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Evaluation of Glycoconjugates on the K562 Cell Surface by Means of Lectin Binding — Comparison with Human Erythrocytes

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The binding of five radiolabelled lectins (*Vicia graminea*, peanut, *Phaseolus vulgaris* isolectins E-PHA and L-PHA, *Evonymus europaeus*) to untreated and desialylated K562 cells and human erythrocytes was compared. The number of glycophorin A receptors recognized on the K562 cells by anti-blood group N *V. graminea* lectin was comparable to that found on the MN or NN erythrocyte surface. However, K562 cells had a several-fold higher number of oligosaccharide chains (presumably *O*-glycosidic) which after desialylation became high-affinity receptors for peanut agglutinin, and of complex type *N*-glycosidic chains available for the reaction with E-PHA and also with L-PHA (the latter lectin was not bound to erythrocytes). Moreover, K562 cells not treated with neuraminidase had a significant amount of extremely low affinity receptors for peanut agglutinin, whereas binding of this lectin to untreated erythrocytes was undetectable. On the other hand, the untreated K562 cells did not bind anti-blood group B and H *E. europaeus* lectin, but a small amount of binding by the desialylated cells was observed. Some other differences observed in the mode of lectin binding to K562 cells and erythrocytes are discussed.

A pluripotent human leukemia cell line (K562) is particularly interesting, since it shows spontaneously or after induced differentiation various markers of erythrocytic, myeloid, megakariocytic, and even lymphocytic progenitors [1] and is the commonly used target for testing *in vitro* the activity of natural killer (NK) cells [2]. Therefore, this cell line offers an excellent model to study the mechanisms of biosynthesis, cell differentiation and natural killing. Characterization of cell surface glycoconjugates is important for such studies, since many of them are markers of cell differentiation or function. Glyco-

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proteins and glycolipids of K562 cell membranes were studied by immunological and chemical methods. K562 cells contain some surface components identical or similar to those present on erythrocyte membranes, including glycophorin A [3, 4], erythroglycan-type oligosaccharide chains [5], fetal antigen i [6-8], and the I antigen which is expressed on a minor cell fraction [7, 8]. On the other hand, K562 cells do not express blood group ABH antigens [8-10] and show different patterns of glycolipids [8, 11], surface glycoproteins [3] and smaller size *N*-glycosidic oligosaccharide chains [12] compared to erythrocytes. Moreover, some platelet glycoproteins were immunologically detected on K562 cells [13].

Limited information is available about the interaction of K562 cells with lectins. It was found by fluorescent methods that K562 cells failed to react with several blood group ABH-specific lectins [10], but did react with *V. graminea* [10] and peanut [10, 14] lectins. Several surface glycoproteins of K562 cells were bound to wheat germ agglutinin [13]. Studies on the interaction of lectins with K562 cells deserve attention, since lectins may be useful for characterization of membrane glycoconjugates at various differentiation stages, for isolation of membrane components and even elucidation of their function.

In this report we present the results of the binding of five radiolabelled lectins of defined specificity to K562 cells and to human erythrocytes, used as reference cells. The aim of these studies was to obtain information about the existence and amount of receptors for these lectins on the K562 cell surface.

Materials and Methods

K562 Cells

The cells were obtained from Dr. L.C. Andersson (University of Helsinki). They were maintained as a stationary suspension culture, using standard tissue culture conditions in RPMI 1640 medium (Flow, U.K.) supplemented with 10% heat-inactivated fetal calf serum, 2mM glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml). Cell cultures were free of mycoplasma and the cells were harvested when their concentration approached 0.8-1 $\times$ 10^6 cells/ml. The cells were used for experiments on the same day that the culture was terminated, and their viability was over 90%.

Erythrocytes

Human blood samples were collected into an anticoagulant solution from healthy volunteers with known blood groups and were stored at 4°C for up to one week. Erythrocytes were washed with phosphate-buffered saline (PBS: 0.075 M phosphate buffer pH 7.4 and 0.075 M NaCl) directly before using and were adjusted to the required concentration by counting in a hemocytometer.

Desialylation of Cells

K562 cells or erythrocytes were treated with *Vibrio cholerae* neuraminidase (Serva, W.Germany) for 1 h at 37°C under conditions used by Sadler *et al.* [15]. The released sia-

lic acid was determined in the supernatant fluid by the periodate/resorcinol method [16], using *N*-acetylneuraminic acid (Koch-Light, U.K.) as a standard.

Lectins

Purification of all lectins was performed with the use of human asialoglycophorin A (coupled to Sepharose or Affi-Gel) as affinity adsorbent. Details of purification of *V. graminea* [17, 18], peanut [19] and *E. europaeus* [20] lectins have been described previously. Leuko- (L-PHA) and erythroagglutinin (E-PHA) from *P. vulgaris* (isolectins L_4 and E_4, respectively) were purified from the commercial preparation of Phytohemagglutinin P (Difco, U.S.A.). The lectins were first fractionated on an asialoglycophorin-Sepharose 4B column, equilibrated with 6.7 mM phosphate buffer pH 7.2 containing 0.15 M NaCl. L-PHA (not bound) was eluted with the column buffer, the bound isolectins were eluted with 0.05 M glycine-HCl buffer pH 3.0 containing 0.5 M NaCl, which has been used previously for elution of PHA isolectins from thyreoglobulin-Sepharose adsorbent [21]. Both the lectin fractions were then fractionated separately on a SP-Sephadex C-50 (Pharmacia, Sweden) column under conditions reported by Leavitt *et al.* [22]. As expected, L-PHA was eluted as an unretarded peak with the equilibration buffer (0.05 M phosphate pH 6.0). The isolectins of the second fraction were bound to SP-Sephadex, and E-PHA (E_4) was obtained in the last protein peak eluted with an NaCl gradient. Purity of the L_4 and E_4 isolectins obtained was assessed by polyacrylamide gel electrophoresis [22]. L-PHA agglutinated mouse thymic lymphocytes at a concentration of 7 μg/ml and did not agglutinate erythrocytes at a concentration of 1 mg/ml, whereas E-PHA agglutinated erythrocytes and lymphocytes at a concentration of 2 μg/ml.

Protein content in lectin solutions was determined by the Lowry method. The purified lectins were stored frozen in small portions.

Radio-iodination of Lectins

Labelling the lectins was carried out by the chloramine T method [23], using carrier-free $Na^{125}I$ (Radiochemical Centre, U.K.) under conditions described in detail previously [18]. The labelled lectins were separated from an excess of reagents by Sephadex G-25 gel filtration. Some lectins were additionally purified by affinity chromatography: *V. graminea* lectin on a Concanavalin A-Sepharose column [18] and E-PHA and peanut agglutinin on an asialoglycophorin-Sepharose column under the same conditions as those used for purification of unlabelled lectins. The recovery of the labelled lectins was evaluated by determination of their agglutinating activity and by inhibition of binding of labelled lectins to cells by the respective cold lectins. Usually 50 μg lectin was used for radio-iodination, the recovery was over 80%, and the specific radioactivity was in the range of 300 - 1300 cpm/ng.

Binding Assay

The assay was performed in PBS buffer containing 0.1% human serum albumin (PBS-alb). K562 cells and erythrocytes were washed twice with PBS and once with PBS-alb and were suspended in the latter buffer at the desired concentration. The labelled lectins

Table 1. The lectins used and their specificity

Lectin	Specificity	References
Vicia graminea	Glycopeptides with clusters of Gal ß1-3 GalNAc- chains. Reaction is enhanced by a hydrophobic residue and weakened by sialylation of the disaccharide chains	24 and refs. therein
Arachis hypogaea	Gal ß1-3 GalNAc-, weakly Gal-, lack of reaction with sialylated chains	25
Phaseolus vulgaris erythroagglutinin (E_4, E-PHA)	Complex type biantennary N-glycosidic chains, containing bisecting GlcNAc ß1-4- residue linked to ß-mannosyl residue	26
Phaseolus vulgaris leukoagglutinin (L_4, L-PHA)	Complex type tri- or tetraantennary N-glycosidic chains, necessary condition: the third antenna linked to C6 of Man α1-6- residue	26, 27
Evonymus europaeus	Fuc α1-2 Gal- R R = Gal α1-3- preferable over R = H (i.e. prefers blood group B to H)	28

were dialyzed (if in another buffer) against PBS-alb. Test sampled of volume 80 - 200 μl, containing the numbers of cells and amounts of labelled lectins indicated in the figure legends, were incubated for 1 h at 20°C with occasional shaking, the cells were then washed twice with 2 ml PBS-alb and their radioactivity was measured. In the case of peanut agglutinin and *E. europaeus* lectin the control samples containing 0.1 M galactose or 0.5 M lactose, respectively, were included in the test. The unspecific binding in the presence of inhibitor was negligible at lower lectin concentrations and did not exceed 5% of the total binding at the highest lectin concentrations used. Other details of the binding assay were as described earlier [18]. The results of binding are presented in the form of Scatchard plots, each point is a mean of values obtained for two identical samples.

Results

The lectins used for characterization of K562 cell surface glycoconjugates were selected on the basis of their defined specificity [24-28] and are listed in Table 1. They react with a high affinity with either *O*-glycosidic or *N*-glycosidic oligosaccharide chains, and three of them (*V. graminea* and the two PHA isolectins) have an extended binding site and are not inhibited effectively by monosaccharides.

V. graminea lectin is known to react with only a few glycoproteins, including human and horse glycophorins which show the structural features indicated in Table 1 [24]. The presence of hydrophobic residues in the receptor seems to be a prerequisite for the reaction of sialylated glycoproteins with *V. graminea* lectin, since this lectin reacts with blood group N glycoprotein and does not react with M sialoglycoprotein [18, 24, 29] due to the presence of an N-terminal leucine versus serine residue in N and M glycoprotein, respectively [30-32]. It is shown in Fig. 1A that binding of *V. graminea* lectin enabled a distinct discrimination between human homozygous NN and heterozygous MN erythrocytes due to the dosage effect of N glycoprotein. Binding of *V. graminea* lectin to K562 cells (Fig. 1B), which most probably occurred exclusively via glycophorin receptors, was similar to binding to MN erythrocytes with two pools of the cells studied. The third pool of K562 cells showed an approximately twofold higher binding, quantitatively resembling the binding to NN erythrocytes and showing a distinct positive cooperation effect. Desialylation of erythrocytes and K562 cells caused a severalfold increase in *V. graminea* lectin binding to both kinds of cells (Fig. 2). The effect of desialylation was more pronounced for MN than for NN erythrocytes, due to the "de novo" lectin binding to blood group M asialoglycoprotein [18, 24, 29]. The blood group of K562 cell glycophorin was determined as MN by Gahmberg *et al.* [4], and as N by Horton *et al.* [10]. The enhancing effect of desialylation on the *V. graminea* lectin binding to K562 cells was closer to that observed for NN erythrocytes (Table 2). Therefore, the results obtained suggested that K562 cells contained blood group N glycophorin in amounts comparable to those present on MN or NN red cells. However, this conclusion must be treated with caution (e.g. the presence of blood group M glycophorin in K562 cells cannot be definitely ruled out), because the course of binding of *V. graminea* lectin to K562 cells and erythrocytes was not identical, especially when the binding to desialylated cells was compared (Fig. 2). The binding to asialo K562 cells showed an apparently lower affinity, which is discussed later.

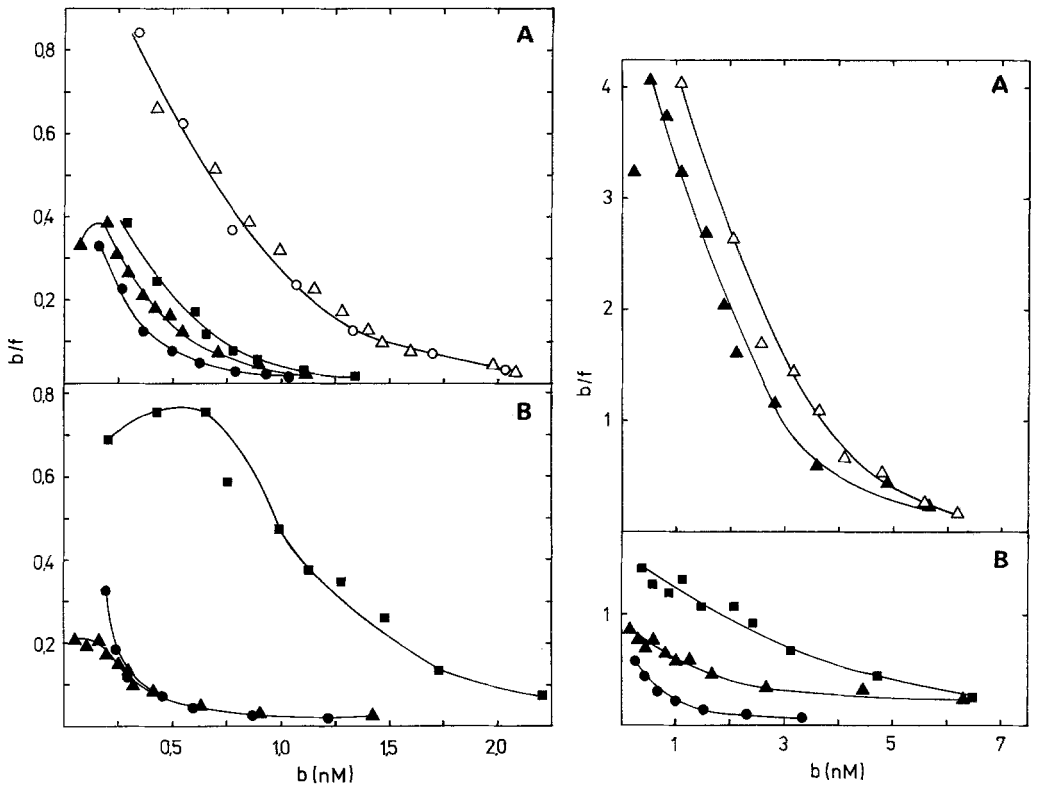

Figure 1. Binding of *V. graminea* lectin to erythrocytes (A) and K562 cells (B). A: Binding to MN (closed symbols) and NN (open symbols) erythrocytes obtained from different persons. B: Different symbols denote binding to different pools of K562 cells. The concentration of cells in all experiments was 6.25 × 10^6/ml; the range of the toal lectin concentration was 0.4 - 60 nM; b = bound lectin, f = free lectin.

Figure 2. Binding of *V. graminea* lectin to desialylated erythrocytes (A) and K562 cells (B). A: Asialoerythrocytes NN (△) and MN (▲). B: Different symbols denote K562 cells from different pools. Other details as in Fig. 1.

Peanut agglutinin reacts with all asialoglycophorins on human erythrocytes in a less specific manner than *V. graminea* lectin, but it does not react at all with sialylated glycoproteins, and therefore neither agglutinates, nor binds to untreated human erythrocytes [29, 33]. Our results of binding of peanut agglutinin to human asialoerythrocytes (Fig. 3A) were similar to those reported by Carter and Sharon [33], and were almost identical using erythrocytes from two different persons. In contrast to erythrocytes, a significant binding of peanut agglutinin to untreated K562 cells was found, but it occurred with extremely low affinity and an unusually extended positive cooperation effect (insert in Fig. 3B). Binding of this lectin to desialylated K562 cells (Fig. 3B) showed in turn the presence of high-affinity receptor sites in six- to tenfold higher amounts than on asialoerythrocytes (Table 2). The "unmasking" of such large amounts of peanut lectin receptors by desialylation was in line with a ten- to twentyfold greater number of sialic

Table 2. Number of lectin molecules bound to one erythrocyte (blood group O) or one K562 cell at the highest lectin concentration used[a]

Lectin	Number of molecules $\times 10^{-6}$	
	Erythrocytes	K562 cells[b]
V. graminea	MN 0.11 NN 0.20 MN 0.54[c] NN 0.59[c]	0.12—0.21 0.32—0.62[c]
Peanut	n.d.[d] 1.8[c]	0.9 10—18[c]
E-PHA	0.4	4.6—7.5
L-PHA	n.d.	2.4—2.6
E. europaeus	0.12 0.17[c]	n.d. 0.02[c]

[a] The saturation of cells with lectins was not achieved, except for the binding of peanut agglutinin and E-PHA to erythrocytes; the real numbers of receptors are therefore higher than the values given.
[b] For K562 cells the range of values obtained with different pools of cells is given (see Figs. 1—6).
[c] binding to desialylated cells.
[d] n.d.: binding was not detectable.

acid residues released from K562 cells by *V. cholerae* neuraminidase, compared to erythrocytes (Table 3). It should be noted that an apparent affinity of binding of peanut agglutinin to asialo K562 cells, albeit high, was several times lower than the affinity of binding to erythrocytes (K_a for the latter binding was calculated to be about 4×10^7 M^{-1}).

As was expected, the binding of L-PHA to erythrocytes was not detectable, but this lectin was bound with a relatively low affinity (K_a about 10^5 - 10^6 M^{-1}) to K562 cells (Fig. 4). The value of over 2×10^6 lectin molecules bound to one cell was calculated for the points far from cell saturation (Table 2) and the real number of oligosaccharide chains able to react with L-PHA must have been much higher.

E-PHA gave a linear Scatchard plot of binding to erythrocytes (Fig. 5A), showing the affinity constant (K_a) 2.2×10^8 M^{-1}, similar to the results of Egorin *et al.* [34]. Surprisingly, binding of E-PHA to K562 cells was at least ten- to twentyfold higher than to erythrocytes (Fig. 5B and Table 2). However, the affinity of binding E-PHA to K562 cells was apparently lower.

Binding of *P. vulgaris* isolectins to erythrocytes and K562 cells was not significantly increased by desialylation of cells, and therefore it is not shown. As in the case of *V. graminea* lectin, the binding of peanut agglutinin and *P. vulgaris* isolectins showed some discrepancies between various pools of K562 cells.

The interaction of *E. europaeus* lectin with erythrocytes occurred with a high affinity, was increased by red cell desialylation in agreement with the earlier findings of Petryniak *et al.* [35], and showed a distinct positive cooperation effect (Fig. 6). Binding of this lectin to untreated K562 cells was undetectable, and barely detectable binding to desialylated K562 cells was found (Fig. 6, Table 2).

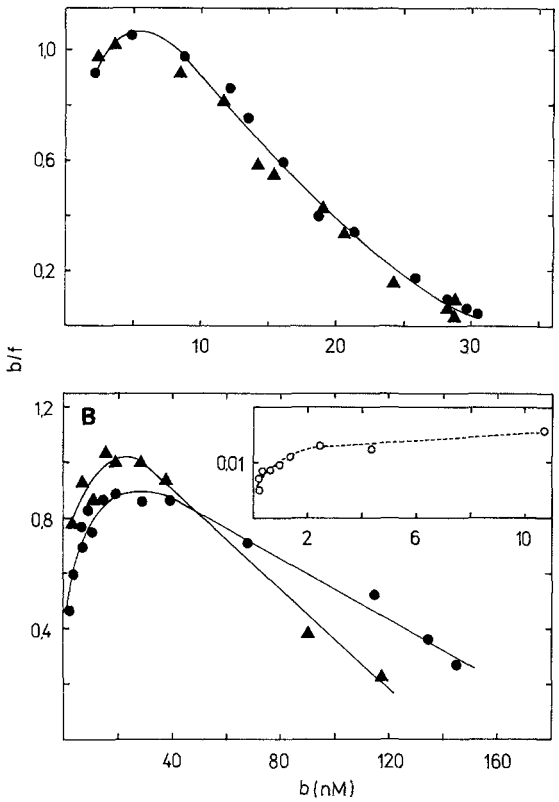

Figure 3. Binding of peanut agglutinin to asialoerythrocytes (A) and K562 cells (B). A: The different symbols refer to asialoerythrocytes from different persons (concentration 10^7 cells/ml). B: Desialylated K562 cells from two different pools at concentrations 5×10^6 cells/ml (●) or 7×10^6 cells/ml (▲). Insert: Binding to untreated K562 cells at concentration 7×10^6 cells/ml. The range of total peanut agglutinin concentration was 5 - 600 nM, the labelled lectin was diluted twentyfold with the unlabelled lectin.

Table 3. The amounts of sialic acid released from K562 cells by *V. cholerae* neuraminidase

Cells	Sialic acid released from one cell	
	nmoles	molecules
K562 pool 1	7.5×10^{-7}	4.5×10^8
pool 2	15.8×10^{-7}	9.5×10^8
pool 3	8.3×10^{-7}	5.0×10^8
Human erythrocytes[a]	$7.0\text{-}8.4 \times 10^{-8}$	$4.4\text{-}5.0 \times 10^7$

[a] The range of many estimations is given

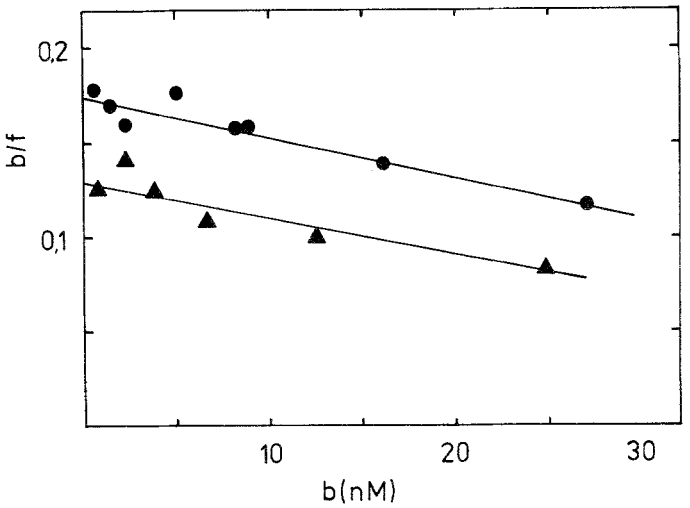

Figure 4. Binding of L-PHA to K562 cells from two different pools. 6.25 × 10^6 cells/ml, the range of lectin concentration was 5 - 300 nM.

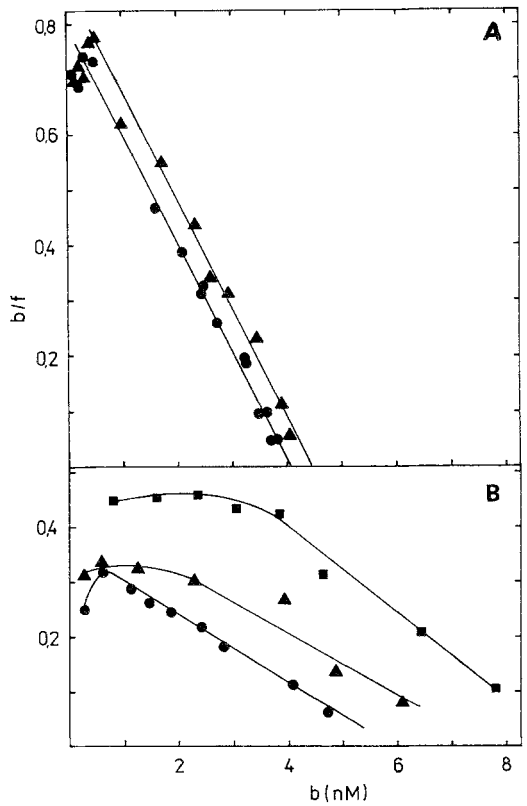

Figure 5. Binding of E-PHA to erythrocytes (6.25 × 10^6 cells/ml) from two individuals (A) and K562 cells (6.25 × 10^5 cells/ml) from three different pools (B). The range of lectin concentration was 1 - 70 nM.

Discussion

The results of lectin binding showed that K562 cells, which are much larger than erythrocytes, have at least a severalfold higher number of *N*-glycosidic oligosaccharide chains able to react with *P. vulgaris* isolectins and of presumably *O*-glycosidic chains reacting (after desialylation) with peanut agglutinin. On the other hand, the comparatively low binding of *V. graminea* lectin to K562 cells indicated that glycophorin A must be a minor glycoprotein component of these cells.

The high content of surface sialic acid in K562 cells and a significant difference in binding of *V. graminea* lectin and peanut agglutinin to native and asialo K562 cells indicated a high degree of sialylation of their *O*-glycosidic oligosaccharide chains. The extremely low affinity of binding of peanut agglutinin to untreated K562 cells suggested that this binding did not result from the presence of a number of nonsialylated Galβ1-3GalNAc-chains, but rather occurred due to the weak interaction of this lectin with other oligosaccharide chains terminated with galactose residues. The high-affinity receptors for peanut agglutinin in desialylated K562 cells may be Galβ1-3GalNAc- chains of glycophorin A and glycolipids terminated with this sequence. However, despite the relatively high content of gangliosides (including G_{M1} in K562 cells [8, 11], their amount is too low [11] to account together with glycophorin for the extensive binding of peanut agglutinin. Most probably K562 cells contain a large amount of other glycoprotein(s) with sialylated Galβ1-3GalNAc- chains.

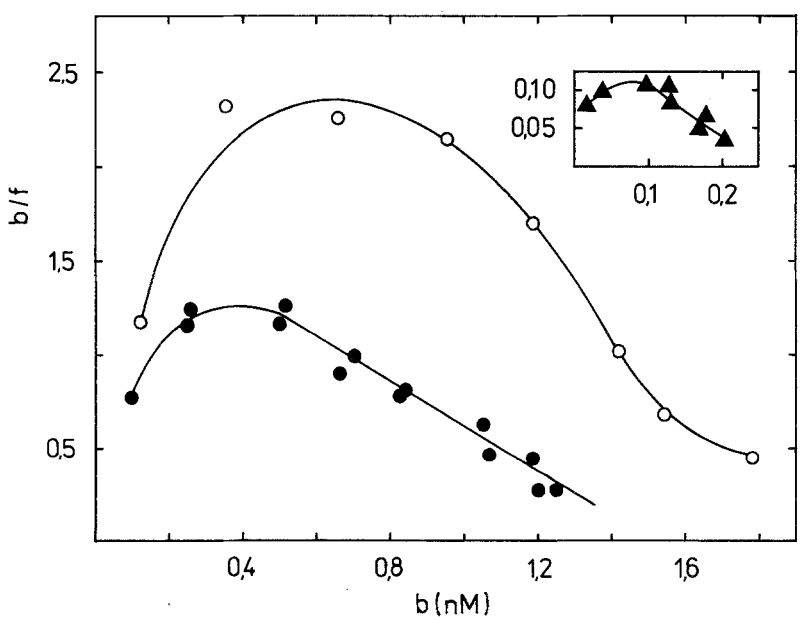

Figure 6. Binding of *E. europaeus* lectin to native (●) and desialylated (○) group O erythrocytes. Insert: Binding of the same lectin to desialylated K562 cells. Concentration of erythrocytes or K562 cells was 6.25×10^6 cells/ml, the range of the total lectin concentration was 0.2 - 6 nM.

The complex type *N*-glycosidic chains of K562 cells represent a variety of bi-, tri- and tetra-antennary structures [12], including those with the third antenna on the Manα1-6-residue, required for the reaction with L-PHA (Table 1). On the other hand, the oligosaccharide chains with "bisecting" *N*-acetylglucosamine residues bound to a β-mannosyl residue, typical for human erythrocyte glycoproteins [12, 36, 37] and found to be receptors for E-PHA [26], were not detected in K562 cells [12]. Therefore, we expected that E-PHA and L-PHA would show a specific binding to erythrocytes and K562 cells, respectively. However, this appeared to be true only for L-PHA, whereas E-PHA was bound in much higher amounts to K562 cells than to erythrocytes. It suggested that the presence of a bisecting *N*-acetyl glucosamine residue is not a necessary condition for the reaction of an oligosaccharide with E-PHA and that this lectin may have a broader specificity than L-PHA.

The lack of binding of *E. europaeus* lectin to K562 cells generally confirmed the known fact that these cells do not express the blood group ABH antigens [8-10]. However, the very small but specific binding of this lectin to asialo K562 cells indicated the possibility that tiny amounts of such structures are present. Similarly, Kannagi *et al.* [8] found a very small amount of blood group H glycolipid in K562 cells.

In addition to the aspects discussed above, some persistent differences in the binding of lectins to K562 cells and erythrocytes were noticed. Firstly, in contrast to reproducible results of lectin binding to erythrocytes of different individuals, the distinct variations (up to twofold differences) in binding of the same lectin to various pools of K562 cells were observed. Since the cells derived from the same clone and were cultured under identical conditions, these variations may be due to cell cycle fluctuations in the expression of surface glycoconjugates. Moreover, in the case of three lectins reacting with both K562 and red cells (*V. graminea*, peanut, E-PHA), the binding to K562 cells usually showed an apparently lower affinity (less steep Scatchard plots) and in some cases a more distinctly pronounced positive cooperation effect. The positive cooperation occurs frequently in lectin binding [38-40] and is not restricted to the interaction with cells which show the functional responsiveness to specific external contacts, but may occur in binding to erythrocytes (Figs. 3 and 6), or even to receptors incorporated into artificial lipid bilayers [40]. The positive cooperation demonstrates either the increase in binding constants, or increasing the number of available binding sites, as the extent of occupancy of receptors increases [38, 39]. The apparent differences in affinity may not only have reflected the differences in structure of lectin receptors on erythrocytes and K562 cells (which is quite probable for E-PHA receptors), but may have also resulted from their different topography or availability for the reaction. The important factor could be also the agglutination of erythrocytes by the lectins studied, whereas K562 cells were not agglutinated, probably due to their large cell size. A strong agglutination, beginning at low lectin concentration may interfere with binding and lead to underestimation of binding sites, as we noticed with two preparations of *V. graminea* lectin differing in agglutinating potency [41]. Due to the fact that binding of lectins to cell surface receptors is a process which may be mediated by many factors, the possibility of definite interpretation of binding plots is sometimes limited. Nevertheless, the results described in this report give some information about the lectin receptors existing at the K562 cell surface and should be helpful in identification and characterization of membrane components carrying these receptors.

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