PHYTOHEMAGGLUTININ PURIFICATION: A GENERAL METHOD INVOLVING AFFINITY AND GEL CHROMATOGRAPHY

W. BESSLER and I.J. GOLDSTEIN
Department of Biological Chemistry, The University of Michigan, Ann Arbor, Mich. 48104, USA

Received 14 May 1973

1. Introduction

Proteins agglutinating erythrocytes and other types of cells have been studied since 1888 when Stillmark observed that extracts from *Ricinus communis* clumped red blood cells [1]. However, it is only since the experiments of Boyd [2, 3] in 1945 on the specificity of a large number of phytohemagglutinins (lectins) [4] that these seed proteins became a subject of general interest [5]. Research on lectins has increased markedly within the last few years after it was shown that they interact with normal and cancerous cells [6–9].

Because of the remarkable interest generated in the many unique biological properties of lectins and the large number of procedures required to purify individual phytohemagglutinins, we have tried to develop a more general method for their purification (cf. [10]). For this purpose, we have taken advantage of the demonstrated capacity of concanavalin A to interact with most phytohemagglutinins of the family *Leguminosae* [11] and of the high resolving power of long columns of Sephadex G-200 superfine gel. This communication describes the purification of four lectins employing essentially three purification steps: i) $(NH_4)_2SO_4$ fractionation of the crude extracts, ii) affinity chromatography on Con A-Sepharose and iii) chromatography on Sephadex G-200 superfine gel. The plant agglutinins investigated were from lima bean (*Phaseolus lunatus*), soy bean (*Glycine max*), wax bean (*Phaseolus vulgaris*) and *Bandeiraea simplicifolia*. Preliminary results on attempted purification of other lectins are also reported.

2. Materials and methods

Lima beans (5111 Carolina or Sieva Pole Lima Bean) were obtained from W. Atlee Burpee Co., Clinton, Iowa; defattened soy flakes were kindly supplied by Dr. W. Youngquist of Procter and Gamble Co. (Batch UKO-70 V-3216); wax beans were obtained from Dr. E. Liener, University of Minnesota, Minneapolis; seeds of *B. simplicifolia* were obtained from C.H. Samanen, University of Michigan. Additional studies were carried out with wheat germ agglutinin on a sample from Calbiochem, San Diego, Calif.; *Dolichos biflorus* agglutinin (kindly supplied by Dr. M. Etzler, University of California, Davis, Calif.); and concanavalin A (jack bean hemagglutinin; Calbiochem Lot. 210 073). Human red blood cells (types A and B) were kindly provided by Dr. H. Oberman, University of Michigan. All chemicals used in this study were of reagent grade or best quality available.

2.1. Preliminary purification of lectins

Partially purified lectins were prepared by $(NH_4)_2SO_4$ fractionation of the crude extracts as described by the various authors: lima bean lectin with modifications [12] according to Galbraith and Goldstein [13], soy bean lectin according to Pallansch and Liener [14], wax bean lectin according to Takahashi et al. [15] and *B. simplicifolia* according to Samanen and Goldstein [16].

2.2. Chromatographic methods

Affinity chromatography was carried out using Con A–Sepharose gel (Pharmacia Fine Chemicals, Lot

 North-Holland Publishing Company – Amsterdam

No. 5014) in glass columns containing 5–30 ml of gel, according to the amount of substance applied. The gels were equilibrated with 0.1 M phosphate buffer in 0.15 M NaCl, pH 6.8 (PBS) containing 0.1 mM each of $CaCl_2$, $MgCl_2$ and $MnCl_2$. The precipitate from the $(NH_4)_2SO_4$ fractionation was dissolved in the minimum volume of PBS required, centrifuged in a refrigerated Sorvall RC-2 centrifuge at 5000 *g* for 20 min to remove any turbidity, and applied to the affinity column. The column was washed thoroughly with PBS, and the bound substances were eluted with methyl α-D-mannopyranoside [17, 18] (Pfanstiehl Laboratories, Inc., Waukegan, Ill.; 10–50 mg/ml) and concentrated by vacuum dialysis.

For the gel chromatographic purification step we used columns (110 × 3.4 cm) of Sephadex G-200 superfine gel (Pharmacia Fine Chemicals, Lot No. 2885) and PBS as eluant.

2.3. Analytical procedures

2.3.1. Protein analyses

Protein in crude extracts was estimated by the biuret method [19]. The purified lectins were determined by absorbance at 279 or 280 nm, using for lima bean lectin $E^{1\%}_{1\,cm,\ 280\,nm} = 12.3$ [20], for soy bean lectin $E^{1\%}_{1\,cm,\ 279\,nm} = 15.7$ [14]; for wax bean lectin $E^{1\%}_{1\,cm,\ 280\,nm} = 12.0$ (determined by the biuret method), and for *B. simplicifolia* lectin $E^{1\%}_{1\,cm\ 280\,nm} = 14.1$ [16].

2.3.2. Electrophoresis

Polyacrylamide disc gel electrophoresis was performed as described by Reisfeld [21] using Coomassie Blue stain [22].

2.3.3. Hemagglutination assays

Hemagglutination assays were conducted by serial dilution of the lectins in phosphate buffered saline (0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.0). The activity was expressed as titer, the reciprocal of the greatest dilution at which agglutination occurred. Specific activity was defined as titer per mg protein per ml.

3. Results and discussion

3.1. Purification

Results on the purification of the four lectins of our study are summarized in table 1. It is apparent that a major purification step in all cases involves adsorption to the Con A–Sepharose column. The subsequent purification step for lima bean lectin, gel chromatography on Sephadex G-200 superfine gel, showed 2 peaks with hemagglutinating activity, the high molecular weight component having about four times the specific activity of the low molecular weight component. These results are in agreement with the results of Gould and Scheinberg [20] and Galbraith

Table 1
Lectin purification schemes.

	Crude extract	$(NH_4)_2SO_4$ ppt	Con A–Sepharose eluant	Sephadex G-200 superfine eluant		
Lectin	Specific activity[a]	Specific activity	Specific activity	Specific activity	Fold[b] purification	Yield[c] (%)
Lima bean	42	287	804	HMWC[d] 2534	60	21
				LMWC[d] 656	15.6	12
Soy bean	1.0	–	14.8	59	59	44
Wax bean	44.5	100	147	839	18.9	53
B. simplicifolia	7.2[e]	32[e]	70.6	109	15.1	29

[a] Specific activity: titer per mg protein per ml.
[b] Relative to the specific activity of the crude extract.
[c] Percent yield of recovered activity after entire purification process.
[d] High molecular weight component (component II) and low molecular weight component (component III) (see text) [13, 20, 23].
[e] According to Samanen and Goldstein [16].

and Goldstein [13, 23]. Sephadex G-200 superfine gel filtration of the soy bean lectin, wax bean lectin, and *B. simplicifolia* lectin showed in each case a single, symmetric peak containing all the hemagglutinating activity. Figs. 1 and 2 present the elution profiles of affinity and gel chromatography, respectively, for the lima bean lectin.

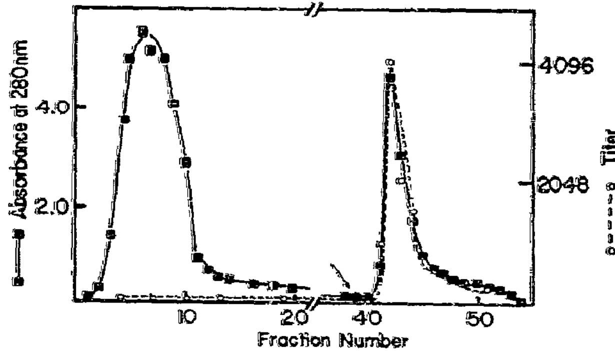

Fig. 1. Affinity chromatography of lima bean lectins. 50 ml solution as obtained by $(NH_4)_2SO_4$ fractionation (see text) and containing 8 mg protein per ml PBS was applied to a column (11 × 2.5 cm) of Con A–Sepharose. Flow rate: 100 ml/hr; 8 ml fractions. The column was eluted by a solution of methyl α-D-mannopyranoside (10 mg/ml) in PBS (arrow).

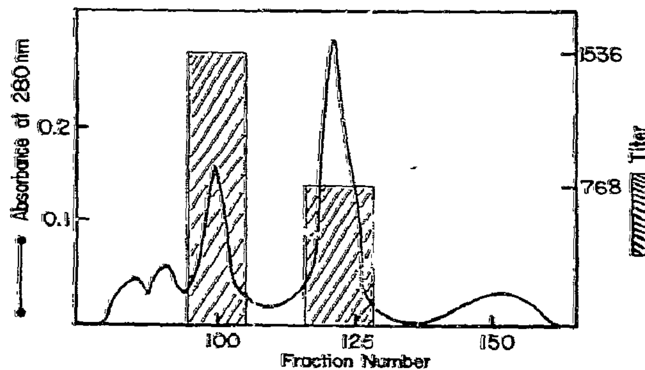

Fig. 2. Gel chromatography of lima bean lectins. 3.5 ml solution as obtained by affinity chromatography and containing 30 mg protein per ml PBS were applied to the column (110 × 3.4 cm) of Sephadex G-200 superfine. Flow rate: 0.5 ml/cm²/hr; 6 ml fractions. Maxima of hemagglutinating activity eluted at 600 ml and 720 ml. Hatched areas represent titer of pooled fractions.

3.2. Purity

The purity of each lectin was determined by disc gel electrophoresis at pH 4.3. The two lima bean lectin components, as well as the purified soy bean and *B. simplicifolia* lectins each gave a single band. Wax bean lectin, however, ran as a spectrum of several closely migrating bands indicating the presence of a series of components differing in charge (see fig. 3).

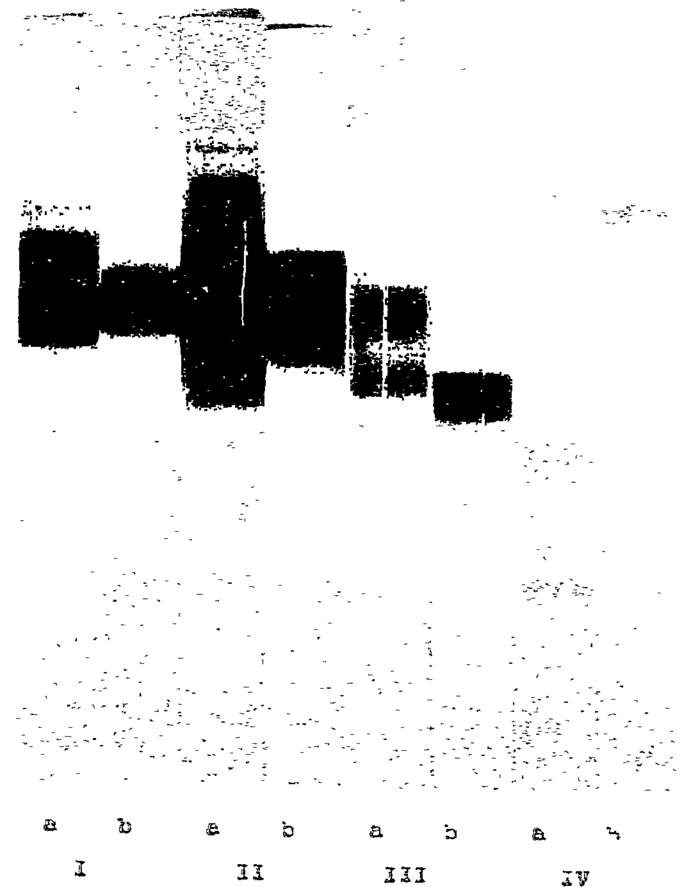

Fig. 3. Polyacrylamide gel electrophoresis at pH 4.3 on 3.75% gels. Current: 5 mA/gel. Stain: Coomassie Blue. I: Lima bean lectin after affinity chromatography (a) and after gel chromatography (b) (low molecular weight component). II: Wax bean lectin after affinity (a) and gel (b) chromatography. III: Soy bean lectin after affinity (a) and gel (b) chromatography. IV: *B. simplicifolia* lectin after affinity (a) and gel (b) chromatography.

3.3. Activity and yield

The activity of the lectins was measured by hemagglutination studies. Lima bean lectin, soy bean lectin and wax bean lectin exhibited specificity for type A red blood cells, whereas *B. simplicifolia* lectin was tested against type B red blood cells [16]. It can be seen (table 1), that in all cases approx. 30–60% of the original activity was recovered, the fold of purification ranging from about 15–60. These values correspond approximately to the results of other purification methods recorded in the literature.

3.4. Other lectins

Under the conditions described above, partially purified wheat germ lectin and concanavalin A were not adsorbed whereas the lectin from *Dolichos biflorous* was only partially bound to Con A–Sepharose gel.

3.5. Limitations of method

A critical step in the purification of the four lectins described above involves affinity chromatography on Con A–Sepharose. Any glycoprotein possessing multiple α-D-mannopyranosyl or α-D-glucopyranosyl end groups [11, 17, 18, 24] or internal 2-O-linked α-D-mannopyranosyl residues [25] will bind to concanavalin A. Many more lectins fulfilling these requirements could probably be enriched by this procedure. It should be noted that all glycoproteins with the structural features noted above will bind to the Con-A–Sepharose gel; it is the gel filtration step which allows isolation of the individual active phytohemagglutinin components in pure form.

Wheat germ agglutinin [26] and concanavalin A [27, 28] which are not glycoproteins are not bound to columns of Con A–Sepharose gel. The *Dolichos biflorous* lectin which is a glycoprotein and gives a precipitin curve with concanavalin A [29] is only partially retained by Con A–Sepharose gel.

In summary, we have developed a procedure for the isolation of plant lectins of glycoprotein nature which appears to be of rather general application.

Acknowledgements

This research was supported by NIH Research Grant AM-10171. We thank Tim Ross for excellent technical assistance.

References

[1] Stillmark, H. (1888) Über Rizin, ein giftiges Ferment aus dem Samen von *Ricinus communis* L. und einigen anderen Euphorbiaceen, Inaug. Dis., Dorpat.
[2] Boyd, W.C. (1962) Introduction to Immunochemical Specificity, pp. 66–70, Wiley and Sons, New York.
[3] Boyd, W.C. and Reguera, R.M.J. (1949) J. Immunol. 62, 333.
[4] Mäkelä, O. (1957) Ann. Med. Exp. Biol. Fenn. 35, suppl. 11.
[5] Sharon, N. and Lis, H. (1972) Science 177, 949.
[6] Aub, J.C., Tieslau, C. and Lankester, A. (1963) Proc. Natl. Acad. Sci. U.S. 50, 613.
[7] Burger, M.M. (1969) Proc. Natl. Acad. Sci. U.S. 62, 994.
[8] Inbar, M. and Sachs, L. (1969) Proc. Natl. Acad. Sci. U.S. 63, 1418.
[9] Sela, B.A., Lis, H., Sharon, N. and Sachs, L. (1970) J. Memb. Biol. 3, 267.
[10] Matsumoto, I. and Osawa, T. (1972) Biochem. Biophys. Res. Commun. 46, 1810.
[11] Goldstein, I.J., So, L.L., Yang, Y. and Callies, Q.C. (1969) J. Immunol. 103, 695.
[12] Bessler, W. and Goldstein, I.J., in preparation.
[13] Galbraith, W. and Goldstein, I.J. (1970) FEBS Letters 9, 197.
[14] Pallansch, M.J. and Liener, I.E. (1953) Arch. Biochem. Biophys. 45, 366.
[15] Takahashi, T., Ramachandramurthy, P. and Liener, I.E. (1967) Biochim. Biophys. Acta 133, 123.
[16] Samanen, C. Hayes and Goldstein I.J. (1973) Federation Proc. 32, abstract 4175.
[17] Goldstein I.J., Hollerman. C.E. and Smith, E.E. (1965) Biochemistry 4, 876.
[18] So, L.L. and Goldstein, I.J. (1967) J. Immunol. 99, 158.
[19] Gornall, A.G., Bardawill, C.W. and David, M.M. (1949) J. Biol. Chem. 177, 751.
[20] Gould, N.R. and Scheinberg, S.L. (1970) Arch. Biochem. Biophys. 137, 1.
[21] Reisfeld, R.A., Lewis, U.J. and Williams, D.E. (1962) Nature 195, 281.
[22] Chrambach, A., Reisfeld, R.A., Wyckoff, M. and Zaccari, J. (1967) Anal. Biochem. 20, 150.
[23] Galbraith, W. and Goldstein, I.J. (1972) Biochemistry 11, 3976.

[24] Goldstein I.J., Hollerman, C.E. and Merrick, J.M. (1965) Biochim. Biophys. Acta 97, 68.
[25] Goldstein, I.J., Reichert, C.M., Misaki, A. and Gorin, P.A.J. (1973) Biochim. Biophys. Acta, in press.
[26] Allen, A.K., Neuberger, A. and Sharon, N. (1973) Biochem. J. 131, 155.
[27] Agrawal, B.B.L. and Goldstein, I.J. (1967) Biochim. Biophys. Acta 133, 376.
[28] Olson, M. and Liener, I.E. (1967) Biochemistry 6, 105.
[29] Goldstein, I.J. and Etzler, M.E., unpublished results.