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Blood Group A and B Glycosyltransferases Synthesize A and B Determinants on Different Acceptor Polyglycosyl Peptides *in Vitro*

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The glycosylation of polyglycosyl chains from human erythrocytes by human plasma blood group A and B glycosyltransferases was studied in order to clarify why human blood group AB erythrocyte polyglycosyl peptides carry only either A or B determinants [Eur J Biochem (1981) 113:259-65].

The blood group A transferase was able to add radioactive *N*-acetylgalactosamine from labeled UDP-*N*-acetylgalactosamine to B-type erythrocytes which had been treated with α-galactosidase in order to cleave the B determinant sugar from the erythrocytes. This suggests that the enzymes specified by the *A* and *B* genes utilize the same acceptor molecules on erythrocyte membranes. Polyglycosyl peptides isolated from blood group B erythrocytes acted as acceptors for blood group A glycosyltransferase and the generation of hybrid structures containing both A and B determinants was demonstrated. When blood group O polyglycosyl peptides were used as acceptors in the simultaneous presence of both blood group A and B glycosyltransferases, however, the A and B determinant sugars were found in different polyglycosyl peptides. It is suggested that the enzyme-acceptor complex does not dissociate until the final number of determinants has been added.

Human blood group A and B glycosyltransferases from the plasmas of A- and B-type individuals have been characterized [1-8]. These enzymes have also been found as membrane enzymes in human erythrocytes [2, 9-12]. As shown by Karhi *et al.* the blood group determinants can be identified on cell membranes in basophilic normoblasts and in later stages during the maturation of an erythrocyte [13]. The α-3-*N*-acetylgalactosaminyltransferase corresponding to the blood group *A* gene and producing the blood group A determinants by adding *N*-acetylgalactosamine residues from UDP-*N*-acetylgalactosamine to the H-determinant structure (Fucα1-2Galβ1-) [14] has been purified from human plasma by affinity chromatography [5, 6]. The *A* gene specified enzyme has a molecular weight of 90-100 000 and consists of two subunits having equal molecular weights; it has a K_M of 0.1 mM for 2'-fucosyllactose [3] and of 20 μM for UDP-*N*-acetylgalactosamine [15]. The corresponding α-3-galactosyltransferase specified by the blood group *B* gene

has also been purified and characterized from human plasma by conventional methods [7] and by biospecific adsorption to blood group O stroma [8]. This enzyme converts H determinants to blood group B determinants by transferring a galactosyl residue from UDP-galactose to the acceptor H determinant [14]. The K_M value of the B enzyme for 2'-fucosyllactose is 0.5 mM and for UDP-galactose 10μM [8].

In addition to the H determinant-containing oligosaccharides, human erythrocytes [16-18] and erythrocyte membranes [19] have also been used as acceptors for the human blood group A glycosyltransferase. In human erythrocytes the quantitatively most prominent fractions containing the acceptor structures are the band 3 and 4.5 protein molecules [16-18]. The glycans of bands 3 and 4.5 are composed of repeating lactosamine units [20-22] and the carbohydrate chains can be isolated after proteolytic digestion as polyglycosyl peptides [23-25]. In agreement with the acceptor studies, lectin-binding experiments [26, 27] and studies using chemical methods [23-25] show that the polyglycosyl chains in bands 3 and 4.5 carry about 70% of the total amount of ABH determinants found in human erythrocytes.

The requirement for the acceptor, Fucα1-2Galβ1-, is shared by blood group A and B transferases and the determinant structures are found in the same classes of molecules [26]. Moreover, in human blood group AB both A and B determinant-containing polyglycosyl peptides were isolated from both band 3 and 4.5 proteins [28]. As each blood group active polyglycosyl peptide carries on the average three to four determinants [23], the finding that in blood group AB the A and B determinants are carried by different polyglycosyl peptides [28] was surprising. In the biosynthesis of carbohydrates no mechanism that could cause this kind of regulation is known [29], and the following possibilities can be suggested.
a) That A and B transferases use a different class of acceptor molecule.
b) The first formed A and B determinant prevents the action of the other transferase on the acceptor molecule.
c) The enzyme-acceptor complex does not completely dissociate before the transferase enzyme has formed A or B determinants on all the available H determinant sites.

The present study was performed to elucidate which type of interactions were involved in producing the differential glycosylation of polyglycosyl peptides by blood group A and B transferases in human blood group AB erythrocytes.

Materials and Methods

Reagents

α-Galactosidase (EC 3.2.1.22) purified from green coffee beans, *Streptomyces griseus* protease (EC 3.4.24.4) type XVI, galactose-oxidase (EC 1.1.3.9) from *Dactylium dendroides*, bovine serum albumin, UDP, unlabeled UDP-galactose, UDP-*N*-acetylgalactosamine and 2'-fucosyllactose were obtained from Sigma Chemical Co. (St. Louis, MO, USA). UDP-[^{14}C]galactose (300 Ci/mol), [^{14}C]acetic anhydride (114 Ci/mol), [^{3}H]acetic anhydride (500 Ci/mol) and NaB^3H_4 (11.0 Ci/mmol) were obtained from Amersham Internatio-

nal (Amersham, UK). *Vicia cracca*-lectin and *Bandeiraea simplicifolia* I B_4-isolectin were coupled to Sepharose 4B as described previously [28]. The immobilized lectins were kindly supplied by Dr. Kimmo Karhi (Department of Biochemistry, University of Helsinki).

Radioactive UDP-*N*-[^{14}C]- and [^{3}H]acetylgalactosamine were prepared by *N*-acetylation [30] of UDP-galactosamine obtained by the uridyltransferase reaction as described by Maley [31]. Reagents and enzymes for preparation of UDP-galactosamine were obtained from Sigma Chemical Co., except calf liver galactose-1-phosphate uridyltransferase (EC 2.7.7.12) which was obtained from Boehringer Mannheim (W. Germany). UDP-*N*-acetyl-[6-^{3}H]galactosamine (1 μmole) was prepared by incubation with galactose oxidase (50 U) in 1 ml of 10 mM sodium potassium phosphate buffer pH 7.4 containing 133 mM NaCl for two hours at 37°C. The aldehyde groups thus generated in the *N*-acetylgalactosamine residues were subsequently reduced with NaB^3H_4 (2.0 mCi in 125 μl of 10 mM NaOH) at room temperature for 1 h. All the radioactive UDP-*N*-acetyl-galactosamine preparations were purified by ion exchange chromatography on a column (1 × 20 cm) of AG® 1-X2, acetate form (Bio-Rad Laboratories, Richmond, CA, USA), eluting the column with a linear 0.1-2.0 M gradient of pyridine-acetic acid buffer pH 5.0. Radioactive UDP-*N*-acetylgalactosamine was eluted from the column with a 1.3-1.5 M concentration of the buffer, lyophilized and dissolved to 1 mM concentration.

Polyglycosyl peptides

These were prepared from blood group B and O outdated erythrocytes as described previously [28, 32, 33]. Erythrocyte membranes were prepared [34] and delipidated [35, 36]. Delipidated membrane residue was treated with protease and the glycopeptides isolated by gel filtration [37]. Polyglycosyl peptides were isolated after mild alkaline treatment [38] by gel filtration on Sephadex G-50 Fine as described [28, 32, 33].

Blood group B polyglycosyl peptides were labeled in the peptide moiety by *N*-acetylation with [^{3}H]acetic anhydride as described [30] and purified by gel filtration. The blood group B polyglycosyl peptides were fractionated by lectin affinity chromatography on *B. simplicifolia* I lectin with a gradient of galactose (0-0.250 mM) and finally with 1 mM galactose as previously described for blood group A_1 and A_2 polyglycosyl peptides [32]. Three fractions were obtained: B 0 (unbound), B I ("weakly" bound, eluted with the galactose gradient) and B II ("strongly" bound, eluted with 1 mM galactose). The fractions were desalted by gel filtration.

The monosaccharide composition of the isolated glycopeptide fractions wasdetermined after methanolysis as described [32, 39, 40].

Erythrocytes

Erythrocytes for the labeling experiments were obtained from healthy pretyped individuals using citrate as anticoagulant. The cells were washed three times with 0.150 M NaCl at room temperature. α-Galactosidase treatment of the erythrocytes was performed as described by Goldstein *et al.* [41]. The extent of the treatment was monitored by hemagglutination assay using *B. simplicifolia* I B_4 isolectin. The hemagglutination titer

of blood group B erythrocytes was found to decrease at least three double dilutions as a consequence of the α-galactosidase treatment.

Blood Group A- and B-Glycosyltransferases

Blood group A *N*-acetylgalactosaminyltransferase (EC 2.4.1.40) was purified from sera of blood group A individuals by adsorption to Sepharose 4B as performed by Nagai *et al.* [6]. The enzyme was eluted from the Sepharose 4B column with 20 μM UDP in 0.1 M disodium cacodylate buffer pH 7.0 containing 0.2 M NaCl at 4°C and immediately adjusted to a bovine serum albumin concentration of 0.1 mg/ml to decrease inactivation of the transferase due to the low protein concentration. The enzyme preparation was concentrated to 0.5% of the original serum volume by vacuum dialysis as suggested by Karhi [18]. This procedure has been reported to yield an apparently pure enzyme producing 360 pmoles of A determinant structure per ml plasma in 1 h under saturating conditions of 2-fucosyllactose and UDP-*N*-acetylgalactosamine [6].

Blood group B α-galactosyltransferase (EC 2.4.1.37) was purified as described by Carne and Watkins [8]. The enzyme was adsorbed to blood group O stroma in the presence of UDP-galactose and detached from the membranes with 5 mM 2'-fucosyllactose. The stroma were removed by centrifugation and bovine serum albumin was added to the B enzyme containing supernatant to a final concentration of 0.1 mg/ml. The B enzyme preparation was dialysed and concentrated by vacuum dialysis as described for the A transferase above. The method yields a 10 000-fold purification of the B enzyme and it converts 31 pmoles of 2-fucosyllactose to a B determinant tetrasaccharide at saturating substrate concentrations [8].

The A and B transferase preparations were stored at -60°C and activities were found to be stable for at least three weeks.

Labeling of the Polyglycosyl Peptides with Blood Group A and B Transferases

Blood group O polyglycosyl peptides were labeled radioactively with UDP-*N*-acetyl-[6-^{3}H]galactosamine and UDP-[^{14}C]galactose by incubation with blood group A α-3-*N*-acetylgalactosaminyltransferase and blood group B α-3-galactosyltransferase. The incubation mixture contained in a total volume of 1 ml: polyglycosyl peptides containing 2 nmol of fucose from blood group O erythrocytes, 10 nmol of UDP-*N*-acetyl-[6-^{3}H]galactosamine (4×10^4 cpm), 10 nmol of UDP-[^{14}C]galactose (10×10^4 cpm), 20 μmol of $MnCl_2$, 100 μmol of disodium cacodylate buffer pH 6.3 and appropriate amounts of A and B transferases in a volume of 100 μl. The incubation was carried out at 37°C for 6 h and stopped by lyophilization. The glycopeptide-bound radioactivity was separated from the radioactive sugar nucleotide substrates by gel filtration on a Bio-Gel P4 column (bed volume 1 ml) eluted with 0.1 M pyridine acetic acid buffer pH 5.0. The Bio-Gel P4 columns were upon precalibration with UDP-galactose and labeled polyglycosylpeptides found to have a baseline separation of these two compounds.

The polyglycosyl chains were fractionated by lectin affinity chromatography on Sepharose 4B-bound blood group A specific *V. cracca* lectin and blood group B specific *B. simplicifolia* I B_4 isolectin as described [28]. The *V. cracca* column (0.5 ml bed volume)

was eluted with 10 mM sodium phosphate buffer pH 7.0 containing 0.15 M NaCl and 0.1 mM $CaCl_2$ and $MgCl_2$ and the adsorbed glycopeptides were eluted with the same buffer containing 10 mM of *N*-acetylgalactosamine. The *B. simplicifolia* I B_4 column (bed volume 0.5 ml) was eluted with 10 mM sodium phosphate buffer pH 7.0 containing 0.15 M NaCl and 0.1 mM $CaCl_2$ and the bound glycopeptides were eluted with the same buffer containing 10 mM of galactose. Fractions of 250 μl were collected and analysed for [^{3}H] and [^{14}C] radioactivity.

Blood group B polyglycosylpeptide subfractions B 0, B I and B II were labeled with UDP-*N*-[^{14}C]acetylgalactosamine using the purified blood group A transferase. Equal molar amounts of glycopeptides of each of the subfractions were incubated with 10 nmol of UDP-*N*-[^{14}C]acetylgalactosamine (25 × 10^4 cpm), 2 nmol of $MnCl_2$, 10 nmol of disodium cacodylate buffer pH 6.3 and 10 μl of the blood group A *N*-acetylgalactosaminyltransferase in a total volume of 100 μl at 37°C for 16 h. The polyglycosyl peptides were separated from the labeled substrate as above and analysed for radioactivity. The B I subfraction was fractionated on *V. cracca*-Sepharose and the B II subfraction on *B. simplicifolia* I B_4-Sepharose.

Labeling of Human Erythrocytes with A Transferase

Packed typed erythrocytes (50 μl) were incubated with 10 nmol of UDP-*N*-[^{3}H]acetylgalactosamine (2.5 × 10^6 cpm), 2 μmol of $MnCl_2$, 5 μmol of disodium cacodylate buffer pH 6.8 and 10 μl of the A-enzyme preparation in a total volume of 100 μl. The incubation was carried out at 37°C for 1-3 h after which the erythrocytes were washed three times with 0.15 M NaCl. The erythrocyte membranes were prepared as described by Dodge *et al.* [34] and the incorporation was calculated as cpm per mg of membrane protein.

Results

Labeling of the Blood Group O Polyglycosyl Peptides

Purified human serum blood group A and B glycosyltransferases incorporated monosaccharides from the corresponding sugar nucleotides into polyglycosyl peptides purified from blood group O erythrocytes. Moreover, under the assay conditions with both UDP-*N*-[^{3}H]acetylgalactosamine and UDP-[^{14}C]galactose present, the A transferase incorporated only *N*-acetylgalactosamine and the B transferase only galactose (Table 1). The amounts of the blood group A and B enzymes in the different incubations were not the same and the activities were adjusted to transfer equal amounts of A and B determinant sugars to the acceptors in the experiments where both enzymes were present. No label was found in the high molecular weight fraction obtained after Bio-Gel P4 chromatography if the glycosyltransferases or the acceptor molecules were omitted from the incubation mixture (data not shown).

The labeled and purified glycopeptides were fractionated by affinity chromatography using blood group A specific *V. cracca* lectin and blood group B specific *B. simplicifolia* I B_4 isolectin. *V. cracca* specifically adsorbed glycopeptide-bound radioactivity only if the blood group A transferase had been present in the incubation mixture (Fig. 1A),

Table 1. Labeling of human blood group O polyglycosyl peptides with A and B transferases. Experimental details are given in the Materials and Methods section.

Blood group glycosyltransferase	nmol monosaccharide incorporated	
	N-acetylgalactosamine	galactose
A	0.6	<0.03
B	<0.03	0.3
A and B	0.6	0.6

whereas all the radioactive material generated in the glycopeptide fraction by the B transferase was eluted from the column with the buffer (Fig. 1B). The opposite was observed when the radioactive glycopeptides were passed through a *B. simplicifolia* I B_4 lectin column: all the radioactivity in the A transferase-treated preparation was eluted from the column with the buffer (Fig. 2A) and a portion of the radioactivity introduced by the B transferase to the polyglycosyl peptides was bound to the column and eluted specifically with the sugar hapten (Fig. 2B). The unbound fraction from Fig. 1A was rechromatographed on the *V. crassa* column and from Fig. 2B on the *B. simplicifolia* I B_4 column (data not shown) but no material was adsorbed to the columns. The low affinity of the unbound glycopeptides containing radioactive *N*-acetylgalactosamine in Fig. 1A and radioactive galactose in Fig. 2B probably reflects the low amount of determinants (probably only one per polyglycosyl chain) in these glycopeptides.

When both blood group A and B transferases were present in the incubation mixture, a proportion of the radioactivity in the newly generated A and B determinants was bound to both *V. crassa* and *B. simplicifolia* I B_4 columns (Figs. 1C and 2C). However, no $[^{14}C]$-labeled galactose was found in the A lectin-reactive glycopeptides and no $[^{3}H]$-labeled *N*-acetylgalactosamine was found in the B lectin-reactive material.

The polyglycosyl peptides are known to carry up to six or seven A determinants in blood group A_1. 30% of the chains carry 0.9 determinants per molecule, 55% carry 4.2, and 15% carry 6.3 determinants per molecule [32]. Assuming that H determinants in O type glycopeptides are distributed in a similar fashion to the A determinants in A_1 erythrocytes, the random distribution of 0.6 mol of A determinants and 0.6 mol of B determinants on to 2 mol of H determinants (Table 1) would generate at least 30% of the total B determinants on polyglycosyl chains carrying two or more A determinants. Correspondingly, 30% of the A determinants would be generated on chains carrying two or more B determinants. As these hybrid structures would be eluted in the *V. cracca*-bound fraction containing a total of 50 cpm of labeled galactose in the *B. simplicifolia* I B_4-bound fraction containing 50 cpm of labeled *N*-acetylgalactosamine, they should be detected without difficulty. Thus, the results suggest that A and B determinants are not generated on the same polyglycosyl chain.

Labeling of the Blood Group B Polyglycosyl Peptides with Blood Group A Transferase

In order to study if the blood group A transferase would be incapable of using blood group B determinant-containing polyglycosyl peptides as an acceptor, blood group B

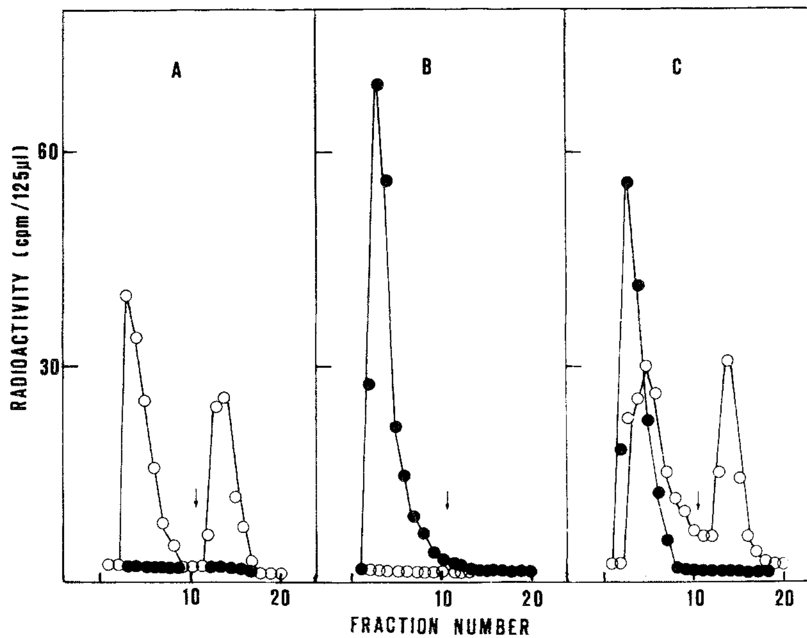

Figure 1. Fractionation of the blood group O-derived polyglycosyl peptides on *Vicia cracca* lectin after labeling with blood group A and B transferases. The blood group O polyglycosyl peptides were radioactively labeled and chromatographed as described in the Materials and Methods section. Half of the preparations were chromatographed on a *V. cracca* lectin column. The arrow indicates the point at which 10 mM *N*-acetylgalactosamine was included in the buffer. Incubation of the glycopeptides was carried out in the presence of A transferase only (A), B transferase only (B) and both A and B transferases (C). Fractions of 250 µl were collected and analysed for [^{3}H]-radioactivity (*N*-acetylgalactosamine), ○; and for [^{14}C]-radioactivity (galactose), ●.

polyglycosyl peptide subfractions were incubated with A transferase and UDP-*N*-[^{14}C] acetylgalactosamine. The blood group A *N*-acetylgalactosaminyltransferase incorporated radioactive *N*-acetylgalactosamine into all of the polyglycosyl peptide subfractions (Table 2). Thus, the presence of B determinant structures in a polyglycosyl chain permits the A transferase to use this glycopeptide as an acceptor molecule. A portion of the labeled B I subfraction was adsorbed to *V. cracca* lectin demonstrating that it contained A determinants after treatment with the blood group A transferase (Fig. 3A). In the B II subfraction, a portion of the labeled galactosamine was bound to the *B. simplicifolia* B_4 lectin indicating that it contained both blood group A and B determinants (Fig. 3B).

Labeling of Blood Group B Erythrocytes with Blood Group A Transferase

To study if human erythrocyte membranes would have two different classes of acceptors for blood group A and B transferases, B erythrocytes were labeled with A transfe-

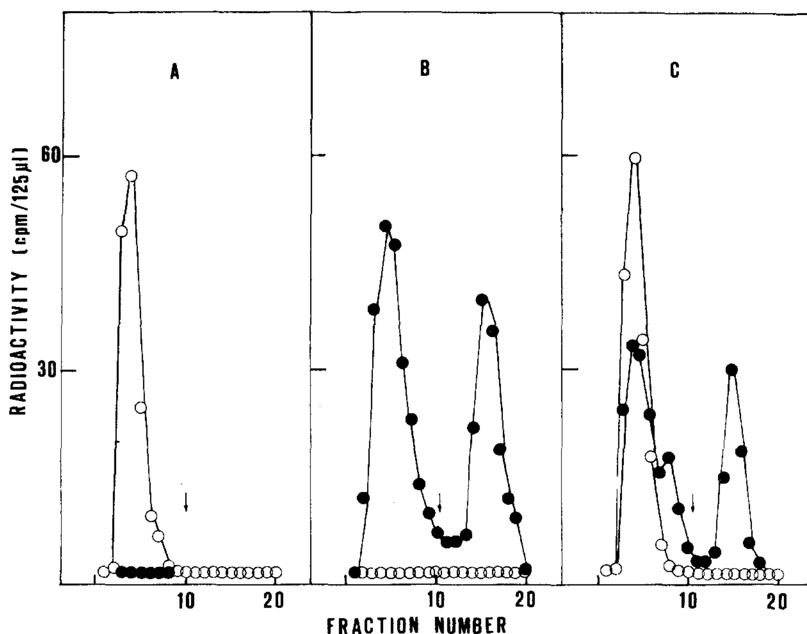

Figure 2. Fractionation of the blood group O-derived polyglycosyl peptides on *Bandeiraea simplicifolia* I B_4 isolectin after labeling with blood group A and B transferases. The blood group O polyglycosyl peptides were labeled and chromatographed as described in the Materials and Methods section. Half of the preparations were chromatographed on a *B. simplicifolia* I B_4 lectin column. The bound glycopeptides were eluted from the column with 10 mM galactose in buffer (arrow). Incubation of the glycopeptides was carried out in the presence of A transferase only (A), B transferase only (B) and both A and B transferases (C). Fractions of 250 µl were collected and analysed for [^{3}H]-radioactivity (*N*-acetylgalactosamine), ○; and for [^{14}C]-radioactivity (galactose), ●.

rase and radioactive UDP-*N*-acetylgalactosamine. Blood group B erythrocytes accepted under the assay conditions one fourth of the amount of label incorporated into blood group O erythrocytes (Table 3). Treatment of B erythrocytes with α-galactosidase to remove some of the B determinants and to create new H determinants increased the incorporation of radioactive *N*-acetylgalactosamine by blood group A transferase to almost twice the level found in the untreated B erythrocytes. This suggests that H determinants unmasked by α-galactosidase treatment of B determinants can be utilized by the blood group A transferase.

Discussion

The blood group A *N*-acetylgalactosaminyltransferase and blood group B galactosyltransferase are able to use the donor substrates for either enzyme [15, 42]. The pH optimum of the *A*-gene-specified *N*-acetylgalactosaminyltransferase activity is 6.0 and for

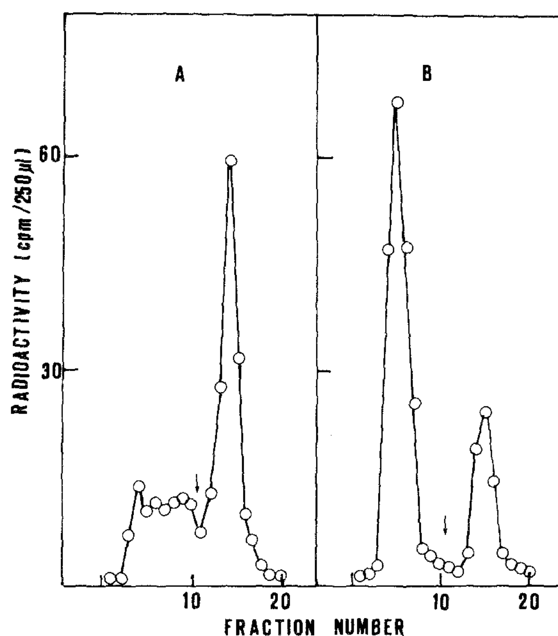

Figure 3. Fractionation of the blood group B polyglycosyl peptide subfractions on *Vicia cracca* lectin and *Bandeiraea simplicifolia* B_4 isolectin after treatment with blood group A transferase. The glycopeptide subfractions B I (A) and B II (B) were radioactively labeled and chromatographed as described in the Materials and Methods section and the legends to Figs. 1 and 2. (A) The *V. cracca* column, and (B) the *B. simplicifolia* I B_4 column. Fractions were analysed for [^{14}C]-radioactivity (*N*-acetylgalactosamine).

the galactosyltransferase activity, 7.0 [15]. The α-3-*N*-acetylgalactosaminyltransferase activity of the B enzyme has a pH optimum of 8.0 whereas the pH optimum for the α-3-galactosyltransferase activity is 6.5 [42]. The transferase assays using both blood group A and B glycosyltransferases were performed at pH 6.3 and as shown in Table 1 only very little, if any, "wrong" transferase activity was detected.

Both blood group A and B glycosyltransferases used isolated polyglycosyl peptides derived from blood group O erythrocytes as acceptor molecules. Since almost all of the fucose residues in polyglycosyl peptides are linked to position 2 of galactose units [24, 28], the amount of fucose can be regarded as the approximate amount of H determinants in the polyglycosyl peptides. The maximal amount of monosaccharides incorporated by the A and B transferases into O glycopeptides was 1.2 nmoles per 2 nmoles of fucose molecules in the glycopeptides, suggesting that a large proportion (at least 60%) of the H determinants in O-type polyglycosyl peptides are used as acceptors by the transferases.

The activity of the *A* and *B* gene-specified enzymes using polyglycosyl peptides as acceptors is comparable with previous studies [6, 8] if the low concentration of Fucα1-2Gal acceptor structures and of donor substrates is taken into account. The concentration of

Table 2. Labeling of blood group B polyglycosyl peptides with blood group A transferase. Human blood group B polyglycosyl peptide (0.25 nmoles of glycopeptide) subfractions isolated by affinity chromatography on *Bandeiraea simplicifolia* I lectin were incubated with blood group A glycosyltransferase as described in the Materials and Methods section.

Acceptor glycopeptide	Incorporation of *N*-[^{14}C]-acetylgalactosamine (cpm/nmol glycopeptide)
B.0	245
B.I	410
B.II	750

Table 3. Labeling of human blood group B and O erythrocytes with blood group A transferase. Human erythrocytes were incubated with UDP-*N*-[^{3}H]acetylgalactosamine and blood group A transferase as described in the Materials and Methods section. α-Galactosidase treatment was performed as described by Goldstein [41]. The isolated membranes were analysed for radioactivity.

Erythrocytes	Incorporation	
	cpm/mg membrane protein	% maximal
O	9900	100
B	2700	28
B treated with α-galactosidase	4800	49

the donor substrates was close to the K_M values because higher concentrations of UDP-*N*-acetylgalactosamine are known to inhibit the B enzyme [8]. The K_M values of the A and B enzyme for the polyglycosyl chain acceptors are not known. As estimated by the amount of fucose on the polyglycosyl peptides, the H determinant concentration in the incubation mixture was 2 μM. This value is much lower than the reported K_M values of the blood group A and B transferase for 2'-fucosyllactose (100 μM and 500 μM respectively) [3, 8].

A proportion of the glycopeptide-bound radioactivity was also adsorbed to blood group A and B specific lectin columns (Fig. 1 and 2) and eluted with the corresponding monosaccharide indicating that the generated structures contained blood group A and B determinants.

The finding that in human blood group AB erythrocytes polyglycosyl chains contained either A or B determinants and that no glycopeptides containing both A and B determinants were present [28], suggested that A and B transferases would use different carbohydrate chains as acceptors. If this were the case, the blood group A transferase could not use the same H determinant structures as the blood group B transferase and *vice versa*. When blood group B erythrocytes were treated with α-galactosidase to remove α(1-3)-linked galactose residues from the blood group B determinants new acceptor sites for the action of the A transferase were generated (Table 3). This indicates at least, that the blood group A transferase uses the same class of acceptor molecules as the B transferase. Polyglycosyl peptides derived from blood group B erythrocytes could also be labeled with *N*-acetylgalactosamine using UDP-*N*-[^{14}C]acetylgalactosamine and the A transferase (Table 2). The B determinants in the polyglycosyl peptides do not seem to prevent the A transferase from using the free H determinant sites in these glycopeptides as acceptors; the presence of both A and B determinants in the generated structures is suggested by their affinity to both *V. cracca* and *B. simplicifolia* I B_4 lectins (Fig. 3).

When blood group O polyglycosyl peptides were labeled simultaneously with blood group A and B glycosyltransferases, both radioactive *N*-acetylgalactosamine and galactose were incorporated (Table 1). The acceptor preparation contained both *V. cracca*, blood group A specific lectin-reactive polyglycosyl peptides, and *B. simplicifolia* I B_4, blood group B specific isolectin-reactive polyglycosylpeptides (Fig. 1C and 2C). However, the glycopeptides bound to the *V. cracca* column contained no labeled galactose residues and correspondingly the *B. simplicifolia* I B_4 column-bound material contained no labeled *N*-acetylgalactosaminyl residues. The results indicate that under competitive conditions between *A* and *B* gene-specified transferases the blood group A and B determinant structures are formed on different polyglycosyl chains. This result is in agreement with the previous finding that blood group A and B determinants are located in different glycopeptides in blood group AB erythrocytes [28].

The finding that A and B sites are formed on different blood group O-type polyglycosyl chains under the *in vitro* conditions with both A and B transferases present requires a mechanism for keeping the acceptor molecules separate. It is suggested that the enzyme-acceptor molecule complex does not dissociate before the final number of H determinant sites are substituted with the transferase-determined monosaccharide, GalNAcα(1-3)- or Galα(1-3)-. Whether A and B transferases have affinity for the polylactosamine backbone of the polyglycosyl peptides or for the H determinants remains to be established. As the molecular weight of the blood group *A* and *B* gene-specified enzymes is 80-100 000 [6, 7] and the molecular weight of one polyglycosyl chain is 6-10 000 [24], it seems reasonable that the enzyme might contain affinity sites which are able to hold the complex together. Attempts have also been made to isolate the blood group A or B transferase-blood group O polyglycosyl peptide complex by gel filtration, but so far without success.

The data above do not eliminate the possibility of intracellular compartmentalization of the blood group A and B transferases during the biosynthesis of ABO determinants, that is that the location of the two transferases in different parts of the cell could contribute to the differential glycosylation of the polyglycosyl chains in blood group AB, but they definitely indicate the possibility that such compartmentalization may be unnecessary.

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