DISTRIBUTION OF CELL SURFACE SACCHARIDES ON PANCREATIC CELLS

I. General Method for Preparation and Purification of Lectins and Lectin-Ferritin Conjugates

MARIE-FRANCE MAYLIÉ-PFENNINGER and JAMES D. JAMIESON

From the Section of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510. Dr. Maylié-Pfenninger's present address is the Laboratory of Developmental Genetics, Sloan-Kettering Institute for Cancer Research, New York 10021.

ABSTRACT

We describe here a simple, general procedure for the purification of a variety of lectins, and for the preparation of lectin-ferritin conjugates of defined molar composition and binding properties to be used as probes for cell surface saccharides. The technique uses a "universal" affinity column for lectins and their conjugates, which consists of hog sulfated gastric mucin glycopeptides covalently coupled to agarose. The procedure involves: (*a*) purification of lectins by chromatography of aqueous extracts of seeds or other lectin-containing fluids over the affinity column, followed by desorption of the desired lectin with its hapten sugar; (*b*) iodination of the lectin to serve as a marker during subsequent steps; (*c*) conjugation of lectin to ferritin with glutaraldehyde; (*d*) collection of active lectin-ferritin conjugates by affinity chromatography; and (*e*) separation of monomeric lectin-ferritin conjugates from larger aggregates and unconjugated lectin by gel chromatography. Based on radioactivity and absorbancy at 310 nm for lectin and ferritin, respectively, the conjugates consist of one to two molecules of lectin per ferritin molecule. Binding studies of native lectins and their ferritin conjugates to dispersed pancreatic acinar cells showed that the conjugation procedure does not significantly alter either the affinity constant of the lectin for its receptor on the cell surface or the number of sites detected.

KEY WORDS lectin affinity purification · mucin peptides · lectin-ferritin conjugates · conjugate binding characteristics

Because of their high specificity of binding to the carbohydrate moieties of glycoproteins and glycolipids, lectins have proven to be powerful tools for examination of the architecture of the plasmalemma, comparison of the surface saccharide patterns of normal and transformed cells, and detection of cell surface changes during development (15). For these purposes, a variety of lectins with different sugar specificities have been purified by either conventional procedures or by affinity chromatographic (15) techniques applicable to individual lectins. In addition, several general affinity adsorbents have been proposed for isolation of groups of lectins. For example, fixed erythrocytes

of suitable blood types (3, 23), concanavalin A-(ConA)[1]-Sepharose (4) and fetuin-Sepharose (26) have been shown to bind several lectins. However, ConA-Sepharose does not really constitute a general adsorbent, because not all agglutinins are glycoproteins and are bound by ConA, and not all glycoproteins which will be adsorbed are lectins. Fetuin is lacking fucose residues and therefore will not bind Lotus and Ulex lectins. Fixed erythrocytes would appear more appropriate as a general adsorbent, although for each lectin to be purified one is required to determine the blood type that gives optimal lectin adsorption.

In this paper we describe a simple, rapid, and general method for the isolation and purification of lectins with a wide spectrum of saccharide binding properties, and for the conjugation of these lectins to ferritin for use as electron microscope tracers. Central to this procedure is the use of an affinity adsorbent for lectins and their conjugates which consists of sulfated glycopeptides derived from hog gastric mucin (21, 27) and is covalently linked to agarose beads. The sulfated glycopeptides (27) possess a wider complement of saccharides than other adsorbents previously used and therefore qualify as general affinity adsorbents for sugar binding proteins.

For use as cell surface saccharide probes, lectins have been visualized by electron microscopy with a variety of markers, including hemocyanin, peroxidase, polysaccharide-iron complexes, and ferritin. The attachment of the marker to the lectin has employed either noncovalent specific interaction between polysaccharide residues on the marker and residual saccharide binding sites on the target-bound lectin, as in the case of hemocyanin, peroxidase, or polysaccharide-iron complexes, or covalent coupling of lectin and tracer as in the case of ferritin and peroxidase (for review, see Nicolson [16]). We report here on the preparation of seven lectin-ferritin conjugates by a modification of the method originally described by Nicolson and Singer (19, 20). Our method results in conjugates of defined molecular composition and of systematically quantitated binding properties which, to our knowledge, has not been previously reported. These conjugates have been used to examine the cell surface saccharides of dispersed cells from the mammalian pancreas as will be presented in the following paper (13).

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; ConA, concanavalin A; PBS, 0.05 M K-phosphate, pH 6.8, containing 0.15 M NaCl; RCA I and II, Ricinus communis agglutinins I and II; WGA, wheat germ agglutinin.

MATERIALS AND METHODS

Materials

Reagents and chemicals were from these sources: Hog gastric mucin, α-methyl mannoside, L-fucose, N-acetyl glucosamine, N-acetyl galactosamine, and glucose oxidase from Sigma Chemical Co., St. Louis, Mo.; horse spleen ferritin (twice crystallized and cadmium free) from Miles Laboratories, Inc., Miles Research Products, Elkhart, Ind., papain and lactoperoxidase from Calbiochem, San Diego, Calif.; 8% glutaraldehyde from Electron Microscopy Sciences, Fort Washington, Pa.; Bio-Gel A5m (200–400 mesh) from Bio-Rad Laboratories, Richmond, Calif.; Sephadex G75 and G100 and Sepharose 4B from Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.; cyanogen bromide from Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.; $Na^{125}I$, carrier free, from New England Nuclear, Boston, Mass. All other reagents were of the purest grades commercially available.

Methods

PREPARATION OF MUCIN GLYCOPEPTIDES: Sulfated glycopeptides were prepared from hog gastric mucin basically by the method of Pamer et al. (21), except that the starting material consisted of 100 g of hog gastric mucin rather than mucosal scrapes. This amount of mucin should yield ~1.5 g of lyophilized sulfated mucin glycopeptides.

PREPARATION AND USE OF THE MUCIN GLYCOPEPTIDE ADSORBENT: Sepharose 4B (48 g wet wt) was activated with cyanogen bromide (30 g) (6) and reacted overnight at 4°C with mucin glycopeptides[2] (1.2 g) dissolved in 0.2 M $NaHCO_3$, pH 9.5. The mucin glycopeptide-Sepharose was filtered, washed with 0.2 M $NaHCO_3$, incubated with 1 M glycine for 30 min at 23°C, and washed extensively with distilled water. The adsorbent was then washed successively with 200 ml each of 6 M guanidine-HCl, distilled water, and 8 M urea, equilibrated with phosphate-buffered saline (PBS) which contained 0.02% Na-azide, and packed in a column of 2 × 40 cm. After each affinity chromato-

[2] Recent experiments have shown that some batches of mucin glycopeptides possess higher contents of sialic acid than those previously used by us, resulting in affinity columns which bind WGA so tightly as to prevent desorption. We suggest that before coupling to Sepharose 4B, the lyophilized mucin peptides be desialylated by incubation in 120 ml of 1 N H_2SO_4 for 1 h at 80°C followed by neutralization to pH 9.5 with 1 N NaOH and addition of solid $NaHCO_3$ to 0.2 M.

graphic run, the column was unpacked, washed in bulk with guanidine-HCl, water, and urea as above, equilibrated with PBS plus 0.02%-Na azide, and repacked before use. The amount of glycopeptide covalently bound was usually found to range from 50 to 70%.

The first steps for purification of all lectins involved PBS extraction of ground seeds or flour followed by ammonium sulfate precipitation (except for Ulex lectin) (9, 14, 24). The precipitates were resuspended and exhaustively dialyzed against PBS. The dialysates were clarified by centrifugation, adsorbed to the mucin-Sepharose column, and eluted with 0.2 M of appropriate hapten sugar at flow rates of 15 ml/h.

All lectins from the affinity column were dialyzed against PBS and concentrated to 5–10 mg/ml in an Amicon ultrafiltration cell fitted with a PM-10 filter (Amicon Corp., Scientific Sys. Div., Lexington, Mass.) and stored at −20°C. ConA (Miles Laboratories, Inc.) was used without further purification or on occasion after repurification on the affinity column.

For all lectins purified by affinity chromatography by the above procedures, 100 g of crude starting material yielded 100–150 mg of lectin protein.

IODINATION: The purified lectins were enzymatically radioiodinated by the method of Hubbard and Cohn (12). 100 mg of protein was incubated for 1 h at 37°C with 0.1 M glucose, 0.05 M specific hapten sugar, 0.3 U lactoperoxidase, 0.3 U glucose oxidase, 2 mM KI, and 1 mCi Na ^{125}I in a volume of 10 ml. After extensive dialysis of the reaction mix against PBS to remove unreacted ^{125}I and hapten sugar, active lectins were separated from those inactivated during iodination by chromatography on the mucin peptide column.

FERRITIN PURIFICATION: Twice-crystallized ferritin was further purified by centrifugation at 85,000 *g* for 3 h at 5°C followed by gel filtration of the resuspended pellet on a Bio-Gel A5m column (2 × 180 cm) to remove polymers. The ferritin pellet was resuspended in 0.1 M K-phosphate buffer, pH 7.0; the column was equilibrated and run in the same buffer at 4°C with a flow rate of 15 ml/h. The fractions of monomeric ferritin were pooled and concentrated to 100 mg/ml by pressure ultrafiltration.

CONJUGATION OF LECTINS TO FERRITIN: The iodinated agglutinins were coupled to ferritin by a modification of the glutaraldehyde conjugation procedure of Avrameas (2) and of Nicolson and Singer (19, 20).

Glutaraldehyde in a 200-fold molar excess was added to a solution of 2×10^{-5} M lectin and 2×10^{-5} M ferritin in PBS containing 0.1 M hapten sugar. After 1 h of incubation at room temperature with stirring, the reaction was stopped by addition of 0.1 M $NaBH_4$ for 15 min. After this, any remaining aldehyde groups were quenched with 0.1 M glycine again for 15 min. Excess reagents, including bound hapten sugar, were removed by dialysis against PBS. The conjugates were then purified by affinity chromatography and gel filtration as described under Results. The purified lectin conjugates were dialyzed against PBS, concentrated to 1 mg/ml by pressure ultrafiltration, and stored at 4°C under toluene vapors to retard bacterial growth. Before use, the conjugates were centrifuged for 10 min at 48,000 *g* to remove aggregates.

RESULTS

Purification of the Agglutinins

For most of the lectins used, the purification procedure involved three steps: extraction of the ground seeds, fractionation with ammonium sulfate, and affinity chromatography on Sepharose 4B to which mucin peptides had been covalently bound. The ammonium-sulfate-precipitation step was omitted during purification of the Ulex lectin because this appeared to destroy activity. For each lectin the bulk of the protein was eluted with the void volume of the column; no hemagglutinating activity was detected under this peak. The lectin was then eluted with 0.2 M hapten sugar. No further purification of lectins was carried out except for Ricinus communis agglutinins (RCA) I and II. These lectins, sequentially eluted with 0.1 M N-acetyl galactosamine and 0.4 M galactose (17, 18), were rechromatographed on Sephadex G100 to reduce cross contamination. Each lectin appeared to be pure by SDS-gel electrophoresis.

Preparation of Lectin-Ferritin Conjugates

To trace the lectins quantitatively during the conjugation procedure, they were first radioiodinated as described in Methods. After iodination, denatured lectins were eliminated from the radioactive preparation by an additional affinity chromatography step.

To protect the binding sites of lectins during conjugation to ferritin with glutaraldehyde, 0.1 M hapten sugar was present during the reaction. The conditions required to obtain maximal yields of monomeric conjugate were determined empirically by systematically varying the concentrations of lectin, ferritin, and glutaraldehyde in the reaction mixture. These conditions were obtained when a 200-fold excess of glutaraldehyde was added to low (2×10^{-5} M) concentrations each of ferritin and lectin.

After the coupling reaction, lectin-ferritin conjugates were separated from unconjugated lectin and ferritin, and from lectin-lectin, ferritin-ferritin, and larger aggregates by two successive chromatographic steps. In the first step, the conjugation reaction mixture was applied to the mucin

peptide-Sepharose column, which should retain all active lectins and lectin-ferritin conjugates, but exclude unconjugated ferritin, ferritin oligomers, and denatured lectins.

In the second step, lectin activity eluted from the affinity column was subjected to gel-filtration chromatography to select monomeric conjugates of lectin-ferritin. In both steps the elution profiles for ferritin and lectin were monitored by absorbancy at 310 nm and by gamma-spectrometry (Beckman Biogamma Counter, Beckman Instruments Inc., Fullerton, Calif.), respectively.

Figs. 1 and 2 show typical examples of the purification of ConA-ferritin and wheat germ agglutinin-(WGA)-ferritin conjugates. Fig. 1*A* and *B* shows the chromatographic profile of the conjugation reaction mixture applied to the mucin-peptide affinity column after dialysis to remove bound hapten sugar. Ferritin (monomeric and polymeric), which was not retained by the affinity column, carried little radioactivity with it. Hence, the majority of the lectin (conjugated, free, and polymeric) was bound to the absorbent, which suggests that there was little destruction of lectin binding sites by exposure to glutaraldehyde. All adsorbed lectins and lectin-ferritin conjugates were subsequently desorbed by the appropriate hapten sugar, concentrated by pressure ultrafiltration, and sized on a Bio-Gel A5m column. As can be seen in Fig. 2*A* and *B*, a peak of polymeric conjugates characterized by a low lectin-to-ferritin ratio appeared in the void volume of this column, and was followed by a peak of mainly monomeric conjugates characterized by a lectin-ferritin ratio of from 1:1 to 2:1, depending on the preparation.[3] Conjugates with lower or higher molar ratios were regarded as contaminated with polymeric ferritin or lectin, respectively, and were discarded. An advantage of this procedure compared to that of others (16) is that the extent of cross contamination of the conjugate by unconjugated lectin can be assessed, and conjugates essentially devoid of free lectin can be collected.

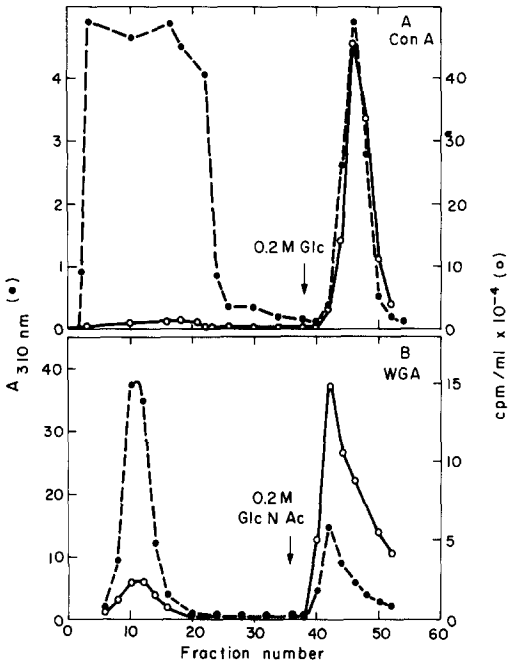

FIGURE 1*A* AND *B* Affinity chromatographic purification of lectin-ferritin conjugates. ●——●, absorbancy of ferritin at 310 nm; ○——○ radioactivity of ^{125}I-labeled lectins. Both lectin-ferritin conjugates were prepared as described under Methods, dialyzed against PBS to remove bound hapten sugar, and applied to the mucin peptide affinity column. The unadsorbed material consists primarily of unconjugated ferritin with a small amount of presumably denatured lectin. Lectin-ferritin conjugates and unconjugated lectins were eluted by the appropriate hapten sugar in PBS, i.e., 0.1 M glucose (Glc) for ConA conjugates and 0.2 M N-acetyl glucosamine (Glc NAc) for WGA. Fraction volumes were 10 ml; elution rates were 15 ml/h.

Binding Properties of Ferritin-Lectin Conjugates: Studies with Erythrocytes

The binding properties of the conjugates were compared to those of the native lectins in a series of pilot experiments with guinea pig erythrocytes. In a typical experiment, 2×10^7 guinea pig erythrocytes/ml in PBS were incubated with increasing concentrations of radioiodinated ConA and ConA-ferritin conjugates at 4°C for 30 min. After three rinses in PBS, the amount of lectin and of lectin-ferritin conjugate bound to the cells was measured by gamma-spectrometry. The amount of nonspecific binding was determined by including 0.2 M α-methyl mannoside in the incubation medium. As shown in Fig. 3*A*, the extent of nonspecific binding in the presence of the hapten sugar was higher for the conjugate than for the native lectin, i.e., 38 and 10% of total binding, respectively.

[3] We have observed that the recovery of ConA-ferritin conjugates after gel filtration is increased if the chromatography in the Bio-Gel A5M column is carried out in the presence of 0.1 M glucose.

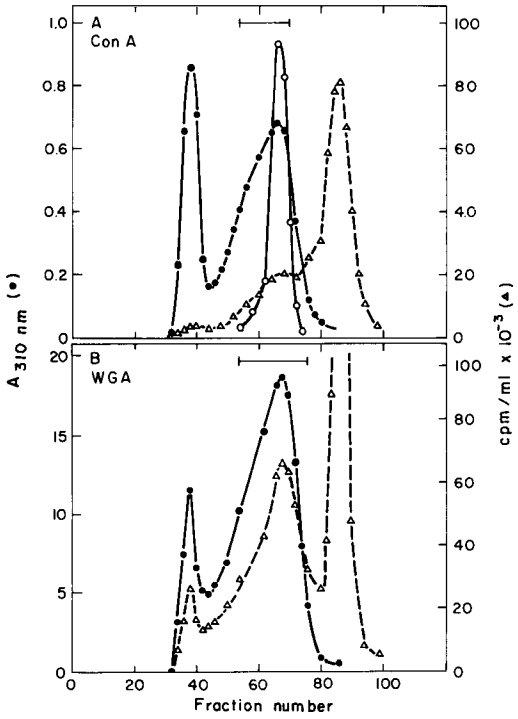

FIGURE 2*A* AND *B* Separation of lectin-ferritin conjugates from unlabeled lectins by gel filtration on a Bio-Gel A5m column (1.5 × 180 cm). ●——● absorbancy of ferritin in the conjugate at 310 nm; ○——○ absorbancy of unconjugated ferritin at 310 nm run separately for column calibration; △——△, radioactivity of ^{125}I-labeled lectin. The first peak from each column represents polymeric conjugates characterized by low lectin-to-ferritin ratios. The second conjugate peak is characterized by a lectin:ferritin ratio ranging from 1:1 to 2:1 (0.9 for ConA-ferritin and 1.8 for WGA-ferritin in this experiment). This conjugate peak contains mainly monomeric ferritin as indicated by its elution position in relation to free ferritin. The horizontal bars indicate the portion of the conjugate peak pooled for use. Fraction volumes were 2 ml; elution rates were 7 ml/h, with PBS as the eluting buffer for WGA-ferritin conjugate, and PBS containing 0.1 M glucose for ConA-ferritin conjugate.

The higher nonspecific binding of the conjugate could be caused by the presence of incompletely quenched aldehyde groups in the conjugate which could react with amino groups on the cell surface, or by nonspecific interaction of ferritin with the plasma membrane. The first possibility is unlikely, because the conjugate had been incubated with sodium borohydride and glycine which we have found to eliminate residual aldehydes (unpublished observations).

To test for the second possibility, ferritin alone was radioiodinated and tested for binding. Guinea pig erythrocytes were incubated with increasing concentrations of radioactive ferritin under the same conditions as described for the conjugate, washed with PBS to remove unbound ferritin, and counted. As shown in the upper curve of Fig. 4*A*, saturable binding of ferritin was obtained which, when plotted by the procedure of Steck and Wallach (28) (Fig. 4*B*, lower line), indicated that about 3×10^5 ferritin molecules were bound per erythrocyte with an affinity constant of 1×10^6 M^{-1}. On the basis of the long-standing observation that bovine serum albumin (BSA) reduces nonspecific binding of a variety of ligands, we re-

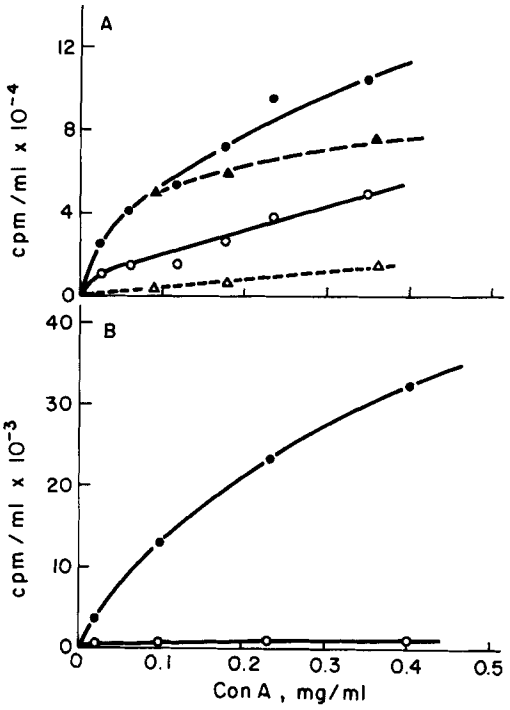

FIGURE 3 Binding of ^{125}I ConA and its ferritin conjugate to guinea pig erythrocytes. (*A*) ●——● ^{125}I ConA-ferritin conjugate; △——△ ^{125}I ConA; ○——○ ^{125}I ConA-ferritin conjugate in the presence of 0.2 M α-methyl mannoside. Note the high level of nonspecific binding of the conjugate under these conditions. (*B*) Binding of ^{125}I ConA-ferritin conjugate in the presence of 1% BSA. Note that the level of non-specific binding is now reduced to 5% of total binding compared to 38% nonspecific binding in the absence of BSA (Fig. 3*A*). ○——○ ^{125}I ConA-ferritin in the presence of 0.2 M α-methyl mannoside; ●——● ^{125}I ConA-ferritin conjugate.

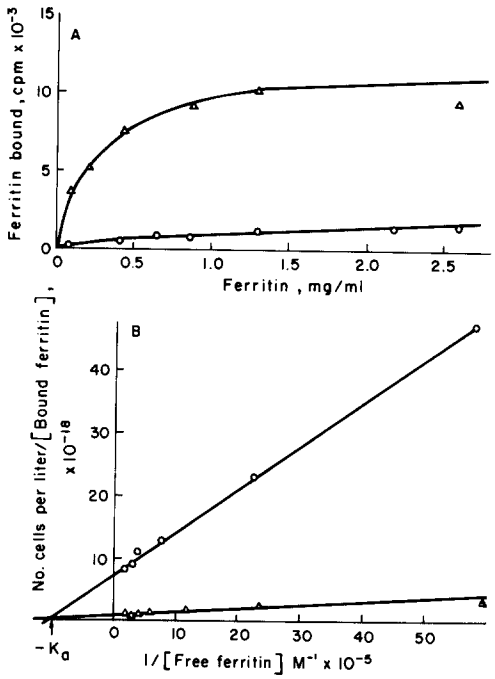

FIGURE 4*A* AND *B* Binding of ^{125}I-ferritin to guinea pig erythrocytes. (*A*) △——△, binding of ^{125}I-ferritin to erythrocytes in PBS; ○——○ binding of ^{125}I-ferritin in the presence of 1% BSA in PBS. The lower curve indicates that nonspecific binding of ferritin is reduced by ~85% in the presence of BSA. (*B*) The same data as shown in Fig. 4*A* plotted by the method of Steck and Wallach (28). No. cells per liter/[Bound ferritin] is the concentration of erythrocytes/liter (2×10^{10}) divided by the molar concentration of ferritin bound to the cells calculated from bound radioactivity; 1/[free ferritin] is the calculated free ferritin remaining in the binding assay after subtraction of the amount of ferritin bound. K_a = −1/[free ferritin] when number of cells per 1/[bound ferritin] = 0; extrapolation of 1/[free ferritin] to zero provides a value for 1/number of moles of ferritin bound per cell. Symbols as in Fig. 4*A*.

peated the binding of labeled ferritin to erythrocytes in the presence of 1% BSA. As can be seen in the lower curve of Fig. 4*A*, the binding of ferritin was markedly reduced and was a linear function of the concentration of ferritin in the assay. The upper line in Fig. 4*B* indicates that BSA in the assay lowered binding of ferritin to about 7×10^4 molecules per cell without altering the K_a of nonspecific binding. Based on this experiment, all subsequent binding studies of lectin-ferritin conjugates were carried out in the presence of BSA. A representative experiment, shown in Fig. 3*B*, indicates that nonspecific binding of ConA-ferritin to erythrocytes is reduced to 5% of total binding in the presence of 0.2 M hapten sugar plus 1% BSA.

Binding Properties of Lectins and their Conjugates: Studies with Dispersed Pancreatic Acinar Cells

As will be presented in more detail in the following paper (13), the lectin-ferritin conjugates were prepared for use as probes for cell-surface-saccharide topography on isolated pancreatic cells. Because populations of dispersed pancreatic acinar cells can be obtained in high yields and with reasonable homogeneity (1), we have used this cell preparation, rather than erythrocytes, to characterize quantitatively the binding properties of all lectin-ferritin conjugates described here. These data will be presented in the following paper where we will show that the binding sites for all lectins and their conjugates appear to be of a single class and do not exhibit cooperativity. In addition, it will be shown that both the number of lectin molecules bound per pancreatic cell and their average affinity constants are of the same order of magnitude for each lectin and its conjugates. From this, we conclude that our conjugation procedure does not markedly affect the binding properties of the lectins examined.

DISCUSSION

We describe here a general method to purify large quantities of lectins of various specificities and to obtain well-defined lectin-ferritin conjugates. The purification procedure involves an affinity chromatography step on sulfated glycopeptides from gastric mucin coupled to Sepharose 4B. These peptides, released by papain digestion of hog gastric mucin and purified by ethanol and cetylpyridinium chloride precipitation, appear to contain the full complement of sugars found on glycoproteins (27). The lectins ConA, WGA, soybean agglutinin, RCAs, Lotus lectin, and Ulex lectin, previously mentioned in this paper, as well as lima bean agglutinin, Limulin (26), and a lectin from lobster hemolymph (10, 11), have been found by us to bind to these insolubilized glycopeptides and can be eluted by their hapten sugars or by increases in ionic strength, as in the case of Limulin and lobster lectin (10, 11, 26). Other agglutinins have also been reported to bind to hog gastric mucin. *Dolichos biflorus* lectin binds to A- and H-blood-group substances from hog gastric

mucin insolubilized by treatment with N-carboxyanhydroleucine (7), and *Sophora Japonica* lectin is adsorbed to insolubilized A blood substance from the same source (22).

A potential disadvantage of the mucin glycopeptide adsorbent is the presence of sulfated groups on the peptide which may cause it to function also as an ion-exchange resin. This, however, should not interfere with its use as an affinity adsorbent for lectins, because the ionic strength and pH of the eluting buffer are maintained constant, and elution relies exclusively on competitive displacement by hapten sugars.

The conjugation procedure described here uses glutaraldehyde as a coupling agent (2, 19, 20). Besides glutaraldehyde, several other bifunctional reagents such as p,p'-difluoro-m'm-dinitrodiphenylsulfone (29), bis-diazotized 3,3'-diamethoxybenzidine (5) and toluene, 2,4-diisocyanate (25) were tested for the preparation of lectin-ferritin conjugates, but usually induced extensive polymerization of lectin-ferritin. Our procedure for preparation of lectin-ferritin conjugates differs from that previously reported by Nicolson and Singer (19, 20) in several respects. First, we have systematically explored the ratio of reacting partners in the procedure (ratio of lectin to ferritin and glutaraldehyde concentrations) and have found that, by reducing the concentration of lectin and ferritin by 5- to 10-fold, by maintaining ferritin and lectin in equimolar amounts in the reaction mixture, and by using a 10-fold higher molar concentration of glutaraldehyde, our yield of monomeric conjugates ranges from 6–12% of the lectin initially present. A further advantage of our conjugation procedure involves the use of radioiodinated lectins during conjugation, because it provides a convenient monitor for coupled lectins during subsequent purification and enables us to estimate and collect conjugates of defined molar ratios of lectin to ferritin. Further, because the conjugates are radioiodinated, binding studies can be carried out on cells before processing for electron microscopy, which enables one to correlate number of binding sites as determined radiochemically with number of sites detected by counting ferritin molecules in thin sections of cells.

Quantitative binding studies in which a comparison was made between native lectins and their conjugates with regard to affinity constants and number of conjugate molecules bound to cells indicate that the conjugation procedure does not interfere seriously with the binding properties of the lectins. To our knowledge, the effect of conjugation on the binding of lectins to their receptors has not been evaluated quantitatively before. Defined lectin-ferritin probes should be of general usefulness for precise analysis of membrane glycoconjugates in a wide variety of cell systems.

Addendum

Nicolson, et al. (Nicolson, G. L., J. R. Smith, and R. Hyman. 1978. Dynamics of toxin and lectin receptors on a lymphoma cell line and its toxin-resistant variant using ferritin-conjugated ^{125}I-labeled ligand. *J. Cell Biol.* **78**:565–576.), have recently described a technique for preparation of ^{125}I-labeled ferritin conjugates. While their procedure is in some ways similar to the one described here (and previously published by us in abstract form [Maylié-Pfenninger et al. 1975. Interaction of lectins with the surface of dispersed pancreatic cells. *J. Cell Biol.* **67**:333*a* (Abstr.)]), it does not provide information on the molecular composition of the conjugate or the equilibrium-binding parameters, and does not allow for separation of monomeric ferritin conjugates from polymers.

The authors wish to express their gratitude to Dr. George E. Palade for his continued interest, support, and helpful criticism during the course of this work. We wish to thank Dr. Motowo Tomita, Showa University, Tokyo, for kindly supplying us with initial samples of RCA I and II.

This work was supported in part by United States Public Health Service grant AM-17389 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, and by a grant from The National Foundation-March of Dimes.

Received for publication 30 May 1978, and in revised form 28 August 1978.

REFERENCES

1. Amsterdam, A., and J. D. Jamieson. 1974. Studies on dispersed pancreatic exocrine cells. I. Dissociation technique and morphologic characteristics of separated cells. *J. Cell Biol.* **63**:1037–1056.
2. Avarameas, S. 1969. Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry*. **6**:43–52.
3. Avrameas, S., and B. Guilbert. 1971. Biologically active water-insoluble protein polymers. Their use for the isolation of specifically interacting proteins. *Biochimie (Paris)*. **53**:603–614.
4. Bessler, W., and I. J. Goldstein. 1973. Phytohemagglutinin purification: A general method involving affinity and gel chromatography. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **34**:58–62.
5. Borek, F. 1961. A new two-stage method for cross-linking proteins. *Nature (Lond.)*. **193**:1293–1294.
6. Cuatrecasas, P., and C. B. Anfinsen. 1971. Affinity chromatography. *Methods Enzymo.* **22**:345–378.
7. Etzler, M. E., and E. A. Kabat. 1970. Purification and characterization of a lectin (plant hemagglutination) with blood group A specificity from Dolichos Biflorus. *Biochemistry*. **9**:869–877.
8. Frost, R. G., R. W. Reitherman, A. L. Miller, and J. S. O'Brien. 1975. Purification of Ulex europeus hemagglutinin I by affinity chromatography. *Anal. Biochem.* **69**:170–179.

9. GINSBURG, V. 1972. Purification and properties of carbohydrate binding proteins. *Methods Enzymol.* **28**:313–395.
10. HALL, J. L., and D. T. ROWLANDS, JR. 1974. Heterogeneity of lobster agglutinins. I. Purification and physiochemical characterization. *Biochemistry.* **13**:821–827.
11. HALL, J. L., and D. T. ROWLANDS, JR. 1974. Heterogeneity of lobster agglutinins. II. Specificity of agglutinin-erythrocyte binding. *Biochemistry.* **13**:828–832.
12. HUBBARD, A. L., and Z. A. COHN. 1972. The enzymatic iodination of the red cell membrane. *J. Cell Biol.* **55**:390–405.
13. MAYLIÉ-PFENNINGER, M. F., and J. D. JAMIESON. 1979. Distribution of cell-surface saccharides on pancreatic cells. II. Lectin labeling patterns on mature mammalian pancreatic cells. *J. Cell Biol.* **80**:77–95.
14. NAGATA, Y., and M. M. BURGER. 1974. Wheat germ agglutinin and molecular characteristics and specificity of sugar binding. *J. Biol. Chem.* **249**:3116–3122.
15. NICOLSON, G. L. 1974. Interaction of lectins with animal cell surfaces. *Int. Rev. Cytol.* **39**:89–190.
16. NICOLSON, G. L. 1978. Ultrastructural localization of lectin receptors. *In* Advanced Techniques in Biological Electron Microscopy, Vol. II. J. K. Koehler, editor. Springer-Verlag Inc., New York, New York. 1–38.
17. NICOLSON, G. L., and J. BLAUSTEIN. 1972. The interaction of Ricinus communis agglutinin with normal and tumor cell surfaces. *Biochim. Biophys. Acta.* **266**:543–547.
18. NICOLSON, G. L., J. BLAUSTEIN, and M. E. ETZLER. 1974. Characterization of two plant lectins from Ricinus communis and their quantitative interaction with a murine lymphoma. *Biochemistry.* **13**:196–204.
19. NICOLSON, G. L., and S. J. SINGER. 1971. Ferritin-conjugated plant agglutinins as specific saccharide stains for electron microscopy: Application to saccharides bound to cell membranes. *Proc. Natl. Acad. Sci. U. S. A.* **68**:942–945.
20. NICOLSON, G. L., and S. J. SINGER. 1974. The distribution and assymetry of mammalian cell surface saccharides utilizing ferritin conjugated plant agglutinins as specific saccharide stains. *J. Cell Biol.* **60**:236–248.
21. PAMER, T., G. B. J. GLASS, and M. I. HOROWITZ. 1968. Purification and characterization of sulfated glycoproteins and hyaluronidase-resistant mucopolysaccharides from dog gastric mucosa. *Biochemistry.* **7**:3821–3829.
22. PORETZ, R. D., H. RISS, J. W. TIMBERLAKE, and S. M. CHIEN. 1974. Purification and properties of the hemagglutination from Sophora japonica seeds. *Biochemistry.* **13**:250–256.
23. REITHERMAN, R. W., S. D. ROSEN, and S. H. BARONDES. 1974. Lectin purification using formalinised erythrocytes as a general affinity absorbant. *Nature (Lond.).* **248**:599–600.
24. RICE, R. H., and M. E. ETZLER. 1975. Chemical modification and hybridization of wheat germ agglutinins. *Biochemistry.* **14**:4093–4099.
25. SCHICK, A. F., and S. J. SINGER. 1961. On the formation of covalent linkages between two proteins molecules. *J. Biol. Chem.* **236**:2477–2485.
26. SELA, B., J. L. WANG, and G. M. EDELMAN. 1975. Isolation of lectins of different specificities on a single affinity adsorbent. *J. Biol. Chem.* **250**:7535–7538.
27. SLOMIANY, B. L., and K. MEYER. 1972. Isolation and structural studies of sulfated glycoproteins of hog gastric mucosa. *J. Biol. Chem.* **247**:5062–5070.
28. STECK, T. L., and D. F. H. WALLACH. 1965. The binding of kidney-bean phytohemagglutinin by Ehrlich ascites carcinoma. *Biochim. Biophys. Acta.* **97**:510–522.
29. TAWDE, S. S., and J. S. RAM. 1962. Conjugation of antibody to ferritin by means of p,p'-difluoro-m,m'-dinitro diphenylsulphone. *Arch. Biochem. Biophys.* **97**:429–430.