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REVIEW

Polyacrylamide-based glycoconjugates as tools in glycobiology

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This review describes the synthesis, physicochemical characteristics and application for studying carbohydrate-binding proteins of polyacrylamide (PAA) type neoglycoconjugates. An approach to the synthesis of conjugates based on the interaction of activated polyacrylic acid with ω-aminoalkyl glycosides has been developed. Both the molecules of Glyc-PAA and the conjugates bearing various labels and effectors, as well as sorbents, and glycosurfaces can be designed using this method. Examples of the application of the conjugates as tools for the study of lectins, antibodies, and glycosyltransferases in glycobiology, cytochemistry and histochemistry are described along with the prospects of the further development of the presented approach in glycotechnology and medicine.

Keywords: **antibodies, assays, carbohydrates, cytochemistry, diagnostics, glycoconjugates, glycosyltransferases, histochemistry, immunoassay, lectins, polyacrylamide**

Abbreviations: **A_{tri}, GalNAcα1-3(Fucα1-2)Galβ; biotbiotin; BSA, bovine serum albumin; B_{di}, Galα1-3Galβ; B_{tri}, Galα1-3(Fucα1-2)Galβ; DMF, dimethylformamide; DMG, 3,6-di-*O*-methyl-β-D-glucopyranose; DMSO, dimethylsulphoxide; ELISA, enzyme-linked immunosorbent assay; flu, fluorescein; αGal-T, α-galactosyltransferase; Glyc, glycosyl- or glycosyl-spacer residue (mono or oligosaccharide); M_r, relative molecular weight; NC, nitrocellulose; p4NP, poly(4-nitrophenylacrylate); PA, polyacrylic acid; PAA, polyacrylamide; PAA^e, poly(*N*-hydroxyethylacrylamide); PE, phosphatidylethanolamine; SERS, surface enhanced Raman scattering; $SiaLe^a$, Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ; $SiaLe^x$, Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ**

Introduction

Heterogeneity of natural glycoconjugates, in particular glycoproteins, complicates their use as the tools for the study of carbohydrate-binding molecules, so the synthetic analogues of glycoconjugates play special and sometimes indispensable roles in glycobiology research. Chemical synthesis gives the possibility both to produce oligosaccharides in different states and in large quantities and, moreover, to perform directed modification of biomolecules when this is necessary for the amplification of a biological effect, selection of one activity from a number of activities, introduction of labels, stabilization against enzyme action, etc.

One of the peculiarities of carbohydrate–protein and carbohydrate–carbohydrate recognition is that the high affinity and specificity of these interactions are achieved by polyvalency, whereas at the level of a monovalent interaction the affinity can be very low or practically undetected [1, 2]. It is convenient to reveal, study, and design the multipoint cooperative processes with the help of polyvalent glycoconjugates (neoglycoconjugates) having predetermined properties – such as molecular weight, solubility, matrix flexibility, stability, distance between the carbohydrate ligands, etc. To synthesize conjugates having predetermined composition and properties, it is necessary to attach the ligands to a matrix with the guaranteed quantitative yield, as only under this condition it is possible to provide the required content and ratio of ligands. These criteria are satisfied by the reaction of aminoalkyl glycosides with activated polyacrylic acid [3–5] resulting in N-substituted polyacrylamides. This article reviews the properties and bioanalytical application of polyacrylate-type glycoconjugates synthesized by this approach.

Synthesis and physicochemical properties of carbohydrate-acrylate conjugates

Two alternative possibilities of glycosylated polyacrylamides synthesis have been described: the first one is condensation

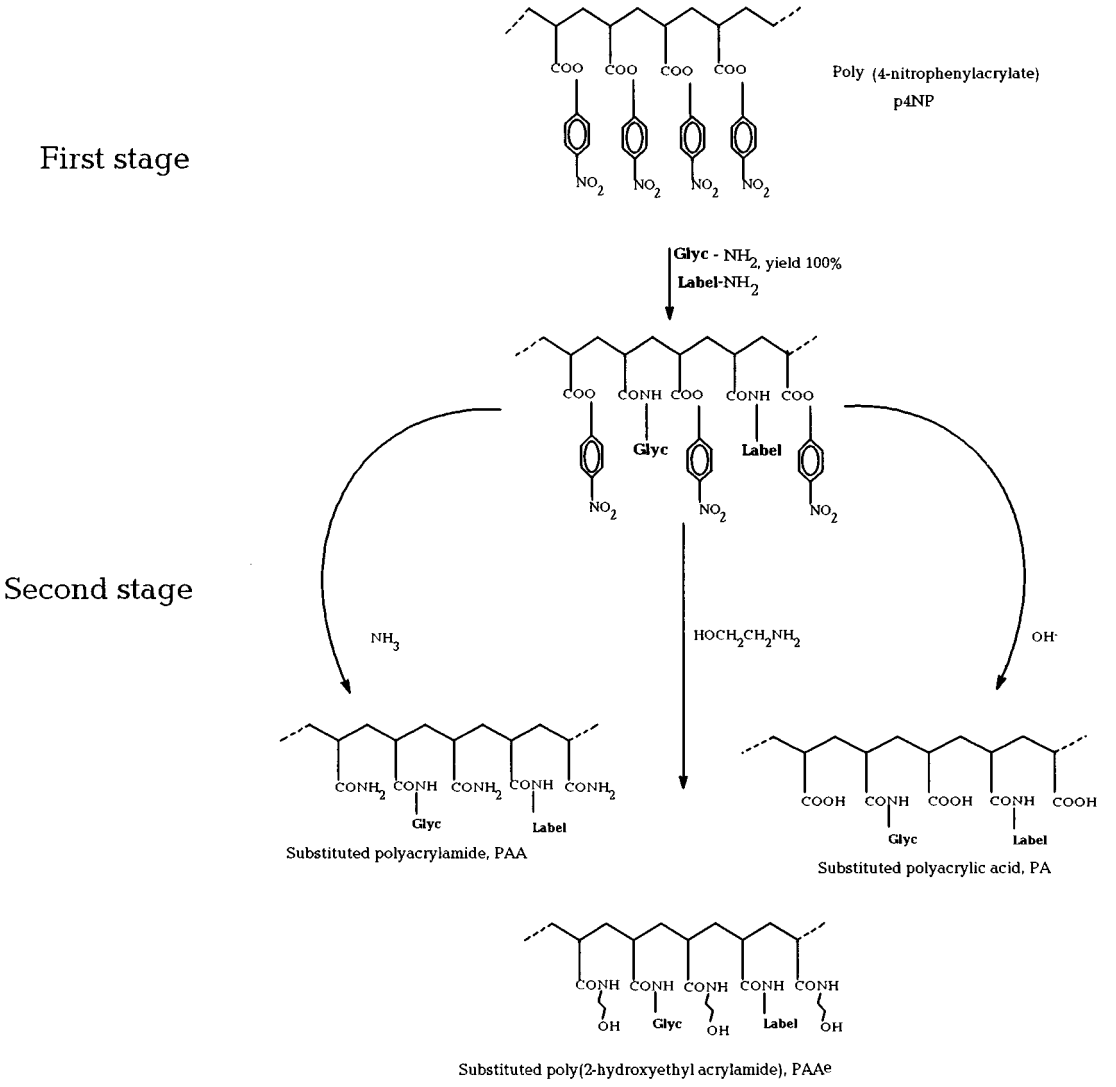

Figure 1. Scheme of soluble conjugates synthesis. First stage: aminoligands (Glyc-NH_2, Label-NH_2) attachment to activated polymer (p4NP). Second stage: carrier modification resulting in the final polymer, *ie* amide-, ethanolamide- or acid-type conjugates (PAA, PAA^e, PA, respectively). Glyc-NH_2 is aminoalkyl glycoside of mono- or oligosaccharide, the latter can be sialylated or sulphated one, N-chain of glycoprotein, etc.

of aminoalkyl glycosides with activated polyacrylic acid [3–8], the second one is polymerization of glycosides, containing the acryloyl group as part of the spacer [9–17]. The main feature of the first approach is its universality: a wide variety of neoglycoconjugates as pseudopolysaccharides, probes bearing different label types, neoglycolipids, adsorbents, and more complex constructions can be obtained by the same principal scheme (Figure 1) [6, 8].

In most of the described methods [18] for carbohydrate conjugation with a carrier-matrix, the ligand to be attached (mono- or oligosaccharide) is an electrophile and the matrix is a nucleophile. When using this approach, there are side reactions, in particular the decay and solvolysis of the electrophile, and as a result, the loss of a part of carbohydrate ligand is unavoidable. In contrast, where the ligand to be attached is a nucleophilic component, and the matrix is electrophilic (Figure 1), the ligand is not involved in any side reactions and it is quantitatively attached to the matrix. To perform this condensation both quantitatively and rapidly, it is desirable to have an excess of activated groups in relation to the ligand (nucleophile). This can be achieved by the activation of all carboxylic groups of the polymer. This is the reason why completely activated polyacrylic acid is the starting compound in the synthesis of these conjugates.

Synthesis of the activated polymers

The activation is performed at the monomer stage, activated acrylic acid ester is synthesized followed by its polymerization resulting in completely activated polyacrylic acid, poly(4-nitrophenylacrylate), p4NP. Polymerization of

4-nitro-phenylacrylate [6, 8, 19] has been performed both on 100 mg and 100 g amounts of monomer, and the characteristics of the obtained polymers were in both cases identical. Independent of its molecular weight the polymer is soluble in DMF and DMSO and these are convenient solvents for further condensation. Recently, the similar activation of soluble (not cross-linked) polyacrylic acid (PA) with *N*-hydroxysuccinimide has been described [5].

Condensation of poly(4-nitrophenylacrylate) with ω-aminoalkyl glycosides

The fact that all carboxylic groups in the initial polymer are activated gives many possibilities for their modification by the compounds having a primary amino group (Figure 1), in some cases secondary aliphatic or primary aromatic amines were used in biological studies (see above and [6]). Usually, conjugates with 5–20% molar substitution have been used, therefore, the remainder of the activated groups have been condensed with other (non-carbohydrate) amines. Amino ligand attachment within the limit of 20 mol % (molar fraction of the acrylate units modified by a ligand is regarded hereafter as molar %) proceeds quantitatively at room temperature; heating to 40–50 °C increased the degree of substitution. The reaction proceeds in DMF faster than in DMSO; methanol can be used as a co-solvent, the only requirement for the solvent is the absence of primary or secondary amine impurities; traces of water do not affect the reaction. The maximum amount of saccharide introduced depends on its size; for bulky tri- and tetrasaccharides this is within the range of 30–35 mol % at 40 °C, whereas with monosaccharides, this could be as high as 80%, with bulky lysogangliosides only 15% is attached. The degree of attachment can be easily controlled using TLC by detecting the non-reacted amine with ninhydrin. This method can detect less than one per cent of the unreacted amine. The mode of the attachment has been confirmed by other methods [6], *ie* by spectrophotometry, monosaccharide composition and ^{13}C-NMR spectroscopy.

The nature of NH_2-group in a ligand (or its spacer) and the length of a spacer did not affect the result of attachment to activated polymer. 3-Aminopropyl served as the spacer group in most of the studies cited in this review because a short spacer minimized the possible non-specific interactions. Due to the flexibility of the polyacrylamide chain (in fact, it serves as an additional spacer), a short aminopropyl linkage was found to be sufficient (we did not observe any exceptions) for the interaction of oligosaccharide with all carbohydrate-binding proteins, such as lectins, antibodies, or glycosyltransferases. This occurred even in cases where BSA-conjugates of the same saccharide require an elongated spacer. Similar results were obtained when glycosides bearing 2-aminoethyl, 4-aminobutyl, 6-aminohexyl, or 4-aminophenyl spacers, and glycosylamines, and N-glycosides of Glyc-$NHCOCH_2NH_2$ type were reacted with p4NP. It was convenient to obtain the derivatives of the Glyc-$NHCOCH_2NH_2$ type from reducing oligosaccharides (milk oligosaccharides or glycoprotein N-chains cleaved from the protein core). These were converted to glycosylamines followed by chloroacetylation and conversion to the amine derivatives Glyc-$NHCOCH_2NH_2$ at the final stage by the action of ammonia [20, 21].

It is necessary to remove the remaining activated groups after the aminoglycosides attach to the polymer; and it is simultaneously possible to modify the polymer matrix. PAA-derivatives were obtained by the action of ammonia (conversion of -$COOC_6H_4NO_2$ to -$CONH_2$), PAA^e-derivatives were obtained by the action of ethanolamine (conversion of -$COOC_6H_4NO_2$ to -$CONHCH_2CH_2OH$), and polyacrylic acid derivatives were obtained in the presence of alkali (conversion of -$COOC_6H_4NO_2$ to -COOH) Figure 1. Introduction of a negative charge and variations in amide nitrogen substituents are additional possibilities for modulation of the physico-chemical properties of the polymers. Glycoconjugates of the 'ethanolamine type', PAA^e, have found most applications of the three types of conjugates (see below).

The conjugate is purified by separation from nitrophenol and the excess of NH_3 (or ethanolamine) and it is performed by chromatography on TSK-gel or Sephadex LH-20 or by re-sedimentation. In a number of cases, *eg* when the conjugate was used for the activation of polystyrene plates or nitrocellulose membranes, no purification was necessary; it was sufficient to dilute the reaction mixture with the appropriate buffer solution. The only potential danger in the latter approach is the possibility of incomplete removal of active groups from pNPA during the final treatment. Even two to three -$COOC_6H_4NO_2$ groups on the conjugate can be sufficient for its covalent binding to protein during an assay which will lead to erroneous results. This danger really exists only for conjugates that bear bulky or tightly introduced substituents that limit the polymer mobility and thus, can spatially shield -$COOC_6H_4NO_2$ residues, making them inaccessible within the molecule. This problem can be avoided by prolonged incubation with 2-ethanolamine or repeated treatment with it. The easiest way of controlling the presence of activated groups in the final conjugate after its purification is treatment with a strong alkali followed by spectrophotometric determination of the nitrophenol produced.

Molecular weight and some features of the conjugates

The relative molecular weights (M_r) of conjugates were evaluated using two approaches. The first used gel chromatography on different columns including HPLC, with calibration against standard protein markers, dextrans, and polyethylene glycols. The second was ultrafiltration by membranes having protein exclusion limits of 30, 100, and 300 kDa. These methods gave values that were in the same

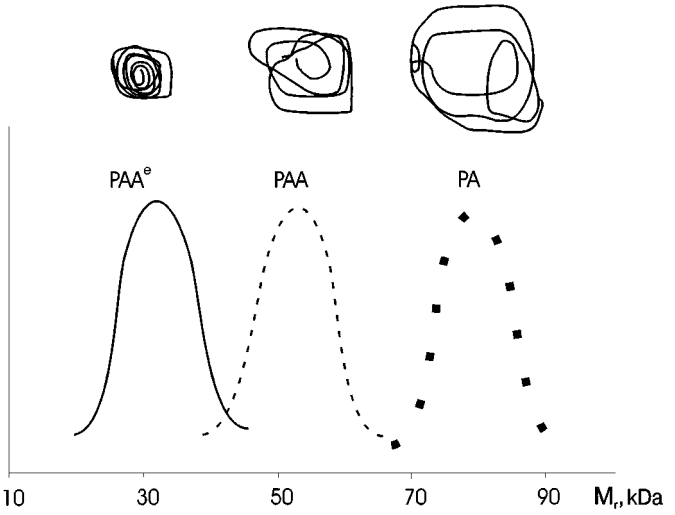

Figure 2. Schematic representation of the sizes of the three types of conjugates. The conjugate Glyc-PAA^e (30 kDa) is presented as a compact globule, the conjugate of polyacrylic acid with the same ligand Glyc-PA has $M_r \sim 80$ kDa and thus it is presented as a friable globule, the conjugate Glyc-PAA is an intermediate variant (~ 50 kDa). The elution profiles of the conjugates according to gel chromatography are presented below.

range, *ie* the average M_r differed by less than two-fold. The degree of polymerization of the final water-soluble conjugates was similar to those of initial p4NP polymers (established on HPLC in comparison with polystyrene standards) [22]. Therefore, the relative molecular weights of conjugates were close to the real ones. M_r values of the conjugates were set by the degree of polymerization of the initial poly(4-nitrophenylacrylate). Biotinylated probes and pseudopolysaccharides of PAA^e type (M_r about 30 kDa), PAA type (M_r about 50 kDa), and PA type (M_r about 80 kDa) were obtained from the activated polymer that was synthesized in the conditions described in [6] (Figure 2, proteins of known M_r were used as standards).

M_r values determined for the polymers modified by oligosaccharides were higher than that of the unsubstituted matrix due to a real increase in mass, although the increase in mass was not exactly equal to the amount of ligand added. For example, the attachment of 20 mol % of trisaccharide should lead to a two-fold increase in the conjugate's mass, but the observed M_r value differed from the initial one only by 10–20%. The real size of conjugates was evaluated with the help of electron microscopy: the molecules had an almost regular spherical form with a diameter about 150 Å [23] this being in accordance with the hydrodynamic size determined by indirect methods.

The principal difference in polyacrylamide conjugates from neoglycoproteins having BSA as a matrix should be emphasized. The carbohydrate part in BSA-conjugates is not more than 15% weight (*eg* for 20 trisaccharide residues it is about 12%), and the conjugate is a big globular protein bearing rare carbohydrate residues on its surface. On the other hand, the PAA^e-conjugate is more like a thin thread with heavy 'beads' attached to it, and the carbohydrate part could be 80–90% of the mass of the molecule. The protein-carrier is a rigid molecule and oligosaccharides are attached to the amino acid residues in fixed positions on the molecule. The polyacrylamide carrier is a random coil, however, in which the distance between two oligosaccharides is not fixed, and the conjugate can adjust itself to a target molecule or cell.

Three basic constituents are necessary for specific carbohydrate-protein recognition: besides the carbohydrate and protein themselves, the complementary clusterization of these groupings on the interacting membranes is necessary [24]. Interacting components that are presented on membranes that are not optimally expressed can ignore each other. Glyc-BSA is not ideal as a model compound for the study of carbohydrate-protein interaction due to the fact that the carbohydrate residues are rigidly fixed on BSA and they can interact with a multivalent partner with high affinity only as the result of random collisions. In contrast, Glyc-PAA can adjust themselves to any partner, due to flexibility of their chain, though with some loss in affinity at the expenses of enthropy.

It was also interesting to discover how Glyc residues were placed along PAA chain: randomly, in a regular way, or in clusters. In the case of Glyc-PAA obtained by radical copolymerization, clusters were the most probable arrangement [5], but in the approach described an even arrangement of ligands was most probable, especially in cases where there was a low degree of substitution. In support of this idea was the evidence that in some cases conjugates having a smaller percentage of substitution had higher activity in comparison to more heavily substituted ones (see below) and also SERS spectrocopy data [25].

Interactions of conjugates with the changing carbohydrate content

Using a series of conjugates where the carbohydrate ligand content was changing (*eg* 2, 5, 10, 15, 20% mol) it was possible to investigate the degree of cooperativity in the carbohydrate-protein interaction. Increasing ligand concentration (and so decreasing distance between neighbouring residues) did not always lead to an increase in binding with a complementary protein. Thus, during the interaction of conjugates Neu5Ac-PA with influenza virus hemagglutinin a sharp activity maximum was observed for 10–12% substitution, whereas conjugates bearing 5 and 20% mol of Neu5Ac were considerably less active [3]. E-selectin bound equally with SiaLe^x-PAA^e conjugates of different carbohydrate density, whereas some antibodies interacting with the same tetrasaccharide SiaLe^x bound only with the conjugates having a higher hapten density [26]. Similarly, when two conjugates, SiaLe^x-PAA^e (10%) and SiaLe^x-PAA^e (20%) were coated onto polystyrene plates (in an ELISA with the

CSLEX-1 antibody) the 20% conjugate gave an absorbance value five times higher than that given by the 10% conjugate for equal mol amounts of $SiaLe^x$ [27].

Based on such data it is possible to select the optimal carbohydrate content for use in different assay systems. Thus, in an assay for detecting antibodies to the blood group trisaccharide B antigen, the conjugate B_{tri}-PAA^e (5% mol) is the optimum concentration. This conjugate gives a signal:background ratio better than conjugates containing 10- and 20%. However, a conjugate containing 15% mol of saccharide proved to be optimum for detecting antibodies to the structurally very similar trisaccharide A [28].

A conjugate bearing 4% of hapten (3,6-di-*O*-methyl-D-glucose, DMG) demonstrated better correlation with the morphological pattern of leprosy compared to conjugates of higher concentration for detecting antibodies to a carbohydrate epitope of phenolic glycolipid-I, in this case the optimum ligand density improved both the signal:background ratio and the diagnostic specificity of the test-system [6]. It should be noted that the specific epitope of phenolic glycolipid-I is a DMG-containing trisaccharide and it was sufficient to attach only the DMG monosaccharide residue to the polymeric matrix to reveal antibodies to this glycolipid.

Similar results were obtained in assay for human antibodies to streptococcus of group A. In this case, the antigen is a polysaccharide with the tetrasaccharide repeating unit; the residues of *N*-acetyl-β-D-glucosamine are attached to linear polysaccharide core. An optimum conjugate of 10% was chosen from a series of GlcNAcβ-PAA^e with a variable content of monosaccharide. This was used for the diagnostics based on quantification of GlcNAc antibodies [29]. Both in the latter two situations the polyacrylate matrix in combination with conjugates containing optimum hapten concentrations gave the possibility to enormously simplify the antigen structure (from tri- or tetrasaccharide to monosaccharide) without deterioration of diagnostic characteristics.

Glyc-PAA^e as coating reagents

Glyc-PAA^e conjugates adsorbed on polystyrene are the substrates for glycosyltransferases. When αGal-T glycosylates Galβ1-4GlcNAc-PAA^e coated on the surface of a well, the Galα1-3Galβ1-4GlcNAc-PAA^e formed can be easily detected using biotinylated αGal-specific lectin (Figure 3). This convenient method gives the possibility not only to measure the amount of enzyme in solution but also to study the glycosylation kinetics [30]. As with other ELISA, a series of Glyc-PAA with varying Glyc content enables one to optimize the assay. Moreover, using Glyc-PAA^e it is possible to demonstrate that the pattern of binding with a αGal-specific lectin reflects the glycosylation process. To do this a series of $Glyc^1$-PAA-$Glyc^2$ is synthesized, in this case $Glyc^1$ is Galβ1-4GlcNAc, $Glyc^2$ is a trisaccharide. The $Glyc^1/Glyc^2$ ratio changes within this series as follows 100/0, 95/5, 90/10, etc. The wells of the plate are coated with the series of conjugates followed by the interaction with the lectin. This determines the range of trisaccharide content over which the ELISA signal is linear, here 0–20%. This permits one to measure the glycosylation kinetics within this range. It should be noted that this ELISA-based system, being similar in sensitivity to radioactive label-based assays, is convenient and permits simultaneous analysis of many samples.

The examples mentioned above describe a version of ELISA where polystyrene is used as a solid phase, usually as 96-well plates. Long-term experience show that NUNC Maxisorb plates are the optimal material for coating Glyc-PAA^e, whereas the most typical concentration range of Glyc-PAA^e for coating is 1–5 $\mu g\,ml^{-1}$. Polyacrylamide conjugates of carbohydrates are also well adsorbed on nitrocellulose porous membranes. This latter finding allows wider application of PAA^e-conjugates in solid phase assays, as nitrocellulose gives the possibility to improve the assay, due to the considerably higher absorption capacity of nitrocellulose in comparison with polystyrene. This approach was successfully used for the analysis of epitope specificity and cooperativity of the binding of anti-A, anti-B and anti-H monoclonal antibodies [1, 2]. The ability of polyacrylamide conjugates to bind to nitrocellulose also made it possible to use immunoblotting to study the clonal diversity of human antibodies to A and B blood group antigens (R. Rieben, personal communication). A special version of PAA^e conjugate for coating onto NC-membranes, that contains HS-groups, is described later.

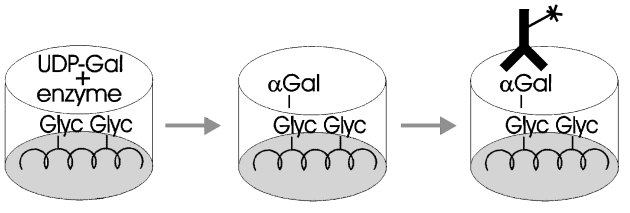

Figure 3. Glycosyltransferase assay based on Glyc-PAA. Enzymatic reaction proceeds in a 96-well plate. UDP-Gal is uridinediphosphogalactose (glycosyl donor), the enzyme is αGal-transferase, Glyc is glycosyl acceptor Galβ1-4GlcNAc-PAA^e coated on the plate, a 'catapult' with asterisk is labelled antibody or lectin.

Conjugates with two or more different ligands

Glycoprobes (the second ligand is a label)

To obtain carbohydrate probes, *ie* reagents for the study of carbohydrate-binding molecules, biotin, fluorescein, mercury or radioactive labels were introduced into the polymer in addition to the carbohydrate (Figure 4) [6]. Biotin (as 6-aminohexylamide) was incorporated at 5 mol %, fluorescein (flu) or rhodamine (rho) derivatives were incorporated of 1 mol %. The relatively low label content (compared to 20% mol. for carbohydrate) was selected to avoid

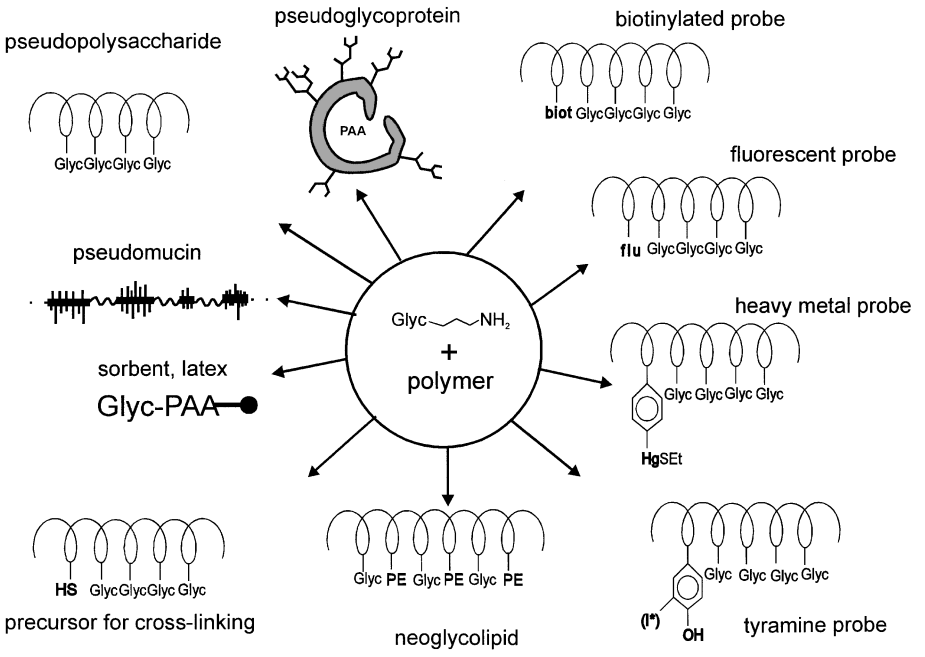

Figure 4. Diversity of PAA-based glycoconjugates.

hydrophobization and its effect on the probe solubility. At the same time these amounts were adequate to probe bound to a cell, lectin or antibody. 4-EtSHg$C_6H_4CONH(CH_2)_6NH_2$ was attached to the polymer for the electron microscopy studies (Figure 4). Radioactive labels were used in surface proximity assays (SPA) (Game SM, Rajapurohit PK, Clifford M, Bird MI, Priest R, Bovin NV, Nifant'ev NE, O'Beirne G, Cook ND, Anal. Biochem, 1998, in press), for the study of pharmacokinetics and the degree of binding to surfaces. These labels were introduced either by direct attachment of a labelled amino acid to the activated polymer (Figure 4) or by the synthesis of a conjugate with tyramine followed by iodination with ^{127}I (Figure 4) [33]. The study of the specificity of the purified lectin from the *Butea frondosa* plant [34] is an example of the solid phase assay where the probe was not absorbed onto the solid surface. The absorption of Glyc-PAA^e followed by the addition of labelled lectin in presence of inhibitor did not work in this case due to the high non-specific absorption of the lectin onto polystyrene. Thus, the lectin was coated onto plastic and then a biotinylated conjugate with the ligand, GalNacα1-3Galβ (A_{di}-PAA^e-biot), and carbohydrate inhibitors were added (Figure 5).

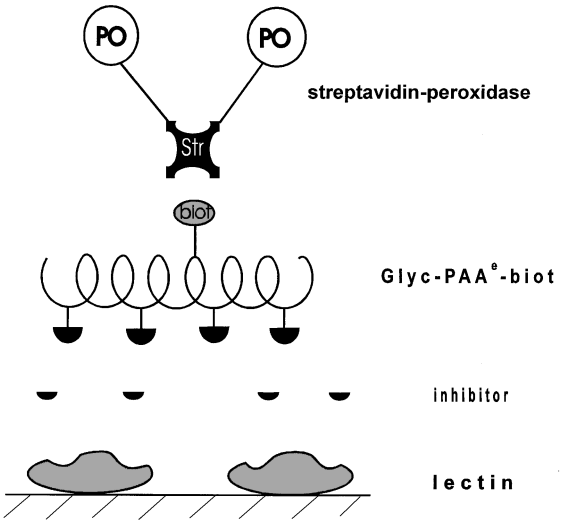

Figure 5. Scheme of solid phase competition assay for the study of lectin specificity. The lectin is adsorbed on polystyrene followed by the addition of a biotinylated probe (Glyc-PAA^e-biot) in the presence of the inhibitor. Binding is measured with the help of streptavidin (Str) conjugated with peroxidase (PO).

An analogous scheme was used to develop a sensitive selectin/ligand assay system (Figure 6) [35]. The probe SiaLe^a-PAA^e-biot was used as the *in situ* complex with avidin-peroxidase conjugate. A comparison of the two lectin assays is given in [36].

The competitive ELISA, similar to those already described, though including antibodies instead of lectin, are rather promising for the diagnosis of carbohydrate tumour-associated antigens such as SiaLe^a (CA 19-9), Neu5Acα2-3Galβ1-3GlcNAc (CA 50), and ganglioside FucGM_1. They provide the opportunity to determine not only the polyvalent antigens but also monovalent ones, such as gangliosides. More conventional sandwich ELISA determines only polyvalent antigens, as the antibodies used to bind to two sites on the antigen: both as catcher and as tracer. The

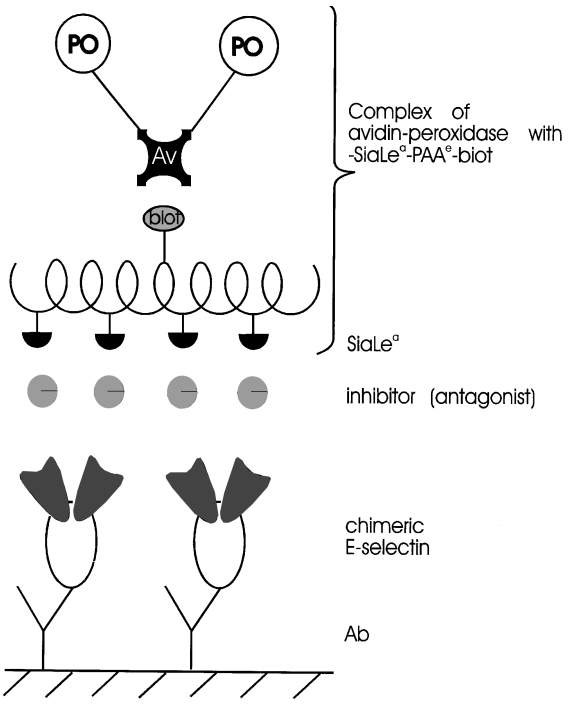

Figure 6. Scheme of solid phase competition assay for the screening of E-selectin antagonists. Antibodies of E-selectin are adsorbed on plastic followed by addition of selectin. The latter specifically interacts with the probe SiaLe^a-PAA^e-biot that had been preliminary complexed with avidin-peroxidase. If the studied substance (represented as a ball) reacts with selectin (*ie* it is its antagonist or agonist), the decrease in the specific signal is registered.

second advantage is that only in a competitive format is it possible to adequately replace a natural antigen (non-standard and unstable) with a synthetic one.

Polymeric probes, especially biotinylated ones have been most widely used for studying endogenous lectins in histochemical and cytochemical experiments [37–47]. Their ability to bind to carbohydrates was established for a number of important cell surface proteins, such as clusters of differentiation antigens (CD) and adhesion factors. Among these proteins are E-, P- and L-selectins, sialoadhesins, calmodulin, and many others [48]. It is likely that the property to bind to carbohydrates is inherent for wide range of proteins. Furthermore, practically all cells investigated with the help of probes Glyc-PAA^e-biot [40] or analogous probes [49], showed the ability to selectively bind mono- or oligosaccharide(s).

A carbohydrate probe for histo- or cytochemistry should satisfy several main requirements: (1) it must be a polyvalent substance that contains several carbohydrate ligands (usually several dozens) on a carrier molecule, as the binding constant of individual ligands with individual carbohydrate-recognizing domains is rather low; (2) the carrier itself, as well as the label should not bind with cells; (3) a convenient label should be introduced into a probe. The probes Glyc-PAA^e-biot and Glyc-PAA^e-flu satisfy these requirements. The cytochemical staining technique has been previously described [8, 40, 43]: the cells (native or those minimally fixed by formaldehyde) were exposed to the biotinylated probe in a suitable buffer solution, washed, and the bound probe was revealed with a (strept)avidin-peroxidase conjugate. Colour intensity reflected the degree of carbohydrate ligand binding, the number of stained cells reflected the specificity of binding for a given species, and the nature of dye distribution displayed the lectin localization. Conclusions about the linkage specificity (that is, about the availability of lectin or a group of lectins binding the given carbohydrate on the given cells) were made by comparison of the cell binding for a group of probes, as well as by carrying out inhibition experiments by adding low molecular weight carbohydrate. The ability to bind to cells using these approaches were demonstrated for the majority of synthesized probes [37–47, 49–53]. The list of carbohydrate ligands included monosaccharides (-Glcα, -Glcβ, -Galα, -Galβ, -Manα, -Fucα, -GlcNAcβ, -GalNAcα, -GalNAcβ, -Rhaα, -Neu5Acα, -Man6Pα, 3-HSO$_3$Galβ), blood group related antigens (ABH, Lewis, i, P), differentiation antigens (Le^x, Le^y), selectin ligands (SiaLe, HSO$_3$Le), tumour associated antigens (TF, T$_{\beta\beta}$, T$_{\alpha\alpha}$, Fs, SiaLe^a, T$_n$, SiaT$_n$), carbohydrates having immunomodulating properties (MDP, N-chains of α_1-acid glycoprotein).

Normal and transformed human cells (both leukemic and carcinomatous), were studied with these probes. The main goal of these studies was to search for quantitative and qualitative differences between the normal and tumour cells in their ability to bind complex carbohydrate molecules. The ability of endogenous lectins to bind to the monosaccharide probes Glyc-BSA-label was previously studied [54, 55], binding with oligosaccharides was demonstrated only on a limited number of examples [56–61]. However, it was shown with the help of the probes Glyc-PAA^e-biot (where Glyc were more than 30 different oligosaccharides) that the oligosaccharide specificity of endogenous lectins was a general phenomenon. It was noted that different cell types preferred different probes. Furthermore, the binding was inhibited by specific low molecular weight glycosides, for example, the binding of Galβ1-3GalNAcα-PAA^e-biot to cell lines P-338 and EL-4 was inhibited by disaccharide Galβ1-3GalNAc as an α-glycoside, but it was not inhibited by the same disaccharide as a β-glycoside or GalNAcαOR [37, 40].

Of particular interest is the binding of white blood cells to probes bearing blood group oligosaccharides A$_{tri}$ and B$_{tri}$. Despite their wide distribution, the function of these antigens is still unknown, it is possible, however, discovering their ligands will help to clarify the function of these antigens. It was found that probes having specificity for A and H(type 1) antigens selectively bound to some tumour cells, and particularly, metastasing cells [37–47]. This data suggest that A- and H-binding lectins (galectins?) may be

utilized by cancer cells as adhesins interacting with histo-antigens A and H during metastasis.

It was shown in experiments with SiaLe^a-PAA^e-biot, that only carcinomatous (but not leukemic) cells bound specifically to the tumour-associated tetrasaccharide SiaLe^a [42]; it is interesting to note that accumulation of the tetrasaccharide SiaLe^x is a typical process in carcinomatous cells, while the accumulation of the tetrasaccharide SiaLe^x is characteristic for leukemic cells [62]. These results along with the similar results from other probes [63], as well other data in the literature [57, 60] suggest that accumulation of carbohydrate chains and complementary lectins on the surface of the same cell, is a general phenomenon. Accumulation of both carbohydrate tumour-associated antigens, and complementary lectins on the cell surface may be one reason for the absence of contact inhibition in tumour cells [64]. Flow cytofluorimetry in combination with Glyc-PAA^e-flu probes opens up the possibility of studying cell surface endogenous lectins in living cells, particularly sub-populations of leukocytes, to evaluate quantitative binding to measure the number of binding sites and to monitor the simultaneous binding of two probes [65]. The results of a cytofluorimetry study of Glyc-PAA^e-flu binding to monocytes are shown in Figure 7 [66], which demonstrates that several probes interact intensely with monocytes whereas other probes bind only at the background level.

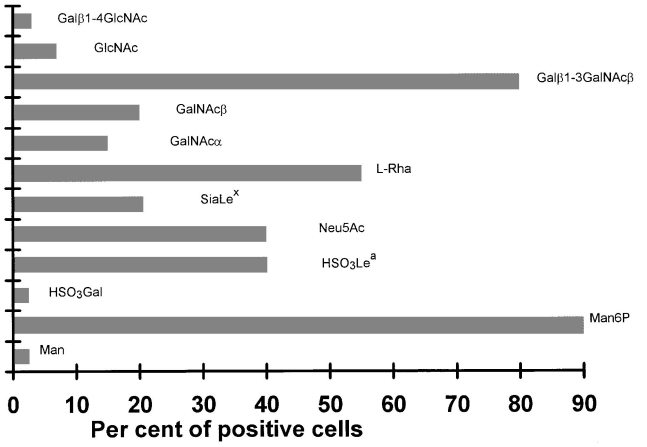

Figure 7. Cytofluorometry study of Glyc-PAA^e-flu binding with human monocytes. The maximum level of probe-positive cells is observed with probes Man6P and Galβ1-3-GalNAcβ, whereas the probes Man, HSO$_3$Gal, and Galβ1-4GlcNAc give only a background value. Binding with selectin ligands (SiaLe^x, HSO$_3$Le^a) is reliable but not intense.

Biotinylated probes in combination with a dot blotting technique revealed the presence of new lectins in human blood. It was shown that blood contained molecules that bound Man6P, Galβ1-3GlcNAcβ and Fucα1-2Galβ and this led to the isolation of new human lectins [50, 67].

A third class of carbohydrate-binding proteins, besides lectins and antibodies, which it is possible to study with the help of Glyc-PAA-biot is glycosyltransferases. Quantitative determination of these enzymes is important both for study of their properties and for diagnosis in some diseases in which their levels change [68]. Incubation of Glyc-PAA^e-biot with enzyme and radioactively-labelled glycosyl-donor results in a labelled product; the latter is rapidly and quantitatively adsorbed from the solution on to streptavidin-coated plate; the amount of bound label being proportional to the activity of the enzyme studied. A more promising approach is the radioactivity-free version of this assay where the enzymatic reaction product is determined quantitatively with the help of a monoclonal antibody or lectin (Figure 8) [30].

The Glyc-PAA^e-biot and Glyc-PAA^e-flu probes were used for the study of a new, recently described type of biological recognition – carbohydrate–carbohydrate interaction [69–71]. Calcium-dependent carbohydrate–carbohydrate interaction can be registered only in multivalent cell–cell, cell–liposome, liposome–liposome binding; a high density of carbohydrate residues is also necessary for this type of interaction [72]. Therefore, polyvalent probes bearing flexible and readily adjustable ligands, can be convenient tools for the detection and modelling of carbohydrate–carbohydrate recognition. The interaction between a yeast polysaccharide complex (zymosan) and mannosylated

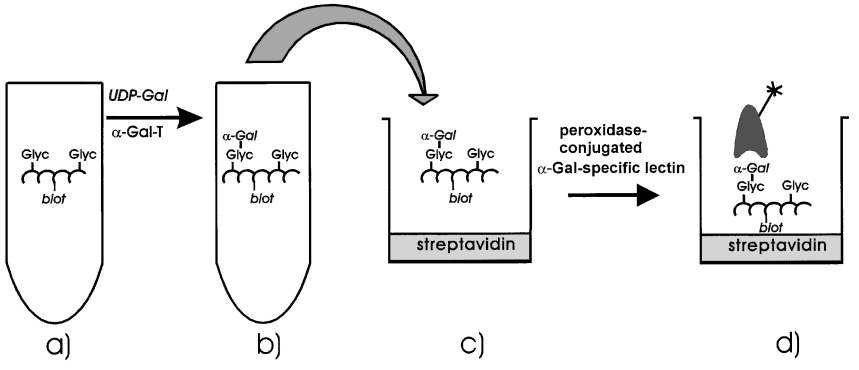

Figure 8. Quantitative determination of *a*-galactosyltransferase with the help of a biotinylated probe. Glyc-PAA^e-biot (a) is incubated with UDP-Gal and the analysed enzyme; the reaction product (b) is transferred to the plate with the pre-adsorbed streptavidin (c); the amount of the reaction product is measured by the labelled lectin (d).

glycoconjugates was demonstrated with the help of Glyc-PAA probes (Glyc is αMan or mannose-rich N-chains). The binding was calcium-dependent and carbohydrate-specific; β-glucan proved to be the component of zymosan responsible for the interaction with mannosylated glycoconjugates [70, 71].

Glyc-PAA-biot conjugates give the possibility to rapidly modify, and with quantitative yield, any commercially-available material that is coated with streptavidin or avidin, such as particles and matrix carriers that are used as absorbents. Using this technique, magnetic beads coated with neoglycoconjugates have been synthesized that permit separation of cell populations, *ie* isolation from complex cell mixtures of a hybridoma that is specific for a given carbohydrate antigen [73].

Polymeric neoglycolipids

Neoglycolipids (in this case the second ligand is a lipophilic residue) were synthesized by the attachement to the polymer of phosphatidylethanolamine (PE). In some cases a radioactive (see below) or fluorescent label was also attached to the polymer besides PE. Radioactively-labelled derivatives (the label was entered either as amino acid, or as a 'hot' PE) were used for the quantitative evaluation of neoglycolipid insertion in biological membranes.

Neoglycolipids with various physicochemical properties were obtained by variation in molecular weight of initial p4NP: (1) oligomeric derivatives ($M_r < 10$ kDa), poor solubility in water and good solubility in organic solvents, including ethanol and $CHCl_3$/MeOH, (2) polymeric derivatives, soluble in water and aqueous methanol. The difference in solubility properties of these two forms can be explained by formation of an intramolecular hydrophobic core in polymeric conjugates and the absence of internal structures in the oligomeric conjugates (Figure 9). The introduction of polymeric glycolipids into the cell membrane is a mild method of surface modification using carbohydrate ligands; the introduced carbohydrate chains imitate the glycosphingolipids clusters (patches) found on cell surfaces, due to their close proximity.

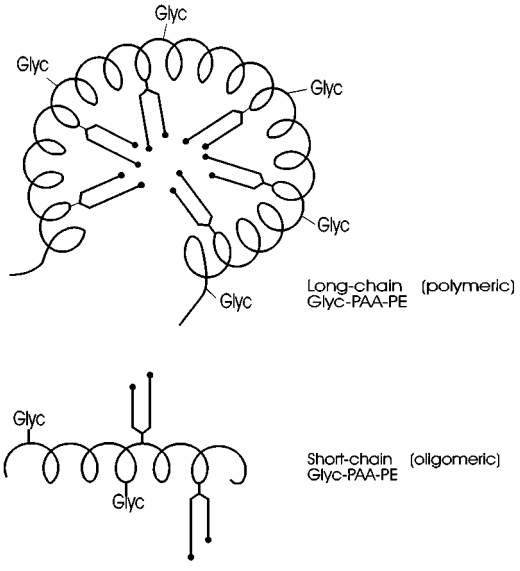

Figure 9. Spatial organization of high- and low molecular weight neoglycolipids. Above: the formation of hydrophobic nucleus (of micelle type) by the polymeric conjugate. Below: flexible amphiphilic structure of an oligomeric conjugate. Glyc, carbohydrate residues; ●—, phosphatidyl ethanolamine residues.

Glyc-PAA-PE probes can be easily introduced into liposomes. As it has been noted above, tumour cells bear a specific set of lectins so glycosylated ('vectored') liposomes can be used as selective cytotoxic agents for cancer cells (Vodovozova E, Gayenko G, Razinkov V, Korchagina E, Bovin N, Molotkovsky J, Biochem Mol Biol Int **44**(3), 543–553 (1998)).

Whereas the usual (non-lipophilic) glycopolymers of polyacrylate type are weak immunogens [14], neoglycolipids can cause a strong immune response. Neoglycolipids bearing haptens Le^y, $SiaLe^a$, $SiaLe^x$ adsorbed on *Salmonella minnesota* (a mutant strain of this bacteria having a low carbohydrate content) were used as immunogens for obtaining monoclonal antibodies [6, 74, 75]. The adjuvant properties of the bacteria provided the immune response, and the adsorbed conjugate provided sufficient quantities of clones producing antibodies of the required specificity. For example, antibodies against tetrasaccharide Le^y [74], do not cross-react with the structurally close trisaccharides Le^x and H (type 2). Moreover, monoclonal antibodies obtained with the help of the synthetic immunogen Le^y-PAA-PE revealed that this oligosaccharide chain is a component of a natural antigen of the cell membrane [51]. An attempt to obtain antibodies using another immunogen, Le^y-BSA has led to several antibodies which interacted with synthetic antigen but poorly recognized the natural Le^y [76]. The difference in the results of immunization by Le^y-BSA and Le^y-PAA-PE can be explained in the following way. During immunization by the neoglycoprotein the response to the glycopeptide epitope takes place due to the immunogenicity of BSA itself. In contrast, the polyacrylamide matrix is non-immunogenic, so the response is only to the carbohydrate epitope, which in this case, is the same on both the synthetic and natural antigens. Another example of this kind is the method used to obtain antibodies to $SiaLe^x$-PAA-PE [75], that are capable of recognizing the natural epitope on the cell surface (Figure 10). There are two other advantages of immunization with Glyc-PAA-PE. The first one is the possibility of obtaining antibodies to crypted (masked) and immunologically 'silent' antigens (*eg* alloantigens). The second one is the possibility of obtaining antibodies to precisely defined topographical epitopes: antibodies could be raised against an oligosaccharide (for example, a tetrasaccharide, all the four monosaccharide residues of which are in direct contact with a protein) that

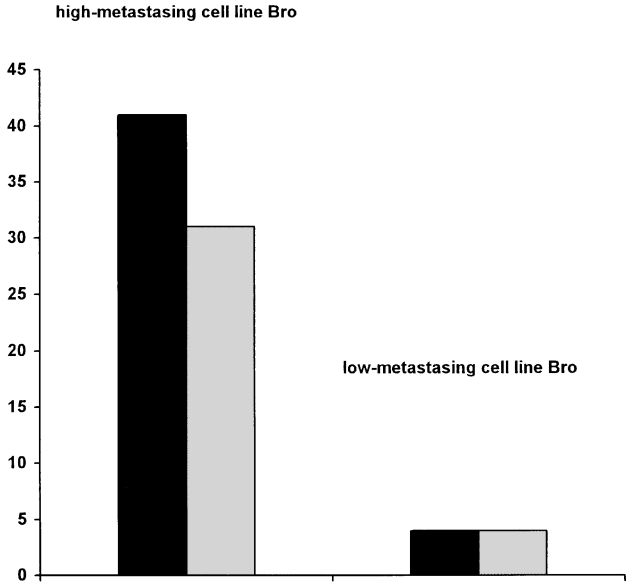

Figure 10. Binding of anti-SiaLe^x antibodies and anti-LAMP antibodies with a melanoma cell line Bro (high- and low-metastasing variants) as % of antigen-positive cells in flow cytofluorometry. Second antibody: rabbit anti-mouse-flu; black bars, anti-LAMP; dashed bars, anti-SiaLe^x. Anti-SiaLe^x monoclonal antibody was obtained against synthetic immunogen SiaLe^x-PAA-PE.

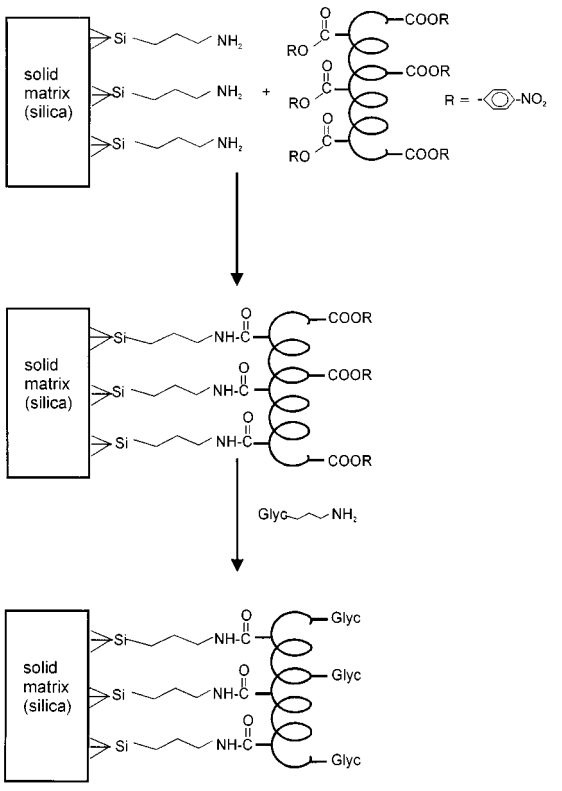

Figure 11. Synthesis of affinity glycosorbent. pNPA is attached to the aminated silica matrix; then the aminoligand is attached to the obtained activated surface. The chemistry of the coupling is the same as in soluble conjugates, see Figure 1.

would normally only be recognized from a specific side or angle. The latter advantage has also particular value wherein the proximity of adjacent molecules may cause steric hindrance of the desired epitope. It is obvious that an antibody recognizing an antigen from the front can usually detect an antigen, even if the latter is located beside a bulky neighbour or is a part of a glycolipid 'cap'. On the other hand, antibodies, interacting obliquely, may encounter spacial difficulties. This was confirmed by the following experiments: from nine anti-A monoclonal antibodies that were investigated only three agglutinated the erythrocytes of all A subgroups. Just these three antibodies interacted with the maximally loaded (30 mol%) synthetic antigen A_{tri}-PAA^e considerably better than other ones. (Deriugina EI, unpublished results).

Thus, one can expect, that a correctly designed synthetic immunogen can be used to perform preferential selection of B-cells bearing the antibodies of necessary topological specificity. It appears, that using more intricate immunogen constructions, it could be possible to obtain antibodies with various topographical specificity, such as those simultaneously recognizing the cluster of two or three closely located haptens.

Glycoparticles (adsorbents and latexes, the second 'ligand' is an insoluble particle) and glycosurfaces

With the help of p4NP, it is possible to activate, and then to modify by a carbohydrate, any surface decorated by primary amino groups. The approach is used most widely for the synthesis of glycosorbents [6, 28, 67, 77–82] on a base of aminoalkylated macroporous glass (Figure 11) or Sepharose. The synthesis is performed as follows [6]: the aminopropylated matrix is treated by p4NP solution, thus giving rise to the activated carrier; then the aminoalkyl glycoside is attached to an immobilized polymer, afterwards the excess of active groups is transformed into amides by the action of ethanolamine. The conditions of the attachment are similar to those in case of soluble probe synthesis. Glycosorbents are convenient for affinity isolation of lectins and antibodies [34, 83]. Due to polymeric binding between the carrier and ligand these adsorbents give favourable properties for affinity purification: proteins bind more rapidly and can be eluted under milder conditions compared to conventional adsorbents, where the ligand is attached directly to the carrier.

Coloured latex particles permit the visual monitoring of probes. Coloured latex probes were prepared as normal except that polystyrene-polyacrolein microparticles were covered with albumin as a source of aminogroups and for the prevention of spontaneous flocculation. The latex-label (usually brightly coloured) permitted the non-instrumental

semiquantitative evaluation of antibody levels (Figure 12) for example, the determination of IgM and IgG antibody titres against blood group A and B antigens [84]. A second example is the latex-diagnosticum for detecting anti-DMG IgM in sera of lepra patients [85].

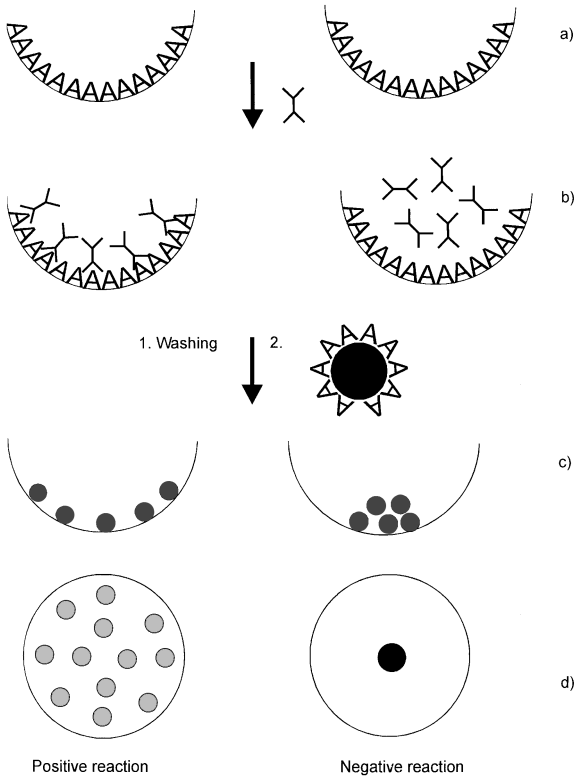

Figure 12. Determination of titre of antibodies to blood group specific antigen A with the help of latex-agglutination on 96-well round bottom plates. (a) Pseudopolysaccharide A_{tri}-PAA^e (designated as capital letters A) is applied to plastic; (b) anti-A antibodies are bound to the modified surface (left), non-specific antibodies are left in solution (right); (c) after washing the latex bearing immobilized antigen A on its surface is added to the wells; the latex specifically bound with antibodies covers uniformly the well surface (left), unbound latex is 'trickled' down to the well bottom; (d) the visible result reflects negative and positive results of the reaction respectively. A – A_{tri}-PAA^e; ⟩⟨ – antibodies; ● – modified latex particles.

An alternative approach to the design of glycobeads and glycosurfaces is the modification of the material with avidin or streptavidin (or the use of commercially-available materials), followed by the attachment of Glyc-PAA^e-biot, as described with magnetic beads [73].

Pseudoglycoproteins

Uromodulin, α_1-acid glycoprotein, and other glycoproteins modulate the immune response, affecting the activity of immunocytes. It is supposed that the interaction of glycoprotein carbohydrate chains with target lectins of cells occurs cooperatively, with the participation of several identical or different chains. In order to clarify the contribution of N-chains into the immunomodulatory effect of glycoproteins and to study the cooperativity of this effect, a convenient approach was developed, namely, the synthesis of 'pseudoglycoproteins'. The latter were obtained [65, 86, 87] by transfer of carbohydrate chains from a glycoprotein on to PAA. Carbohydrate chains of the glycoprotein were totally cleaved from the protein followed by their conversion to glycosylamines or aminoalditols and attached to p4NP via amino groups. The quantitative nature of the yield from the coupling reaction permitted, firstly, the obtaining of pseudo-glycoproteins having the same carbohydrates content as the initial glycoprotein, and secondly, the preservative of the same ratio of the different carbohydrate chains as found in the prototype (Figure 13). Thus, the conjugate completely mimicked the carbohydrate 'coat' of the glycoprotein.

This approach showed, that only the carbohydrate N-chains were responsible for the immunomodulating activity (action on lymphocytes proliferation and production of cytokines [65]) in α_1-acid glycoprotein, and that the arrangement of chains was not essential for the observed effects, as the activity of pseudoglycoprotein (where the chains were shuffled) was similar or higher than that of the natural glycoprotein.

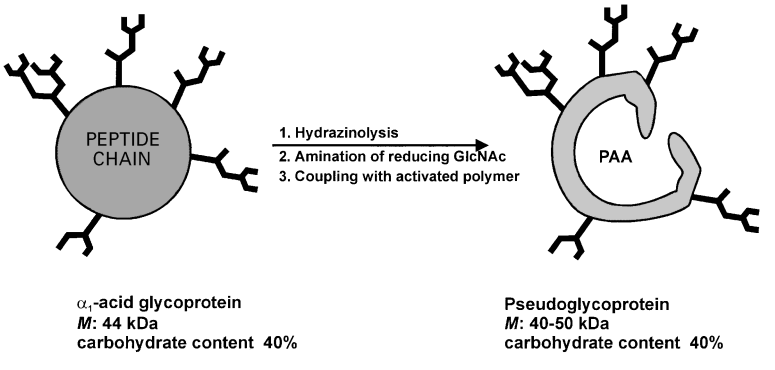

Figure 13. Translocation of N-bound carbohydrate chains of α_1-acid glycoprotein onto polyacrylamide (synthesis of pseudoglycoprotein). The pool of aminated glycans (as glycosylamines or aminoalditols) is attached to the activated polymer (pNPA). An additional label (*eg* flu) can be introduced. The pseudoglycoprotein is designed as a molecule similar to the parent glycoprotein in respect of molecular weight and carbohydrate content.

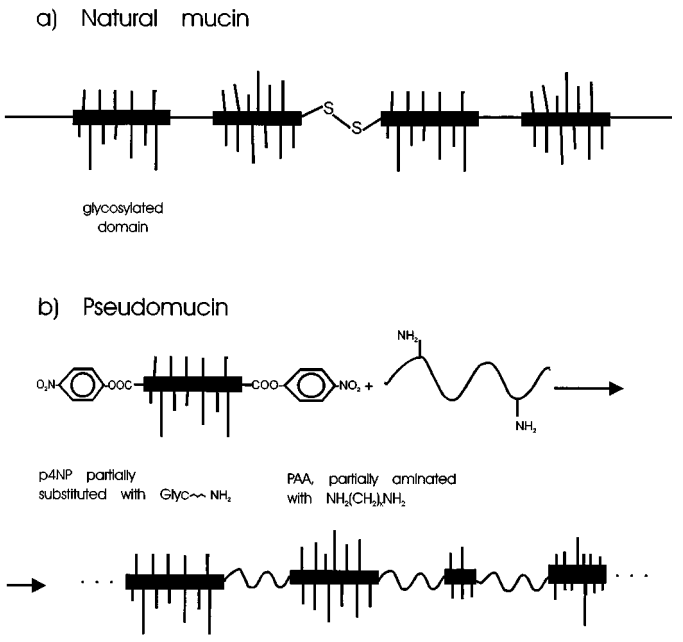

Figure 14. Schematic presentation of the natural mucin (a) and a pseudomucin obtained from partially substituted activated first polymer and second, a non-glycosylated one containing a limited number of amino groups (b).

One more example of the applicability of pseudoglycoproteins should be noted. A glycoprotein may bear only one carbohydrate chain, but several copies of this glycoprotein closely situated on a cell membrane can form a multivalent ensemble of carbohydrate chains. When studying the biological function of these chains in solution (where it is monovalent) therefore, important information can be lost if its effects depend upon cooperativity. With pseudoglycoprotein this problem can be overcome by constructing a molecule bearing several carbohydrate chains. This approach has been applied to the study of carbohydrate-carbohydrate interaction [71].

Synthesis of pseudoglycoproteins was notable with respect to the attachment of sialylated N-chains. The conditions of attachment were the same as for neutral saccharides; sialylation was not affected. Individual sialooligosaccharides could also be condensed with p4NP without any complications and synthetic oligosaccharides, such as selectin ligands $SiaLe^x$ and $SiaLe^a$, were attached via O-glycosidic spacers [88]. Oligosaccharides from human milk and glycoprotein N-chains were converted either to aminoacldditols, or to glycosylamines or glycyl derivatives Glyc-$NHCOCH_2NH_2$, lysogangliosides were attached directly to the polymer. All the four types of amino derivatives were quantitatively attached to the polymer [20].

Pseudomucins

The characteristic features of typical mucins are high molecular weight (up to several million daltons), high degree of glycosylation (up to 90 weight % of carbohydrates, mainly attached to Ser and Thr), presence of negatively charged groups (Neu5Ac and HSO_3Glyc), alternative highly glycosylated and 'naked' repetitive peptide motifs and, frequently, the occurrence of subunits due to numerous S-S bonds (Figure 14). Because of these structural features, mucins have an open threadlike form, so their solutions have high viscosity in low physiological concentrations. Viscosity and heterogeneous glycosylation are the reasons for their excellent protective properties against microorganisms. Viscosity makes a non-specific contribution, and the carbohydrate chains a specific one, as they mimic the bacterial and viral receptors on the host cell. In addition, some mucins play a major role as specific molecules for intercellular adhesion [48]. All these properties make mucins an attractive molecule for modelling synthetic glycoconjugates, *ie* synthesis of 'pseudomucins'. The synthetic approach, based on the use of p4NP permitted the synthesis of conjugates having both average M_r (30–40 kDa) and high M_r [90]. The synthetic scheme is presented in Figure 14. An oligosaccharide (which can be sialylated or sulfated, as is the case in many mucins) was attached to an activated polymer, followed by the condensation with a second macromolecule, bearing a limited number of amino groups. A soluble conjugate having a molecular weight of several thousand kDa was formed as the result of limited cross-linking. As only the resultant polymer is soluble in water, and the reaction is carried out at the interphase of water/organic solvent, greater cross-linking was not observed.

The glycosylated polymer of the resulting pseudomucin mimics the Ser/Thr-rich sites, and the non-glycosylated one mimics the carbohydrate-free repetitive motifs of mucin. Such high molecular weight soluble conjugates that contain *N*-acetylneuraminic acid show considerably higher virus-neutralizing activity stet with the 30–100 kDa conjugates [91]. Another, 'biomimetic' approach was applied to obtain mucin-like molecules. A limited number of SH-bonds was introduced into the polymer with the help of $NH_2CH_2CH_2SH$ followed by mild oxidation [90]. As expected, a high molecular weight product was accumulated due to intermolecular S-S bonds formation, however it was basically insoluble. Nevertheless, this approach proved to be useful for another purpose *ie* the modification of the nitrocellulose carrier (Figure 15). Regular Glyc-PAA^e (see above) are convenient reagents for the assay of lectins and antibodies on nitrocellulose membranes, however the introduction of SH-groups into Glyc-PAA^e gave considerably better results. After applying conjugates to nitrocellulose they were oxidized by air giving rise to a cross-linked 'superconjugate' that was permanent and stable. For example, the sensitivity of blot analysis for blood group A and B antibodies was improved up to a level only previously observed with natural mucins [91].

As it has been already mentioned, mucin antimicrobial activity is associated with the heterogeneity of their

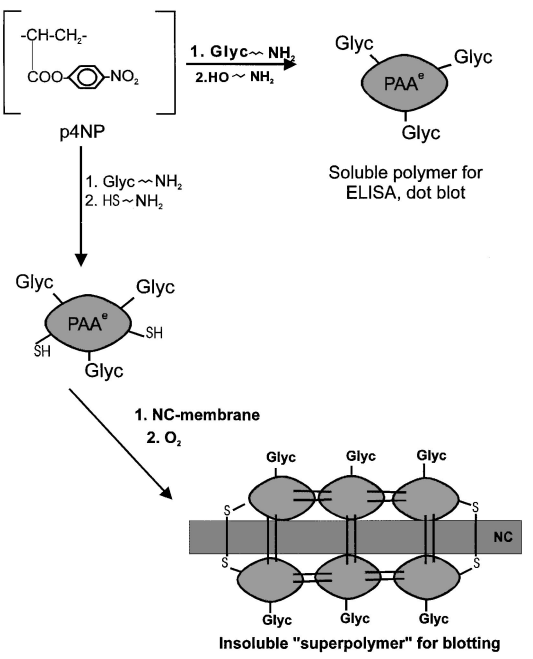

Figure 15. Synthesis of two types of glycosylated polymers for solid phase analysis. The introduction of a limited number of SH groups into PAA^e backbone gives possibility to cross-link 30 kDa polymers giving rise to a 'superpolymer' on the porous material (*eg* nitrocellulose). There is no need to premodify porous material with any functionals.

carbohydrate chains. However, several examples are known in which a microorganism (*eg M. pneumoniae*) has two different carbohydrate-binding receptor sites. By introducing into 'pseudomucin' both active oligosaccharide ligands, it is possible to increase the antimicrobial action of a synthetic analogue. It should be noted, that the synthetic method permitted the introduction of the two ligands both uniformly distributed on a macromolecule, or in blocks, one ligand situated on the primary polymer, and the second one on the linking polymer.

Conclusion remarks

In summary, it is possible to formulate the following advantages of polyacrylamide-type conjugates and the way in which they are synthesized.

(1) Condensation proceeds quantitatively, that giving the possibility to use small amounts of oligosaccharides without loss, the synthesis of conjugates with a predesigned composition; the synthesis of conjugates with mixed ligands that is controlled by the amounts introduced into the reaction mixture.

(2) It is possible to introduce into polymer other ligands besides carbohydrate ones, thus obtaining various probes, immunogens and glycoparticles. The method permits the formation of 'pseudoglycoproteins', 'pseudomucins' and other complex constructions.

(3) It is possible to regulate the molecular weight, charge, hydrophobicity, solubility and other properties of the conjugates.

(4) The synthesis is very simple to carry out; it is performed under routine conditions, not requiring low temperatures or anhydrous solvents; it is possible to control the reaction by TLC.

(5) The technique's simplicity and quantitative yields permit the synthesis of microquantities of the conjugates (about 0.1 mg).

(6) Due to the inert matrix, the conjugates display low non-specific absorption on to proteins and cells; the matrix itself does not display immunogenic properties.

(7) Being a flexible polymer, the polyacrylamide serves as an additional spacer, a three-carbon spacer was sufficient for saccharide-protein interaction in all cases investigated. A flexible polymeric coil seems to provide optimal adjustment of the carbohydrate residue for carbohydrate-binding protein in cooperative processes.

(8) A series of conjugates with varying content of carbohydrate ligand permits the evaluation of the sensitivity of interaction to the epitope density of ligand, as well as selecting the optimal conjugate for a given assay.

This review shows the possibilities of polyacrylate type glycoconjugates for the study of carbohydrate-binding proteins. However, this approach, based on the reaction of p4NP with amino ligands, could be considerably more general, giving the possibility to synthesize the conjugates of practically any low molecular weight compounds, if they have readily available or specially introduced amino group (*ie* peptides).

This review also suggests that polyacrylate glycoconjugates, have possible therapeutic applications. For example, possible prophylactic influenza virus neutralization during epidemics. As shown [3, 5, 8, 91], the conjugates of Neu5Ac with polyacrylic acid inhibited at low concentrations the binding of the virus to target cells. Electron microscopy studies [90] have shown (Figure 16), that pseudopolysaccharide not only bound to virion, but also distorted its major surface protein, haemagglutinin, thus it was not only virostatic, but also had viricidal properties. The second prospective application field of high M_r, pseudopolysaccharides and pseudomucins could be the neutralization of gastrointestinal infections caused by pathogenic or conditionally-pathogenic bacteria, having the lectins on their surface such as some strains of *E. coli*, and *Helicobacter pylori*. Another possible application in this field is bacterial toxin neutralization.

Possibilities also exist for the creation of a new generation of babies' nutrition [92, 98]. As sialyllactose plays a role as a viral and bacterial neutralizing agent in natural milk it would seem possible to replace free oligosaccharide with more efficient glycoconjugates.

Recently the basic role of complex oligosaccharides (in this case Le^b) in the fertilization process has been shown

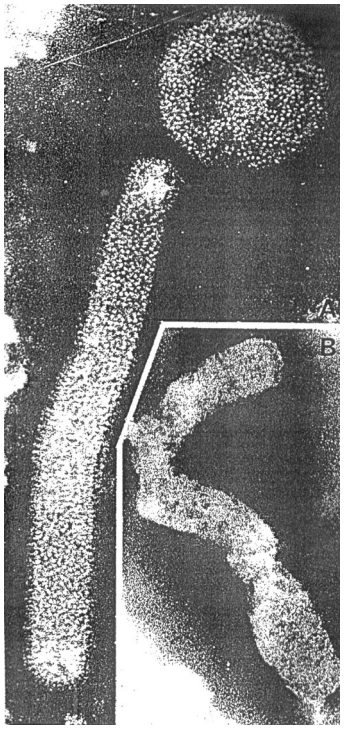

Figure 16. Electron micrograph of influenza virus, magnification 230 000. A. Native virions; B. Destruction of virion's haemagglutnin as the result of treatment with sialylated neoglycoconjugate.

[56], thus opening up the opportunity for new approaches to contraception and infertility treatment. Synthetic glycoconjugates may be developed that can be used in this way.

The conjugates of tumour-associated oligosaccharides, such as Neu5Acα2-6GalNAc, with keyhole limpet haemocyanin have been previously used as anti-cancer vaccines [93]; also glycoconjugates on a base of polylysine have been shown to inhibit metastasis [94]. In all these cases of potential or already used therapeutic applications of glycoconjugates, especially when a preparation is intended for peroral use, polyacrylate matrices would seem to make useful carriers for biologically active carbohydrate ligands due to their inertness and stability as well as the possibilities described above of giving practically any necessary properties to the conjugate. Polyacrylates have already passed a series of tests in this capacity [95, 96].

One more promising application area of polyvalent glycoconjugates and glycosorbents is transplantation. Human kidney or liver transplantation requires the very precise selection of donor/recipient pairs in relation to histocompatibility antigens, however the situation is frequently observed where there is incompatible blood groupings, in particular in the ABO system. Therefore, antibodies against carbohydrate antigens of donor kidney can be presented in the recipient's blood. It may be possible to resolve this situation either by adsorption of interfering antibodies on an appropriate glycosorbent, or by their neutralization with the help of a carbohydrate introduced into the blood [97]. A similar situation can be developed during xenotransplantation (pig-to-human) [82], as the cytotoxic anti-Galα1-3Gal antibodies causing fast rejection are present in a high concentration in the blood. Synthesis of a multivalent form of an oligosaccharide-neutralizor, acting in low concentration and slowly removable from the blood stream, could resolve the problem of both allo- and xenotransplantation. Whether poly- (or rather oligo-) acrylamides possess the complex properties necessary for intravenous introduction, need to be clarified.

It is impossible as yet to answer unequivocally whether the approaches listed above will be realized, as therapeutic application will require more investigation than that needed for diagnostic applications. However, from the point of view of the technology, the synthesis of biologically active polyvalent carbohydrates by the conjugation of Glyc-NH_2 with an activated polymer, seems to have potential, as it corresponds to the main requirements for a technology *ie* consistency and reproducibility.

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