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Immunology

Repetitive A epitope (type 3 chain A) defined by blood group A_1-specific monoclonal antibody TH-1: Chemical basis of qualitative A_1 and A_2 distinction

(subgroups/erythrocyte/glycolipid/methylation analysis/^{1}H NMR)

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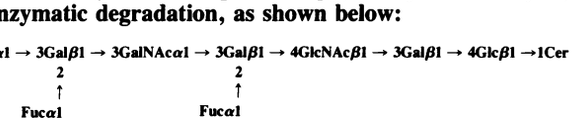
ABSTRACT **The IgG2a monoclonal antibody TH-1, which reacts specifically with blood group A_1 but with neither A_2 nor O erythrocytes, has been established. The antibody reacted only with A_1 erythrocytes in hemagglutination and antibody absorption assays; it did not react with A_2 erythrocytes, even after trypsin or sialidase treatment. This antibody detected, on TLC immunostaining, a series of glycolipids from A_1 erythrocytes but virtually none or very weak bands from A_2 erythrocytes. It did not react with type 1 or type 2 chain A, or with globo-A. The simplest reactive component was isolated from a previously assigned A^b fraction by HPTLC of acetylated compounds. The structure of the reactive component was characterized by ^{1}H NMR spectroscopy, methylation analysis, and enzymatic degradation, as shown below:**



The structure is essentially a repetitive A epitope attached to type 2 chain and is hereby called type 3 chain A. The determinant can be carried on extended and/or branched structures, but it was not detectable in glycoproteins. The structure was characteristic of A_1 erythrocytes and present in only trace amounts in A_2 erythrocytes. The precursor H (Fucα1 → 2Galβ1 → 3GalNAcα1 → 3[Fucα1 → 2]Galβ1 → 4GlcNAcβ1 → R; type 3 chain H) was present in greater quantity in A_2 erythrocytes than in A_1 erythrocytes, but it was absent in both O and B erythrocytes. The A_1 transferase apparently can transfer α-GalNAc to type 3 chain H, while the A_2 transferase may not have this ability.

Blood group A has been divided into the major subgroups A_1 and A_2 since it was first described by von Dungern and Hirszfeld in 1911 (1). Although the serology and genetics of A_1 and A_2 have been well-established (2), the chemical basis of the distinction between A_1 and A_2 has been an unresolved problem. Some investigators have concluded that A_1 erythrocytes contain greater quantities of A determinants than do A_2 erythrocytes (3); others have suggested a qualitative difference between A_1 and A_2 based on the production of specific antibodies reacting with A_1 but not A_2 erythrocytes (4), and on binding kinetics of A_1 and A_2 blood group glycoproteins with insolubilized anti-A antibodies (5). The glycolipid fraction with low TLC mobility separated from blood group A_2 erythrocytes was weakly A active, in contrast to the same slow-migrating fraction separated from A_1 erythrocytes (6). We have found a monoclonal antibody that reacts specifically with A_1 erythrocytes. This antibody defines clearly the A_1-associated A determinant carried by a different type of glycolipid structure from that previously known.

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MATERIALS AND METHODS

Antibodies and Determination of Antibody Specificity. The A_1-specific monoclonal antibody TH-1 was obtained by immunization with A_1 erythrocytes followed by fusion of spleen cells with Sp2 myeloma cells (7) and selection of hybridoma by strong reactivity with A^b and A^c glycolipids (for designation of A variants, see Table 1). The original plan of this experiment was to isolate a monoclonal antibody directed to branched A (A^c and A^d species) but not to unbranched A^a and A^b species. The original attempt failed, but this hybridoma, secreting an antibody that specifically distinguishes A_1 and A_2 erythrocytes, was isolated. The monoclonal antibodies AH16, which defines both type 1 and type 2 chain A (9); AH21, which defines type 1 chain A (9); MBr1, which defines the globo-H structure Fucα1 → 2Galβ1 → 3GalNAcβ1 → 3Galα (it is now clear from this work that MBr1 antibody also reacts with the sequence Fucα1 → 2Galβ1 → 3GalNAcα1 → 3Galβ) (11); BE2, which defines type 2 chain H (Fucα1 → 2Galβ1 → 4GlcNAc); and 1B2, which defines *N*-acetyllactosamine (Galβ1 → 4GlcNAc) (12) were established as described. The MBr1 antibody was a gift from Sandro Sonnino (Universita Degli Studi di Milano, Milano, Italy). Reactivity of glycolipids with monoclonal antibodies was tested by immunostaining on TLC (13, 14) and by solid-phase radioimmunoassay as described (14).

Glycoproteins and Glycolipids. A total erythrocyte glycoprotein profile was determined by immunoblotting (15). The upper neutral glycolipid fraction was prepared and purified, and the desired fraction was rechromatographed on HPLC through an Iatrobeads 6RS-8010 column as described (16). The A^b glycolipid fraction (6) was acetylated and separated into components on Merck HPTLC plates (silica gel 60; Merck, Darmstadt, F.R.G.) with a solvent of dichloroethane/acetone/water (40:60:2.5, vol/vol/vol) or chloroform/methanol/water (89:11:0.01, vol/vol/vol). The location of glycolipids was detected by Primulin spray (Aldrich) followed by observation under UV light (17), and glycolipid acetates were extracted from silica gel and deacetylated.

Direct Immunostaining of Glycolipid Acetates Separated on HPTLC. To identify which components of an acetylated glycolipid separated on a HPTLC plate were reactive to a specific antibody, the HPTLC plate (Baker), after development and drying, was transferred into methanol containing 2% sodium methoxide (wt/vol). After 15 min, HPTLC plates were immersed in phosphate-buffered saline (0.9% NaCl/10 mM Na phosphate, pH 7.0) containing 5% bovine serum albumin, and the immunostaining procedure was performed according to the method of Magnani *et al.* (13) as modified by Kannagi

Table 1. Blood group A determinant carried by four types of carbohydrate chains in human erythrocytes

(A) Type 1 chain A[a*]: GalNAcα1 → 3Galβ1 → 3GlcNAcβ1 → 3Galβ1 → 4Glc
2
↑
Fucα1

(B) Type 2 chain

A[a†]: GalNAcα1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glc
2
↑
Fucα1

A[b†]: GalNAcα1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glc
2
↑
Fucα1

A[c†]: A branched type 2 chain (for the structure, see refs. 6 and 8)

A[d†]: Same as A[c], but the unit Galβ1 → 4GlcNAc in β1 → 3 side chain is repeated twice (8)

(C) Type 3 chain A[b] (repetitive A)[‡]

GalNAcα1 → 3Galβ1 → 3GalNAcα1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glc
2 2
↑ ↑
Fucα1 Fucα1

(D) Type 4 chain (A[x]; Globo-A[§])

GalNAcα1 → 3Galβ1 → 3GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glc
2
↑
Fucα1

*From human gastric cancer cell line MKN45 (9) and from human erythrocytes with Le[a⁻b⁻] blood group status. A variant with internal Fucα1 → 3GlcNAc substitution (ALe[b]) was detected in Le[a⁻b⁺] erythrocytes (unpublished data).
†From human erythrocytes (6).
‡From human erythrocytes, this paper.
§From human erythrocytes (10).

et al. (14). Solid-phase radioimmunoassay on a vinyl strip was carried out as described (14).

Glycolipid Characterization. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy in deuterated dimethyl sulfoxide with 2% ^2H_2O (18), and methylation analysis with methane chemical ionization mass spectrometry of partially *O*-methylated alditol or hexosaminitol acetates were carried out using selected ion mass chromatography as described (see refs. 16 and 19 and refs. therein). To obtain additional structural information on the glycolipids, TLC immunostaining (13) by monoclonal antibodies was performed before and after successive enzymatic degradation. Enzymatic degradation was carried out as described (16, 19).

Table 2. Reactivities of monoclonal antibody TH-1 with A_1 and A_2 erythrocytes

	Intact			Trypsin-treated†		Sialidase-treated‡	
	A_1	A_2	O/B	A_1	A_2	A_1	A_2
	TH-1 Hemagglutination*						
TH-1 supernatant	32	0	0	32	0	128	0
TH-1 ascites	3200	0	0	ND	ND	12,800	0
AH16 ascites§	320	28	0	448	ND	224	ND
	TH-1 Absorption¶						
TH-1 supernatant							
Before absorption	32	32	32				
After absorption	0	32	32				

*Numbers indicate the reciprocals of the maximum dilution that caused obvious hemagglutination. 0, No agglutination by undiluted antibody.
†Treated with 0.05% trypsin at 37°C for 1 hr.
‡Treated with sialidase (*Cl. perfringens* type VI; Sigma), 0.1 unit/ml at 37°C for 1 hr. ND, not determined.
§Titer of the AH16 antibody with ABO erythrocytes as reported (9) is corrected.
¶A_1 hemagglutination titer of supernatant before and after absorption with A_1 erythrocytes. Erythrocytes were washed 5 times with phosphate-buffered saline; 1 vol of packed erythrocytes was mixed with 2 vol of TH-1 antibody and incubated at 4°C overnight. After absorption, erythrocytes were centrifuged, and agglutination titer of the supernatant was determined as described above.

RESULTS

A_1 Specificity of Monoclonal Antibody TH-1. The TH-1 antibody (IgG2a) specifically agglutinated A_1 erythrocytes

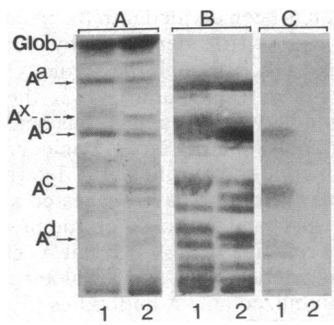


FIG. 1. Immunostaining pattern of A-active glycolipids separated on HPTLC from the upper neutral glycolipid fraction of A_1 and A_2 erythrocytes. Solvent was chloroform/methanol/water (60:45:10) on Baker's silica gel plates. (*A*) Glycolipids stained by 0.5% orcinol in 10% sulfuric acid; (*B*) stained by monoclonal antibody AH16 (ascites); (*C*) stained by monoclonal antibody TH-1. Lanes 1, glycolipids from A_1 erythrocytes. Lanes 2, glycolipids from A_2 erythrocytes. Positions of globoside, A[a], A[b], A[c], A[d] (9), and A[x] (10) are shown on the left.

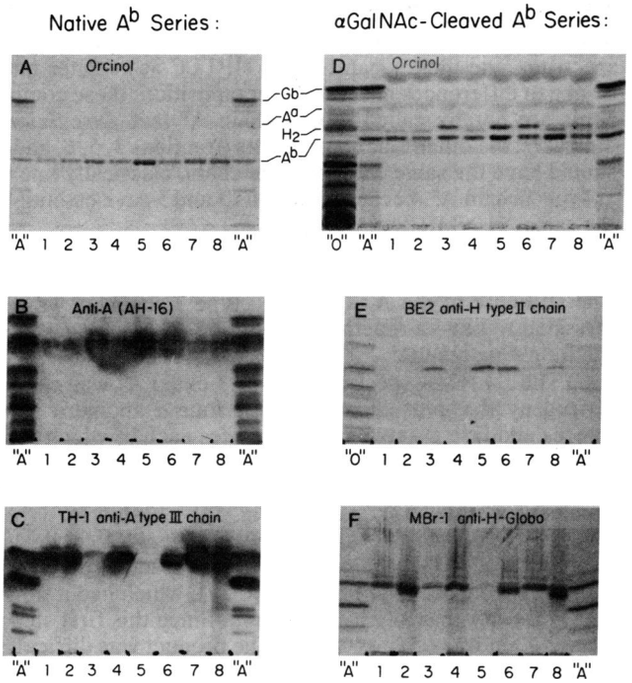
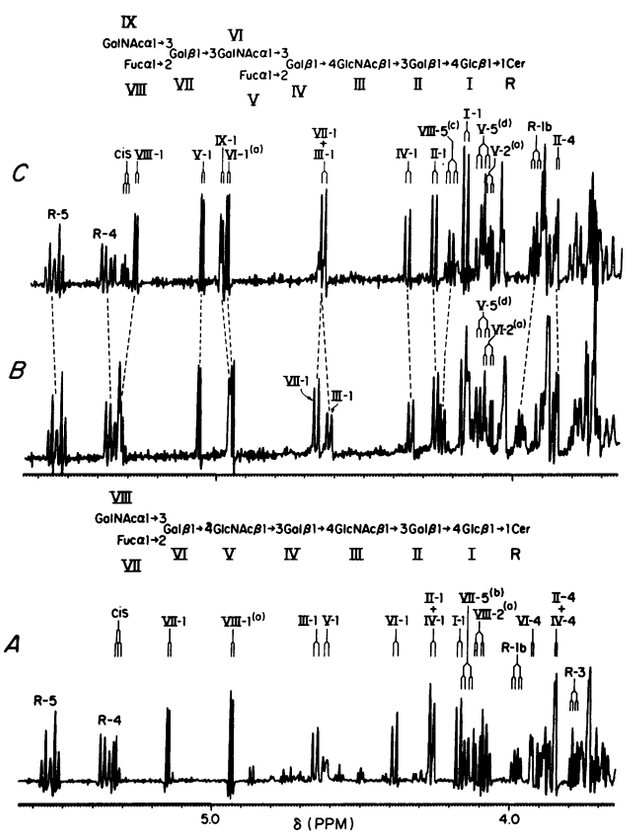


FIG. 2. Immunostaining of A^b subfractions and their degradation products by α-*N*-acetylgalactosaminidase. (*A*) Subfractions 1–8 of A^b stained by orcinol/sulfuric acid; (*B*) stained by AH16 monoclonal antibody; (*C*) stained by TH-1 monoclonal antibody. (*D*) Subfractions of A^b treated with α-*N*-acetylgalactosaminidase of *C. lampas*. Note that fractions 3, 5, 6, and 8 were degraded to H_2, but fractions 1, 2, 4, and 7 were degraded to a component with a slower mobility than H_2. The figure shows only a partial conversion, but complete conversion is possible by repeated incubation. Fractions 1, 2, 4, and 7 were more difficult to hydrolyze. (*E*) Enzyme-degraded product stained by monoclonal antibody BE2. (*F*) Enzyme-degraded product stained by MBr1 antibody.



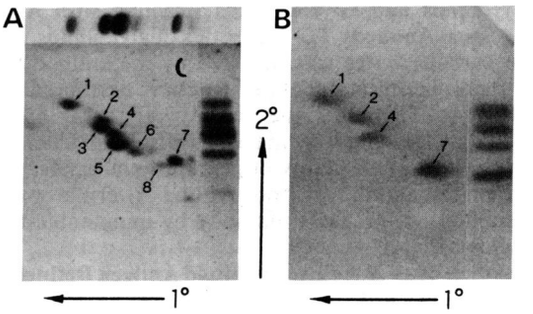


FIG. 3. Two-dimensional HPTLC of A^b fraction separated as peracetylated derivative. First dimension in dichloroethane/acetone/water (40:60:2.5) and second in chloroform/methanol/water (89:11:0.01). (*A*) Orcinol stain; (*B*) *in situ* deacetylation and immunostaining with TH-1. Fractions 1, 2, 4, and 7 were reactive with the antibody TH-1.

but not A_2, O, or B erythrocytes. The antibody was absorbed by A_1 but not by A_2 or O erythrocytes, and it did not react with A_2 erythrocytes even after sialidase or trypsin treatment (Table 2).

FIG. 4. ^1H NMR spectrum of type 3 chain A^b as compared to type 2 chain A^b obtained on a Bruker WM-500 spectrometer equipped with an Aspect 2000 Data System operating in the Fourier-transform mode and with quadrature detection. Approximately 300 μg of each sample was ^2H exchanged and dissolved in 0.4 ml of deuterated dimethyl sulfoxide containing 2% ^2H_2O and 1% tetramethylsilane as a chemical shift reference. (*Lower*) Spectrum of type 2 chain A^b at 303 K; (*Middle*) spectrum of type 3 chain A^b at 303 K; (*Upper*) Spectrum of type 3 chain A^b at 323 K. Nine hundred free induction decays were accumulated per spectrum at a given probe temperature. These spectra are fraction 5 (type 2) and fraction 7 (type 3). Identical spectra were obtained for fraction 3 (type 2) and for fractions 1 and 2 (type 3). (a), α-GalNAc H-1, H-2 connectivities established by continuous irradiation decoupling; (b), α-Fuc H-5 established by decoupling from Me doublet at 1.080 ppm ($^3J_{5,6}$ = 6.7 Hz); (c), α-Fuc H-5 established by decoupling from Me doublet at 1.024 ppm; and (d), α-Fuc H-5 established by decoupling from Me doublet at 1.076 ppm. (*Lower*) Two α-anomeric protons at 4.933 ppm ($^3J_{1,2}$ = 3.7 Hz) and at 5.146 ppm ($^3J_{1,2\,3}$ 4.3 Hz) were assigned, respectively, to α-GalNAc and α-Fuc. Five other β-anomeric resonances are assigned as shown above, based on the spectra of A^a glycolipids and decoupling experiments. Assignments for other resonances are determined in analogy to those found in the spectrum of type 2 chain A^a. (*Middle* and *Upper*) Resonances have been identified based on the following rationale: (*i*) Of the four α-resonances ($^3J_{1,2} \cong 4$ Hz) at 4.947, 4.955, 5.061, and 5.329 ppm, separated only at increased temperature, the furthest upfield must be an α-GalNAc, because it is decoupled by irradiation of the α-GalNAc H-2 at 4.084 ppm. In the same experiment, the resonance at 4.955 ppm is partially decoupled, indicating that it is also on α-GalNAc H-1, coupled to an α-GalNAc H-2 that is near the one irradiated, but hidden; (*ii*) there are two α-Fuc residues, indicated by H-5 quartets at 4.104 and 4.237 ppm that are coupled to α-Fuc Me doublets at 1.076 and 1.024 ppm ($^3J_{5,6}$ = 6.7 Hz); therefore, the other two α signals are α-Fuc H-1 resonances; (*iii*) while the spectrum of type 2 chain A^b contains two *N*-acetyl signals at 1.824 and 1.820 ppm (β-GlcNAcs) and one at 1.846 ppm (α-GalNAc), that of type 3 chain A^b shows one *N*-acetyl signal at 1.818 (β-GlcNAc) and two at 1.843 ppm (α-GalNACs). These α-resonances, and the five β-H-1 resonances ($^3J_{1,2} \cong$ 7–8 Hz) are readily decoded by comparison with the NMR spectrum of the precursor type 3 chain H structure (the same compound minus the terminal α-GalNAc) isolated from A erythrocytes (unpublished observations). Thus, the only major changes in the anomeric region brought about on addition of the GalNAc ($\alpha 1 \rightarrow 3$) residue are (*i*) the appearance of the additional α-GalNAc H-1; (*ii*) the shift of an α-Fuc H-1 from 5.229 ppm downfield 0.1 ppm to its position in the A^b type 3 spectrum; and (*iii*) the shift of a resonance at 4.548 ppm downfield to 4.663 ppm ($^3J_{1,2}$ = 7.9 Hz), which is therefore assigned to the subterminal Gal$\beta 1 \rightarrow 3$.

Glycolipids and Glycoproteins Reacting with A_1-Specific Monoclonal Antibody TH-1. A series of glycolipids with long-chain carbohydrates was detected by the antibody TH-1 in the upper neutral glycolipid fraction extracted from A_1 erythrocytes (Fig. 1*C*, lane 1); only very weak bands were detected by this antibody in the same fraction from A_2 erythrocytes (lane 2). A series of glycoprotein bands, including band 3 and band 4.5, in both A_1 and A_2 erythrocytes were not stained by the TH-1 antibody by immunoblotting (data not shown).

Isolation of an A-Active Glycolipid Antigen Defined by Antibody TH-1 (Type 3 Chain A) from the A^b Fraction. The simplest glycolipid species showing specific reactivity with TH-1 antibody was isolated from the A^b fraction by acetylation followed by HPTLC separation. At least eight bands, termed fractions 1–8, were separated from the A^b fraction by one-dimensional HPTLC (data not shown). These components, separated on preparative HPTLC, were indistinguishable on HPTLC after deacetylation (Fig. 2*A*), and all of them were stained by anti-A antibody AH16 (Fig. 2*B*), but none of them was stained by anti-A type 1 chain AH21 or by the new monoclonal antibodies directed to difucosyl A—i.e., anti-ALe^b or anti-ALe^y (data not shown). Some fractions (fractions 1, 2, 4, and 7) were stained by TH-1 antibody (Fig. 2*C*) and were hydrolyzed by α-*N*-acetylgalactosaminidase to a component with a slower TLC mobility than H_2 (Fig. 2*D*) that was strongly reactive with MBrl antibody[2] (13) (Fig. 2*F*) but was not reactive with BE-2 (Fig. 2*E*). Other fractions (fractions 3 and 5) were only weakly stained by TH-1 antibody (Fig. 2*C*) and were converted by α-*N*-acetylgalactosaminidase to the component classically known as H_2 (Fig. 2*D*), which is reactive with BE-2 antibody (Fig. 2*E*), but not with MBrl antibody. Fractions 6 and 8 were revealed to be a mixture of type 2 chain A^b and type 3 chain A^b, and repeated TLC purification was needed to obtain a pure substance. Complete separation of the A^b fractions was demonstrated by two-dimensional HPTLC followed by *in situ* deacetylation and immunostaining. Eight components were clearly separated (Fig. 3*A*), four of which were reactive with TH-1 and four of which were not reactive with TH-1 (Fig. 3*B*). The four components that were reactive with TH-1 antibody (fractions 1, 2, 4, and 7) gave essentially the same ^1H NMR spectrum, and their separation on HPTLC as acetates must be due to differences in ceramide composition; these components are hereby called type 3 chain A^b (see *Discussion*). Similarly, the other four components (fractions 3, 5, 6, and 8) should have the same carbohydrate chain, classically known as type 2 chain A^b, because fractions 3 and 5 gave essentially the same ^1H NMR spectrum.

Structural Characterization of the A-Active Glycolipid (Type 3 Chain A^b) Defined by A_1-Specific TH-1 Antibody. The structure of the A_1-associated type 3 chain A^b was determined to have a repetitive A epitope (Table 1), based on the following results:

(*i*) The ^1H NMR spectrum of type 3 chain A^b was characterized by the unusual presence of four α anomeric resonances and five β anomeric resonances each of which is assigned as shown in Fig. 4.

(*ii*) The structure described above is fully supported by the results of methylation analysis (Fig. 5).

(*iii*) Type 3 chain A^b was hydrolyzed by α-*N*-acetylgalactosaminidase of *Charonia lampas* to an H-active precursor, defined by the MBrl antibody (Fig. 2*F*), which had a slower TLC mobility than the H_2 glycolipid. Since this first step of degradation was so sluggish, and the product was difficult to obtain in sufficient quantity, the same precursor glycolipid reactive with MBrl antibody, which is present in A but not in B or O erythrocytes, was isolated from A erythrocytes for further degradation analysis. This H-active precursor was degraded to an A-active glycolipid having the same TLC mobility as A^a glycolipid by successive hydrolysis with α-L-fucosidase (bovine liver) and β-galactosidase (*C. lampas*) (16).

Reactivity of Various A-Active Glycolipids with A_1-Specific Monoclonal Antibody TH-1. To confirm the specificity of the A_1-specific TH-1 antibody, its reactivity with various A-active glycolipids was tested. Only type 3 chain A^b was highly reactive in solid-phase radioimmunoassay, as shown in Fig. 6, and only type 3 chain A was immunostained on TLC (data not shown). A weak cross-reaction with globo-A (type 4 chain A; Table 1, part D) was observed.

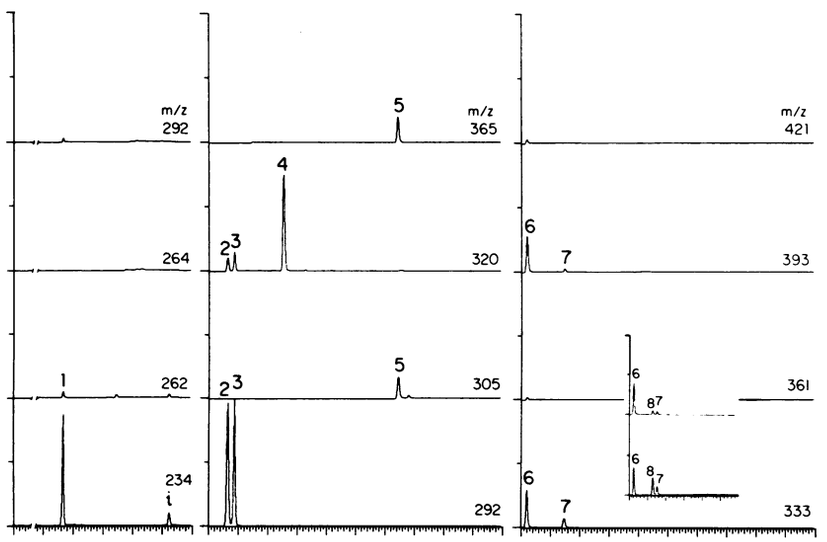


FIG. 5. Selected ion chromatogram of partially *O*-methylated alditol and hexosaminitol acetates from the hydrolyzate of type 3 chain A^b glycolipid. Partially methylated sugar acetates were separated on a DB-5 capillary column (temperature programmed from 140 to 250°C at 4°C/min) and identified by methane chemical ionization mass spectrometry with selected ion monitoring on a Finnigan 3300 gas chromatograph/mass spectrometer with a 6110 Data System (16, 19). Ordinate, intensity of each ion at the mass number indicated. Abscissa, scan number; each smallest unit represents five scans, 1 sec per scan. Peaks identified were as follows: (1) 2,3,4-tri-*O*-Me-Fuc; (2) 2,3,6-tri-*O*-Me-Glc; (3) 2,4,6-tri-*O*-Me-Gal; (4) 4,6-di-*O*-Me-Gal; (5) 3,4,6-Tri-*O*-Me-GalNAcMe; (6) 3,6-di-*O*-Me-GlcNAcMe; and (7) 4,6-di-*O*-Me-GalNAcMe. (*Inset*) Pattern of di-*O*-Me-hexosaminitols co-injected with 4,6-di-*O*-Me-GlcNAcMe (8) is shown (m/z 333, 393), indicating the separation between 4,6-Di-*O*-Me-GlcNAcMe and 4,6-di-*O*-Me-GalNAcMe.

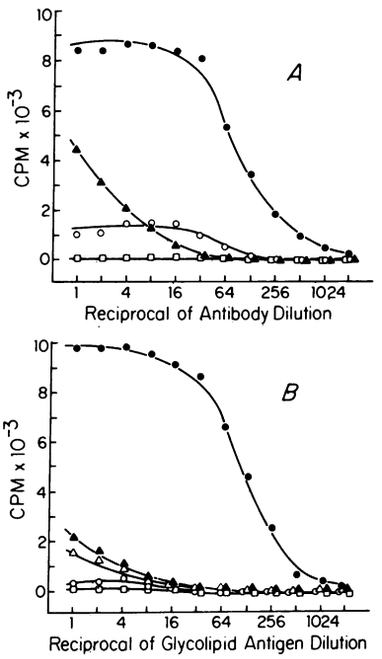


FIG. 6. Binding specificity of TH-1 antibody to purified glycolipids according to the method previously described (14), but with omission of secondary antibody since ^{125}I-labeled protein A combined directly with TH-1 antibody (IgG2a). (*A*) Binding activity with antibody dilution. Each well contains 10 ng of glycolipid antigen, 30 ng of cholesterol, and 50 ng of lethicin. (*B*) Binding activity of antibody with antigen dilution. The initial glycolipid concentration was 100 ng with 300 ng of cholesterol and 500 ng of lecithin. Concentration of antibody applied to each well as a 1:10 dilution of culture supernatant of TH-1. ●, A^b type 3 chain; ○, A^b type 2 chain; □, A^a type 2 chain; ▲, A^x (globo-A); △, H_2 type 3 chain. Additional glycolipids tested, which gave negative results (□) were H_2 type 2 chain, ALe^b, Le^y, Forssman, ALe^y, and A^a type 1 chain. ALe^b and ALe^y were donated by J. M. McKibbin (for structure, see ref. 20).

DISCUSSION

The chemical basis of blood group A_1 specificity, if it exists, has not been demonstrated unequivocally (3–6). A single glycolipid carbohydrate chain displaying a specific reactivity with the A_1-specific monoclonal antibody was isolated and characterized unequivocally as described in *Results*. The A_1-specific structure identified is a repetitive A determinant shown below:

$$\begin{array}{l} \text{GalNAc}\alpha 1 \rightarrow 3\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}\alpha 1 \rightarrow 3\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc} \\ \qquad\qquad\quad 2 \qquad\qquad\qquad\qquad 2 \\ \qquad\qquad\quad \uparrow \qquad\qquad\qquad\qquad \uparrow \\ \qquad\qquad\quad \text{Fuc}\alpha 1 \qquad\qquad\qquad\quad \text{Fuc}\alpha 1 \\ \beta 1 \rightarrow 3\text{Gal}\beta 1 \rightarrow 4\text{Glc} \end{array}$$

Since the internally located hexosamine of the terminal A determinant is α-GalNAc, it is hereby called type 3 chain A, in contrast to type 1 and type 2 chain A (for reviews, see refs. 20 and 21). Previously, the term type 3 chain was used by Donald (22) for an A-active short chain oligosaccharide, $\text{GalNAc}\alpha 1 \rightarrow 3[\text{Fuc}\alpha 1/2]\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}\alpha 1 \rightarrow \text{R}$, linked directly to serine or threonine of protein. Although no clear cut difference was found between A_1 and A_2, this oligosaccharide was found in a higher concentration in A_1 than in A_2 glycoproteins (22). Recently, we found globo-A structure (Table 1, part D) to be present exclusively in A_1 but not in A_2 erythrocytes (10). More recently, Samuelsson and co-workers† observed a conversion of globo-H to globo-A by the A_1 transferase, but not by the A_2 transferase. The structure of one of the A-active glycolipids (A^x) contains the sequence $\text{GalNAc}\alpha 1 \rightarrow 3[\text{Fuc}\alpha 1 \rightarrow 2]\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}\beta 1 \rightarrow 3\text{Gal}\alpha$, although the internal GalNAc residue is a β-structure in contrast to an α-structure in type 3 chain A. In this regard, we hereby classify A^x as globo-A or type 4 chain A (Table 1, part D). Interestingly, the A_1-specific antibody TH-1 does not react with this structure, although the internal $\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}$ is similar to type 3 chain A. Since globo-A is an extremely minor component of human erythrocytes (10), contribution of this structure to A_1 specificity could be of secondary importance as compared to the type 3 chain A structure described in this paper. Thus, the essential qualitative difference between A_1 and A_2 status could be ascribed to the ability of the A_1 enzyme, and the inability of the A_2 enzyme, to convert type 3 chain H to type 3 chain A.

†Breimer, M., Jacobsson, A., Larson, G. & Samuelsson, B. E. (1984) Proceedings of the 18th Congress of the International Society of Blood Transfusion, July 22–27, 1984, Munich.

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