Some Physical and Chemical Properties of Concanavalin A, the Phytohemagglutinin of the Jack Bean*

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ABSTRACT: Three-times-crystallized concavanalin A, prepared by the method of J. B. Sumner and S. F. Howell [*J. Bacteriol. 32*, 227 (1936a)], was found to contain 4% carbohydrate. Disc gel electrophoresis revealed one major and one minor component. Carbohydrate could be removed by treatment with 1 M acetic acid, and further purification could be achieved by selective adsorption on Sephadex G-100 followed by elution with dilute acid. The direct purification of concanavalin A from an aqueous extract of jack bean meal could be accomplished by a sequence of steps involving adsorption and elution from Sephadex, treatment with acetic acid, and a second cycle of adsorption and elution from Sephadex. Concanavalin A had an $s_{20,w}$ value of 6.0 S and a molecular weight of 71,000 based on sedimentation velocity and equilibrium studies, respectively, at pH 7. The most notable feature of its amino acid composition was the absence of cystine residues. The finding that there were 1.5 N-terminal alanine residues suggests a multichain structure.

The insoluble product formed by the interaction of concanavalin A with glycogen contained carbohydrate and protein in the ratio of 2:1. This insoluble complex could be solubilized under acid (pH < 4) and alkaline (pH > 9) conditions. Chromatographic evidence indicated that complete dissociation of the complex had occurred under these conditions.

Proteins having the unique ability of being able to agglutinate the red blood cells of various species of animals are widely distributed in the plant kingdom (see reviews by Boyd, 1963; Liener, 1964). Among these so-called phytohemagglutinins the one occurring in the jack bean (*Canavalia ensiformis*) was first isolated by Sumner (1919) who gave it the name concanavalin A. In addition to its hemagglutinating activity, con A[1] forms a precipitate with certain polysaccharides such as glycogen, amylopectin, yeast mannan, and dextrans (Sumner and Howell, 1936a; Sumner and O'Kane, 1948; Cifonelli and Smith, 1955; Cifonelli *et al.*, 1956; Manners and Wright, 1962; Goldstein *et al.*, 1965a,b) as well as blood serum glycoproteins (Nakamura and Suzuno, 1965).

The reaction of con A with such a variety of polysaccharides thus provides a unique opportunity for studying protein–carbohydrate interactions. Although the structural prerequisites of the carbohydrate participating in this interaction have been studied in considerable detail (Cifonelli *et al.*, 1956; Goldstein *et al.*, 1965a,b; Goldstein and So, 1965; Goldstein and Iyer, 1966), little is known about the structural features of the protein molecule which provides a receptor site for the carbohydrate. Con A had been obtained in crystalline form by Sumner in 1919, but a rigorous examination of its purity and physicochemical properties by presently available techniques has not been heretofore reported. The only data relevant to the physical properties of con A are the reports (Sumner and Howell, 1936a; Sumner *et al.*, 1938) that it has an isoelectric point of pH 5.5, a sedimentation coefficient ($s_{20,w}$) of 6.0, and a molecular weight of 96,000.

This paper reports a reevaluation of the purity and properties of crystalline con A which had been prepared by the procedure described by Sumner and Howell (1936a). Since this preparation proved to be inhomogeneous, steps designed to enhance the purity of this protein are described. The purified con A has also been studied with particular regard to the quantitative aspects of its interaction with glycogen.

Experimental Section

Materials. The starting material for all of the preparations of con A described here was defatted jack bean meal purchased from the Sigma Chemical Co., St. Louis, Mo. The glycogen employed in these studies was of oyster origin obtained from Nutritional Biochemical Corp., Cleveland, Ohio. Three-times crystallized con A was prepared by the method of Sumner and Howell (1936a). Sephadex G-100 and SE-Sephadex, C-25, were products of Pharmacia, Piscataway, N. J.

Purification of Con A. Inert impurities could be removed from three-times crystallized con A by taking advantage of the specificity of the reaction of con A with dextrans (Goldstein *et al.*, 1965a,b). Crystalline

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[1] Abbreviation used: con A, concanavalin A.

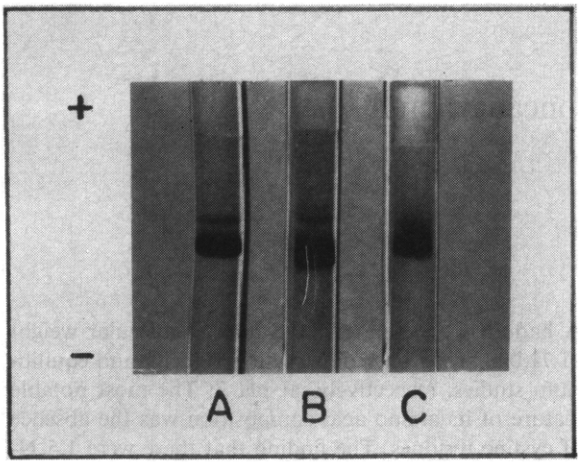

FIGURE 1: Disc gel electrophoresis of various preparations of con A. (A) Three-times crystallized con A. (B) Active component from crude extract of jack bean meal after adsorption and elution from Sephadex (see Figure 2B). (C) Three-times crystallized con A after treatment with 1 M acetic acid and adsorption–elution from Sephadex (see Figure 2A). Active component from crude extract after treatment with 1 M acetic acid and recycling through Sephadex (see Figure 2C) gave pattern identical with C.

con A which had been treated with acetic acid (see below) was dissolved in 0.01 M Tris buffer, pH 7.4, containing 10^{-3} M $CaCl_2$ and 10^{-3} M $MgCl_2$ and applied to a column (1 × 12 cm) of Sephadex G-100 equilibrated against the same buffer. Calcium and magnesium ions were included in this buffer system because of the report (Sumner and Howell, 1936b) that these divalent metals enhance the activity of con A. Fractions (3 ml) were collected at a flow rate of 10 ml/hr. The column was eluted with the Tris buffer until the absorbance (280 mμ) of the eluate was <0.02, at which point this buffer was replaced by 0.02 M glycine–HCl buffer, pH 2.0. The effluent at this time was collected in tubes containing 1 ml of 0.075 M Tris buffer, pH 8.6, which served to neutralize the acid effluent. An aliquot of each tube was assayed for hemagglutinating activity against trypsinated rabbit erythrocytes and the activity was expressed as hemagglutinating units as defined by Liener (1955). Those tubes displaying activity were pooled, dialyzed against deionized water, and lyophilized.

Con A could also be purified directly from an aqueous extract of jack bean meal by adsorption and elution from a large column of cross-linked dextran. Jack bean meal (200 g) was suspended in 500 ml of 0.01 M Tris buffer, pH 7.4, and stirred for 2 hr at room temperature. The suspension was filtered through cheese cloth and the residue was reextracted twice with the same volume of buffer. All of the filtrates were combined and centrifuged to remove any remaining insoluble material. The clarified solution (1500 ml) containing a total of 13.5 g of protein was applied to a column (6 × 20 cm) of Sephadex G-100 equilibrated against the pH 7.4 buffer. Fractions (10 ml) were collected at a flow rate of 60 ml/hr. When no more protein could be detected in the effluent as measured by adsorption at 280 mμ, 0.02 M glycine–HCl buffer, pH 2.0, was introduced into the column. Each collection tube receiving the acid effluent contained 3 ml of 0.075 M Tris buffer, pH 8.6. The contents of those tubes displaying hemagglutinating activity were pooled, dialyzed, and lyophilized. When this preparation was recycled through Sephadex G-100 to effect further purification, the experimental details were essentially the same.

Crystalline con A is soluble only in solutions of fairly high salt concentration (Sumner and Howell, 1936a), a factor which poses certain problems in attempting to study its properties. It was found, however, that con A could be easily dissolved in distilled water if it were first treated with acetic acid in the following manner. Crystalline con A, or the active component obtained from a crude extract after adsorption and elution from Sephadex as described above, was stirred into 1 M acetic acid to a final concentration of 1.25%. After stirring for 20 min, the mixture was clarified by centrifugation. The acetic acid was removed by dialysis and the protein was lyophilized. The hemagglutinating and glycogen-precipitating activities of con A were unimpaired by this procedure. In addition to a drastic change in the solubility characteristics of con A, treatment with acetic acid served to eliminate completely a small amount of carbohydrate which was invariably present in untreated preparations.

Sedimentation and Molecular Weight Determination. Sedimentation velocity and equilibrium experiments were performed in a Spinco Model E ultracentrifuge. Velocity studies were carried out in 2-mm single-sector aluminum cells at a rotor speed of 59,780 rpm at 20°. Runs were made on solutions of three different concentrations of protein (0.2, 0.5, and 0.8%) prepared by dissolving the protein directly in 5% NaCl (equivalent to 1.15 M), pH 5.5, or, in some cases, 0.1 M phosphate buffer, containing 0.1 M NaCl adjusted to a final pH of 7.0. The Svedberg equation (Svedberg and Pederson, 1940) was used to calculate sedimentation coefficients, and the $s_{20,w}$ was obtained by graphical extrapolation to zero concentration. Sedimentation equilibrium experiments were carried out in the 8-channel short-column system described by Yphantis (1960). Four concentrations of protein ranging from 0.2 to 0.8% were exhaustively dialyzed against 0.1 M sodium phosphate buffer containing 0.1 M NaCl, pH 7.0. The concentrations were determined as refractive index differences between the solvent and protein solution using a Phoenix differential refractometer. Runs were made at 12,590 rpm for 384 min. In calculating the molecular weight a partial specific volume of 0.727 was employed. This value was based on the amino acid composition of con A and the partial specific volumes of the individual amino acids (McMeekin *et al.*, 1949). Apparent molecular weights were plotted against protein concentration and extrapolated to zero concentration.

Disc Gel Electrophoresis. Disc gel electrophoresis

TABLE I: Purification and Recovery Data on Con A.

Fraction	Sp Act. (HU[a]/mg of protein)	Purifcn Factor	Total Act. (HU × 10^{-5}/100 g of meal)[a]	Recov of Act. %	Composition (%) Carbohydrate	Composition (%) Protein	Yield of Protein (g/100 g of meal)
Crude extract	1032	1.0	70.0	—	—	—	6.8
Active component (Figure 2B)	2280	2.2	52.0	75	0.8	15.2	2.3
Active component (Figure 2C)	2340	2.3	36.8	53	0	15.6	1.6
Three-times crystallized con A	2200	2.1	17.5	25	4.0	14.8	0.8
Active component (Figure 2A)	2300	2.2	13.8	20	0	15.4	0.6

[a] HU = hemagglutinating units as defined by Liener (1955).

was conducted at pH 4.5 in the manner described by Reisfeld *et al.* (1962). Photographs of the stained gels were taken with a Polaroid camera using the technique suggested by Burns and Pollak (1963).

Amino Acid Analysis. Amino acid analyses were determined with a Spinco Model 120 analyzer according to Spackman *et al.* (1958). Aliquots (0.5 ml) of a stock solution containing 8 mg of protein/ml were mixed with an equal volume of concentrated HCl and hydrolyzed in sealed, evacuated tubes at 110° for 8, 18, 24, 44, and 66 hr. Average values were taken for all of the amino acids with the following exceptions: maximum values were taken for isoleucine and valine; and threonine and serine values were extrapolated to zero time. Dry weight, nitrogen (Lanni *et al.*, 1950), tryptophan (Spies and Chambers, 1949), amide nitrogen (Hirs *et al.*, 1954), and cysteic acid (Hirs, 1956) were determined independently on aliquots of the stock solution.

Amino-Terminal Groups. N-Terminal amino acid residues were determined by the dinitrophenylation technique as described by Fraenkel-Conrat *et al.* (1955) and Biserte *et al.* (1959). Quantitative estimates of the DNP-amino acids which appeared in the chromatograms were made by eluting the excised spots with 4 ml of 1% $NaHCO_3$ and measuring their absorbance at 360 mμ.

Reaction with Glycogen. The interaction of con A with glycogen was studied by mixing 5 mg of con A with various levels (0–10 mg) of glycogen in a total volume of 1.5 ml of 0.05 M sodium phosphate buffer, pH 7.0. After 2 hr at room temperature, the insoluble con A–glycogen complex was removed by centrifugation and washed twice with 1-ml portions of the same buffer. The insoluble residue was dissolved in 1 ml of 0.1 M NaOH and analyzed for carbohydrate (Dubois *et al.*, 1956) and nitrogen (Lanni *et al.*, 1950). The latter value was converted to protein using a factor of 6.25.

The effect of pH on the solubility of the con A–glycogen complex was studied by suspending the precipitate obtained with 0.5 mg of con A and 10 mg of glycogen in 1-ml portions of buffer having pH values ranging from 2 to 12 (Britton and Welford, 1937). The tubes were allowed to stand for 18 hr at 4° with intermittent shaking. Any insoluble material that remained after this time was recovered by centrifugation, dissolved in 1 ml of 0.1 M NaOH, and analyzed for carbohydrate and protein as before.

Results

Purification of Con A. When three-times-crystallized con A which had been prepared by the method of Sumner and Howell (1936a) was examined by disc gel electrophoresis, one major and one minor component could be observed (Figure 1A). This material contained 4% carbohydrate which could be removed by treatment with 1 M acetic acid without any diminution of hemagglutinating or glycogen-precipitating activity. The acetic acid treated material likewise presented a disc electrophoretic pattern identical with the untreated protein. In contrast to its previous insolubility in dilute buffer, however, the acetic acid treated protein could be readily dissolved in 0.01 M Tris buffer, pH 7.4. This buffer was therefore employed in an attempt to further purify crystalline con A on Sephadex G-100. As shown in Figure 2A the active component was retained on the column permitting a small amount of inert protein to pass rapidly through the column. Subsequent elution with 0.02 M glycine–HCl buffer, pH 2.0, displaced this active component which proved to be electrophoretically homogeneous (Figure 1C) and somewhat more active than three-times crystallized con A (Table I).

In view of the purification achieved by adsorption and elution from Sephadex G-100, this technique was investigated as a possible means of purifying con A directly from a crude extract of jack bean meal. Figure 2B shows that a considerable amount of inert protein may be removed from the crude extract by a single adsorption elution cycle on Sephadex. About 75% of the total activity in the crude extract could be recovered with a twofold enhancement of activity (Table I). This active fraction, however, was still grossly heterogeneous as evidenced by the electrophoretic pattern shown in Figure 1B. It also contained 0.8% carbohydrate but this could be eliminated by treatment

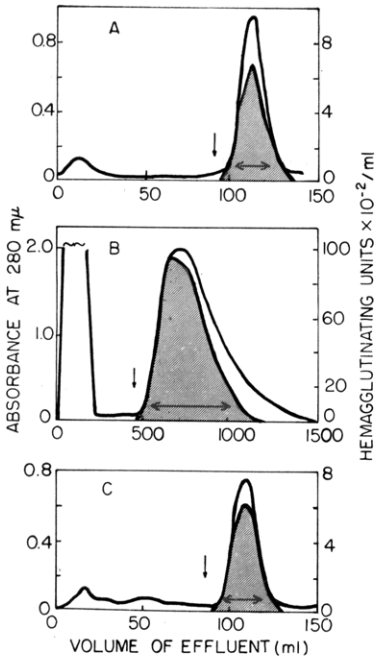

FIGURE 2: Purification of con A on Sephadex G-100. Solid curve is adsorbance measured at 280 mμ. Hemagglutinating activity is denoted by shaded portion of the curve. Vertical arrow denotes point at which 0.01 M Tris buffer, pH 7.4, was replaced by 0.02 M glycine–HCl buffer, pH 2.0. Double-headed arrows indicate tubes which were pooled, dialyzed, lyophilized, and saved for further study. (A) Three-times crystallized con A (15 mg) which had been pretreated with 1 M acetic acid. (B) Crude extract of jack bean containing 1.8 g of protein. (C) Active fraction (10 mg) from B after treatment with 1 M acetic acid. Other experimental details are given in text.

with 1 M acetic acid. A second adsorption–elution cycle on Sephadex G-100, as depicted in Figure 2C, yielded an active component which showed only one component on gel electrophoresis. As noted in Table I, its activity was likewise comparable to that obtained with the purified crystalline con A. Because of the ease with which purified con A could be prepared in good yield directly from a crude extract of the jack bean meal, most of the studies dealing with the physical and chemical properties of this protein were carried out with material prepared in this manner.

Sedimentation Behavior and Molecular Weight. Unless specified otherwise the observations recorded here were obtained with preparations of con A which had been dissolved in 1.15 M NaCl, pH 5.5. In contrast to the heterogeneity of three-times crystallized con A revealed by disc electrophoresis (Figure 1A), only one component could be detected in the ultracentrifuge patterns of this protein. Treatment of three-times crystallized con A with 1 M acetic acid followed by adsorption and elution from Sephadex yielded a preparation which likewise displayed a single component. The $s_{20,w}$ value was approximately 3.8 S in both cases. Identical results were obtained with a preparation of con A which had been purified directly from the crude extract as described in the preceding section. However, when this preparation was dissolved in 0.1 M phosphate buffer, pH 7.0, containing 0.1 M NaCl, an $s_{20,w}$ value of 6.0 S was calculated for the single component which was observed in the ultracentrifuge pattern. Sedimentation equilibrium experiments performed in the same buffer gave a value of 71,000 for the molecular weight of this preparation of con A.

Amino Acid Analyses. Data pertaining to the amino acid composition of con A are presented in Table II. The recovery in terms of dry weight and nitrogen was essentially quantitative, thus indicating the absence of any appreciable amount of nonprotein material. The molecular weight value of 68,000 calculated from the amino acid composition may be compared with the value of 71,000 obtained from the sedimentation equilibrium data.

Amino-Terminal Residues. DNP-alanine was the

TABLE II: Amino Acid Composition of Con A.[a]

Amino Acid	Amino Acid Residues (g/100 g of protein)	Nitrogen[b] (g/100 g of N)	Amino Acid Residues[c] (mole/71,000 g of protein)
Alanine	4.75	6.02	47.4 (47)
Arginine	3.68	8.49	16.6 (17)
Aspartic acid	15.35	12.02	94.8 (95)
Glutamic acid	5.58	3.89	30.7 (31)
Glycine	3.40	5.37	42.3 (42)
Half-cystine[d]	0	0	0 (0)
Histidine	3.96	7.81	20.5 (21)
Isoleucine	6.13	4.87	38.4 (38)
Leucine	6.89	5.53	46.1 (46)
Lysine	5.64	8.09	31.2 (31)
Methionine	0.88	0.63	5.0 (5)
Phenylalanine	5.82	3.56	28.1 (28)
Proline	3.66	3.40	26.8 (27)
Serine	9.13	9.43	74.4 (74)
Threonine	6.93	6.18	48.7 (49)
Tryptophan	3.12	3.02	11.8 (12)
Tyrosine	4.14	2.29	18.1 (18)
Valine	6.04	5.44	43.3 (43)
Amide		8.18	64.6 (65)[e]
Total	97.1	101.12	624
Molecular weight			67,940

[a] All values are based on dry weight of protein. [b] Based on an N content of 15.6%. [c] Numbers in parentheses indicate the number of residues to the nearest whole integer. [d] Determined as cysteic acid. [e] Not included in total number of amino acid residues.

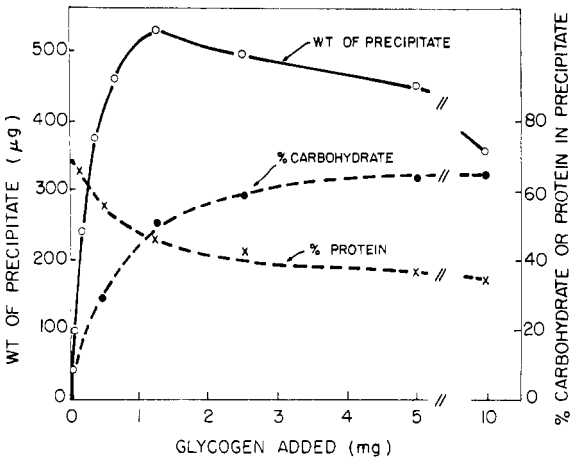

FIGURE 3: Reaction of con A with glycogen. Various levels of glycogen (0–10 mg) were added to 0.5 mg of con A in a total volume of 1.5 ml of 0.1 M sodium phosphate buffer, pH 7.0. —O—, weight of precipitate (sum of protein and carbohydrate); - -O- -, % carbohydrate in precipitate; - -x- -, per cent protein in precipitate.

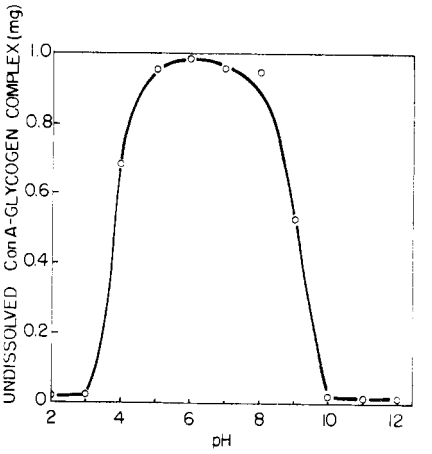

FIGURE 4: Effect of pH on solubility of con A–glycogen complex. The precipitate formed by adding 10 mg of glycogen to 0.5 mg of con A was dispersed in buffers of a given pH. The residue which remained, designated as "undissolved con A–glycogen complex," was analyzed for carbohydrate and nitrogen from which was calculated its total weight.

only amino acid which could be detected in chromatograms of the ether extract of a 16-hr hydrolysate of DNP-con A. When the time of hydrolysis was reduced to 6 hr, however, trace amounts of glycine and serine could be detected as well. The quantity of DNP-alanine was determined to be 1.16 moles/71,000 g of protein, and DNP-serine and DNP-glycine were present in amounts less than 0.05 mole. Assuming a recovery value of 80% for DNP-alanine (Biserte *et al.*, 1959), the value for this amino acid becomes 1.45 moles/mole of con A.

Reaction of Con A with Glycogen. The reaction of con A with varying levels of glycogen is depicted in Figure 3. Maximum precipitation was obtained when approximately 2.5 mg of glycogen was added/mg of con A. As the level of glycogen exceeded this value, however, the amount of precipitate gradually decreased, an observation similar to that reported for the reaction of con A with dextran (Goldstein *et al.*, 1965b). Changes that occur in the composition of the insoluble con A–glycogen complex are shown in Figure 3. The precipitate did not attain a constant proportion of carbohydrate to protein until con A had been treated with at least an eight- to tenfold level of glycogen. At this point the precipitate contained about 65% carbohydrate and 35% protein.

The effect of pH on the solubility of the con A–glycogen complex is shown in Figure 4. The complex remained insoluble over a pH range of 5–9 but could be readily solubilized on either side of this region. In order to ascertain whether dissociation of the complex accompanies the process of solubilization, the precipitate which was obtained at neutral pH was dissolved in the pH 2 buffer and applied to a column of SE-Sephadex. As can be seen from Figure 5, a distinct separation of the glycogen and con A could be effected. The glycogen was not retained on the column, and the con A which remained on the column could be eluted with 1.0 M acetate buffer, pH 5.0. Based on the carbohydrate and protein content of the original insoluble complex, over 70% of the glycogen was recovered in the hold-up volume of the column, and the protein was quantitatively recovered in the peak eluted with the pH 5.0 acetate buffer. The specific activity of the recovered protein, in terms of hemagglutinating units per milligram of protein, was essentially unchanged from its activity prior to its complexation with glycogen. It is evident therefore that the solubilization of the con A–glycogen complex, at least under acid conditions, is simply the result of a dissociation of the complex into its original soluble components.

Discussion

While this work was in progress, Agrawal and Goldstein (1965) also reported the specific binding of con A to cross-linked dextran gel. These authors used glucose to elute con A from a column of Sephadex G-200, and subsequently eliminated the glucose from the eluate by dialysis. Unless completely removed, the presence of glucose could prove troublesome in efforts to determine whether con A contains covalently bound carbohydrate. This is an important point since most of the phytohemagglutinins which have been isolated to date have been shown to be glycoproteins containing 5–10% carbohydrate. These include preparations from the black bean (Jaffé and Hannig, 1965), certain varieties of peas (Huprikar and Sohonie, 1965; Salgalkar and Sohonie, 1965), the soybean (Lis *et al.*, 1966), and the wax bean (T. Takahashi, P. Ramachan-

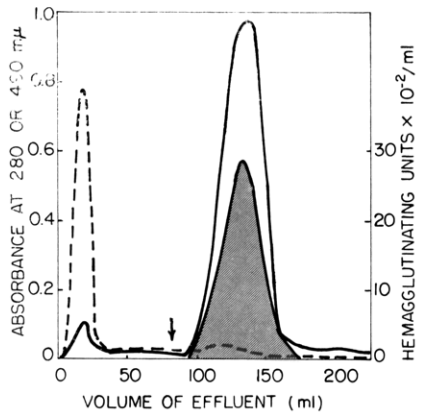

FIGURE 5: The separation of glycogen and con A from an acid solution of the complex. Con A–glycogen complex (15 mg) formed at pH 7 was dissolved in 3 ml of 0.2 M glycine–HCl buffer, pH 2.0, and applied to a column (1 × 12 cm) of SE-Sephadex equilibrated against the same buffer. Vertical arrow indicates point at which 1.0 M acetate buffer, pH 5.0, was introduced into the column. Solid curve, absorbance at 280 mμ; broken curve, carbohydrate as determined by absorbance at 490 mμ using phenolsulfuric acid (Dubois *et al.*, 1956). Shaded area denotes tubes showing hemagglutinating activity.

dramurthy, and I. E. Liener, 1966, unpublished data). The complete absence of carbohydrate in con A which had been purified by the procedure described here clearly distinguishes this phytohemagglutinin from others which have thus far been characterized.

The $s_{20,w}$ value of the various preparations of con A used in these studies was found to be 3.8 S when the protein was dissolved in 1.15 M NaCl, pH 5.5. In 0.1 M phosphate buffer, pH 7.0, however, the $s_{20,w}$ is considerably higher, 6.0 S, a value which agrees with that reported earlier by Sumner *et al.* (1938). The molecular weight calculated from sedimentation equilibrium experiments performed in the phosphate pH 7 buffer was 71,000 which is much lower than the value of 96,000 obtained by Sumner. The reason for this discrepancy in molecular weights is not obvious unless it is related to the fact that Sumner's data were obtained with only one concentration of protein. Our own sedimentation equilibrium data indicate that the molecular weight of con A is concentration dependent. In any event, it would appear that at pH 7 con A most likely exists as molecular species having a sedimentation coefficient of 6 S and a molecular weight of 71,000. Under somewhat different conditions of salt concentration and pH, dissociation into smaller subunits having a sedimentation coefficient of 3.8 S may take place. In experiments to be reported elsewhere, it was noted that the apparent molecular weight of con A in 8 M urea is 42,000, indicating that urea is also instrumental in causing molecular disaggregation.

The most striking feature of the amino acid composition of con A is the absence of half-cystine residues derived from disulfide bonds or thiol groups. A low content of cystine seems to be a characteristic feature of the amino acids which make up the structure of phytohemagglutinins (Rigas and Johnson, 1964; Huprikar and Sohonie, 1965; T. Takahashi, P. Ramachandramurthy, and I. E. Liener, 1966, unpublished observations).

The fact that almost 1.5 moles of DNP-alanine could be detected/71,000 g of protein strongly suggests the presence of two polypeptide chains in the molecule. This concept is supported by experiments which indicate that con A, in the presence of 8 M urea, can be dissociated into two nonidentical subunits, each of which has an N-terminal alanine residue. These studies will be the subject of a future communication.

Virtually nothing is known regarding the nature of the binding forces involved in the interaction of con A with various polysaccharides, although it has been suggested that this reaction is analogous to an antigen–antibody reaction (Goldstein *et al.*, 1965a). Quantitative aspects of this interaction as reported here show that glycogen and con A form a complex in which the carbohydrate and protein are present in a weight ratio of approximately 2:1. Since estimates for the molecular weight of glycogen vary from 270,000 to several million (White *et al.*, 1964), whereas the molecular weight of con A is under 100,000, it is obvious that the number of molecules of con A involved in the formation of this complex far exceeds the number of molecules of glycogen. This means that the glycogen molecule must possess a large number of binding sites capable of interacting with relatively few receptor sites on the protein molecule. Goldstein *et al.* (1965b) believe that the interaction of con A with polysaccharides involves the nonreducing ends of the chain of the latter. The large number of such nonreducing ends in a highly branched molecule such as glycogen would provide sufficient binding sites to accommodate a great many con A molecules. The number of receptor sites on the con A molecule is still unknown, but, if a three-dimensional lattice is indeed involved in the formation of a precipitate between con A and polysaccharides, there must be at least two such sites in each molecule of con A.

The observation that the glycogen–con A complex can be dissociated under acid (pH < 4) and alkaline conditions (pH > 9) would suggest the participation of ionizing groups at the receptor site of the protein molecule. Since the polysaccharides with which con A can combine need not possess any charged group, an electrostatic interaction between these two reactants does not seem likely. What appears to be a more reasonable explanation would be that the effect of pH is to alter the charge distribution of the protein molecule to the point where a drastic conformational change takes place which in turn renders the binding sites of con A less complementary to the polysaccharide. This conformational change appears to be reversible since the con A which had been released from the complex under acid conditions was fully active upon recovery from SE-Sephadex (Figure 5).

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