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Equilibrium Dialysis Studies on Two Lima Bean Lectins

In 1945 W. C. Boyd (1) first described the presence of a type A blood group-specific hemagglutinating principle in the lima bean (*Phaseolus lunatus*). Subsequently, two groups (2–5) working independently resolved the lima bean hemagglutinating activity into two molecular species: a low-molecular-weight component of M_r 124,400 [LBL III, Refs. 2–5] and a high-molecular-weight component of M_r 247,100 (LBL II, Refs. 2–5). Upon Na dodecylsulfate–polyacrylamide electrophoresis in the presence of dithiothreitol or mercaptoethanol, both lectins afforded a single protein band of M_r 31,000. These data suggested that lima bean lectins III and II were composed of 4 and 8 subunits, respectively.

The development of a new isolation procedure (6) involving successive affinity chromatography on concanavalin A–Sepharose and gel filtration chromatography on Sephadex G-200 superfine makes it possible to prepare 100-mg quantities of the two lima bean lectins. In conjunction with a study of the biologic activity of these lectins, we have begun a detailed study of the physical and chemical properties of the two proteins. In this communication we report on the number of combining sites of the two lima bean phytohemagglutinins.

Finely ground Sieva lima beans *P. lunatus* (500 g, W. Atlee Burpee Co., Clinton, IA) were stirred for 1 hr at 2°C with 4 liters of 0.1 M phosphate buffer, pH 6.8. The suspension was filtered through cheesecloth and centrifuged in a Sorvall RC-2 refrigerated centrifuge for 1 hr at 12,000*g*. To the supernatant was added $(NH_4)_2SO_4$ (197 g/liter) with gentle stirring and the pH adjusted to 4.4 with 2 N HCl. After standing 2 hr at 2°C, the precipitate was centrifuged (12,000*g*, 1 hr) and discarded. The pH of the supernatant was adjusted to 7.0 with 50% NaOH and $(NH_4)_2SO_4$ (90 g/liter) was added. After standing overnight at 2°C, the precipitate was dissolved with stirring in the minimum volume (ca. 100 ml) of phosphate-buffered saline (0.1 M phosphate buffer in 0.15 M NaCl, pH 6.8, containing 0.1 mM each of $CaCl_2$, $MgCl_2$, and $MnCl_2$). Insoluble residue was centrifuged and the supernatant solution chromatographed on a concanavalin A-Sepharose column followed by a Sephadex G-200 superfine column to resolve components II and III as described previously (6).

^{14}C-labeled methyl-2-acetamido-2-deoxy α-D-galactopyranoside was prepared by T. Adamson of this university by reaction of [1-^{14}C]acetic anhydride (Amersham) with methyl-2-amino-2-deoxy α-D-galactopyranoside according to Roseman and Ludwieg [7]. Conditions for equilibrium dialysis as described by Kharush (8) are in the legend of Fig. 1. Samples (50 μl) were mixed with 20 ml scintillation fluid (six parts Triton X-100 to seven parts toluene containing 3.8 g/liter Omnifluor) and counted in a Packard Tricarb Liquid Scintillation Spectrometer. Protein concentration was determined spectrophotometrically ($E^{1\%}_{1cm}$ = 12.3 at 280 nm; Ref. 2). Triton X-100 was a product of Research Products International Corp, Elk Village, IL. Omnifluor was purchased from New England Nuclear, Boston, MA.

Figure 1a and 1b show the results of the binding experiments of ^{14}C-labeled methyl-2-acetamido-2-deoxy-α-D-galactopyranoside to the two lima bean lectins, plotted according to the method of Scatchard (9). The ratio of the molar concentration of bound sugar to that of protein is represented by r; c is the molar concentration of free sugar, n the number of binding sites, and K the association constant. Molecular weights of 124,400 and 247,100, respectively, were employed in the calculations (5). For both lectins, essentially linear plots were obtained, with n equal to 2 for LBL III and to 4 for LBL II. The calculated binding constants were 1.01×10^3 liters/mole and 0.93×10^3 liters/mole respectively.

The equilibrium dialysis experiments described in this study provide an exact measure of the protein–ligand interactions of the two lima bean lectins, thus quantitating the results obtained previously by inhibition studies of the lima bean lectin–type A blood group substance interaction (15). Methyl-2-acetamido-2-deoxy-α-D-galactopyranoside was chosen as the binding ligand for this study because it is one of the most potent inhibitors of the lima bean lectin interaction examined (3, 5).

Sulfhydryl group titration and dissociation experiments indicated that the lima bean lectins are composed of dimers consisting of two subunits (M_R 31,000) joined together by a disulfide bond (4, 5). Each subunit within the dimer contains a single sulfhydryl group which is required for carbohydrate binding. Component III consists of two dimers and component II four dimers held together by noncovalent forces. The demonstration that LBL components III and II contain 2 and 4 carbohydrate-binding sites, respectively, suggests that a binding site is constituted of two disulfide-linked subunits. The lentil lectin (10) and the soy bean lectin (11) also have been reported to contain carbohydrate-binding sites composed of two subunits.

Both lima bean lectins have approximately the same association constants (1.01×10^3 liters/mole for LBL III, 0.93×10^3 liters/mole for LBL II) for

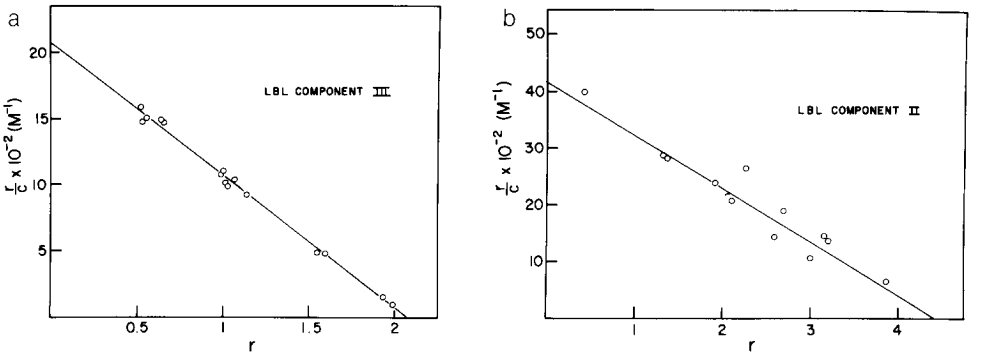

FIG. 1. (a) Scatchard plot of the binding of ^{14}C-labeled methyl-2-acetamido-2-deoxy-α-D-galactopyranoside to lima bean lectin component III (M_r 124,400) at 2°C and pH 6.8. Equilibrium dialysis was performed as described by Kharush (8) in phosphate-buffered saline at 2°C. Dialysis casing (Union Carbide Corporation, Chicago, IL) was boiled and washed exhaustively in distilled water prior to use. Dialysis cells (3 ml) were purchased from Bellco Glass, Inc., Vineland, NJ. Two milliliters of the lectin solution containing approximately 10 mg protein/ml were placed in one compartment of the cell; the other compartment was filled with 2 ml of the desired concentration of the sugar ligand. The cells were placed on a multipurpose rotator (Scientific Industries, Inc., Queens Village, NY) rotating at a rate of 5 rpm at 2°C. After equilibrium was reached (72 hr), samples (3 x 50 μl) were removed for counting. (b) Scatchard plot of the binding of ^{14}C-labeled methyl-2-acetamido-2-deoxy-α-D-galactopyranoside to lima bean lectin component II (M_r 247,100) at 2°C and pH 6.8. Experimental procedure was as described in the legend to Fig. 1a.

methyl-2-acetamido-2-deoxy-α-D-galactopyranoside. These values are one order of magnitude lower than that of concanavalin A [12, 13] for methyl α-D-glucopyranoside, soy bean lectin (11) for *N*-acetyl-D-glucosamine, and the isolectins from *Lotus tetragonolobus* for L-fucose (14), but are considerably higher than the association constants for the binding of methyl-α-D-glucopyranoside and D-mannose to the lentil lectin (10).

The fact that the association constants for both lima bean lectins are virtually identical suggests that the carbohydrate binding sites of LBL II (M_r 247,100) are not altered in their affinity for ligand from LBL III (M_r 124,400) when higher aggregates are formed.

Studies in progress (15) indicate LBL II is a rather potent mitogen toward human lymphocytes compared to LBL III, indicating that the valence of a lectin molecule may be an important parameter in relation to its activity as a mitogenic agent.

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