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A Latex Agglutination Test for Lectin Binding

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Blood group A + H substance purified from hog gastric mucin was fixed to polystyrene particles by physical absorption to produce a lectin-agglutinable latex. Agglutination of this latex by soybean agglutinin, concanavalin A, and wheat germ agglutinin is specifically inhibited by the respective monosaccharide haptens and is not affected by known noninhibitory sugars. The sensitivity of the assay, a function of particle loading and latex concentration, approaches that of the hemagglutination test. NaCl, $NaHCO_3$, NH_4CO_3, NH_4-acetate, and Tris–HCl up to a concentration of 1 M do not interfere with the test. The latex suspension is stable for several months and can be stored in freeze-dried form.

Lectins, carbohydrate-binding proteins that are found in several plant and animal tissues, have been used extensively as reagents in polysaccharide and glycoprotein biochemistry and also in serological tests (for a review, see (1–3)). The most extensively used methods for the study of lectin-mediated interactions are based on cell agglutination phenomena. These assay procedures (for a review, see (4)) are simple, sensitive, and make it possible, through inhibition tests, to determine the carbohydrate specificity of the interaction studied. Interpretation of cell agglutination patterns is hampered, however, by the facts that (a) cell surfaces contain a number of different moieties that can bind molecules other than lectins (lipids, polyions); (b) nonspecific agglutination thus produced may be inhibited by carbohydrates or glycoproteins (5); and (c) agglutination caused by "true" lectins depends on receptor density and, particularly in the case of receptors associated with glycolipids, the topological distribution of receptors in the membrane may be of importance (6).

For these reasons, results derived from cell agglutination experiments have to be corroborated by other techniques such as precipitation reactions or affinity separation methods (cf. (1)). Although well defined in the chemical sense, these methods are much less sensitive than the hemagglutination assay and can sometimes be adapted only with difficulty to the lectin/ligand interaction to be studied. One possible solution to this problem is to attach purified ligands to particles that could not otherwise be agglutinated by lectins. Glycolipid ligands such as natural gangliosides (7–12) and synthetic, cholesterol-based glycolipids provided with spacer arms of different lengths (6) have been incorporated into liposomes to serve as receptors for various lectins. Bovine erythrocytes have been rendered Con-A[2] agglutinable by attaching D-mannose residues to their outer membrane (13,14).

The present study was undertaken to apply a similar approach to lectin/glycoprotein in-

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[2] Abbreviations used: BGA, blood group A + H substance; BSA, bovine serum albumin; Con A, concanavalin A; GBS, glycine-buffered saline; SBA, soybean agglutinin; WGA, wheat germ agglutinin; Gal, D-galactose; Man, D-mannose; Glc, D-glucose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine.

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teractions. We describe the fixation of a glycoprotein, blood group A + H substance to the surface of polystyrene particles to produce a lectin-agglutinable latex. Originally described in 1956 by Singer and Plotz (15), latex techniques have become increasingly popular for the study of various antigen/antibody interactions (18). Since lectins are similar in many respects to antibodies, we adapted this method for the detection of lectins and the study of lectin-mediated interactions.

MATERIALS AND METHODS

Polystyrene latex, a 3% w/w suspension (particle diameter 0.3–3 μm) was obtained from HUMAN, Budapest.

BGA was purified from hog stomach by the phenol–ethanol procedure as described in Ref. (16). The crude product was used to coat the latex for routine purposes. The product used in this study was further purified by affinity chromatography on Con A–Sepharose (Pharmacia) as follows: 200 mg BGA was dissolved in 20 ml of GBS and the solution was passed through a 25-ml Con A–Sepharose column. The column was washed with GBS until the absorbance at 280 nm decreased below 0.1, and then the wash was changed to 0.1 M α-methylmannoside (Sigma) in PBS (50 ml). The eluted material was dialyzed against distilled water (6× 4 liters) and subsequently lyophilized. The yield varied between 110 and 160 mg.

Concanavalin A and wheat germ agglutinin (WGA) were obtained from Pharmacia and Sigma, respectively. The soybean agglutinin (SBA) was prepared by affinity chromatography on galactosylated Spheron (Lachema, Brno, Czechoslovakia), as described by Filka *et al.* (17).

Reagent solutions used were glycine-buffered saline (GBS): 0.1 M NaCl, 0.17 M glycine, 0.04 g/l NaN_3, pH adjusted to 7.0 with NaOH (18); GBS–bovine serum albumin (BSA): GBS containing 10 mg/ml BSA (Biograde, Calbiochem); and BGA: GBS containing 10-mg/ml BGA.

The term saline is used throughout this paper for a 0.9% w/v NaCl solution.

Latex coating was carried out as follows. BGA (100 μl) was added to 100 μl of latex suspension, Vortex mixed, and then left to stand at 37°C for 30 min. Then 100 μl of GBS–BSA was added, the mixture was Vortex mixed, incubated at 37°C for another 30 min, centrifuged, and then washed twice with 300 μl of GBS–BSA. The pellet was resuspended in 300 μl of GBS–BSA. The mixture was diluted twice with saline for the assay. If not directly used, the mixture was stored at 4°C or in lyophilized form. The lyophilized latex was resuspended in distilled water before use.

Latex agglutination assay was carried out by spotting equal amounts of coated latex suspension and lectin solution onto microscope slides or microtiter plates. Agglutination was observed after 1 h at room temperature.

Hemagglutinating activity was assayed by the doubling-dilution technique on microtiter plates using a 1% suspension of washed (×4) trypsinized human blood group A erythrocytes. The trypsinized red cells were prepared as described by Turner and Liener (19). The agglutination titer was expressed as the highest dilution of a 1% w/v sample solution showing detectable erythroagglutination.

RESULTS AND DISCUSSION

Coating of polystyrene latex was carried out by physical absorption according to the general principles of antibody fixation tests (18). Under these conditions, BGA is instantly absorbed on the surface of the latex. If latex particles are centrifuged, the supernatant does not inhibit hemagglutination any longer than those tested with WGA, Con A, and SBA, respectively.

BGA-coated latex was found to be strongly agglutinated by Con A, SBA, WGA, and crude extracts of *Helix pomatia* and soybeans. Typical agglutination patterns are shown in Fig. 1. The particulate sediment obtained by this technique is somewhat different from the fibrillar aggregates found with IgG-coated la-

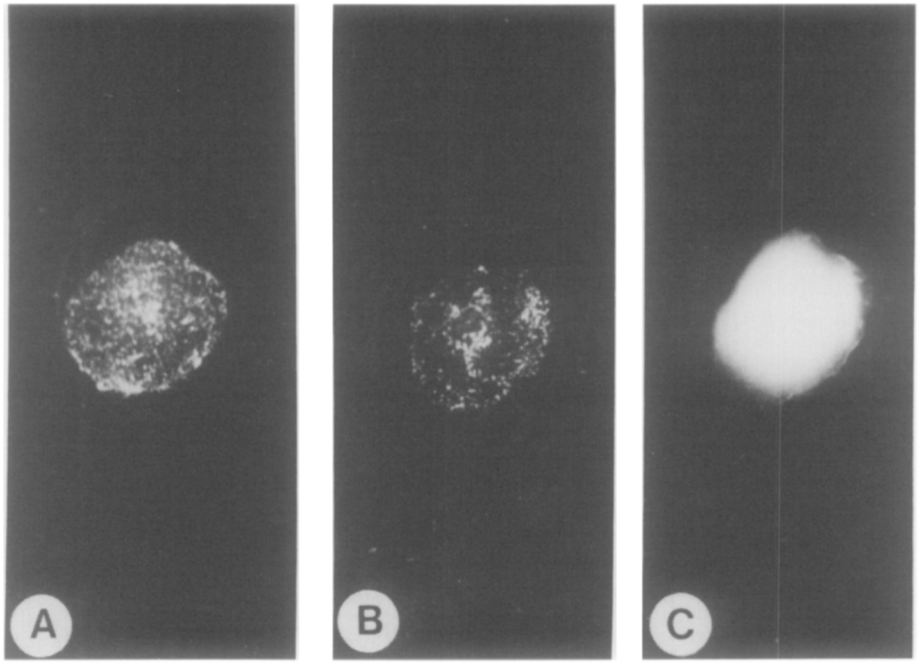

FIG. 1. Agglutination patterns obtained with BGA-coated latex. Magnification is 1.6:1. (A) 2 μg/ml wheat germ agglutinin; (B) 100 μg/ml wheat germ agglutinin; (C) negative pattern (lectins absent or inhibited). 25 μl sample solution + 25 μl latex suspension was spotted onto a microscope slide. The photo was taken after slide had stood 1 h at room temperature.

tex. As with hemagglutination, the picture obtained depends on the shape of the vessel (20). If the test is carried out in concave (U-shaped) wells, nonagglutinated latex will form a circular sediment on prolonged incubation (3–4 h). Positive and negative patterns remain clearly distinguishable, however. No agglutination was found when lectins were incubated with uncoated or BSA-coated latex.

Evaluation was carried out on a semiquantitative basis by serial dilution techniques. The sensitivity of the assay depends on the latex concentration (Fig. 2) and on the amount of ligand bound to the latex (Fig. 3). These dependent variables are similar to those found with antibody-coated latexes, and the "sensitive ranges" appear to be broad enough for analytical applications.

Specificity of the assay was checked by sugar inhibition experiments. The results summarized in Table 1 are in good accordance with the known specificities of the lectins studied, i.e., agglutination was inhibited by the respective monosaccharide haptens, and unaffected by other carbohydrates at similar concentrations. The same was found to be valid for the dissolution of aggregated (not shown), which is the recommended criterion for determining lectin specificity (5).

In a search for nonspecific interfering substances, it was found that sodium chloride, sodium hydrogen carbonate, sodium acetate, ammonium hydrogen carbonate, ammonium acetate, and Tris buffer do not interfere with the test up to a concentration of 1 M at pH 7. Ca^{2+} ions in concentrations beyond 0.1 M were found to form a microparticulate sediment with BGA-coated latex. This phenomenon can be clearly distinguished from the macroscopic aggregates obtained with lectins (Fig. 1) and can be prevented by adding 0.5 M NaCl to the sample solutions. Mg^{2+}, Mn^{3+}, and Zn^{2+} in similar concentrations do not interfere with the test. Various proteinaceous

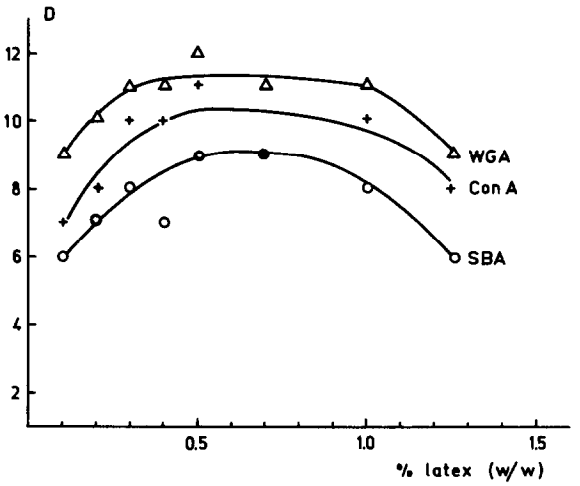

FIG. 2. Agglutination of BGA-coated latex by lectins and the effect of latex concentration (w/w), where D is the number of doubling dilutions showing detectable agglutination. Undiluted lectin solutions contained 10 mg/ml of lectin. The coated latex was prepared as described under Materials and Methods; the latex concentration was varied by adding different volumes of GBS–BSA after the last washing.

materials known to lack erythroagglutinating activity (chicken ovomucoid, soybean trypsin inhibitor, chicken ovalbumin, muscle phosphorylase *b*, wheat meal extract, soybean meal extract inactivated by heat treatment, polyglutamic acid, and polylysine) were checked for nonspecific agglutination. Of the materials tested, only polylysine showed a slight tendency to agglutinate the latex (both BGA-coated and uncoated forms). This phenom-

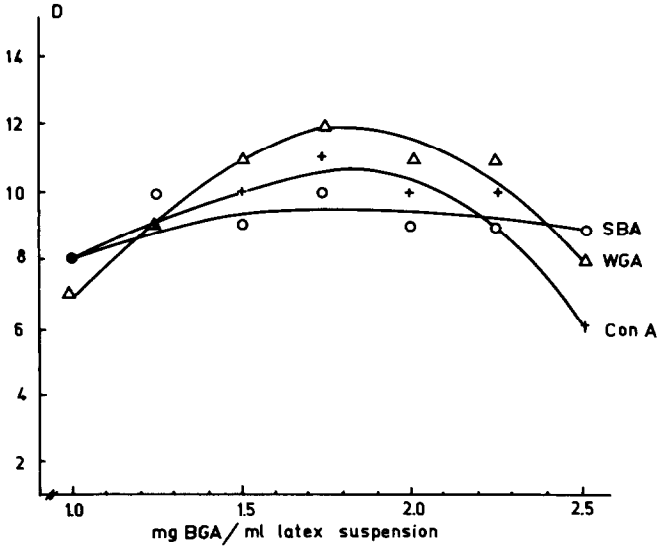

FIG. 3. Effect of different BGA loadings of latex on its agglutination by lectins. Increased BGA loading was obtained by incubating the latex with various BGA concentrations. Concentrations indicated here refer to the final volume of the latex suspension (prior to addition of the lectin sample). D is the number of doubling dilutions showing detectable agglutination.

TABLE 1

SPECIFICITY OF THE BGA–LATEX ASSAY FOR LECTINS[a]

	Con A	WGA	SBA	Saline
BGA-coated latex	10	11	9	—
+GalNAc (20 μM)	10	10	—	—
+Gal (20 μM)	9	9	—	—
+Man (20 μM)	—	10	9	—
+Glc (20 μM)	2	11	8	—
+GlcNAc (20 μM)	10	3	9	—
+BGA (1 mg/ml)	—	—	—	—
+NaCl (0.5 M)	10	12	10	—
+$NaHCO_3$ (0.5 M)	10	11	10	—
+NH_4HCO_3 (0.5 M)	10	12	9	—
+NH_4-acetate (0.5 M)	8	10	8	—
+Tris–HCl (0.5 M)	9	10	9	—
+$CaCl_2$ (0.1 M)	5	7	5	2
BSA-coated latex	—	—	—	—
Human erythrocytes[b]	12	14	11	—

[a] Results expressed as number of doubling dilutions showing detectable agglutination. Starting value of dilution corresponds to 1% lectin by weight.

[b] Blood group A, trypsinized.

enon, probably an effect due to negatively charged groups present on the latex surface (25), could be suppressed by including 0.17 M glycine or 0.5 M NaCl in the test mixture. The above results indicate that, under appropriately selected conditions, BGA-coated latex can be used as a specific probe for lectin-type activity.

The sensitivity of the latex assay described here approaches that of the visually evaluated hemagglutination test (Table 1). The detection limits of these methods are several orders of magnitude lower than those reported for precipitation tests (21–25). Crude BGA purified by the phenol–ethanol procedure appears to be satisfactory for routine applications such as monitoring column effluents. In fact, we could not show any reproducible difference between latex preparations coated with crude and affinity-purified BGA. Affinity-purified BGA was used throughout this study in order to minimize the possibility of nonspecific interferences.

Advantages of the assay proposed here can be summarized as follows. Unlike erythrocytes, coated latex is a homogeneous and chemically well-defined test system where the number and quality of lectin-reactive receptor molecules can be varied at will. This makes it possible, simultaneously, to distinguish nonlectin-type agglutination by applying carbohydrate-free (e.g., BSA-coated) latex as a control. The reagents are chemically stable for long periods of time and their use eliminates many of the problems associated with red cell suspensions such as variability of individual preparations and sensitivity to microbial attack.

The agglutination assay described here may be easily adapted to various lectin–glycoprotein interactions in cases where the glycoprotein can be effectively fixed to the latex surface with its carbohydrate side chain(s) pointing toward the solvent. However, the scope of the method is not necessarily restricted to glycoprotein ligands. According to our preliminary results, it is possible, under the conditions described here, to fix Dextrane D 500 (Serva, average M_r 500,000), which yields a latex agglutinable with Con A but not with SBA. It should be noted that ligands investigated by these techniques can include those that are not precipitated by lectins from solution, such as glycoproteins having only one lectin binding site. Absorptive fixation used in this study may be ineffective with small-molecular-weight or polar ligands. These difficulties may be overcome, however, using covalent fixation techniques such as the coupling of protein with carbodiimide to carboxylated latex (18).

Finally, it should be mentioned that, similar to erythroagglutination, latex agglutination can be quantitated by turbidimetry (23), light-scattering (24), and particle-counting techniques (18). These principles may substantially add to the sensitivity of the method described here.

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