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Isolation and Characterization of a Phytohemagglutinin from the Lentil*

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ABSTRACT: A hemagglutinin from the common lentil has been isolated from crude extracts by precipitation in slightly acidified water followed by either DEAE-cellulose chromatography or elution from a Sephadex G-100 column with 0.1 M D-glucose. The hemagglutinin thus prepared exhibited a single precipitin arc on immunoelectrophoresis and a single symmetrical peak on ultracentrifugation. The sedimentation coefficient did not vary appreciably over a range of concentration from 2 to 8 mg per ml, and the $s^0_{20,w}$ = 4.0 S. Despite the homogeneity indicated by the above criteria, *Lens culinaris* hemagglutinin consistently migrated as a smear upon prolonged electrophoresis on cellulose acetate at pH 8.6. Red cells of different animal species were agglutinated to specific extents by the hemagglutinin; some strongly, others moderately, still others not at all. A saccharide binding site was implicated in the agglutination reaction by the following observations: (a) several soluble saccharides inhibited agglutination to various degrees; (b) D-glucose, a good inhibitor of agglutination, could protect the hemagglutinin against heat denaturation; (c) the hemagglutinin adhered to columns of dextran gels in which the pore size was such that the agglutinin could reach the interior of the gel; (d) the hemagglutinin could be eluted from such gels with D-glucose but not with buffer alone.

Hemagglutinins are proteins which combine with red cell surface structures causing visible clumping. Although the most familiar ones are antibodies (*e.g.*, human blood-group isoagglutinins), there are a large number whose occurrence does not appear to be immunologically induced. Such "naturally occurring" hemagglutinins have been noted in the *Limulus* crab (Marchalonis and Edelman, 1968a) and the lamprey eel, *Petromyzon* (Marchalonis and Edelman, 1968b). Many hemagglutinins are found in plants and are known as phytohemagglutinins or lectins (Boyd, 1954). Structural studies have been performed on only a few phytohemagglutinins (Agrawal and Goldstein, 1968; Olson and Liener, 1967b; Rigas *et al.*, 1966) and little is known about their *in vivo* function or about the nature of the hemagglutinin–erythrocyte bond(s). A number of phytohemagglutinins appear to combine specifically with saccharide-containing structures of the red cell surface (Mäkelä, 1957). It has been suggested that phytohemagglutinins react with their receptor molecules in a manner similar to antibodies (So and Goldstein, 1967). It has also been suggested (Agrawal *et al.*, 1968) that some lectins may serve as ideal models for studying protein–carbohydrate interactions because, unlike antibodies, phytohemagglutinins can be readily obtained in a homogeneous state.

The common lentil, *Lens culinaris* or *Lens esculenta*, was first reported to contain a hemagglutinin, *L. culinaris* hemagglutinin, by Landsteiner and Raubitschek (1908). *L. culinaris* hemagglutinin has been termed an "unspecific" phytohemagglutinin because it agglutinated red cells of all human blood groups equally well (Mäkelä, 1957). The receptor site(s) for *L. culinaris* hemagglutinin probably contained a saccharide moiety since a number of sugars (D-glucose, D-mannose, D-fructose, etc.) specifically interfered with hemagglutination. These sugars fell into the group III saccharides of Mäkelä (1957).

Because *L. culinaris* hemagglutinin was easily obtained from a common source and reacted with simple sugars, it appeared to be a useful material for studying the structure and

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binding involved in protein–polysaccharide interactions. The present paper deals with the purification and properties of the *L. culinaris* hemagglutinin molecule as well as some studies on the reaction of *L. culinaris* hemagglutinin with D-glucose and D-glucose-containing polysaccharides.

Materials and Methods

Purification of L. culinaris Hemagglutinin. Dried lentils (purchased from local food stores) were soaked overnight in distilled H_2O, drained, and homogenized with an equal weight of phosphate-buffered saline[1] in a Waring blender. Seed coats were removed by filtration through cheesecloth and the insoluble residue was centrifuged away at 12,000*g* for 15 min. The supernatant (crude *L. culinaris* hemagglutinin) usually had a titer of 1–32 (with human red cells) and a titer to A_{280} ratio of 3.5. Crude *L. culinaris* hemagglutinin was dialyzed against deionized H_2O (pH 5.8) and most *L. culinaris* hemagglutinin activity precipitated. The precipitate was centrifuged for 15 min at 12,000*g* and the supernatant was discarded. After washing and resuspension in H_2O, the precipitate was lyophilized and stored at 4°. The solid (partially purified *L. culinaris* hemagglutinin) was dissolved in pH 7.5, 0.015 M phosphate buffer and applied to a DEAE-cellulose column (Selectacel type 20, lot no. 1933, Schleicher & Schuell Co., Keene, N. H.) equilibrated against the same buffer at room temperature. Purified *L. culinaris* hemagglutinin came through unretarded and, within the limits of the titer assay, little (if any) activity was lost in this step. After ultrafiltration a typical preparation had a titer to A_{280} ratio of 50 to 100, a purification of 14–28-fold over crude *L. culinaris* hemagglutinin. Yields were 50 mg/kg of dry lentils. Purified preparations were stored frozen, in buffer, at −20°.

Red Cell Ghosts. Red cell membranes were prepared by the method of Dodge *et al.* (1963).

Sugars, Polysaccharides, and Molecular Sieve Gels. Simple sugars were obtained from various commercial suppliers in the highest purity available. Various dextrans and a phosphomannan were kindly provided by Dr. Allene Jeanes (USDA, Peoria, Ill.). Sephadex preparations (G-25, G-50, and G-100) were purchased from Pharmacia Fine Chemicals. The polyacrilamide resin, Bio-Gel P-100, was purchased from Calbiochem.

Concanavalin A. Concanavalin A, purified by the method of Agrawal and Goldstein (1967), was kindly provided by Dr. Myron A. Leon (St. Luke's Hospital, Cleveland).

Protein Concentration. Protein concentration was measured by ultraviolet absorption at 280 mμ using $E^{mg/ml}_{1.0\,cm}$ 1.0 for crude preparations and $E^{mg/ml}_{1.0\,cm}$ 1.25 for purified *L. culinaris* hemagglutinin. The latter value was obtained by measuring A_{280} and nitrogen content of several *L. culinaris* hemagglutinin solutions. Nitrogen analysis was performed by a modified Nessler titration (Koch and McMeekin, 1924). To convert nitrogen into protein concentration a value of 16.6% was taken for *L. culinaris* hemagglutinin. This value was calculated from *L. culinaris* hemagglutinin composition analysis (I. K. McGregor and H. J. Sage, 1969, unpublished data).

Estimation of Molecular Weight. An approximate molecular weight of *L. culinaris* hemagglutinin was determined by comparing the rate of passage of *L. culinaris* hemagglutinin activity through a column of Bio-Gel P-100 (88 × 2.5 cm diameter) to the rates of passage of horseradish peroxidase (mol wt 40,000) and bovine plasma albumin (mol wt 68,000) according to the method of Whitaker (1963). In separate experiments crude *L. culinaris* hemagglutinin (200 mg in 2 ml of phosphate-buffered saline), peroxidase (Worthington Biochemical Corp., 2 ml of an A_{280} 0.536 solution in phosphate-buffered saline), and plasma albumin (Armour, 20 mg in 2 ml of phosphate-buffered saline) were applied to the column equilibrated with phosphate-buffered saline. The proteins were percolated through the column with phosphate-buffered saline (3 ml/min) at room temperature and 1-ml samples were collected and assayed. *L. culinaris* hemagglutinin activity was assayed by the method of Sage and Vazquez (1967), peroxidase activity by a modification of the method of Worthington Biochemical Corp. (1965), and plasma albumin by ultraviolet absorption, A_{280}. Blue Dextran 2000, used to determine the free volume of the column, was measured by colorimetric assay, A_{640}.

L. culinaris Hemagglutinin Titer and Inhibition Assays. *L. culinaris* hemagglutinin activity and inhibition by saccharides were measured with 5% suspensions of various human and animal red cells by the method of Sage and Vazquez (1967).

Immunodiffusion and Immunoelectrophoresis. Ouchterlony double-diffusion and immunoelectrophoresis were done on microscope slides in agar using the LKB Immunophor Model 6800 A (LKB, Stockholm, Sweden) according to standard LKB procedures. Rabbit antisera prepared against crude *L. culinaris* hemagglutinin and against purified *L. culinaris* hemagglutinin were used in these studies. For antisera to crude *L. culinaris* hemagglutinin rabbits were immunized with four courses of 20 mg each in Freund's complete adjuvant. The first course was given half in the toepads, half subcutaneously, and subsequent courses were given subcutaneously. Bleedings were taken 14 days after the first course and 7 days after the subsequent courses. No qualitative differences were noted in immunoelectrophoretic patterns obtained with the sera from any of the four courses. For antisera to purified *L. culinaris* hemagglutinin, rabbits were immunized with a single course of 2 mg of purified *L. culinaris* hemagglutinin given in Freund's complete adjuvant, half in the toepads and half subcutaneously. Bleedings were taken 10, 24, and 33 days after injection. No antibodies were evident in the 10-day sera, but single precipitin lines were seen in Ouchterlony and immunoelectrophoretic analyses of the 24- and 33-day sera.

Cellulose Acetate Electrophoresis. Using a Millipore "Phoroslide" apparatus, electrophoresis of purified *L. culinaris* hemagglutinin was performed in pH 8.6 barbital buffer at 100 V for 45 min. The slides were stained with ponceau S.

Ultracentrifugation. Sedimentation velocity analysis was done on a Spinco Model E ultracentrifuge at 25° either at 59,780 rpm in phosphate-buffered saline or at 56,100 rpm in pH 8.6 barbital buffer.

Heat Denaturation of L. culinaris Hemagglutinin and Protection by Simple Sugars. Solutions of either crude or partially purified *L. culinaris* hemagglutinin with a titer of 1–128 for rabbit red cells were prepared in phosphate-buffered saline.

[1] Phosphate-buffered saline is 0.075 M NaCl–0.075 M phosphate (pH 7.3).

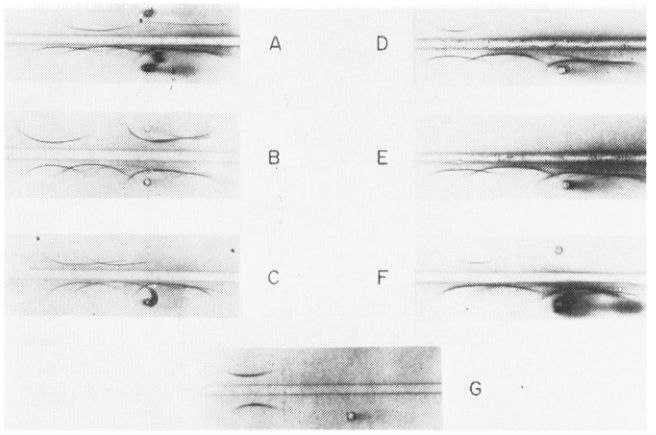

FIGURE 1: Immunoelectrophoretic patterns of *L. culinaris* hemagglutinin purification. All bottom wells: crude *L. culinaris* hemagglutinin. Troughs A–F: antiserum to crude *L. culinaris* hemagglutinin; trough G: antiserum to purified *L. culinaris* hemagglutinin. Top wells: (A) crude *L. culinaris* hemagglutinin absorbed with human O^+ ghosts; (B) partially purified *L. culinaris* hemagglutinin; (C) crude *L. culinaris* hemagglutinin after elution from DEAE-cellulose column; (D) purified *L. culinaris* hemagglutinin; (E) purified *L. culinaris* hemagglutinin after absorption with human O^+ ghosts; (F) purified *L. culinaris* hemagglutinin eluted from the ghosts with 1 M glucose; and (G) purified *L. culinaris* hemagglutinin. Cathodes are to the left.

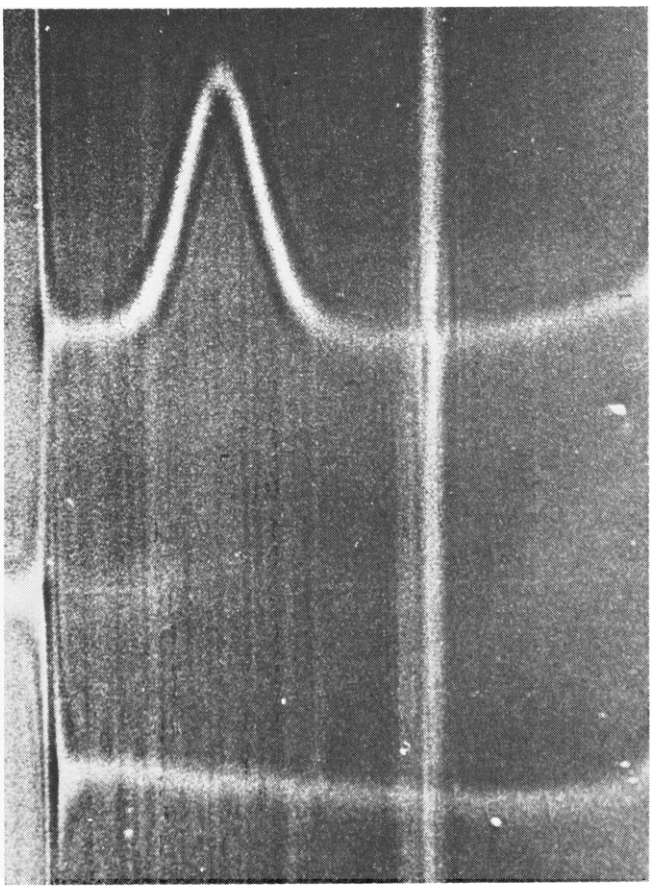

FIGURE 2: Ultracentrifuge patterns of purified and absorbed *L. culinaris* hemagglutinin in phosphate-buffered saline at 59,780 rpm, 25°. Upper pattern: purified *L. culinaris* hemagglutinin (7.0 mg/ml). Lower pattern: purified *L. culinaris* hemagglutinin after absorption with human O^+ ghosts. The photograph was taken 56 min after top speed has been attained.

Samples (4.0 ml) were prepared and various concentrations of test sugars were added. A control, containing no added sugar, was also included. Test and control samples were incubated at 72°. At given time intervals small aliquots were removed, rapidly chilled in an ice bath, and dialyzed against phosphate-buffered saline to remove sugars. The dialyzed *L. culinaris* hemagglutinin preparations were assayed for activity with rabbit red cells as above.

Results

Identification and Purification of L. culinaris Hemagglutinin. Crude *L. culinaris* hemagglutinin contained a number of immunogenic components as shown by immunoelectrophoresis (Figure 1A, bottom). Repeated absorption of crude *L. culinaris* hemagglutinin with human red cell ghosts removed *L. culinaris* hemagglutinin activity and immunoelectrophoresis of the absorbed preparation demonstrated the absence of a single immunophoretic component (Figure 1A, top). The line removed corresponded to that component of crude *L. culinaris* hemagglutinin which moved most rapidly toward the cathode at pH 8.6. The antisera used in this experiment contained antibodies to *L. culinaris* hemagglutinin as demonstrated by removal of *L. culinaris* hemagglutinin activity from the crude extract with immune precipitation. Normal rabbit sera could not remove *L. culinaris* hemagglutinin activity. Dialysis of crude *L. culinaris* hemagglutinin against pH 5.8 H_2O precipitated most of the *L. culinaris* hemagglutinin activity and removed several water-soluble immunophoretic components from the activity-containing fraction (Figure 1B, top). The most significant of these soluble materials was a component moving slightly to the anode side of the suspected *L. culinaris* hemagglutinin component. The solubility of *L. culinaris* hemagglutinin activity was a function of pH. At low ionic strengths *L. culinaris* hemagglutinin was insoluble at pH values below 6.0. At pH 7.5 *L. culinaris* hemagglutinin was soluble under similar conditions. When crude *L. culinaris* hemagglutinin was passed through a DEAE-cellulose column, only two components did not adhere to the column: the component corresponding to suspected *L. culinaris* hemagglutinin and the slightly less basic component removed by dialysis against H_2O (Figure 1C, top). A combination of these two procedures, dialysis and DEAE-cellulose chromatography, yielded purified *L. culinaris* hemagglutinin (Figure 1D, top). When purified *L. culinaris* hemagglutinin was absorbed with red cell ghosts, the single immunophoretic component was removed (Figure 1E, top) along with all activity. The ghosts onto which *L. culinaris* hemagglutinin had been absorbed were washed with phosphate-buffered saline and incubated with 1 M D-glucose. The resultant eluate showed the single immunophoretic component seen in purified *L. culinaris* hemagglutinin (Figure 1F, top). When either crude or purified *L. culinaris* hemagglutinin was examined by immunoelectrophoresis using antisera prepared against purified *L. culinaris* hemagglutinin, a single immunoelectrophoretic component was seen (Figure 1G).

Purified *L. culinaris* hemagglutinin showed a single sedimenting component in the ultracentrifuge (Figure 2, top). Repeated absorption of this preparation with packed human

TABLE I: Molecular Weight Estimation of *L. culinaris* Hemagglutinin by Rate of Passage through a Bio-Gel P-100 Column.

Compound	V_e/V_0[a]	Mol Wt
Dextran 2000	1.000	2,000,000
Bovine plasma albumin	1.133	68,000
Horseradish peroxidase	2.622	40,200
L. culinaris hemagglutinin	2.356	44,050[b]

[a] V_e is the elution volume of the given compound; V_0 is the elution volume of Dextran 2000. [b] Calculated from the data given.

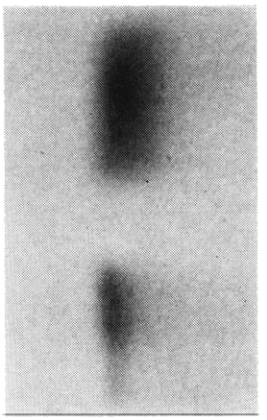

FIGURE 3: Cellulose acetate electrophoresis of hemagglutinins. Top pattern: purified *L. culinaris* hemagglutinin. Bottom pattern: purified concanavalin A.

O^+ red cell ghosts removed all *L. culinaris* hemagglutinin activity and all of the sedimenting material, resulting in a flat base-line pattern (Figure 2, bottom). The single peak was observed at *L. culinaris* hemagglutinin concentrations of 2–8 mg/ml in phosphate-buffered saline at 25°, and the $s_{20,w}$ of 4.0 S was practically independent of protein concentration.

Although the above experiments indicated a homogeneous *L. culinaris* hemagglutinin preparation, cellulose acetate electrophoresis yielded a broad band (Figure 3, top). The entire band was removed by absorption with red cell ghosts. *L. culinaris* hemagglutinin activity could be eluted directly off the strips and was associated only with the broad band. In order to determine whether the broad band was due to a specific binding of *L. culinaris* hemagglutinin to the cellulose acetate support, the same experiment was done in the presence of 0.1 M D-glucose. The resultant pattern was identical with that obtained without D-glucose. Another purified saccharide binding hemagglutinin, concanavalin A, was run simultaneously with *L. culinaris* hemagglutinin. Concanavalin A showed a similar mobility to *L. culinaris* hemagglutinin, and although concanavalin A exhibited a small amount of trailing, the smear appearance noted with *L. culinaris* hemagglutinin was not evident (Figure 3, bottom). Purified *L. culinaris* hemagglutinin eluted from red cell ghosts also exhibited the same broad band. In order to test whether the broad band was due to an effect of the buffer (pH 8.6 barbital), such as dissociation of *L. culinaris* hemagglutinin, an ultracentrifuge run was performed on *L. culinaris* hemagglutinin in this buffer. The resultant pattern was not distinguishable from *L. culinaris* hemagglutinin in phosphate-buffered saline.

In addition to the standard variety of lentil (yellow-brown), a lentil common to India was tested for the presence of a hemagglutinin and its identity with *L. culinaris* hemagglutinin. This variety, called "*Massur dhal*" (bright orange), was obtained from Kalustyan Importers (New York, N. Y.). It contained a phytohemagglutinin which was indistinguishable from *L. culinaris* hemagglutinin by immunoelectrophoresis.

Molecular Weight Estimation. L. culinaris hemagglutinin activity, peroxidase activity, and plasma albumin were all retarded on the molecular sieve Bio-Gel P-100 (Table I). The rate of passage of *L. culinaris* hemagglutinin activity was slightly faster than peroxidase (mol wt 40,000) and considerably slower than plasma albumin (mol wt 68,000). Using the method of Whitaker (1963) an estimate of 44,000 was made for the molecular weight of *L. culinaris* hemagglutinin.

Species Specificity of L. culinaris Hemagglutinin. The extent to which partially purified *L. culinaris* hemagglutinin was able to agglutinate red cells from various animal species varied widely (Table II). A preparation which had a titer of 1–16 with human cells gave a titer of 1–512 with horse cells. In contrast, a concentrated solution of *L. culinaris* hemagglutinin having a titer of 1–64 with human red cells did not agglutinate sheep or goat erythrocytes. The majority of rabbits tested had erythrocytes which were four times more agglutinable than human cells. However, one individual of those tested had red cells which were consistently 64 times more agglutinable. All reactivity toward hog and horse erythrocytes could be removed by absorption with human B^- ghosts, indicating that a single *L. culinaris* hemagglutinin activity was involved in agglutination of the various species.

Saccharide Specificity. The reaction of *L. culinaris* hemagglutinin with red cells was inhibited by a number of simple sugars (Figure 4). D-Mannose was the best inhibitor, while

TABLE II: Agglutination of the Erythrocytes of Various Animal Species by Partially Purified *L. culinaris* Hemagglutinin.

Type of Cells	No. Animals Tested	Titer
Human O^+	2	1–16
Human A^+	2	1–16
Human B^-	1	1–16
Horse	3	1–512
Rabbit	24	1–128 or 1–64
Rabbit 39	1	1–1024
Hog	1	1–32
Sheep	6	0[a]
Goat	2	0[a]

[a] Sheep and goat cells were tested against a solution of partially purified *L. culinaris* hemagglutinin which had a titer of 1–64 with human cells.

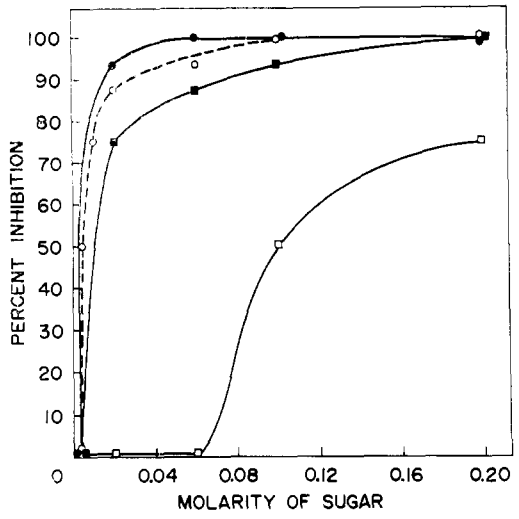

FIGURE 4: Inhibition of *L. culinaris* hemagglutinin by sugars. Galactose (□), mannose (●), fructose (■), and sucrose (○). A similar pattern to that of fructose was obtained with sorbose and glucose; a similar pattern to that of sucrose was obtained with maltose.

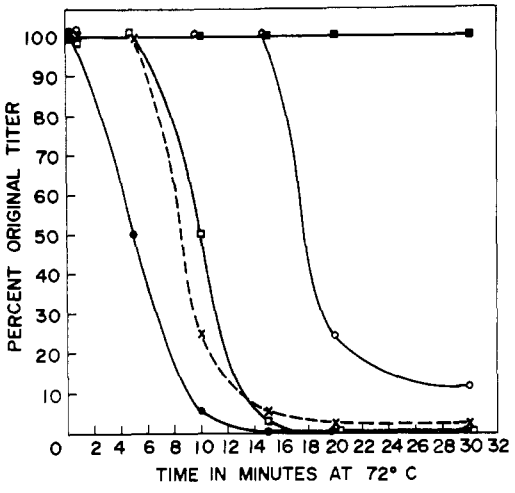

FIGURE 5: Protection against the heat denaturation of *L. culinaris* hemagglutinin by sugars. The "original titer" is the titer which each solution had at 25° and was identical in each case with the zero-time titer measured immediately after the solution reached 72°. Control containing no sugar (●), 0.005 M glucose (□), 0.10 M galactose (×), 0.05 M glucose (○), and 0.10 M glucose (■). A pattern similar to that of the control was obtained with 0.001 M glucose; a pattern similar to that of 0.005 M glucose was obtained with 0.010 M glucose.

D-galactose inhibited poorly. No inhibition was observed with 18-mg/ml concentrations of 14 different dextrans and one phosphomannan. Similar concentrations of Sephadex G-50 or G-100 did not inhibit the *L. culinaris* hemagglutinin red cell reaction. However, *L. culinaris* hemagglutinin activity was absorbed to Sephadex G-50 and G-100 columns during chromatography of partially purified *L. culinaris* hemagglutinin and was eluted with 0.1 M D-glucose. The eluted material was indistinguishable from purified *L. culinaris* hemagglutinin by immunoelectrophoresis. Olson and Liener (1967a) and Agrawal and Goldstein (1965) have observed binding of concanavalin A to various Sephadex gels. The latter authors eluted concanavalin A with glucose as a purification procedure. This procedure could be used to obtain purified *L. culinaris* hemagglutinin from partially purified *L. culinaris* hemagglutinin since none of the other components adhered to Sephadex G-50 and G-100. In sharp contrast Sephadex G-25 had no observable affinity for *L. culinaris* hemagglutinin activity.

Studies were performed on the heat denaturation of *L. culinaris* hemagglutinin in the presence and absence of two sugars. *L. culinaris* hemagglutinin showed heat stability to temperatures as high as 65°, but at 75° all activity was lost in 30 min. Experiments were performed on the heat denaturation of *L. culinaris* hemagglutinin at 72° in the presence of D-glucose (an effective *L. culinaris* hemagglutinin inhibitor) and D-galactose (a poor inhibitor). Figure 5 shows that 0.1 M D-glucose completely protected *L. culinaris* hemagglutinin from heat denaturation over the time studied while 0.1 M D-galactose was only slightly protective. Incubation with only 0.005 M D-glucose gave appreciable protection of *L. culinaris* hemagglutinin.

Discussion

L. culinaris hemagglutinin, prepared by the procedure outlined above, appeared homogeneous by three different criteria: ultracentrifugally, immunophoretically, and immunogenically. In each case the single component could be demonstrated to be active material and identical with *L. culinaris* hemagglutinin eluted from red cell membranes. The immunogenic test (the production of a monospecific antiserum by immunization with purified *L. culinaris* hemagglutinin) was the weakest evidence because only a single immunization course was given. The results with cellulose acetate electrophoresis suggest that *L. culinaris* hemagglutinin may contain more than one component. If this is so, then these components would have to be very similar in size and composition. *L. culinaris* hemagglutinin was a rather basic protein by immunoelectrophoresis. The sedimentation constant $s_{20,w}$ was essentially independent of protein concentration from 2 to 8 mg per ml. These data, together with a molecular weight of approximately 44,000, indicate that *L. culinaris* hemagglutinin is a globular nondissociating molecule in phosphate-buffered saline at 25°.

L. culinaris hemagglutinin could not distinguish among human blood types although it had a distinct species specificity. However, it appears that *L. culinaris* hemagglutinin could distinguish among a rabbit population. Landsteiner and Raubitschek (1908) found rabbit red cells to be 100 times more agglutinable than human erythrocytes. On the other hand, Mäkelä (1957) found rabbit cells to be four times more agglutinable than human erythrocytes. In our experiments 24 rabbits gave titers which were four to eight times more agglutinable and one which was 64 times more agglutinable. Horse red cells were strongly agglutinated by *L. culinaris* hemagglutinin. The present report is in agreement with Landsteiner and Raubitschek (1908) and stands in contrast to the observation of Bird (1954) that *L. culinaris* hemagglutinin did not agglutinate horse red cells appreciably.

Although it is premature to suggest that *L. culinaris* hemagglutinin could distinguish among rabbit blood types, these observations are consistent with such a possibility

Blood group differences might also explain the diverse observations on the agglutinability of horse cells.

L. culinaris hemagglutinin had a specificity for some saccharide-containing moiety. Simple sugars reacted with *L. culinaris* hemagglutinin as indicated by (a) the inhibition of the *L. culinaris* hemagglutinin–erythrocyte reaction, (b) elution of *L. culinaris* hemagglutinin from red cell membranes and from Sephadex G-50 or G-100 columns with D-glucose, and (c) protection of *L. culinaris* hemagglutinin from heat denaturation by D-glucose. Concanavalin A has been shown to precipitate with glycogen (Olson and Liener, 1967a), dextrans (Agrawal and Goldstein, 1967), and the macroglobulins of human serum (Leon, 1967). In contrast *L. culinaris* hemagglutinin did not react visibly with a number of dextrans inculding some primarily 1–6-linked dextrans, primarily 1–4-linked dextrans, and highly branched dextrans. In addition none of these dextrans inhibited the *L. culinaris* hemagglutinin–erythrocyte reaction appreciably at a concentration of 18 mg/ml. Similar concentrations of Sephadex G-50 and G-100 did not interfere with the *L. culinaris* hemagglutinin–erythrocyte reaction. However, columns of G-50 and G-100 absorbed *L. culinaris* hemagglutinin specifically, suggesting that the capacity of G-50, G-100, and perhaps the other dextrans tested was very low.

The nonreactivity of *L. culinaris* hemagglutinin toward Sephadex G-25 as contrasted with its absorption to G-50 and G-100 can be explained by the following. (a) There is a greater percentage of chemical cross-links in G-25. A resultant smaller number of sugar residues with free 3- and 4-hydroxyl positions may result in a much lower capacity of G-25 for *L. culinaris* hemagglutinin. (b) *L. culinaris* hemagglutinin required either a certain steric arrangement of glucose residues or an environment present on the inside of a Sephadex particle. Therefore, only a gel which permitted the entry of *L. culinaris* hemagglutinin could absorb *L. culinaris* hemagglutinin. Sephadex G-50 and G-100 could permit the entry of a particle of mol wt 44,000 while G-25 could not.

Although concanavalin A and *L. culinaris* hemagglutinin were inhibited by similar sugars, the differences in their reactivity with dextrans suggest that either their specificities or their strength of binding were different.

The prime goal of our investigation of *L. culinaris* hemagglutinin is examination of the mechanism of the lectin–erythrocyte reaction and comparison of the antibody–antigen reaction. This includes a study on the structure and number of binding sites of phytoagglutinins. Recently structural studies have been done on concanavalin A (Agrawal and Goldstein, 1968; Olson and Liener, 1967b), *Phaseolus vulgaris* hemagglutinin (Rigas *et al.*, 1966), and *Agaricus campestris* hemagglutinin (H. J. Sage and S. L. Connett, 1969, unpublished data; and H. J. Sage, 1969, unpublished data). These studies have demonstrated noncovalently bonded subunit structures. So and Goldstein (1968) have reported two binding sites for concanavalin A. The present studies on *L. culinaris* hemagglutinin provide a basis for the determination of the subunit structure and the number of binding sites of the *L. culinaris* hemagglutinin molecule.

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