# Interaction of Lectins with Membrane Receptors on Erythrocyte Surfaces

LANPING AMY SUNG, ELVIN A. KABAT, and SHU CHIEN
Departments of Human Genetics and Development, Microbiology, and Physiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

ABSTRACT    The interactions of human genotype AO erythrocytes (red blood cells) (RBCs) with N-acetylgalactosamine-reactive lectins isolated from Helix pomatia (HPA) and from Dolichos biflorus (DBA) were studied. Binding curves obtained with the use of tritium-labeled lectins showed that the maximal numbers of lectin molecules capable of binding to human genotype AO RBCs were $3.8 \times 10^5$ and $2.7 \times 10^5$ molecules/RBC for HPA and DBA, respectively. The binding of one type of lectin may influence the binding of another type. HPA was found to inhibit the binding of DBA, but not vice versa. The binding of HPA was weakly inhibited by a $\beta$-D-galactose–reactive lectin isolated from Ricinus communis (designated RCA1). Limulus polyphemus lectin (LPA), with specificity for N-acetylneuraminic acid, did not influence the binding of HPA but enhanced the binding of DBA. About 80% of LPA receptors (N-acetylneuraminic acid) were removed from RBC surfaces by neuraminidase treatment. Neuraminidase treatment of RBCs resulted in increases of binding of both HPA and DBA, but through different mechanisms. An equal number $(7.6 \times 10^5)$ of new HPA sites were generated on genotypes AO and OO RBCs by neuraminidase treatment, and these new sites accounted for the enhancement (AO cells) and appearance (OO cells) of hemagglutinability by HPA. Neuraminidase treatment did not generate new DBA sites, but increased the DBA affinity for the existing receptors; as a result, genotype AO cells increased their hemagglutinability by DBA, while OO cells remained unagglutinable. The use of RBCs of different genotypes in binding assays with $^3$H-labeled lectins of known specificities provides an experimental system for studying cell–cell recognition and association.

Cell–cell interaction in multicellular organisms is a fundamental phenomenon which includes cell–cell recognition, communication, adhesion, and rearrangement, etc. Most of these interactions are mediated by the cell surface (1–3), which contains a number of surface membrane proteins or glycoproteins responsible for cellular recognition or junction formation (4, 5). The cell surface membrane–bound glycosyltransferases and the sugar acceptors have been implicated in dynamic intercellular adhesion and dissociation (6–8). Lectins are a group of carbohydrate-binding proteins or glycoproteins isolated from plants and animals capable of recognizing specific sugar residues and their linkages. Different types of lectins have been used extensively to study the topology of cell surfaces (2, 9), including cells during differentiation (10, 11) and after transformation (12).

Many lectins can agglutinate human erythrocytes (red blood cells) (RBCs)[1] (2). Indeed, RBC agglutination is the usual method for detecting the presence of lectins in a solution and has contributed to the discovery of many lectins (13). A considerable number of lectins are blood group-specific, and some recognize A, B, H, Le[b], and N determinants (9, 14). In the present investigation, two blood group A–specific lectins, obtained from Helix pomatia (15–18) and Dolichos biflorus (19–21), were used to react with genotype AO human RBCs, with the aim of studying the interaction of these molecules

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[1] Abbreviations used in this paper:   DBA, Dolichos biflorus agglutinin or lectin; GalNAc, N-acetylgalactosamine; HPA, Helix pomatia agglutinin or lectin; LPA, Limulus polyphemus agglutinin or lectin; RBC, erythrocyte of human, genotype AO, unless stated otherwise; RCA1, an agglutinin isolated from Ricinus communis; TSA, Tris-buffered saline containing 2.5 mg/ml of bovine serum albumin, pH 7.4.

with their receptors on the cell surface membrane and elucidating this specific recognition process of cell–cell association and dissociation.

*Helix pomatia* lectin (HPA, A standing for agglutinin), which was isolated from the albumin glands of the edible garden snail (*Helix pomatia*), consists of six subunits, each containing one carbohydrate binding site (15–17). *Dolichos biflorus* lectin (DBA), which was extracted from the seeds of horse gram (*Dolichos biflorus*) (18), consists of four subunits, two type I and two type II; only the type I subunits bind carbohydrates (22). Both HPA and DBA resemble the anti-A antibodies in that they agglutinate human group A but not B or O RBCs, and that they precipitate with blood group A substance and group C streptococcal polysaccharides (16, 19). DBA is even more restricted in its affinity toward blood group substances; it can distinguish subgroup $A_1$ from other subgroups (23). Both lectins were found to have combining sites specific for terminal nonreducing $\alpha$-linked $N$-acetylgalactosamine (GalNAc) (16, 19, 24). These two lectins have overlapping but not identical specificities (25, 26), with HPA lectin having a broader sugar range than DBA.

In the present study, HPA and DBA labeled with [³H] acetic anhydride were used to determine the equilibrium binding of HPA and DBA and the number of their receptor sites on the genotype AO RBC surface. Experiments were designed to evaluate and analyze the factors that may influence the interactions of lectins with their receptors on RBCs, including the competition between lectins for common receptor sites, the possible roles of steric hindrance and charge interference by other lectin molecules, and the effect of removal of $N$-acetylneuraminic acids. The analysis of these experimental data has provided a molecular basis for understanding the mechanism of hemagglutination induced by these lectins under different conditions.

## MATERIALS AND METHODS

*Materials:*    [³H] acetic anhydride (5.4 Ci/mM) was purchased from Amersham Corp. (Arlington Heights, IL). DBA (molecular weight ≈110,000), *Limulus polyphemus* lectin (LPA) (≈400,000), HPA (≈79,000), and an agglutinin isolated from *Ricinus communis* (RCA1) (≈120,000) were purchased from E.Y. Laboratory (San Mateo, CA). HPA was also purified in E.A. Kabat's Laboratory, with polyleucyl hog A + H blood group substances as previously described (15). The purity of these lectin preparations is shown in the SDS PAGE pattern (Fig. 1). PAGE was run according to the method of Fairbank et al. (27).

*Preparation of Tritiated Lectins:*    A break-seal ampoule containing 25 mCi of [³H] acetic anhydride, kept in dry ice with ethanol, was broken at the fine tip by two 4–5-mm diameter glass beads while the ampoule was stoppered with a ground glass cone. The released [³H] acetic anhydride was allowed to equilibrate with the previously added 1.25 ml of acetonitrile for 15 min. A 50-μl volume of the equilibrated solution was pipetted into a glass test tube that contained 1 ml of a lectin solution (1 mg/ml) and 0.1 ml of 1 M carbonate buffer (pH 8.03), giving a [³H]acetic anhydride:lectin ratio of 1 mCi/mg. Acetylation of HPA, DBA, and LPA was allowed to proceed at 4°C for 20 h in the sealed test tubes. The radiolabeled lectin solutions were dialyzed against a large volume of 0.001 M phosphate buffered saline (PBS), pH 7.2, which was changed every 24 h for 8 d to remove free radioactivity.

*The Residual Dialyzable Radioactivity of Lectin Solutions:*    Each radiolabeled lectin solution prepared as above (1 or 2 ml) was dialyzed in a small dialysis bag against an equal volume of PBS in a tightly sealed small vial at 4°C with constant rotation for 8 d without changing the external buffer solution. Every 24 h, 10 μl of solution outside of the dialysis bag was removed, mixed with 3 ml of Soluene 350 and 6 ml of toluene (containing Ominflour, 4.0 g/liter), and counted in a liquid scintillation counter (Siemens Gammasonics Inc., Des Plaines, IL). The equilibrium dialysis was continued until the external radioactivity, recorded as counts per minute (cpm),
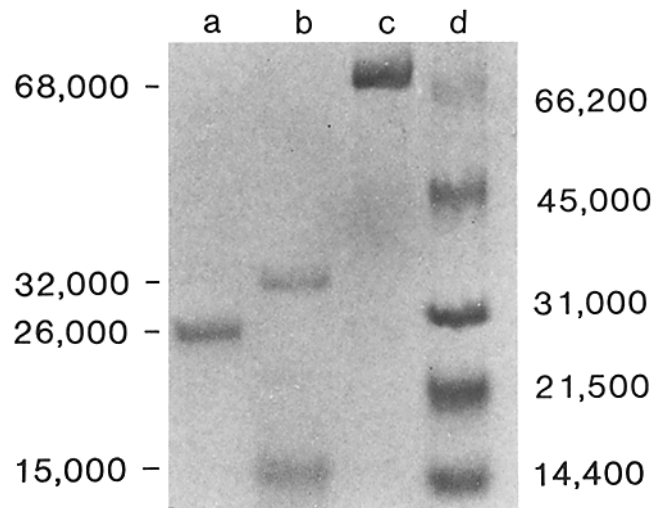
reached a stable reading. The radioactivity of each lectin solution inside the dialysis bag was then determined by the same scintillation counting method. The amount of radioactivity in the external solution at equilibrium was compared to that of the lectin solution inside the dialysis bag at the end of equilibrium dialysis for the calculation of the percentage of dialyzable radioactivity.

*The Specific Activity of the Lectin Solution:*    The concentration of each radiolabeled lectin solution (in μg N/ml) was determined by multiplying the absorbance (O.D.) at 280 nm by a conversion factor, which had been established for each lectin by determining its O.D. at 280 nm and total nitrogen with the ninhydrin method and by assuming nitrogen content to be 16% (28). The radioactivity of each lectin solution at a known concentration (in cpm/μg) was determined by scintillation counting, and the specific activity was calculated.

*Cell Count in 2% RBC Suspension:*    The cell number per ml of the 2% RBC suspension was determined to be $2 \times 10^8$ RBCs/ml with the use of a Coulter Counter (model $Z_B$, Coulter Electronics Inc., Hialeah, FL).

*Hemagglutination Assay:*    Twofold serial dilutions of the lectin solution (25 μl of each) were made in a 96-well microtitrator plate (Cooke Engineering Company, Alexander, VA). An equal volume (25 μl) of a 2% RBC suspension was then added into each well. After 1 h at 25°C, the settling patterns were read to determine the agglutination titer. The highest dilution of the lectin solution showing the detectable agglutination is called the titer.

*Binding of Lectins to RBCs:*    Fresh normal genotype AO human RBCs were washed three times with Tris-buffered saline containing 2.5 mg/ml bovine serum albumin, pH 7.4 (TSA). The washed packed cells were suspended in the same buffer solution to give a 2% RBC concentration by volume. Conical glass centrifuge tubes (3 ml or 5 ml) were used to carry out the cell binding assays. The tubes were acid washed, soaked in 5 mg/ml bovine serum albumin, drained, and dried before use. Varying amounts of radiolabeled lectin solution were mixed with a fixed number of human RBCs ($1 \times 10^7$) suspended in PBS to give a total volume of 200 μl. The mixture was kept at 4°C for 21 h with constant rotation. The cell suspensions were centrifuged, and aliquots of the supernatant were taken for scintillation counting to determine the amount of free lectin at the end of the reaction. The pellets were washed twice with PBS, dissolved in 300 μl of Soluene 350 and 6 ml of toluene-based scintillation fluid, and counted to determine the amount of lectins bound to the RBCs.

*Removal of N-Acetylneuraminic Acids from RBC Surfaces:*    These experiments were performed on fresh normal human RBCs with genotypes AO and OO. $1 \times 10^7$ RBCs were incubated with 250 units of neuraminidase (from *Vibrio Cholerae*, 500 U/ml) (Calbiochem-Behring Corp., San Diego, CA) in 5 ml of TSA at 37°C for 1 h. After this treatment RBCs were washed twice with the TSA solution and prepared as 2% suspensions in the same buffer solution.

*Determination of Electrophoretic Mobility of RBCs:* The electrophoretic mobility of the normal or neuraminidase-treated RBCs (0.1% suspensions) was determined in a cylindrical microelectrophoresis apparatus (Grant Instruments [Cambridge] Ltd., England) at 25°C (29, 30). The velocity of individual cells in the electric field was measured by using a microscope with a water-immersion objective. Twenty such measurements were made in alternate directions with a polarity switch. From the velocity ($\mu$m/s) of the cells and the voltage gradient (V/cm) of the electrical field, the electrophoretic mobility ($\mu$m-s$^{-1}$/V-cm$^{-1}$) of the cells was determined.

## RESULTS

By small volume equilibrium dialysis, the residual dialyzable radioactivities of the lectin solutions radiolabeled with [$^3$H] acetic anhydride were determined to be 0.2, 0.5, and 0.6% of their total radioactivities for HPA, DBA, and LPA, respectively. Their specific activities were $8.5 \times 10^4$ cpm/$\mu$g (for HPA), $8.0 \times 10^4$ cpm/$\mu$g (for DBA), and $2.9 \times 10^3$ cpm/$\mu$g (for LPA). Hemagglutination assays showed that titers of the three lectins were not changed after radiolabeling.

These radiolabeled lectin solutions, unchanged in their agglutinabilities, containing minimum dialyzable radioactivities and having sufficient specific activities, were then used in the cell binding assays. The binding assays were performed to determine the number of lectin molecules bound to each RBC and the number of receptor sites on the RBC for each of the lectins. The RBCs used were genotype AO unless otherwise stated. The binding curve for HPA is shown in Fig. 2a. When HPA lectin was <0.02 $\mu$M, practically all the added molecules were bound to RBCs. With the addition of more HPA, the slope of the binding curve changed to reach a saturation level equivalent to $3.8 \times 10^5$ molecules/RBC. Determination of binding at various time periods between 1

and 24 h indicated that equilibrium was already reached by 1 h.

Fig. 2a also shows the binding curve for DBA. With increasing amounts of DBA, there was a progressive increase in binding to RBCs, but saturation was not reached even with 2.6 $\mu$M of the lectin. To estimate the maximum amount of DBA that can be bound to the RBCs, a reciprocal plot according to Steck and Wallach (31) was used (Fig. 2b). The reciprocal of lectin bound was plotted against the reciprocal of free lectin at the end of reaction. The maximum number of bound DBA was thus estimated to be $2.7 \times 10^5$ molecules/RBC.

A similar binding assay was carried out for $^3$H-labeled LPA in the presence of 25 mM [$Ca^{+2}$] (Fig. 2a). This lectin reacts with $N$-acetylneuraminic acid (32), the sugar residue located mainly at the terminal nonreducing end of the carbohydrate moieties on RBC membrane surfaces. The maximum binding of LPA was estimated, with a reciprocal plot (Fig. 2c), to be $1.5 \times 10^6$ molecules/RBC.

The comparison of the binding curves of these lectins showed that, for a given number of molecules added, there were more molecules of HPA bound to the RBC than DBA, even though these two lectins have a comparable number of receptor sites per RBC ($3.8 \times 10^5$ for HPA and $2.7 \times 10^5$ for DBA). The difference was more dramatic at the lower ends of the curves. The saturation level was reached by the addition of much less HPA (<0.46 $\mu$M) than DBA (>2.6 $\mu$M required). The number of receptor sites for LPA on genotype AO human RBCs was five times as much as those for the other two lectins, and the binding of LPA on RBC exceeds that of the other two lectins for a given number of molecules added. From the numbers of receptor sites for each lectin and the surface area of human RBCs ($\approx$150 $\mu$m$^2$), the receptor density for each of the lectins on RBC surface can be estimated as $2.5 \times 10^3$ receptors/$\mu$m$^2$, $1.8 \times 10^3$ receptors/$\mu$m$^2$, and $10 \times 10^3$ receptors/$\mu$m$^2$ for HPA, DBA, and LPA, respectively.

To test the influence of the binding of one type of lectin on the binding of another, combinations of lectins sharing sugar specificities were added together to a known number of RBCs. $1 \times 10^7$ RBCs were treated with a fixed amount of $^3$H-labeled DBA and varying amounts of unlabeled HPA in a total volume of 200 $\mu$l. The results indicate that the binding of $^3$H-labeled DBA was inhibited by HPA (Fig. 3). When 2.6 $\mu$M of $^3$H-labeled DBA alone was added to $1 \times 10^7$ RBC, $2.4 \times 10^5$ molecules of $^3$H-labeled DBA were bound to each RBC; this number was reduced to one-half when HPA was also added such that $2.3 \times 10^5$ HPA molecules were also bound to each RBC. These findings indicate the existence of overlapping receptor sites for these two lectins. DBA, however, did not cause inhibition of the binding of HPA to RBCs even with ratios of DBA to HPA greater than 100:1. RCA1, a galactose-specific lectin isolated from *Ricinus communis*, caused a weak inhibition of the binding of HPA to RBCs. The presence of 17 $\mu$M of RCA1 with 0.68 $\mu$M of $^3$H-labeled HPA in our test system resulted in a 35% inhibition of the binding of HPA.

To test the possible effect of steric hindrance on the binding of lectins to receptors on RBC surfaces, lectins with no common sugar specificity were paired in the binding assay. LPA was chosen to pair with HPA or DBA; the rationale was that LPA molecules, which are relatively large (molecular weight = 400,000) and bound to $N$-acetylneuraminic acids at nonreducing termini of the sugar moieties at the cell surface, might decrease the accessibility of other lectin molecules to
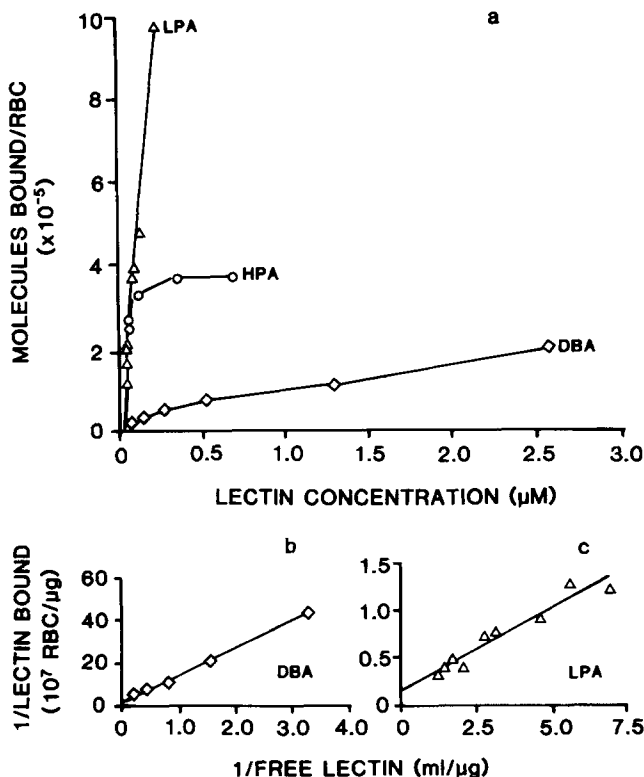


FIGURE 2 (a) Binding of HPA, DBA, and LPA to human genotype AO RBCs. (b) Estimation of DBA receptor sites on human RBCs by Steck-Wallach plot. (c) Estimation of LPA receptor sites on human RBCs.

their receptors. However, LPA (up to 0.3 $\mu$M) had no effect on the binding of 0.02 $\mu$M $^3$H-labeled HPA to human RBCs (Fig. 3), indicating the absence of steric hindrance for this pair of lectins.

There was also no evidence of steric hindrance when LPA was paired with DBA. In fact, the binding of DBA was found to be enhanced by the increasing amounts of LPA added (Fig. 3). Since LPA reacts with $N$-acetylneuraminic acid, a negatively charged residue, it is possible that such an interaction may reduce the electrical charge density of the microenvironment in the vicinity of DBA receptors, thus facilitating the
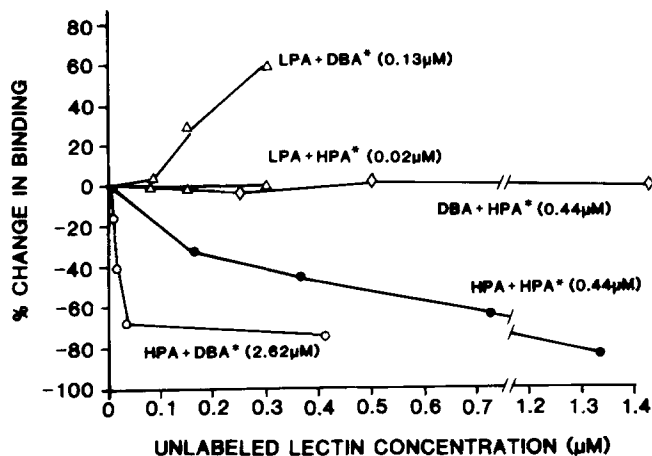


FIGURE 3 Influence of one type of lectin (unlabeled) on the binding of another type of lectin (labeled, marked with *) to human RBCs. Numbers in the parentheses indicate the amount of radiolabeled lectins used in each binding experiment.

binding of the negatively charged DBA molecules (pI = 4.5) as a result of a decreased electrostatic repulsion.

To study the effects of modification of the membrane surface on the binding of HPA and DBA, human RBCs were treated with neuraminidase, which caused the removal of ~80% of $N$-acetylneuraminic acid from genotype AO RBC surfaces, as determined by the $^3$H-labeled LPA binding study (data not shown). The neuraminidase treatment resulted in a threefold increase in the maximum amount of HPA bound, from ~3.8 × $10^5$ to 11.4 × $10^5$/RBC. Neuraminidase also converted OO cells, which had no significant HPA binding prior to treatment, to have HPA binding capacity with a maximum of 7.6 × $10^5$ molecules/RBC (Fig. 4). Therefore, neuraminidase treatment exposed additional sites to allow an additional 7.6 × $10^5$ molecules of HPA to bind to $10^7$ RBCs for both AO and OO cells. These results indicate that, for both cell types, 7.6 × $10^5$ HPA receptor sites were newly generated after neuraminidase treatment.

Neuraminidase treatment also caused an enhancement of DBA binding to AO cells (Fig. 5a). From the Steck-Wallach reciprocal plot, the affinity of binding was found to be increased after neuraminidase treatment, but the maximum number of binding sites per RBC remained unchanged (Fig. 5b), indicating that no new sites were generated in AO cells. In OO cells, no significant binding was found either before or after neuraminidase treatment, and hence there was also no generation of binding sites. The increased affinity of DBA for its receptors on AO cells after neuraminidase treatment may be attributed to the reduction in negative charge density after the removal of $N$-acetylneuraminic acids. The negative charge density of the RBC surface can be determined from the cell mobility in an electrophoretic field (29, 30). Whereas the
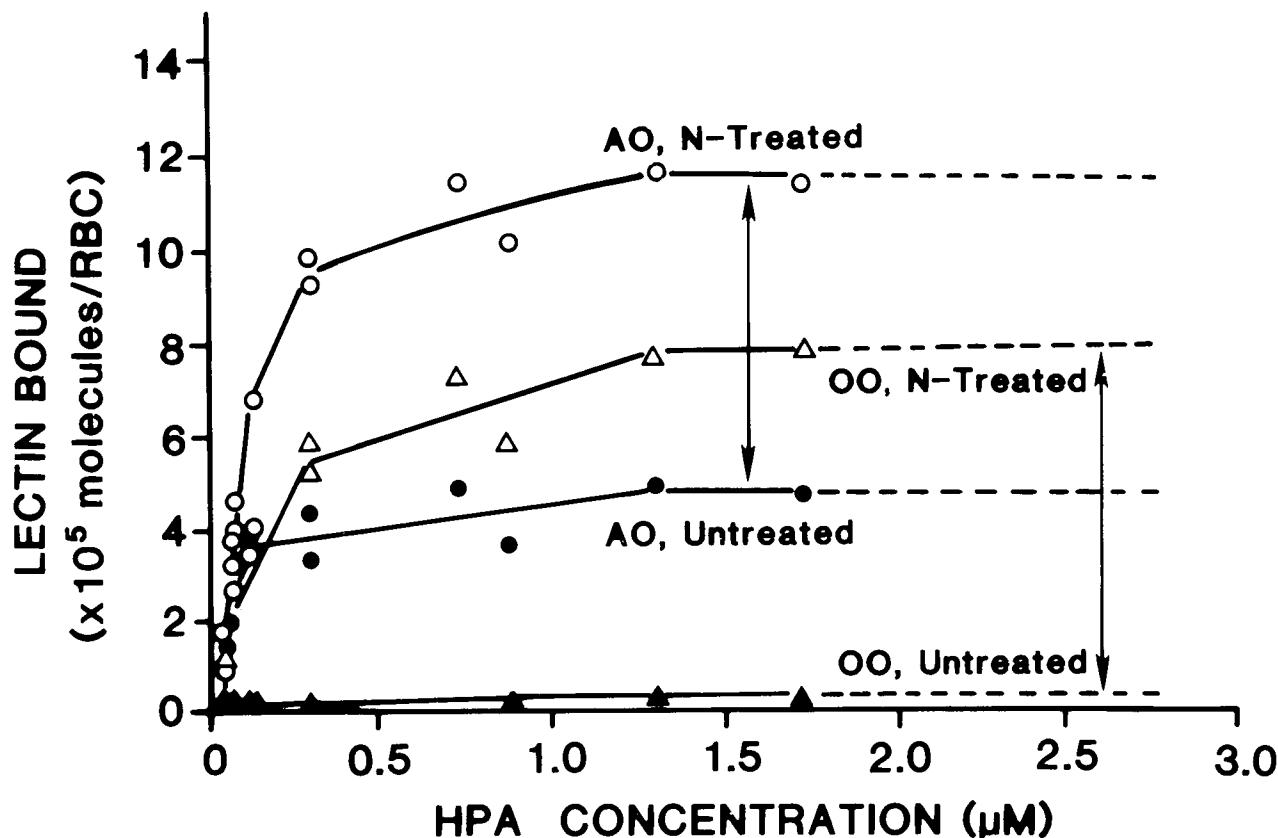


FIGURE 4 Effect of neuraminidase treatment (N-treated) on the binding of HPA to human RBCs.
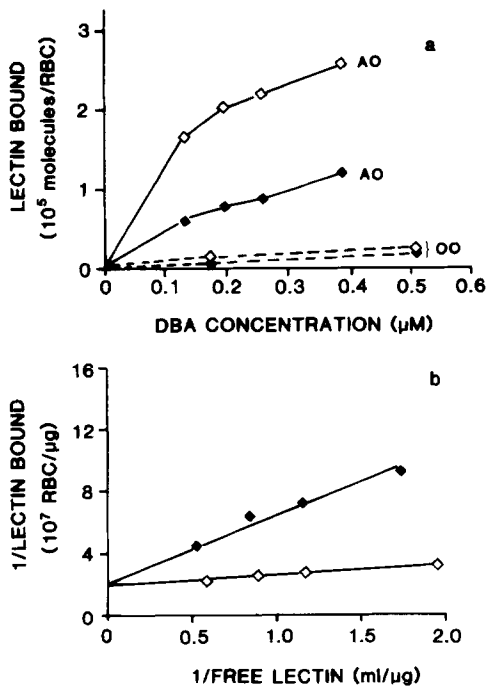
FIGURE 5 (a) Binding curves of DBA on genotype AO and OO cells before (◆) and after (◇) neuraminidase treatment. (b) Estimation of DBA receptor sites on genotype AO human RBCS, before (◆) and after (◇) neuraminidase treatment. The two lines with different slopes coincide at the intercept, indicating no alteration in the number of receptor sites after N-treatment.

TABLE I. *The Agglutinabilities of AO and OO Cells by HPA and DBA before and after Neuraminidase Treatment*

| | | | Titer* of the lectin | |
| | | | Before | After |
| Lectin | Concentration | Cell | treatment | treatment |
|--------|---------------|------|-----------|-----------|
| | $\mu M$ | | | |
| HPA | 14 | AO | 8,192 | 32,768 |
| | | OO | 0 | 4,096 |
| DBA | 7.2 | AO | 64 | 512 |
| | | OO | 0 | 0 |

* See Materials and Methods (*Hemagglutination Assay*).

normal RBC had an electrophoretic mobility of 1.19 $\mu$m-s$^{-1}$/ V-cm$^{-1}$, the neuraminidase-treated RBCs did not move significantly in the electrical field. Molecules of DBA, negatively charged at pH 7.4, would bind more readily to their receptors on the charge-depleted RBC because of a decrease of electrostatic repulsion.

The results of lectin binding assays can be used to explain the hemagglutination findings (Table I): Genotype AO cells treated with neuraminidase became more agglutinable by both HPA and DBA; the titer (the highest dilution of lectin solution showing the detectable agglutination) increased by factors of 4 and 8, respectively. The increase in hemagglutination in both cases is attributable to the number of lectin molecules bound, either by increasing the number of receptor sites (HPA) or by increasing the affinity of binding (DBA). Genotype OO cells, normally not agglutinable by HPA even at an undiluted concentration of 14 $\mu$M, became agglutinable by this lectin, even when the lectin was diluted to $3 \times 10^{-3}$ $\mu$M, after neuraminidase treatment, as $7.6 \times 10^5$ HPA sites/

RBC were generated. Genotype OO cells after neuraminidase treatment remained unagglutinable by DBA, even at the original undiluted concentration of 7.2 $\mu$M, as no DBA receptor sites were generated.

## DISCUSSION

The interactions of two GalNAc–reactive lectins, isolated from *Dolichos biflorus* and *Helix pomatia*, with human genotype AO RBCs have been studied. With the use of tritiated lectins, the number of lectin molecules bound to each RBC was determined as a function of the amount of lectin added. The number and density of receptor sites on human RBC surface for each of the lectins was derived either from the saturation level of the binding curve or from a reciprocal plot analysis. The number of receptor sites for HPA on RBCs of genotype AO obtained in this study using $^3$H-labeled HPA ($3.8 \times 10^5$ sites/RBC) agrees very well with the number of receptor sites on $A_1B$ cells ($3 \times 10^5$ to $5 \times 10^5$ sites/RBC); these sugar residues determined by Hammerstrom (18) using $^{125}$I-labeled HPA. The present finding that genotype AO RBCs have $2.7 \times 10^5$ sites/RBC for DBA is not far from the value of $5.8 \times 10^5$ sites/RBC reported by Carter and Sharon (33) on group A RBC (genotype not specified) using $^{14}$C-labeled DBA and is almost identical to the $2.6 \times 10^5$ sites/cell reported by Roland and Bourrillon (34) for the chick embryo cell. With the use of $^{125}$I anti-A antibody, Economidou et al. (35) found $4.6 \times 10^5$ to $8.5 \times 10^5$ blood group A-specific sites per $A_1B$ cell, which is comparable to the numbers of receptor sites for HPA and DBA. It is noted that these numbers are very close to the copy number of the major RBC membrane glycoprotein, glycophorin ($4 \times 10^5$ copies) (36). Glycophorin molecules, however, are unlikely to be the glycoproteins that react with HPA and DBA, because there is indication (33) that the glycoprotein receptor for DBA is distinct from the peanut agglutinin receptor which is similar to asialoglycophorin. Besides, each glycophorin contains 16 oligosaccharide chains with a total of ~30 terminal sugar residues of $N$-acetylneuraminic acid with galactose and GalNAc in the middle of the chain which may not be available for HPA and DBA to react with (37, 38).

That HPA can inhibit the binding of DBA suggests that these two lectins share some common receptor sites on human genotype AO cells. Both lectins can precipitate blood group A substance, specifically agglutinate blood group A RBCs, and react with $\alpha$-D-GalNAc-(1→3) D-GalNAc, and D-GalNAc (12, 14, 15, 28); these findings further support the suggestion that they share common receptors. However, DBA was found not to inhibit the binding of HPA on RBCs even at DBA:HPA ratios higher than 100:1; this suggests that HPA may have a much higher binding affinity for the primary receptors than DBA and that HPA may be able to bind to other sugar receptors which have little or no affinity for DBA. It has been shown that HPA also reacts weakly with GlcNAc and very weakly with galactose, whereas DBA does not react with either (16, 19, 24). This broader specificity for HPA, together with the finding that RCA1, a galactose-specific lectin, can inhibit weakly the binding of HPA to RBCs, supports the notion that the receptors on RBCs for HPA and DBA are not identical.

The binding of DBA to genotype AO RBCs is enhanced by the removal of $N$-acetylneuraminic acids from the cell surface and the interaction of $N$-acetylneuraminic acid with LPA. These findings suggest that the interaction of DBA and its

receptor is sensitive to the neighboring electric field at the cell surface. Since both $N$-acetylneuraminic acid and DBA (pI = 4.5) are negatively charged, and since the removal of $N$-acetylneuraminic acid does not change the number of receptor sites for DBA (a finding which confirms a report by Carter and Sharon [33]), it is most likely that a reduction in surface electrostatic repulsive potential leads to the increase in the affinity between DBA and its RBC receptor after neuraminidase treatment.

Neuraminidase treatment generated new receptor sites (7.6 × 10⁵ sites/RBC) on both AO and OO cells for HPA but not for DBA. The newly exposed sugar residues are most likely to be galactose, based on the reason that HPA reacts with galactose (in addition to GalNAc), whereas DBA does not. In addition, it has been shown that neuraminidase treatment leads to the generation of new receptor sites (1.8 × 10⁶ sites/RBC) for peanut agglutinin, which is a galactose-binding lectin, on RBCs of both A and B types (33). Thus, the galactose residues exposed after the removal of the terminal $N$-acetyl-neuraminic acid by neuraminidase treatment may serve as new receptors for lectins that react with galactose.

The experiments presented here indicate that removal of the $N$-acetylneuraminic acid residues on RBCs with neuraminidase may reduce surface charge density and expose new sugar residues and that the relative importance of these two mechanisms in influencing lectin binding varies with the lectin. The mechanisms discussed may also be used to explain the different behaviors in hemagglutination after neuraminidase treatment of RBCs bearing different genetically determined antigens (AO and OO cells in this study). By generating new sites for HPA, neuraminidase treatment caused an increase in agglutinability of both AO and OO cells by HPA. By reducing surface charge and electrostatic repulsion, neuraminidase treatment caused an increase in agglutinability by DBA only in AO cells, but not in OO cells since no new sites were generated.

The present study has provided a molecular basis for understanding the interaction of lectins with their receptors on cell surfaces and helps to elucidate the mechanisms by which agglutination occurs. Quantitation of the lectin molecules bound on the cell surface provides useful information for analyzing the energies involved in lectin-induced aggregation (39). The methodology and the mechanisms described may be applied to studies on other cell systems, including those in normal or diseased states and during development and differentiation, and on problems related to cell surface topography, cell–cell recognition, and cell–cell association.

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