available for other metabolic processes, (3) affect the amount of these materials available for destruction, and hence (4) also exert an effect on reaction equilibria.

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Physical and chemical characterization of concanavalin A, the hemagglutinin from jack bean *(Canavalia ensiformis)**

The globulin concanavalin A was isolated in crystalline form by SUMNER[1] and later identified as the hemagglutinating principle[2] in the jack bean. The ability of concanavalin A to form a precipitate with glycogens, mannans, dextrans, levans, and serum proteins has been studied by various workers[3–7]. It has been suggested[3,7,8] that the interaction of concanavalin A with reactive polysaccharides is analogous to that between an antibody and an antigen. Our studies of concanavalin A–polysaccharide interaction have been largely guided by its suitability as a possible model for the antibody–antigen system and as a tool for investigating the fine structure of polysaccharides[9,10].

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The demonstration of the reactivity of concanavalin A with dextrans[7] and the availability of dextran supports (Sephadexes) led us to explore the use of these media for the purification of concanavalin A. We have already reported[11] the specific binding of concanavalin A to dextran gels G-50 through G-200 and its elution by specific low molecular weight carbohydrates. Only sugars shown to be inhibitors of concanavalin A–polysaccharide interaction were found to be effective in displacing concanavalin A bound to Sephadex G-50. Concanavalin A was also displaced from Sephadex by 1 M acetic acid (pH 2.4), by 8.0 M urea, and by 3.0 M $MgCl_2$ (ref. 12). Based on the specific adsorption on Sephadex we have developed a new method for the isolation and purification of concanavalin A. This communication presents the results of various physical and chemical studies of this globulin. Details will follow subsequently.

Concanavalin A was prepared as follows: jack bean meal (200 g) was extracted in the cold (5°, overnight) with two successive portions of 0.15 M NaCl (2 × 1 l). The protein fraction in the NaCl extract which precipitated between 0.30 to 0.80 saturation of $(NH_4)_2SO_4$ contained all the concanavalin A activity as determined by the quantitative assay developed in this laboratory[13]. When this fraction was passed through a Sephadex G-50 column (4 cm × 50 cm) equilibrated with 1.0 M NaCl all inactive protein appeared in the breakthrough peak. When the eluate had reached a constant low absorbance at 280 mμ, concanavalin A was displaced from the dextran bed by a solution of D-glucose (0.10 M in 1.0 M NaCl). The D-glucose was removed by extensive dialysis of the concanavalin A solution against 1.0 M NaCl. Concanavalin A prepared by this method was approx. 95–98 % active as determined by the quantitative analyses of washed concanavalin A–dextran precipitates[13]. This represents a 3-fold purification of concanavalin A over the ammonium sulfate fraction applied to the Sephadex column.

Studies in the analytical ultracentrifuge revealed the presence of a single component in the pH range 2.0–5.0, $s^{\circ}_{20,w}$ being 3.9 S (at pH 5.0; I, 0.45). At pH 7.0 two components (4 S and 7 S) were observed. From sedimentation–diffusion data at pH 5.0 a molecular weight of 68000 was calculated in contrast to the higher values reported earlier[14]. This may represent the molecular weight of a subunit. Gel filtration of concanavalin A on Biogel P-100 (exclusion limit 100000) at pH 7.5 resulted in complete exclusion of the protein. At pH 5.0, concanavalin A (R_F 0.80) was retarded, (ovalbumin, mol. wt. 45000 had R_F 0.81). These results, although approximate, suggest the formation of higher molecular weight species near pH 7, in confirmation with the ultracentrifuge data.

Concanavalin A has an extinction coefficient, $E^{1\%}_{1\,cm}$ of 11.4 ± 0.1 at 280 mμ in 1.0 M NaCl. Its isoelectric point, determined by free-boundary electrophoresis, was found to be pH 7.1 ± 0.1 as opposed to previous reports of pH 5.5 (refs. 3, 15). Below the isoelectric point free-boundary electrophoresis yielded a pattern which was essentially that of a single component. A three-peak pattern at pH 7.4 cannot be rationalized at the present time but may involve the formation of multiple forms in the presence of bound Mn^{2+}. A parallel situation has been observed in the case of the ovalbumin–plakalbumin system[16]. Essentially, a single band was observed upon cellulose acetate electrophoresis at three different pH values (5.0, 7.0, and 8.6), no net migration occurring at pH 7.0. On starch-gel electrophoresis in borate buffer, pH 8.6, an anodically migrating streak was obtained. Incorporation of 0.10 M D-glucose in the starch gel gave a single sharp band which migrated very slowly towards

the cathode. The presence of D-glucose may inhibit interaction between starch and concanavalin A.

Extensive dialysis of concanavalin A against 1 M acetic acid (pH 2.4) resulted in loss of activity. The presence of Mn^{2+} (0.029 %) in native concanavalin A was confirmed by neutron-activation analysis. This tightly-bound metal was found to be essential for activity although it could be replaced by certain other bivalent metallic ions in essential agreement with earlier studies[17].

TABLE I

AMINO ACID COMPOSITION

Amino acid	*Residue (g/100 g protein)*
Lys	5.96
His	3.55
Arg	3.97
Asp	14.38
Ser	7.61
Thr	10.55
Glu	5.96
Pro	4.06
Gly	3.56
Ala	5.02
Cys	0.00
Val	6.43
Met	0.98
Ile	6.37
Leu	8.04
Tyr	4.69
Phe	5.76
Trp	3.01

Total N	15.2 %
Amide N (as % of the total N)	6.3 %
Carbohydrate	Negative
Mn^{2+}	0.029 %
$E_{1\%}^{1\,cm}$ at 280 mμ in 1.0 M NaCl	11.4 ± 0.1
p*I*	7.1 ± 0.1
At pH 5.0	
$s^{\circ}_{20\,w}$	3.9 S
$D_{20,w}$	5.43 F
$\bar{v}$ (assumed)	0.73
Mol. wt.	68 000

Concanavalin A displayed maximum capacity to precipitate specific polysaccharides in the pH range 6.5–8.0. Excess bivalent cations inhibit precipitate formation. Present evidence suggests that the active precipitating unit probably consists of a cluster of several subunits although the monomer also may be active.

Alanine was identified as the N-terminal amino acid residue by both the fluorodinitrobenzene and phenylisothiocyanate methods[18]. Small amounts of serine and glycine were also detected. In contrast to other phytohemagglutinins[19], concanavalin A does not contain any carbohydrate as shown by negative results with several colorimetric reactions for neutral, amino and acidic sugars[20]. Amino acid analysis revealed the absence of both cysteine and cystine, contrary to earlier reports[21].

Acetylation of concanavalin A did not change its activity toward the dextran (B-1355-S) used for its assay[13]. Photooxidation in the presence of methylene blue slowly destroyed its ability to precipitate dextran (90 % loss of activity in 23 h). Treatment of concanavalin A with pronase, chymotrypsin, pepsin, and papain readily

destroyed its activity, whereas trypsin attacked it very slowly. Further modification studies are in progress.

The results of our investigations are in excellent agreement with the studies of OLSON AND LIENER[22] to be published elsewhere.

The results are summarized in Table I.

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