant amorphous powder kept at 4°.

Concn of canavalin solns. Determined from A_{275} (peak). In order to calibrate the method, samples of canavalin were dialyzed exhaustively against twice dist. H_2O, lyophilized and dried in vacuum at room temp. to constant wt. The dried samples were dissolved in 30 mM Tris–HCl buffer, pH 7, and the A of the solns measured. A value of $E^{1\%}_{1cm} = 6.8$ at 275 nm was obtained.

Amino acid composition. Samples of canavalin were hydrolyzed in 6 N HCl and amino acid analysis performed on the hydrolysate. Separate samples were treated with performic acid in order to oxidize cystine to cysteic acid residues and subjected to acid hydrolysis and analysis. Tryptophan was estimated spectrophotometrically [6].

Ultracentrifugation. Performed with a Beckman Model E analytical ultracentrifuge, equipped with electronic drive control and absorption splitbeam photoelectric scanning system. Schlieren optics were used in the sedimentation velocity expts. Scanner absorption optics were used in the sedimentation equilibrium expts.

SDS gel electrophoresis. As ref. [7]. The gels were 7.5% acrylamide with 0.1% SDS. Tube gel electrophoresis was run at room temp. applying a constant current of 8mA per tube. Slab gel electrophoresis was run at 4° applying 120 V (constant). Incubation of canavalin with SDS and mercaptoethanol was as specified in the results. The MW was determined from a calibration curve, obtained using the following proteins: cytochrome *c*, mycoglobin, pepsin, BSA monomer and BSA dimer.

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