Isolation of Lectins of Different Specificities on a Single Affinity Adsorbent*

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SUMMARY

Affinity chromatography on Sepharose-fetuin columns was used in a single step procedure to isolate the lectins concanavalin A, Favin, phytohemagglutinin, wheat germ agglutinin, and Limulus hemagglutinin. New lectins with unknown binding specificities were also purified by the same procedure from extracts of small California white beans, Idaho red beans, and white pea beans. The purified lectins exhibited different cell surface mapping properties on erythrocytes, lymphocytes, and sperm cells. It was particularly striking that neither 125I-labeled concanavalin nor 125I-labeled wheat germ agglutinin had any effect on the binding of the other to mouse spleen cells. In accord with this observation, gel electrophoretic analysis of radiolabeled lymphocyte receptors for these two lectins yielded different patterns. These results indicate that highly purified lectins prepared by affinity chromatography on the same adsorbent can possess strikingly different binding specificities for cell surface receptors.

Lectins interact with specific carbohydrate structures on cell surfaces and are therefore particularly useful in studying alterations in the number, distribution, and mobility of cell surface receptors associated with the control of cell proliferation and with cell-cell interactions (1, 2). Although various multistep procedures exist for purifying different lectins, there is a need for a simple and general method for the rapid isolation of purified lectins of various binding specificities.

Because of the similarity of saccharide structures found in various serum glycoproteins such as fetuin and in cell surface receptors (3-5), carbohydrate-specific ligands such as lectins can potentially recognize similar monosaccharide units, oligosaccharide structures, or the entire saccharide complex on glycoproteins and on cell surfaces. This suggests that affinity columns containing Sepharose covalently coupled to a glycoprotein such as fetuin might be used as a single step procedure for the isolation of lectins that occur naturally in the absence of isolectins. A similar approach has been previously used to isolate the hemagglutinin receptor from influenza virus (6), carbohydrate-specific antibodies (7), as well as the wax bean agglutinin. In the present communication, we describe the use of Sepharose-fetuin for the purification of such lectins and an analysis of the specificities of these proteins. Despite the fact that the various lectins were fractionated by this method on a single affinity adsorbent, they exhibited different specificities in cell surface mapping experiments.

Sepharose 4B (50 ml) was activated with CNBr (8) followed by extensive washing with cold 0.1 N sodium bicarbonate, pH 8.6. Fetuin (Gibco, Spire method, 400 mg) was added to the activated Sepharose and allowed to react overnight at 4°C. The conjugated material was then stirred gently for 2 hours with 2-aminoethanol (0.5 M, pH 8.0), washed with 0.05 M glycine-HCl/0.5 M NaCl, pH 3.0, and finally equilibrated with phosphate-buffered saline, pH 7.4.

Jack beans, fava beans, white pea beans, small California white beans, and Idaho red beans were ground in a Waring Blender to a fine powder, extracted with PBS9 at 4°C overnight, and then centrifuged at 6,000 rpm for 15 min. Ammonium sulfate was added to the supernatant fraction to 50% saturation. After 6 hours at 4°C, this material was centrifuged at 10,000 rpm, and the precipitate was resuspended in water, dialyzed, and lyophilized. In order to prepare wheat germ agglutinin, 30 g of wheat germ lipase (Calbiochem) were suspended in 500 ml of water, heat-inactivated at 63°C for 15 min (9), and then centrifuged at 8,000 rpm. The supernatant from this material was made 50% in ammonium sulfate as described above. For the preparation of Limulus hemagglutinin, the hemolymph of Limulus polyphemus (10) was first centrifuged at 2,000 rpm for 30 min, and the clarified hemo-lymph was used directly in the affinity fractionation procedure. Commercial phytohemagglutinin-P (Difco), which showed eight components in sodium dodecyl sulfate gel electrophoresis, was used directly for affinity chromatography in order to prepare the purified lectin.

In a typical fractionation, 500 to 1000 mg of the lyophilized ammonium sulfate fraction or of the commercial PHA was dissolved in 100 ml of PBS. After centrifugation to remove insoluble material, the supernatant was loaded on a column (2 × 15 cm) of Sepharose-fetuin equilibrated with PBS at room temperature. After extensive washing with PBS at a flow rate of 10 to 12 ml per hour, the bound protein material was eluted with 0.05 M glycine-HCl/0.5 M NaCl, pH 3.0. The effluent fractions were then adjusted to pH 7.0, dialyzed against water, and lyophilized. For the isolation of Limulus hemagglutinin, 100 ml of the hemolymph were used for affinity chromatography, and the bound material was eluted with 1 M NaCl. In those instances in which the binding specificity of a particular lectin for simple saccharides was known, the bound protein could be eluted with the specific sugar. For example, 90% of the protein from extracts of jack beans bound to Sepharose-fetuin could be eluted with 0.1 M α-methyl-d-mannoside.

The protein fractions from various extracts isolated by affinity chromatography were subjected to gel electrophoresis in sodium dodecyl sulfate (11). The gel patterns (Fig. 1) for each of the respective lectins revealed one predominant Coomasie blue-stained component.

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1 R. Lotan, B. Sela, H. Lis, L. Suchs, and N. Sharon, unpublished results.
2 The abbreviations used are: PBS, phosphate-buffered saline (0.9% NaCl solution); WGA, wheat germ agglutinin; PHA, phytohemagglutinin; Con A, concanavalin A.
These results indicate that affinity chromatography using Sepharose-fetuin is a highly efficient one-step procedure for obtaining purified proteins from plant extracts (Table I).

In agreement with previous reports, the purified concanavalin A (Con A), Favin, and PHA-P (14-16) were found to be strongly mitogenic for mouse splenocytes, whereas WGA and Limulus hemagglutinin were not. Lectins from the white pea bean, small California white bean; F, Idaho red beans; G, white pea beans; H, Limulus hemagglutinin. The numbers at the right indicate the migration positions corresponding to molecular weights of 80,000, 30,000, and 18,000, respectively.

Table I

| Lectin              | Yield (mg) | % Recovery from the column | Mitogenic activity | Agglutination of RBC from different species |
|---------------------|------------|---------------------------|-------------------|------------------------------------------|
| Con A               | 14         | 65-90                     | ++                | human (type A) +                        |
| Wheat germ agglutinin | 70         | 70-80                     | -                 | calf                                    |
| Favin               | 12         | 70-80                     | -                 | mouse                                   |
| PHA-P               | 75         | 80                        | -                 | sheep                                   |
| White pea           | 25         | 85                        | ±                 | -                                       |
| Small California white | 17      | 85                        | ±                 | -                                       |
| Idaho red           | 60         | 80                        | +                 | -                                       |
| Limulus hemagglutinin | 96       | 40-50                     | +                 | -                                       |

- The yield is based on affinity chromatography of 100 ml of Limulus hemolymph, 500 mg of commercial PHA-P, and 1 g of the lyophilized ammonium sulfate fraction of all other plant extracts tested.
- The recovery of activity was based on the agglutination of horse erythrocytes for Limulus hemagglutinin, mouse erythrocytes for Favin, and sheep erythrocytes for the other lectins.
- The mitogenic activity was assayed as described elsewhere (17). The incorporation of [rH]thymidine by lectin-treated cultures was compared to that observed in cultures containing only cells, and the results are expressed as (+) for stimulation ratio ≥ 20, (±) for stimulation ratio ~4, and (-) for stimulation ratio ≤ 1 to 2.
- The agglutination assay was performed by serial dilution of the various lectins starting at a concentration of 1 mg/ml, and the results are expressed as (+) for agglutination at >1/64 dilution, (±) for 1/2 dilution, and (-) for no agglutination even at 1 mg/ml. RBC, red blood cells.

The agglutinating activity of both lectins for other cell types can be inhibited by the same monosaccharides (16).

In order to demonstrate further that the lectins isolated using the same affinity ligand recognize different cell surface receptors, the labeling patterns of the lectins conjugated with fluorescein isothiocyanate (18) were analyzed on a highly differentiated and polar cell type, the mouse spermatozoan. The results, summarized in Table II, showed that different lectins bound to different parts of the spermatozoan, classified here into four easily distinguishable regions: the head, the acrosome, the midpiece, and the tail. Only white pea lectin stained the head, the tail, and the midpiece regions of the sperm cells. The binding of Con A was restricted to the acrosome, and that of the lectin from Idaho red beans was found only in the head region. The other lectins were bound mainly to the midpiece (Table II). These findings are similar to results obtained using Con A and other fluorescein-labeled lectins binding to mouse spermatozoa (19).

We have also measured the simultaneous binding to mouse splenic lymphocytes of two lectins isolated on the same affinity column. Because many lectins are themselves glycoproteins, particular care was taken to choose pairs of lectins that did not interact with each other as assayed by the double immunodiffusion test (20). The results of studies using two pairs of radio-labeled lectins (21), Con A with WGA and PHA with WGA, are shown in Fig. 2. The amounts of 125I-labeled Con A and 125I-labeled WGA bound to mouse splenocytes were similar whether they were measured individually or simultaneously. This suggests
that over the concentration range of lectins tested, these two lectins do not share binding sites on the lymphocyte surface. This conclusion is in accord with the observation of Yahara and Edelman (22) that fluorescein-labeled Con A and rhodamine-labeled WGA are not capped simultaneously on the lymphocyte surface.

In contrast, when the binding of PHA and WGA was compared in a similar fashion, it was found that each lectin inhibited the binding of the other (Fig. 2). For example, the amount of PHA bound in the presence of WGA was 75% of that observed in the absence of the second lectin. Conversely, the binding of WGA to lymphocytes decreased by about 50% in the presence of PHA. These results suggest that PHA and WGA may compete for a subpopulation of cell surface receptors having saccharides that can bind each of the two lectins.

Finally, we have found that the gel electrophoresis profiles of glycoprotein receptors on murine lymphocytes for Con A and WGA showed striking differences (Fig. 3). The basic procedures for this analysis have been developed previously (23). Briefly, surface receptors were labeled with 125I using lactoperoxidase and then solubilized from the membrane with the detergent Nonidet P-40. The extracted membrane fraction was treated with lectin, followed by immunoprecipitation with antibodies directed against the lectin. After washing, the immunoprecipitate was subjected to gel electrophoresis in sodium dodecyl sulfate. As shown in Fig. 3, the types and amounts of glycoproteins detectable by this technique are different for Con A and WGA. These results lend further support to the conclusion that these two lectins do not share binding sites on the lymphocyte surface.

A number of recent studies have suggested the hypothesis that the structure of the cell surface receptors for carbohydrate-specific ligands resembles the carbohydrate moiety of glycoproteins such as fetuin (3-5, 24). The isolation by affinity chromatography using Sepharose-fetuin of antibodies (7) and lectins reactive with cell surface carbohydrates lends strong support to this hypothesis. The fact that carbohydrate-specific ligands can be isolated on Sepharose-glycoprotein independent of the particular specificity of the ligand molecule is particularly useful for collecting a large number of cell surface probes having different properties. The present results indicate that lectins isolated on a single affinity adsorbent or lectins exhibiting similar monosaccharide specificities may show striking differences in their binding to cell surface.

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