Occurrence of a novel fucose-containing pentaglycosylceramide with blood-group-B active determinant in *Xenopus* blastula cells: its possible involvement in cell–cell adhesion

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For understanding of the biological function of glycoconjugates during embryogenesis and morphogenesis, *Xenopus laevis* is considered a very useful animal model. We have found that blood-group-active molecules characteristically were distributed in the cell–cell contact region of *Xenopus* blastula cells. The chemical nature of blood-group-active glycoconjugates, including glycosphingolipids, is little known. T.l.c.-immunostaining using anti-blood-group-antigen antibodies showed that many species of blood group-B-active glycosphingolipids existed in the neutral glycosphingolipid fraction extracted from *Xenopus laevis* eggs. Among the B-active glycosphingolipids detected, two major components with the fastest mobility on a t.l.c. plate, tentatively termed XN-1 and XN-2, were isolated, and their chemical structures were characterized by gas chromatography–mass spectrometry, immunological analysis, fast-atom-bombardment mass spectrometry and ^{1}H-n.m.r. spectroscopy. Both XN-1 and XN-2 had an identical pentaoligosaccharide structure, but differed in their ceramide moiety. The chemical structure is:

Galα1-3

Galβ1-3Galβ1-4Glcβ1-1′Cer

Fucα1-2

This is a novel type of pentaglycosylceramide with blood-group B activity, in that it lacks *N*-acetylhexosamine in its core carbohydrate structure. In this paper, a possible involvement of the blood-group antigen in the cell-adhesion process of *Xenopus* embryonic cells is discussed.

INTRODUCTION

Cell adhesion plays crucial roles in morphogenesis and organogenesis during development of multicellular organisms. A variety of cell–cell and cell–substratum adhesion molecules have been identified and characterized in the last decade, and their significant roles in cellular interactions and differentiation have been extensively studied. Most of these cell-adhesion molecules belong to the glycoprotein group, such as cadherins and integrins (for review see [1]), and the biological activities are ascribed to their protein portion, but not to the carbohydrate moiety.

Recent advances in glycobiology show the possibility that cells communicate with each other through a recognition system based on interaction between cell-surface glycoconjugates and counterpart molecules. For example, cell adhesion between lymphocytes and endothelial cells is mediated through interaction between selectins and the carbohydrate moiety of NeuAcα-2-3Galβ1-4(Fucα2-3)GlcNAc (sialyl-Le^x). In this case, the blood-type-related carbohydrate chain is an essential component for cell–cell adhesion in lymphocytes–endothelial interaction in inflammation [1].

We previously showed the presence of Ca^{2+}-dependent cell–cell adhesion systems in frog (*Xenopus laevis*) embryonic cells and somatic cells [2]. To examine the possibility that the Ca^{2+}-dependent cell adhesions are achieved through a recognition system based on molecular interaction between carbohydrate molecules and their counter-receptors, we tested whether or not specific antibodies against carbohydrate antigens related to blood type had any effect on Ca^{2+}-dependent embryonic cell–cell adhesion. Although an antibody against Le^x antigen had no effect on Ca^{2+}-dependent cell adhesion, the antibody against blood-group B antigen completely inhibited Ca^{2+}-dependent cell adhesion of frog blastula cells (K. Nomura, K. Nomura, N. Nakajo, H. Nomura, M. Fujisue, M. Murata, J. Iwashita, K. Yamamoto, Y. Hirabayashi and K. Yamana., unpublished work).

To understand the molecular mechanism of cell adhesion of frog blastula cells mediated by blood-group-B-related carbohydrate molecules, it is of particular importance to know with what molecular species the anti-blood-group-B antibody interacts to inhibit Ca^{2+}-dependent cell adhesion. In spite of their significance, however, the detailed structures of blood-group-active glycoconjugates, including glycosphingolipid, are not well characterized. This paper describes the extraction and structural determination of novel neutral glycosphingolipids recognized by anti-blood-group-B antibody from *Xenopus laevis* unfertilized eggs. Possible roles of the novel antigenic compounds in cell–cell interaction are also discussed.

MATERIALS AND METHODS

Materials

DEAE-Sephadex A-25 was purchased from Pharmacia Fine

Abbreviations used: sialyl-Le^x, NeuAcα-2-3Galβ1-4(Fucα2-3)GlcNAc; paragloboside, nLc$_4$Cer; asialo-G$_{M1}$, Gg$_4$Cer; Le^x-active glycosphingolipid, III^{3}Fucα-nLc4Cer; Gb$_5$Cer, Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer; h.p.t.l.c., high-performance t.l.c.; FAB-m.s., fast-atom-bombardment mass spectrometry.

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Chemicals (Uppsala, Sweden), phenylboranate–agarose (PBA–60) from Amicon Division, W. R. Grace and Co. (Danvers, MA, U.S.A.), high-performance t.l.c. (h.p.t.l.c.) plates (silica gel 60) were from E. Merck (Darmstadt, Germany), and affinity-purified goat anti-human IgG and IgM antibody conjugated with horseradish peroxidase were from Jackson Immunoresearch Laboratories (Avondale, PA, U.S.A.). Mouse anti-blood-group-B monoclonal antibody was obtained from DAKO (Carpinteria, CA, U.S.A.). Blood-group-B-active glycosphingolipids were isolated from human red blood cells of type B. Blood-group-A-active hexaglycosylceramide was kindly given by Dr. S. Ando, Department of Biochemistry, Tokyo Metropolitan Institute of Gerontology, Japan.

Purification of B-active glycosphingolipids from *Xenopus laevis* eggs

Adult *Xenopus laevis* females were stimulated to ovulate by injection of 300 IU of choriogonadotropin (Teikoku Hormone Manufacturing Co., Tokyo, Japan). Unfertilized eggs were de-jellied with 1% sodium thioglycolate in modified Steinberg's solution [5.8 mM NaCl/0.67 mM $Ca(NO_3)_2$/0.83 mM $MgSO_4$/3.0 mM Hepes, neutralized with 1 M HCl to pH 7.3], and then washed with modified Steinberg's solution. The freeze-dried eggs (90 g dry wt.) were treated with 1 litre of acetone to remove neutral lipids. Total glycosphingolipids were extracted from the acetone-dried powder with successively 1 litre of chloroform/methanol/water (5:5:1, by vol.) and 1 litre of chloroform/methanol (1:1, v/v). The extracts were combined and evaporated to dryness. The total glycosphingolipid extract was dissolved in chloroform/methanol/water (15:30:2, by vol.) and applied to a DEAE-Sephadex A-25 column (bed volume 500 ml) which had been equilibrated with the same solvent. The column was washed with 5 litres of the same solvent to obtain the neutral glycosphingolipids. The eluate was evaporated to dryness, and the resulting residue was then incubated in 0.5 M NaOH in methanol at 4 °C for 12 h. After the reaction mixture was evaporated, inorganic salts and glycerol were removed by dialysis. The freeze-dried powder was dissolved in chloroform/methanol (4:1, v/v) and applied to a PBA column (bed volume 50 ml) in accordance with the method of Higashi et al. [3]. After the column had been washed with 1.5 litres of chloroform/methanol (4:1, v/v), the neutral glycosphingolipids were eluted successively with 2 litres of chloroform/methanol/water (5:5:1, by vol.), and 250 ml of chloroform/methanol/water (2:8:1, by vol.). The eluates were combined, and evaporated to dryness. The sample was dissolved in a small volume of chloroform/methanol/water (90:10:0.1, by vol), and applied to a column (8 mm × 250 mm) packed with Aquasil (Senshu Pak AQUASIL SS-652N, Senshu Scientific Co., Tokyo, Japan) which was equilibrated with the same solvent. The neutral glycosphingolipids were eluted for over 120 min with a linear gradient system prepared from a first solvent mixture of chloroform/methanol/water (90:10:0.1, by vol.) and final solvent mixture of chloroform/methanol/water (120:40:1, by vol.). The flow rate was 2.5 ml/min and the eluate was collected in fractions of 2.5 ml per tube. The blood-group-B-active glycosphingolipid-positive fractions detected by t.l.c.–immunostaining were pooled (tubes 61–63 for XN-1 and 67–69 for XN-2) and evaporated.

H.p.t.l.c.

Glycosphingolipids were chromatographed on h.p.t.l.c. plates with solvent system 1 [chloroform/methanol/12 mM $MgCl_2$ (60:35:8, by vol.] or 2 [chloroform/methanol/99.28% ammonia/12 mM $MgCl_2$ (60:35:5.6:2.4, by vol.)]. Glycosphingolipids were made visible with orcinol/H_2SO_4 reagent [4]. The isolated glycosphingolipids were quantified densitometrically (Personal Densitometer, Molecular Dynamics, U.S.A.) on t.l.c. plates by the method of Ando et al. [5].

Compositional analysis

Carbohydrate compositions in the isolated glycosphingolipids were analysed after methanolysis, re-*N*-acetylation and trimethylsilylation by the method of Bhatti et al. [6] with modifications as described previously [7]. The analysis was performed in a Hitachi G-5000 gas chromatograph (Hitachi, Tokyo, Japan) with a capillary column of 3% OV-101 (0.2 mm × 25 m; Shimadzu, Kyoto, Japan), programmed from 150 °C to 240 °C at 3 °C/min. Fatty acid compositions were also determined by g.l.c.

Methylation analysis

Permethylation of glycosphingolipids was carried out by the method of Hakomori [8]. Partially methylated alditol acetates from the permethylated glycosphingolipid were prepared by the method of Yang and Hakomori [9] and analysed by g.l.c. (Hewlett Packard 5890) with a capillary column of DB-1 (0.25 mm × 15 m; J&W, U.S.A.), with temperature programmed to rise from 140 to 170 °C at 6 °C/min, from 170 to 173 °C at 1 °C/min, and from 173 °C to 200 °C at 9 °C/min. Each peak was identified by comparing retention times and mass analysis using an INCOS 50 mass spectrometer (Finnigan MAT) with carbohydrate standards as described by Björndal et al. [10] and Tai et al. [11].

Fast-atom-bombardment mass spectrometry (FAB-m.s.)

Negative-ion FAB-m.s. of the isolated glycosphingolipids was carried out with a JMS-HX 110 mass spectrometer (JEOL, Tokyo, Japan), with triethanolamine as a matrix. The accelerating voltage was 8 kV and the primary beam for bombardment was Xe at 6 kV.

Immunohistochemical staining

Xenopus blastulae were fixed in 3% paraformaldehyde in PBS at room temperature, and 10 μm cryostat sections were made at −25 °C by using a cryostat microtome (Meiwa Shoji Co., Tokyo, Japan). The sections were then soaked in 3% BSA (fraction V, Sigma) in PBS for 1 h and incubated with antibody against blood group B for 2 h. The sections were examined with a laser scanning microscope (LSM410; Zeiss, Oberkochen, Germany) after incubation with fluorescein-labelled anti-mouse IgM antibody for 2 h. In one experiment, the sections were delipidated by treatment with chloroform/methanol (1:1, v.v) for 1 h, and then with chloroform/methanol/water (5:5:1, by vol.) for a further 1 h, followed by further incubation in methanol for 1 h. The lipid-free sections were stained as described above.

Immunochemical analysis

E.l.i.s.a. was performed by the method of Higashi et al. [12] with a slight modification [13]. Enzyme immunostaining on a t.l.c. plate (Polygram Sil G; Macherey-Nagel, Duren, Germany) was performed by the method of Magnani et al. [14], with modifications as described previously [15].

^{1}H-n.m.r. spectroscopy

Spectra of the isolated glycosphingolipids were obtained with a 400 MHz n.m.r. spectrometer (model XL-400, Varian). The sample (0.3 mg) was dissolved in 0.5 ml of [$^2H_6$]dimethyl

sulphoxide containing tetramethylsilane. The operation temperature was 60 °C. Two-dimensional *J*-coupling correlation spectroscopy (COSY) was performed as previously described [16].

RESULTS

Immunohistochemical staining of *Xenopus laevis* blastula cells with anti-blood-group-B antibody

The immunohistochemical staining of *Xenopus laevis* embryonic cells showed that the antigens recognized by anti-group-B monoclonal antibody appeared in oocytes and were present throughout embryogenesis (results not shown). The epitopes recognized by anti-blood-group-B antibody were predominantly distributed in the cell–cell adhesion area between blastula blastomeres. No staining was observed in nuclei of blastula cells (Figure 1A). To examine whether glycolipids were among the immunoreactive components, we carried out delipidation of histological sections of the blastula before immunostaining. Although the delipidation removed all of the immunological staining in intracellular vesicles, signals in the cell–cell contact area still remained significant. After extensive delipidation with chloroform/methanol, the overall signal intensity in the cell–cell contact area of blastula cells was markedly decreased, and the continuous staining pattern at cell–cell adhesion sites changed to

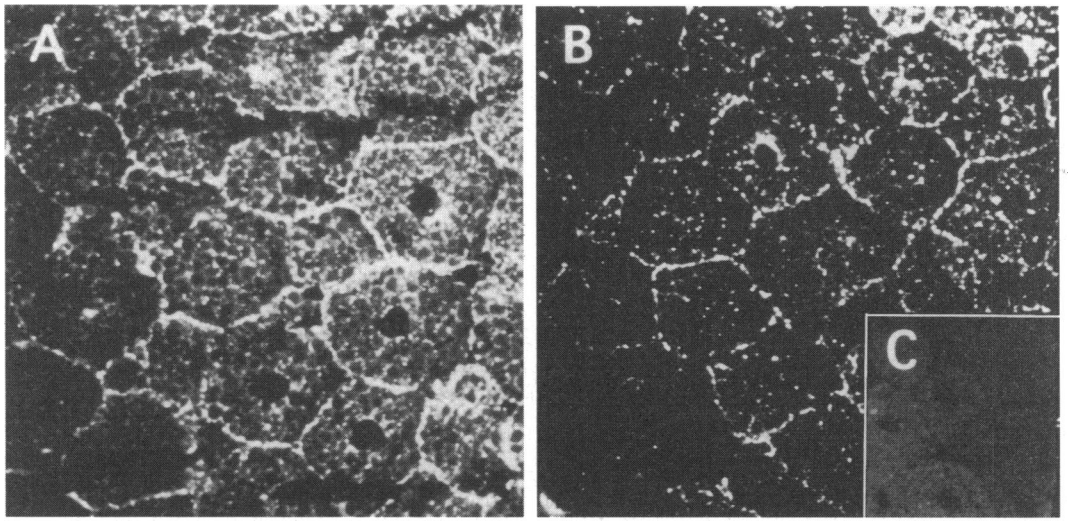

Figure 1 ***Xenopus laevis*** **blastula expressed blood-group-B-active glycoconjugates**

(**a**) Immunochemical staining of blastula cells by anti-blood-group-B monoclonal antibody. Note the staining in cell–cell contact area and intracellular vesicles. (**b**) Immunohistochemical staining of blastula with the same antibody after treatment with chloroform/methanol; the overall staining intensity was decreased, and the signal in intracellular vesicles disappeared. (**c**) Negative control against staining (**b**). It was stained with mouse IgM as a primary antibody. Magnification × 500 for all photographs.

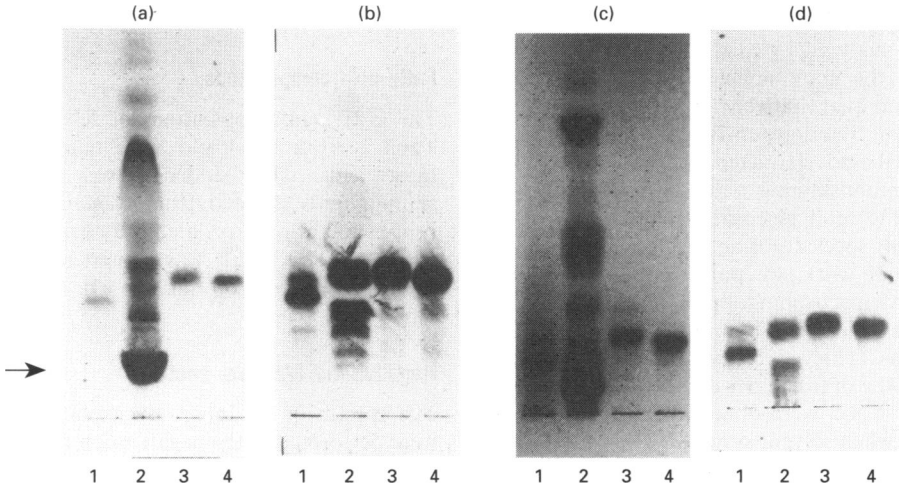

Figure 2 T.l.c. and immunostaining of blood-group-B-active glycosphingolipid of ***Xenopus laevis*** **eggs**

Glycosphingolipids (0.5 μg each) were applied on to Polygram Sil G and developed with solvent system 1 (plates **a** and **b**) or solvent system 2 (plates **c** and **d**). Plate (**a**) and (**c**) were developed with orcinol/H_2SO_4 reagent. Plates (**b**) and (**d**) were immunostained with anti-blood-group-B antibody. Lane 1, B-active hexaglycosylceramide isolated from human erythrocytes; lane 2, total glycosphingolipids extracted from *Xenopus laevis* eggs; lane 3, XN-1; lane 4, XN-2. Arrow indicates an orcinol-positive spot derived from glucose.

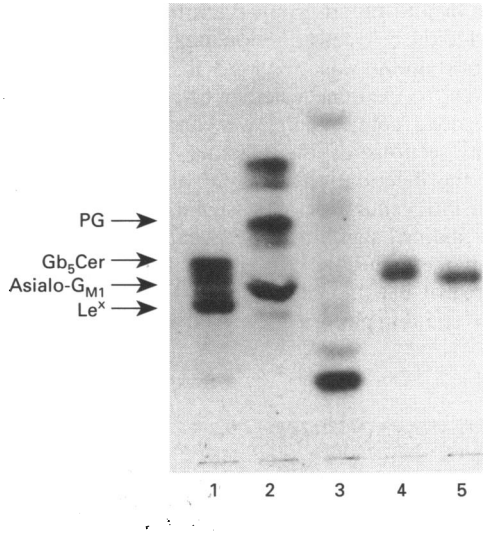

Figure 3 H.p.t.l.c. of purified anti-blood-group-B-active glycosphingolipids isolated from *Xenopus laevis* eggs

The purified glycosphingolipids (1 μg each) were analysed on h.p.t.l.c. with solvent system 1. Lane 1, Gb_5Cer and Le^x-active glycosphingolipid; lane 2, paragloboside (PG) and asialo-G_{M1}; lane 3, total glycosphingolipids of *Xenopus laevis* eggs; lane 4, XN-1; lane 5, XN-2. The glycosphingolipids were revealed with orcinol/H_2SO_4 reagent.

discontinuous staining (Figure 1B). The results suggest that a high proportion of blood-group-B-active molecules in cell–cell contact areas are glycolipids. Also, the results indicate the presence of glycoproteins reactive with anti-blood-group-B antibody. In fact, two-dimensional gel electrophoresis followed by Western-blot analysis of frog embryos revealed three major blood-group-B-active proteins, with molecular masses of 70, 58 and 40 kDa (results not shown). Characterization of the glycoproteins recognized by the antibody will be published elsewhere.

Purification of blood-group-B-active glycosphingolipids from *Xenopus laevis* eggs

Blood-group-B-active lipid antigens from unfertilized eggs to tailbud (stage 28) were analysed by t.l.c.–immunostaining. No significant changes in the immunoactive compounds were observed qualitatively and quantitatively (results not shown). In the present study, major blood-group-B-active glycosphingolipids have been isolated and characterized from unfertilized eggs. T.l.c.–enzyme immunostaining with anti-blood-group-B antibody showed that the total glycosphingolipid of *Xenopus laevis* eggs contained many species of B-active glycosphingolipids (Figure 2). In the present work, we purified the two fastest-moving species of B-active glycosphingolipids, termed XN-1 and XN-2, on a t.l.c. plate, as indicated in Figure 2. Final purification of XN-1 and XN-2 was achieved by preparative t.l.c. using solvent system 1. From 90 g of freeze-dried *X. laevis* eggs, 450 μg of XN-1 and 400 μg of XN-2 were obtained. Both XN-1 and XN-2 gave a single spot when solvent system 1 was used (Figure 2a). Although the presence of minor contaminating species was revealed by immunostaining (Figure 2b) and by using solvent system 2 (Figure 2c), the amount of the contaminating species was so little that the further structural analysis was not affected by them. Figure 3 shows that the isolated B-active glycosphingolipids from *Xenopus laevis* eggs migrated between Gb_5Cer and Le^x-active pentaglycosylceramide, and close to asialo-G_{M1}.

Table 1 Fatty acid composition of glycosphingolipids XN-1 and XN-2 isolated from *Xenopus laevis* eggs

Values are expressed as mol%.

	Glycosphingolipid			
	XN-1		XN-2	
Fatty acid	Non-hydroxy	Hydroxy	Non-hydroxy	Hydroxy
$C_{16:0}$	11.3	–	12.3	4.9
$C_{18:0}$	8.5	–	3.3	–
$C_{18:1}$	–	–	3.1	–
$C_{20:1}$	4.0	–	–	–
$C_{22:0}$	7.2	18.3	5.6	20.3
$C_{23:0}$	–	–	–	6.3
$C_{24:0}$	11.0	9.8	4.8	16.8
$C_{24:1}$	11.7	18.2	4.6	18.0
Subtotal...	53.7	46.3	33.7	66.3

Carbohydrate compositions of XN-1 and XN-2

The carbohydrate compositions of the glycosphingolipids isolated from *Xenopus laevis*, XN-1 and XN-2, were analysed. XN-1 and XN-2 consisted of fucose, glucose and galactose, in the approximate molar proportions 1:3:1. No *N*-acetylhexosamine was detected.

Methylation analysis of XN glycosphingolipids

The methylation analysis of XN-1 and XN-2 was performed. The partially methylated alditol acetates from both glycosphingolipids were identified as 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetylfucitol, 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetylgalactitol, 2,3,6-tri-*O*-methyl-1,4,5-tri-*O*-acetylglucitol, 2,4,6-tri-*O*-methyl-1,3,5-tri-*O*-acetylgalactitol and 4,6-di-*O*-methyl-1,2,3,5-tetra-*O*-acetylgalactitol. This result suggested the presence of a branched structure at the galactose residue with terminal fucose and galactose.

Fatty acid compositions

The fatty acid compositions of XN-1 and XN-2 are shown in Table 1. Both XN-1 and XN-2 mainly contained hydroxylated fatty acids. This tendency was observed in acidic glycosphingolipids isolated from *Xenopus laevis* oocytes [17]. The proportion of hydroxylated fatty acids in XN-2 was higher than in XN-1. This result was consistent with the mobility of the glycosphingolipids on t.l.c.

Negative-ion FAB-m.s. analysis

The spectra of the intact glycosphingolipids, XN-1 and XN-2, were performed in the negative-ion mode (Figure 4). Two major molecular-ion species for XN-1 were seen at m/z 1456 and 1430 (Figure 4a). The sugar chain sequence was deduced from the spectra as Hex-(Fuc)-Hex-Hex-Hex-Cer, and the ceramide moiety was predicted to consist of a combination of sphingenine/fatty acid $C_{24\,h:1}$ and sphingenine/fatty acid $C_{22\,h:0}$ (where 'h' refers to hydroxyfatty acid). The spectrum of XN-2 (Figure 4b) suggests that XN-2 is composed of the same carbohydrate moiety and

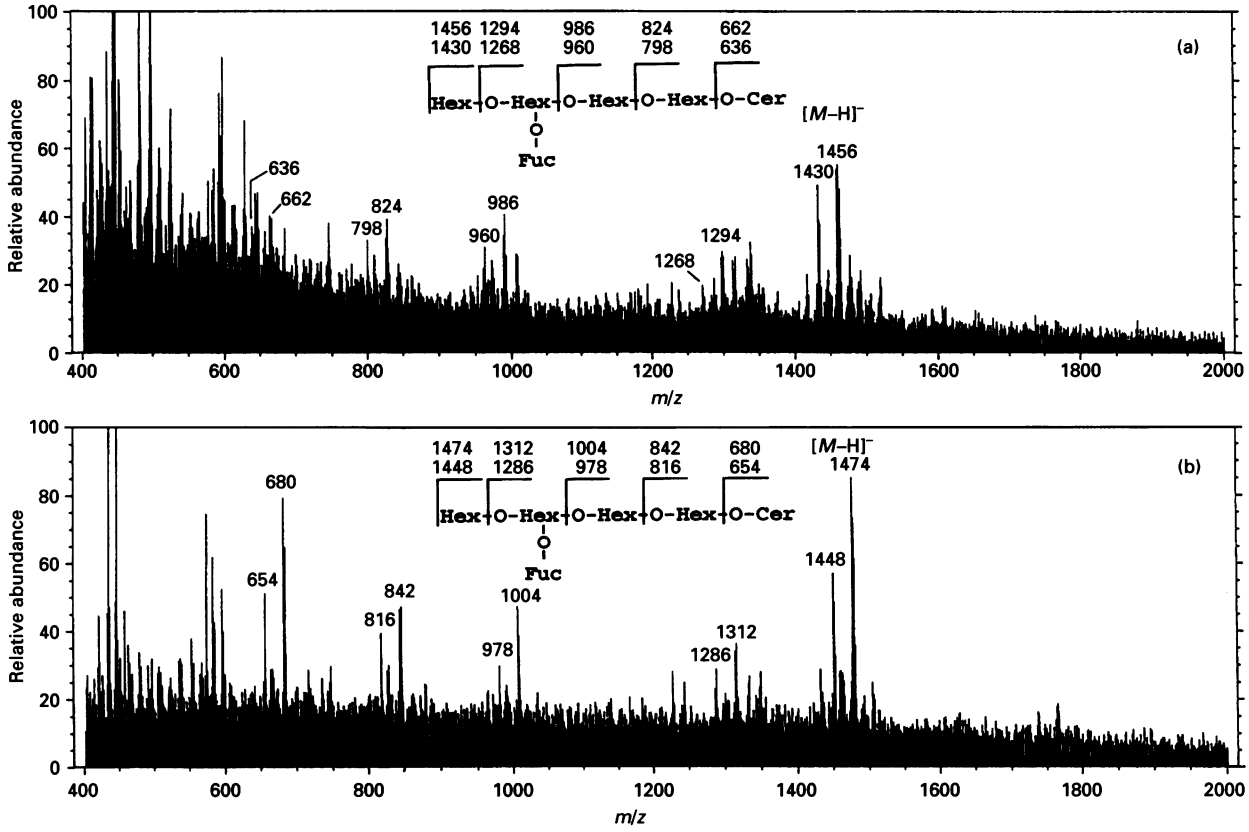

Figure 4 Negative-ion FAB-m.s. of intact glycosphingolipids isolated from *Xenopus laevis* eggs

(**a**) XN-1; (**b**) XN-2.

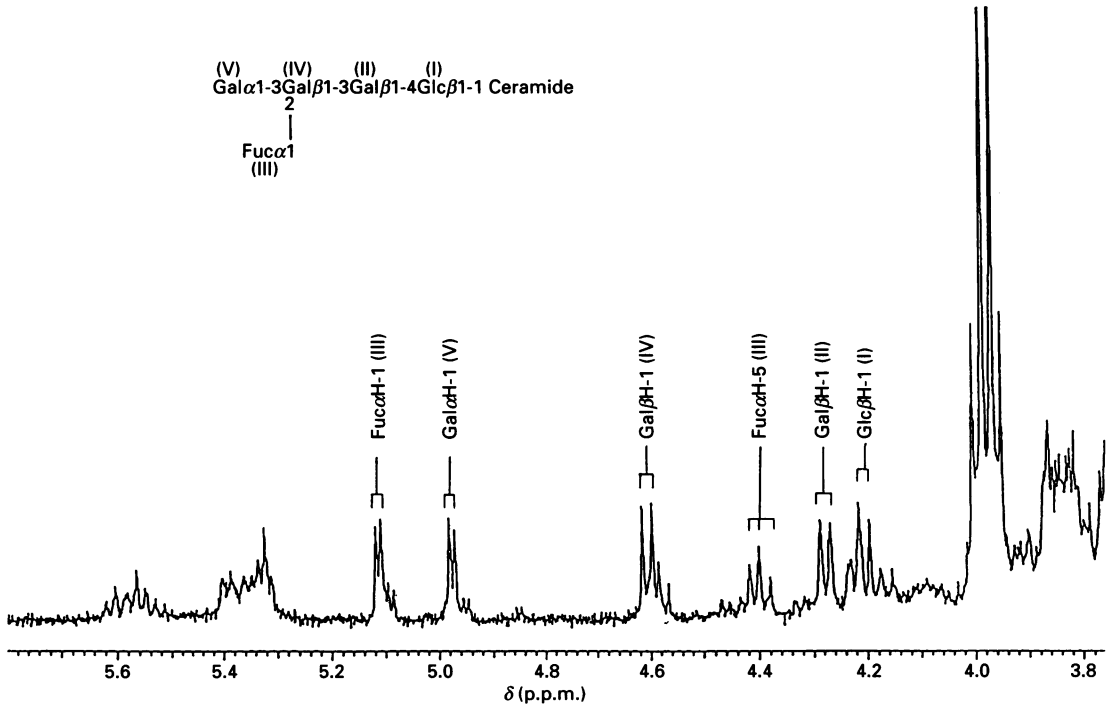

Figure 5 ^{1}H-n.m.r. spectrum of XN-1 at 400 MHz

Table 2 Chemical shifts of the anomeric protons of XN-1 and their coupling constants

	Glc (β1-1Cer)	Gal (β1-4)	Gal (β1-3)	Gal (α1-3)	Fuc (α1-2)
Chemical shift (p.p.m.)	4.2	4.3	4.6	5.0	5.1
Coupling constant, $J_{1,2}$ (Hz)	7.7	7.7	7.7	4.0	3.7

different ceramide composition (combination of trihydroxysphingenine/fatty acid $C_{24h:1}$ and trihydroxysphingenine/fatty acid $C_{22h:0}$).

^{1}H-n.m.r. analysis

XN-1 and XN-2 were subjected to 400 MHz ^{1}H-n.m.r. measurement. The ^{1}H-n.m.r. spectrum of XN-1 is shown in Figure 5. An almost identical spectrum was obtained for XN-2 (not shown). Anomeric proton signals were assigned according to values previously reported in the literature [18–20]. The peaks at 4.2 p.p.m. ($J_{1,2}$ = 7.7), 4.3 p.p.m. ($J_{1,2}$ = 7.7), 4.6 p.p.m. ($J_{1,2}$ = 7.7), 5.0 p.p.m. ($J_{1,2}$ = 4.0) and 5.1 p.p.m ($J_{1,2}$ = 3.7) corresponded to the β-anomeric protons of Glc(β1-1), Gal(β1-4) and Gal(β1-3), and α-anomeric protons of non-reducing terminal galactose and fucose, respectively (Table 2). A proton at Gal(β1-3) residue resonates in a lower field than other β-galactose. This downfield shift may be due to the presence of non-reducing terminal galactose and fucose.

Reactivity of isolated glycosphingolipids with anti-blood-group B antibody

Reactivity of XN-1, XN-2 and B antigen isolated from human erythrocytes was compared by e.l.i.s.a. with anti-blood-group-B antibody. The anti-blood-group B antibody similarly reacted with XN-1 and XN-2 glycosphingolipids (Figure 6).

From the above experimental results, the structure of both XN-1 and NX-2 was determined to be:

Galα1-3

Galβ1-3Galβ1-4Glcβ1-1′Cer

Fucα1-2

DISCUSSION

In this study, we isolated two novel blood-group-B-active glycosphingolipids, named XN-1 and XN-2, from *Xenopus laevis* eggs and determined their structures to be Galα1-3(Fucα1-2)Galβ1-3Galβ1-4Glcβ1-1′Cer by t.l.c.–immunostaining, FAB-m.s., ^{1}H-n.m.r. spectroscopy and permethylation analyses. Both XN-1 and XN-2 had an identical pentasaccharide structure, but they differed in their ceramide portion. Although the presence of blood-group A antigens in mucosa of *Xenopus laevis* was reported in the late 1960s [21], the presence of blood-group type B antigen was not reported before. This is the first report showing the presence of blood-group-B-active glycosphingolipids on the cell-surface membrane of amphibian embryos.

The isolated B-active glycosphingolipids differed from the previously reported B-active glycosphingolipids, since the frog glycosphingolipids have no *N*-acetylhexosamine. That is, the blood-group B determinant, Galα1-3(Fucα1-2)Galβ1-3, is bound to inner galactose instead of *N*-acetylhexosamine. A blood-group-A-active glycosphingolipid lacking *N*-acetylhexosamine has been also reported by Slomiany et al. [22] in hog gastric mucosa. In this case, the trisaccharide core structure is Galβ1-3Galβ1-4Glcβ1-1′Cer, which occurs in mucous tissues [23] and is known as 'muco series' [24]. The present study shows that muco-series glycolipids are distributed in amphibians too. We also detected many minor blood-group-B-active glycosphingolipids, including acidic glycosphingolipids, in *Xenopus laevis* eggs by immunostaining on t.l.c. plates. However, the amounts of the

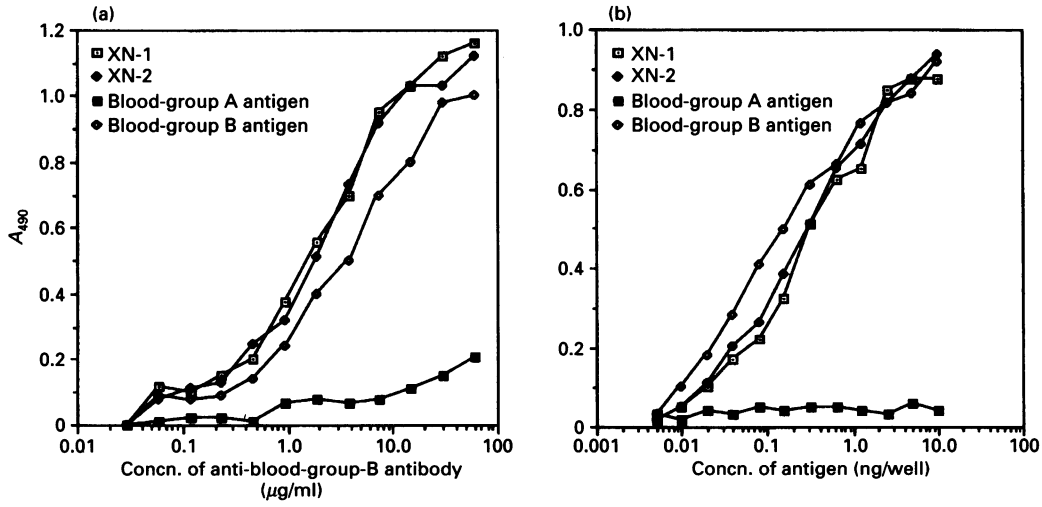

Figure 6 Reactivity of anti-blood-group-B antibody with glycosphingolipids isolated from *Xenopus laevis* eggs and those from human erythrocytes

(**a**) Effect of antibody dilution. A 20 pmol sample of each antigen was coated on a 96-well polystyrene plate and made to react with anti-blood-group-B antibody in e.l.i.s.a. (**b**) Effect of antigen concentration. Anti-blood-group-B antibody as primary antibody was used at a concentration of 0.3 μg/ml.

minor B-active glycosphingolipids were not sufficient to allow further detailed structural characterization.

It has been suggested that glycoconjugates expressing Le^x play a role in cell adhesion of the early mouse embryo, based on the observation that Le^x antigen appears in the developing embryo at the compaction stage [25] and that in some experiments oligosaccharides containing the Le^x sequence inhibit compaction ([26]; for review, see Feizi [27]). Adhesion experiments using liposomes expressing Le^x-active glycolipids have suggested that carbohydrate-mediated Le^x–Le^x interaction occurs [28]. In addition, the participation of cadherin molecules in compaction was reported; the monoclonal antibody ECCD-1 raised against mouse E-cadherin inhibited compaction completely [29]. It is possible that carbohydrate- and cadherin-dependent adhesion systems act co-operatively in Ca^{2+}-dependent cell–cell adhesion in mouse embryos.

Our previous study showed that Ca^{2+}-dependent cadherin-like cell-adhesion systems are present in the frog [2]. Several different cadherins have been detected in early frog embryos [30–32], and some of the antibodies against embryo-type cadherins partially inhibited Ca^{2+}-dependent cell–cell adhesion of early frog blastula cells [31]. Through these findings, significant involvement of cadherin in early-embryonic cell adhesion is apparent.

Although it is well known that blood-group-ABH glycosphingolipids are widely distributed in mammals (for review, see [33], their biological significance remains unclear. For instance, blood-group-active glycosphingolipids, abundantly expressed in human red blood cells, apparently have no biological function. However, for amphibian embryos, our study raises the possibility that blood-group B plays a role in cell–cell adhesion. Immunohistochemical studies using monoclonal antibody against blood-group B showed that B antigens exist on the blastomere cell surface. Recently we found that Ca^{2+}-dependent adhesion of dissociated blastomeres was completely inhibited by anti-blood-group-B antibody; this Ca^{2+}-dependent cell–cell adhesion of blastomeres was also inhibited by the addition of purified blood type B antigens, including protease-digested B-active glycopeptides (K. Nomura, K. Nomura, N. Nakajo, H. Nomura, M. Fujisue, M. Murata, J. Iwashita, K. Yamamoto, Y. Hirabayashi and K. Yamana, unpublished work). Interestingly, no Le^x-active antigens were detected in *Xenopus laevis* embryonic cells (results not shown). These results suggested that, in early amphibian embryos, cell–cell adhesion may be mediated through a recognition system based on interaction between cell-surface blood-group-B-active glycan and a receptor such as a cell-surface lectin-like molecule. Studies along these lines are under way in our laboratory.

Turner et al. [34] reported that a monoclonal antibody M4B, recognizing glyceroglycolipid, disrupts Ca^{2+}-dependent cell adhesion of *Xenopus laevis* blastula cells. However, the immunoreactive component was not characterized. Unlike our anti-blood-group-B antibodies, their antibody, M4B, did not react with proteins of the blastula. We could not detect any alkali-labile glyceroglycolipids reactive with anti-blood-group-B antibody in *Xenopus laevis* eggs. It will be interesting to know the relationship between the M4B reactive component and our blood-group-B-reactive glycosphingolipids in frog cell adhesion.

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