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Chemical characterization of the oligosaccharides in Bactrian camel (*Camelus bactrianus*) milk and colostrum

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ABSTRACT

Bactrian camel milk and colostrum are commonly used as foods in Mongolia, whose people believe that these products promote human health. It has been hypothesized that milk oligosaccharides are biologically significant components of human milk, acting as receptor analogs that inhibit the attachment of pathogenic microorganisms to the colonic mucosa, and as prebiotics, which stimulate the growth of bifidobacteria within the infant colon. To evaluate their biological significance, we studied the oligosaccharides present in samples of Bactrian camel milk and colostrum. Using ^{1}H-nuclear magnetic resonance spectroscopy, we identified and characterized the following oligosaccharides of camel colostrum: Gal(β1–4)[Fuc(α1–3)]Glc (3-fucosyllactose), Gal(β1–3)Gal(β1–4)Glc (3′-galactosyllactose), Gal(β1–6)Gal(β1–4)Glc (6′-galactosyllactose), Neu5Ac(α2–3)Gal(β1–4)Glc (3′-sialyllactose), Neu5Ac(α2–6)Gal(β1–4)Glc (6′-sialyllactose), Neu5Ac(α2–3)Gal(β1–3)Gal(β1–4)Glc (sialyl-3′-galactosyllactose), Neu5Ac(α2–6)Gal(β1–4)GlcNAc(β1–3)Gal(β1–4)Glc (sialyllacto-*N*-tetraose c), Neu5Ac(α2–3)Gal(β1–3)[Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc (sialyllacto-*N*-novopentaose a), Gal(β1–3)[Neu5Ac(α2–6)Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc (sialyllacto-*N*-novopentaose b); and Neu5Ac(α2–6)Gal(β1–4)GlcNAc(β1–3)[Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc (monosialyllacto-*N*-neohexaose). The oligosaccharides in the mature camel milk were characterized as 3′-galactosyllactose, Gal(β1–3)[Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc (lacto-*N*-novopentaose I), and 3′-sialyllactose.

Key words: Bactrian camel, milk oligosaccharide, colostrum, sialyl oligosaccharide

INTRODUCTION

Milk and colostrum from cows, as well as from goats, sheep, horses, and camels, are used as foods by people in various areas of the world. Camel milk is consumed in the Middle East and Mongolia. In Dubai, a chocolate containing camel milk components is produced commercially. Bactrian camel milk is consumed daily by people living in the Gobi region of Mongolia and provides nutrients that are essential for infant development. Since the time of Chinggis Khaan (Genghis Khan), Mongolians have traditionally used camel milk as a health-promoting product. Mongolian nomads like to consume colostrum (which has the consistency of gelatin after it is cooked) and to drink fermented camel milk. Mongolians believe that camel milk and colostrum have medicinal properties, and fermented camel milk has been used to treat edema in pregnant women and as antiscorbutic agent for the elderly (Dubach et al., 2007). Mongolia has 2% of the total world camel population and 30% of total Bactrian camels in the world. The Mongolian camel has a relatively low milk yield, producing on average 320 L of milk (from 175 to 576 L) during the lactation period of 528 d. The daily milk yield ranges from 0.5 to 1.7 L per camel (Dubach et al., 2007).

The composition of Bactrian camel colostrum obtained at 2 h postpartum is estimated to be 14.23% protein, 0.27% fat, 4.44% carbohydrate, and 0.77% minerals (Zhang et al., 2005). That of mature milk at 90 d postpartum is 3.55% protein, 5.65% fat, 4.24% carbohydrate, and 0.87% minerals (Zhang et al., 2005). These results suggest that the concentration of total solids in camel milk is greater than that of bovine milk.

Mammalian milk and colostrum usually contain lactose as the dominant saccharide, in addition to small amounts of a variety of milk oligosaccharides in which a lactose unit is usually located at the reducing end (Jenness et al., 1964). It is generally believed that milk oligosaccharides are biologically significant as receptor analogs that inhibit the attachment of pathogenic mi-

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croorganisms to the colonic mucosa; as prebiotics, which stimulate the growth of bifidobacteria in the colon; and as nerve growth factors (Urashima et al., 2009).

Bovine milk contains only trace amounts of milk oligosaccharides, but colostrum contains over 1 g/L of milk oligosaccharides in which 3′-sialyllactose is predominant (Gopal and Gill, 2000; Martin-Sosa et al., 2003; Nakamura et al., 2003; McJarrow and Amelsport-Schoonbeek, 2004). The question of whether camel milk or colostrum contains milk oligosaccharides and if so, what are their chemical structures, has not been investigated to date. In this study, we characterized the chemical structure of some milk oligosaccharides in Mongolian camel milk and colostrum using ^{1}H-nuclear magnetic resonance spectroscopy (^{1}**H-NMR**), and compared these with oligosaccharides found in bovine milk and colostrum.

MATERIALS AND METHODS

Samples

Colostrum (300 mL) and mature milk samples (300 mL) were collected from 2 lactating animals, at 18 h and 10 mo postpartum, respectively, bred at 2 private farms in Mongolia in March 2009. The samples were collected manually without oxytocin, and stored at −20°C until use. Bovine colostrum (100 mL) was obtained immediately after parturition and mature milk (100 mL) was obtained 120 d postpartum from a healthy Friesian-Holstein dairy cow located at the Field Center of Animal Science in our university (Obihiro, Hokkaido, Japan), during the early summer of 2004 and autumn of 2006, respectively. The samples were stored at −80°C until use.

Materials

3-Fucosyllactose {**3-FL**; Gal(β1-4)[Fuc(α1-3)]Glc} and sialyllacto-*N*-tetraose c [**LST c**; Neu5Ac(α2-6)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc] were purchased from Seikagaku Co. (Tokyo, Japan), where **Gal** = galactose, **Fuc** = fucose, **Glc** = glucose, **Neu5Ac** = *N*-acetylneuraminic acid, and **GlcNAc** = *N*-acetylglucosamine. 3′-Sialyllactose [**3′-SL**; Neu5Ac(α2-3)Gal(β1-4)Glc] and 6′-sialyllactose [**6′-SL**; Neu5Ac(α2-6)Gal(β1-4)Glc] were obtained from Sigma Co. (St. Louis, MO). 3′-Galactosyllactose [**3′-GL**; Gal(β1-3)Gal(β1-4)Glc] and 6′-galactosyllactose [**6′-GL**; Gal(β1-6)Gal(β1-4)Glc] were isolated from caprine colostrum (Urashima et al., 1994), whereas lacto-N-novopentaose I [**novoLNPI**; Gal(β1-3)[Gal(β1-4)GlcNAc(β1-6)]Gal(β1-4)Glc] was from brown capuchin colostrum (Urashima et al., 1999). Monosialyllacto-*N*-neohexaose {**MSLNnH**; Neu5Ac(α2-6)Gal(β1-4)GlcNAc(β1-3)[Gal(β1-4)GlcNAc(β1-6)]Gal(β1-4)Glc} and disialyllacto-N-neohexaose {**DSLNnH**; Neu5Ac(α2-6)Gal(β1-4)GlcNAc(β1-3)[Neu5Ac(α2-6)Gal(β1-4)GlcNAc(β1-6)]Gal(β1-4)Glc} were isolated from harbour seal milk (Urashima et al., 2003).

Colorimetric Assay

The carbohydrate concentration was assayed as total hexose using the phenol-H_2SO_4 method (Dubois et al., 1956), with lactose as the standard. The sialic acid concentration was determined by the periodate-resorcinol method using *N*-acetylneuraminic acid as the standard (Jourdian et al., 1971).

Isolation of Milk Oligosaccharides and Lactose

Samples of camel colostrum (**CC**), bovine colostrum (**BC**), and mature milks (**CM** and **BM** for camel and bovine milk, respectively) were thawed, and 15 mL of each was extracted with 4 volumes of chloroform:methanol (2:1, vol/vol). The emulsion was centrifuged at 4°C and 4,000 × *g* for 30 min, and the lower chloroform layer and the denatured protein were discarded. The methanol was removed from the upper layer by rotary evaporation, and the residue was dissolved in 30 mL of water and freeze-dried. The resulting powder was called the carbohydrate fraction.

The carbohydrate fractions were each dissolved in 2 mL of water and the solution passed through a Bio Gel P-2 column (<45 μm, 2.5 × 100 cm; Bio-Rad Laboratories, Hercules, CA) that had been calibrated with 2 mg each of galactose (monosaccharide), lactose (disaccharide), and raffinose (trisaccharide). Elution was done with distilled water at a flow rate of 15 mL/h, and fractions of 5 mL were collected. Aliquots (0.5 mL) of each fraction were analyzed for hexose with phenol-H_2SO_4 (Dubois et al., 1956) and for sialic acid with periodate-resorcinol (Jourdian et al., 1971). Peak fractions were pooled and freeze-dried. The saccharides in the peak fractions CC-7, separated from camel colostrum (Figure 1a), and CM-2, CM-3, and CM-4, separated from mature camel milk (Figure 2a), were subjected to ^{1}H-NMR. The components in each peak were analyzed by thin layer chromatography (TLC) on Silica Gel 60 (20 × 20 cm; Merck, Darmstadt, Germany) with acetone:2-propanol:0.1 mol/L lactic acid (2:2:1, vol/vol/vol) as a developing solvent. The spots were detected by spraying with 5% sulfuric acid in 94.5% ethanol and heating above a flame. The components in CC-6 were separated into CC-6-1, CC-6-2, and CC-6-3 by preparative TLC (chromatogram in Figure 3) in the same solvent system, and further purified by passage through a Bio Gel

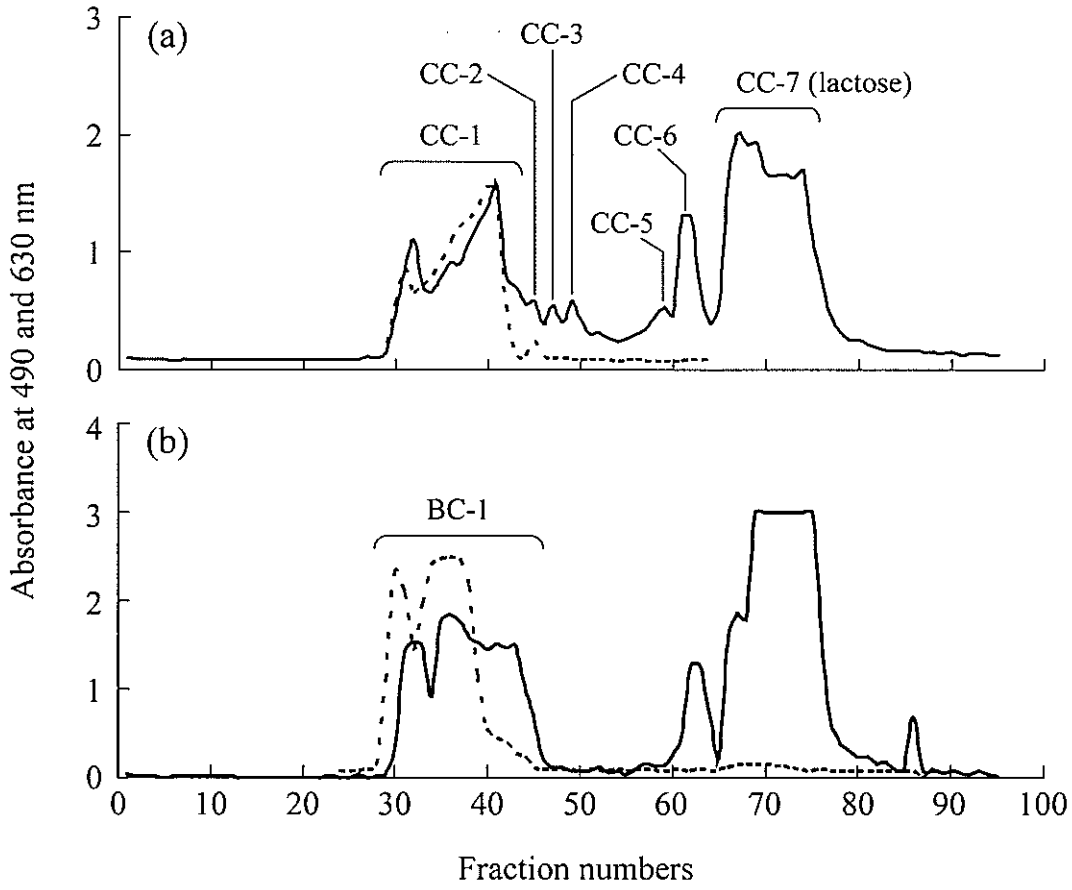

Figure 1. Chromatograms of the carbohydrate fractions from (a) camel colostrum (CC) and (b) bovine colostrum (BC) on a Bio Gel P-2 column (2.5 × 100 cm; Bio-Rad Laboratories, Hercules, CA). Elution was done with distilled water at a flow rate of 15 mL/h and fractions of 5.0 mL were collected. Each fraction was monitored by the phenol-H_2SO_4 method at 490 nm (——) and the periodate-resorcinol method at 630 nm (-----).

P-2 column (2.0 × 35 cm). The components in CC-6-1 (relative migration distance to lactose, R_{Lac} = 0.90), CC-6-2 (R_{Lac} = 0.76), and CC-6-3 (R_{Lac} = 0.66) were characterized by ^{1}H-NMR.

The components of peaks CC-1 and CM-1 from camel colostrum and mature milk, respectively (Figure 1a and Figure 2a), and BC-1 and BM-1 from bovine colostrum and mature milk, respectively (Figures 1b and 2b), which gave positive reactions with periodate-resorcinol (630 nm) and phenol-H_2SO_4 (490 nm), were dissolved in 2 mL of 50 mmol/L Tris hydroxyaminomethane-HCl buffer (pH 8.7) and subjected to anion exchange chromatography on DEAE-Sephadex A-50 column (2.0 × 20 cm; GE Healthcare, Uppsala, Sweden) equilibrated with the same buffer. The unadsorbed components were eluted with 250 mL of the same buffer and the adsorbed components were then eluted with a linear gradient of 0 to 0.5 mol/L NaCl in the Tris buffer solution. Elution was done at a flow rate of 15 mL/h and fractions of 5 mL were collected. Aliquots (0.5 mL) of each fraction were analyzed for hexose using the phenol-H_2SO_4 method. The fractions of CC-1-2 (Figure 4a), CM-1-2 (Figure 5a), BC-1-2 (Figure 4b), and BM-1-2 (Figure 5b) were each pooled, lyophilized, dissolved in 2 mL of water, and passed through a column (2.0 × 35 cm) of Bio Gel P-2 to remove salts, as described above.

The components in CC-1-2 and CM-1-2 (Figures 4a and 5a) were further separated by HPLC on a TSK gel Amide-80 column (4.6 × 250 mm, pore size 80Å, particle size 5 μm; Tosoh, Tokyo, Japan) using a LC-10 ATVP pump (Shimadzu, Tokyo, Japan) (chromatograms in Figure 6). The mobile phase was 50% and 80% (vol/vol) acetonitrile (CH_3CN) in 15 mmol/L potassium phosphate buffer (pH 5.2). Elution was done using a linear gradient of acetonitrile from 80 to 50% at 60°C at a flow rate of 1 mL/min. The eluates were monitored by measuring the absorbance at 195 nm. The peak fractions of oligosaccharides were pooled, concentrated by rotary evaporation, and subjected to ^{1}H-NMR to determine the structures. The components

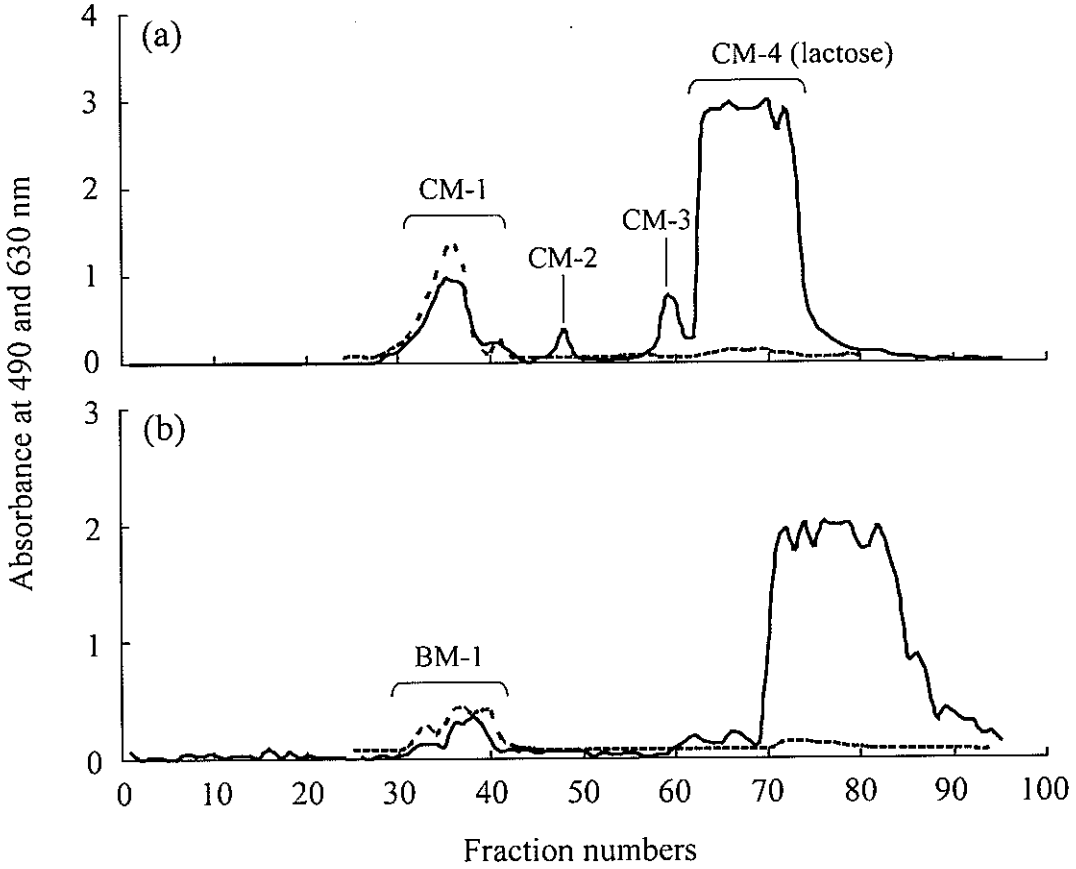

Figure 2. Chromatograms of the carbohydrate fraction from (a) camel mature milk (CM) and (b) bovine mature milk (BM) on a Bio Gel P-2 column (2.5 × 100 cm; Bio-Rad Laboratories, Hercules, CA). Elution was done with distilled water at a flow rate of 15 mL/h and fractions of 5.0 mL were collected. Each fraction was monitored by the phenol-H_2SO_4 method at 490 nm (——) and the periodate-resorcinol method at 630 nm (-----).

in BC-1-2 and BM-1-2 (Figures 3b and 4b) were also separated by HPLC (chromatograms in Figure 7).

^{1}H-NMR Spectroscopy

Nuclear magnetic resonance spectra were recorded in D_2O (100.00 atom D%; Aldrich, Milwaukee, WI) at 500 or 600 MHz for ^{1}H-NMR with a JEOL ECP-500 Fourier transform-NMR (Jeol, Tokyo, Japan) or a Varian INOVA 600 spectrometer (Varian Inc., Palo Alto, CA) operated at 293.1 K. Chemical shifts are expressed as change relative to internal 3-(trimethylsilyl)-1-propane sulfuric acid, sodium salt, but measured by reference to internal acetone ($\delta = 2.225$).

Mass Spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on the oligosaccharide fractions of CC-1-2-3, CC-1-2-5, and CC-1-2-6, which were separated from CC-1-2 (see Figure 4a) by HPLC as shown in Figure 6, using AutoflexII TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The sample solution (0.5 μL) was mixed on a target plate (MTP 384 target plate ground steel T F, Bruker), with an equal volume of 10 mg/mL of 2,5-dihydroxybenzoic acid (DHB) saturated in distilled water. After the solvent dried, the target plate was loaded into the mass spectrometer. Mass spectra were obtained using a reflector positive ion mode optimized to the mass range of 0 to 3 kDa. Sialyl Lewis X {Neu5Ac(α2–3)Gal(β1–4)[Fuc(α1–3)GlcNAc]} and 3′-SL were used as external mass calibrants.

RESULTS

Separation of Oligosaccharides from Colostrum and Mature Milk of Camel and Cow

The carbohydrate concentrations of camel colostrum and mature milk were found to be 4.99 and 5.74 g/100 mL, respectively, and their sialic acid concentrations

were 0.20 and 0.12 g/100 mL, respectively. On the other hand, the carbohydrate concentrations of bovine colostrum and mature milk were found to be 3.05 and 5.17 g/100 mL, respectively, and their sialic acid concentrations were 0.29 and 0.04 g/100 mL, respectively. The carbohydrate fractions from the camel colostrum and mature milk as well as from bovine colostrum and mature milk were further separated by chromatography as shown in Figures 1 and 2. The components in CC-1 (Figure 1a), CM-1 (Figure 2a), BC-1 (Figure 1b), and BM-1 (Figure 2b), which contained sialic acid, were subjected to anion exchange chromatography. The unadsorbed fractions, designated as CC-1-2 (Figure 4a), CM-1-2 (Figure 5a), BC-1-2 (Figure 4b), and BM-1-2 (Figure 5b) were pooled and passed through a Bio Gel P-2 column. Each component in those fractions was then separated by HPLC as shown in Figures 6 and 7. The components in CC-1-1 and CM-1-1, which were assumed to be higher neutral oligosaccharides, were not characterized in this study.

Structural Characterization of Neutral Oligosaccharides in Camel Colostrum and Mature Milk

CC-7, CM-4, CC-6-1, CC-6-2, and CC-6-3. Because the ^{1}H-NMR spectra (Figure 1a and Figure 2a) of the saccharide in CC-7 (chemical shifts in Table 1) and CM-4 (chemical shifts in Table 2) were identical to that of lactose, these were characterized to be Gal(β1–4)Glc.

The ^{1}H-NMR spectra of CC-6-1 and CC-6-2 showed that these fractions contained 2 oligosaccharides. Two oligosaccharides were not completely separated by the preparative TLC. Because the characteristic resonances in the ^{1}H-NMR spectra (chemical shifts in Table 1) were essentially identical to those of each spectrum of authentic 3′-GL and 3-FL, the oligosaccharides in the fractions were characterized to be Gal(β1–3)Gal(β1–4)Glc and Gal(β1–4)[Fuc(α1–3)]Glc.

The ^{1}H-NMR spectrum of the saccharide in CC-6-3 (chemical shifts in Table 1) was identical to that of authentic 6′-GL; thus, it was characterized as Gal(β1–6)Gal(β1–4)Glc.

The saccharides in fractions CC-2 to CC-5 were not characterized in this study, because these spectra contained many resonances caused by substances other than carbohydrates.

CM-2 and CM-3. The ^{1}H-NMR spectrum of CM-2 (chemical shifts in Table 2) was completely identical to that of authentic novoLNPI; thus, it was characterized to be Gal(β1–3)[Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc. The spectrum of CM-3 (chemical shifts in Table 2) was completely identical to that of authentic 3′-GL, and thus it was characterized to be Gal(β1–3)Gal(31–4)Glc.

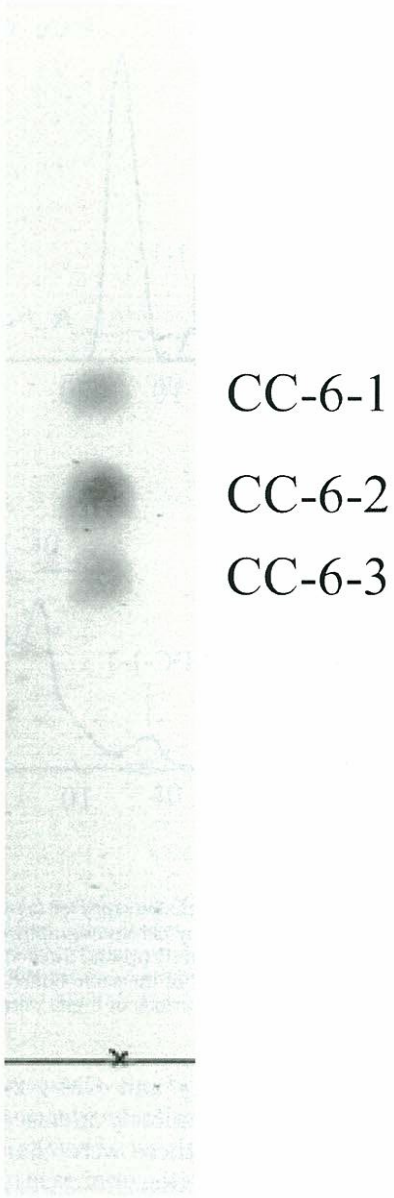

Figure 3. Thin layer chromatogram of the fraction CC-6, separated from camel colostrum (CC) by chromatography on Bio Gel P-2 (Bio-Rad Laboratories, Hercules, CA). The thin layer chromatography was performed on Silica Gel 60 with acetone:2-propanol:0.1 mol/L lactic acid (2:2:1, vol/vol/vol) as a developing solvent. The spots were detected by spraying with 5% sulfuric acid in 94.5% ethanol and heating above a flame.

Structural Characterization of Acidic Oligosaccharides in Camel Colostrum and Mature Milk

CC-1-2-1, CM-1-2-1, and CC-1-2-2. The ^{1}H-NMR spectra of the oligosaccharide in CC-1-2-1

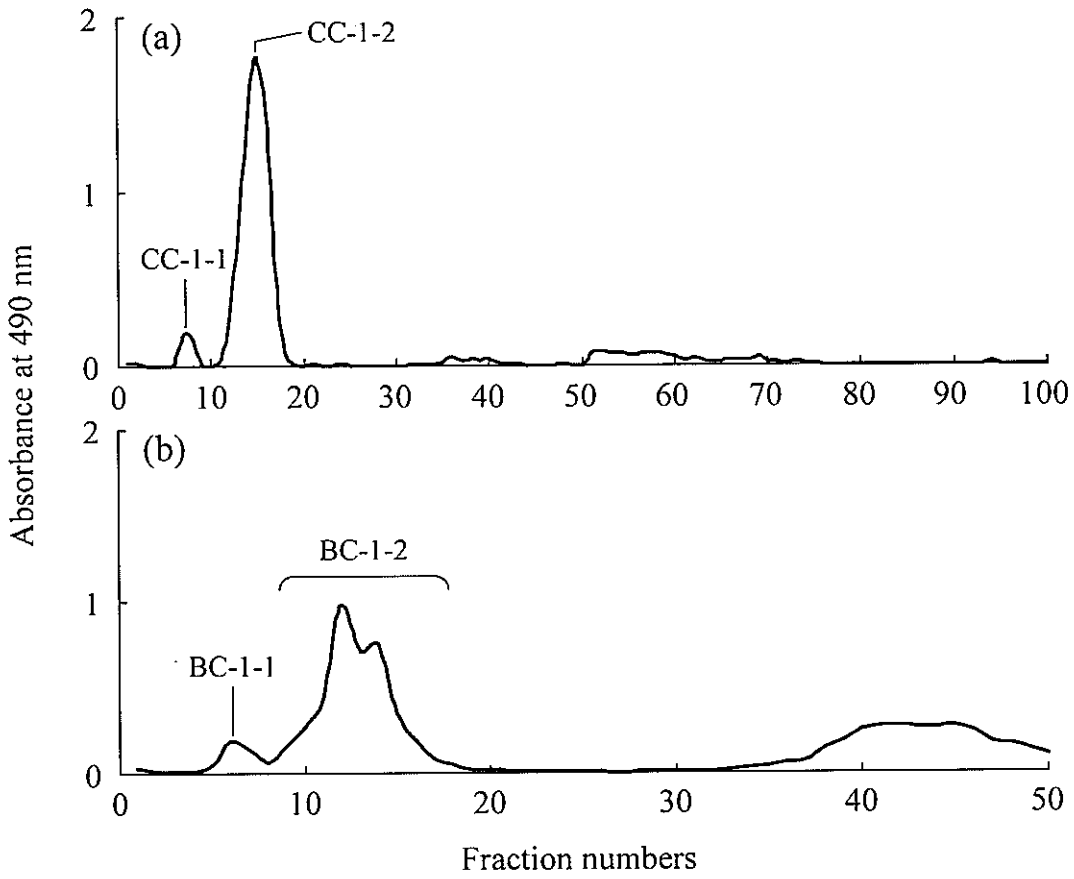

Figure 4. Anion exchange chromatography of (a) CC-1 (Figure 1a) separated from camel colostrum (CC) and (b) BC-1 (Figure 1b) separated from bovine colostrum (BC) by chromatography on Bio Gel P-2 (Figure 1; Bio-Rad Laboratories, Hercules, CA). A DEAE-Sephadex A-50 column (2.0 × 20 cm; GE Healthcare, Uppsala, Sweden) equilibrated with 50 mmol/L Tris hydroxyaminomethane-HCl buffer (pH 8.7) was used. Elution was done first with 250 mL of the same buffer and then with a linear gradient of the same buffer containing NaCl from 0 to 0.5 mol/L. The flow rate was 15 mL/h and fractions of 5 mL were collected. The fractions were monitored by the phenol-H_2SO_4 method.

(chemical shifts in Table 1) and CM-1-2-1 (chemical shifts in Table 2) were completely identical to that of authentic 3′-SL; therefore, these were characterized to be Neu5Ac(α2–3)Gal(β1–4)Glc.

The ^{1}H-NMR spectrum (chemical shifts in Table 1) of the oligosaccharide in CC-1-2-2 was completely identical to that of authentic 6′-SL, and so it was characterized to be Neu5Ac(α2–6)Gal(β1–4)Glc.

CC-1-2-3. The oligosaccharide in CC-1-2-3 was characterized by its ^{1}H-NMR spectrum (spectrum in Figure 8, chemical shifts in Table 1) compared with those of 3′-GL and 3′-SL. The spectrum had the characteristic resonances of H-3 axial, H-3 equatorial, and NAc of Neu5Ac at δ 1.813, 2.763, and 2.029, respectively, and H-3 of β-Gal, which was substituted by Neu5Ac, at δ 4.116, similar to those of 3′-SL; this indicated the presence of Neu5Ac(α2–3)Gal in the structure. The spectrum had the anomer signals of α-Glc, β-Glc, and β(1–4)-linked Gal at δ 5.223, 4.668, and 4.515, and the H-4 resonance of β(1–4)-linked Gal at δ 4.195, which was substituted by β(1–3)-linked Gal; these chemical shifts were close to those of anomers of α-Glc, β-Glc, and β(1–4)-linked Gal and H-4 of β(1–4)-linked Gal of 3′-GL, indicating the presence of reducing Gal(β1–3)Gal(β1–4)Glc unit in the structure. An additional anomeric signal was detected at δ 4.690, which was assigned to H-1 of β(1–3)-linked Gal. We concluded that this resonance shifted to down field compared with that (δ 4.612) of 3′-GL by substitution of this residue by Neu5Ac(α2–3).

From this interpretation of the resonances, the oligosaccharide in fraction CC-1-2-3 was characterized to be Neu5Ac(α2–3)Gal(β1–3)Gal(β1–4)Glc. The ions at 834.3 and 872.3 of its mass spectrum (Figure 9a) corresponded to $[M+K]^+$ and $[M-H+2K]^+$, respectively, supporting the structural characterization.

CC-1-2-4 and CC-1-2-5. The ^{1}H-NMR spectrum (chemical shifts in Table 3) of the oligosaccharide in

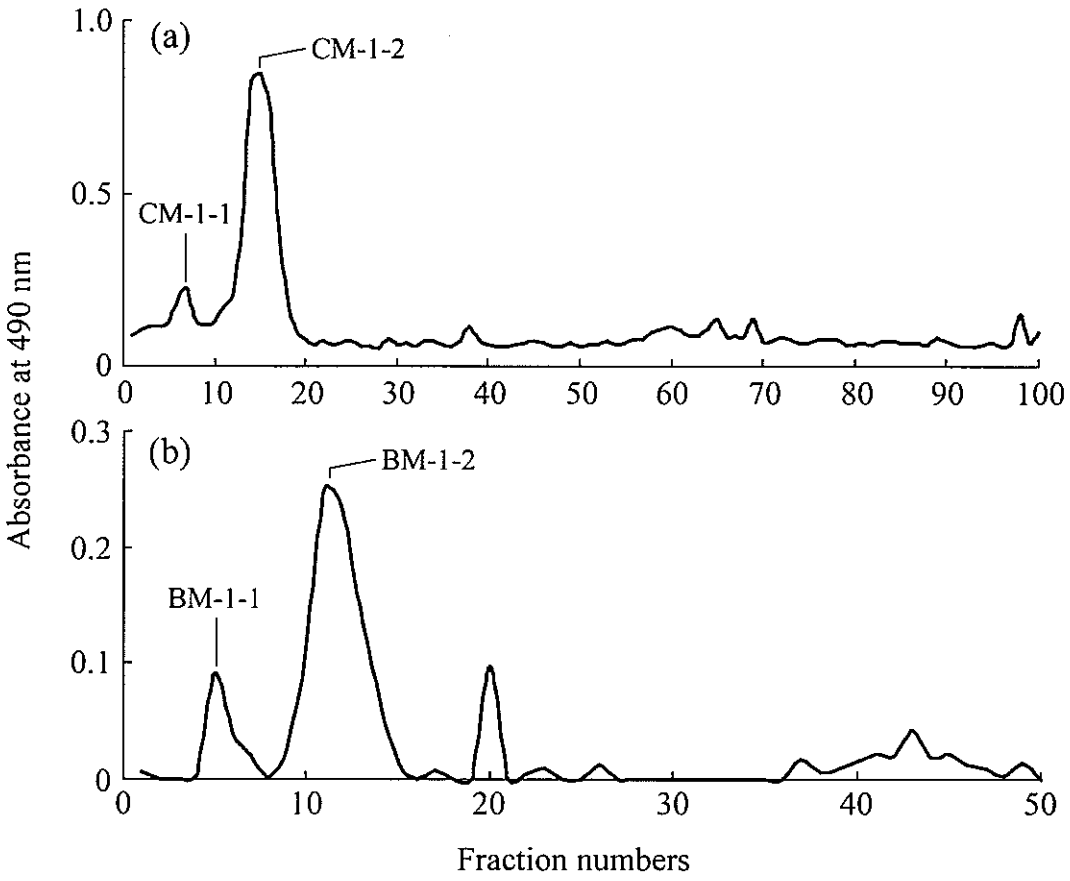

Figure 5. Anion exchange chromatography of (a) CM-1 (Figure 2a) separated from camel mature milk (CM) and (b) BM-1 (Figure 2b) separated from bovine mature milk (BM) by chromatography on Bio Gel P-2 (Figure 2; Bio-Rad Laboratories, Hercules, CA). A DEAE-Sephadex A-50 column (2.0 × 20 cm; GE Healthcare, Uppsala, Sweden) equilibrated with 50 mmol/L Tris hydroxyaminomethane-HCl buffer (pH 8.7) was used. Elution was done first with 250 mL of the same buffer and then with a linear gradient of the same buffer containing NaCl from 0 to 0.5 mol/L. The flow rate was 15 mL/h and fractions of 5 mL were collected. The fractions were monitored by the phenol-H_2SO_4 method.

CC-1-2-4 was identical to that of authentic LST c; therefore, it was characterized to be Neu5Ac(α2–6)Gal(β1–4)GlcNAc(β1–3)Gal(β1–4)Glc.

The oligosaccharide in fraction CC-1-2-5 was characterized by ^{1}H-NMR (spectrum in Figure 10, chemical shifts in Table 3) compared with those of CC-1-2-3 and novoLNPI. The spectrum had the characteristic H-3 axial, H-3 equatorial, and NAc shifts of Neu5Ac at δ 1.802, 2.763, and 2.029, respectively, and H-3 of β-Gal, which was substituted by Neu5Ac, at δ 4.117; this showed the presence of Neu5Ac(α2–3)Gal as in the spectrum of CC-1-2-3. The anomeric signal at δ 4.688 and the signal at δ 4.175 were assigned to H-1 of β(1–3)-linked Gal and H-4 of β(1–4)-linked Gal in the unit of Neu5Ac(α2–3Gal(β1–3)Gal(β1–4), similar to the spectrum of CC-1-2-3.

The anomeric signals at δ 5.225 and 4.671 arose from reducing α-Glc and β-Glc, respectively, whereas those at δ 4.645, 4.504, and 4.471 were assigned to H-1 of β(1–6)-linked GlcNAc and two residues of β(1–4)-linked Gal, respectively, because of the close chemical shifts in the spectrum of novoLNPI. The NAc shift at δ 2.061 also showed the presence of β(1–6)-linked GlcNAc. From these interpretations of the chemical shifts, the oligosaccharide in CC-1-2-5 was characterized to be Neu5Ac(α2–3)Gal(β1–3)[Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc. The ions at 1199.5 and 1237.5 of its spectrum (Figure 9b) corresponded to $[M+K]^+$ and $[M-H+2K]^+$, respectively, supporting the structural characterization.

CC-1-2-6. The oligosaccharide in fraction CC-1-2-6 was characterized by ^{1}H-NMR (spectrum in Figure 11, chemical shifts in Table 3) compared with those of 3′-SL, novoLNPI, and DSLNnH. The spectrum had the characteristic H-3 axial, H-3 equatorial, and NAc shift at δ 1.718, 2.667, and 2.028, which showed the presence of α(2–6)-linked Neu5Ac but not α(2–3)-linked Neu5Ac. The chemical shifts at δ 4.612 and 4.188 arose from H-1 of β(1–3)-linked Gal and H-4 of β(1–4)-linked Gal of Gal(β1–3)Gal(β1–4) unit as in the spectrum of

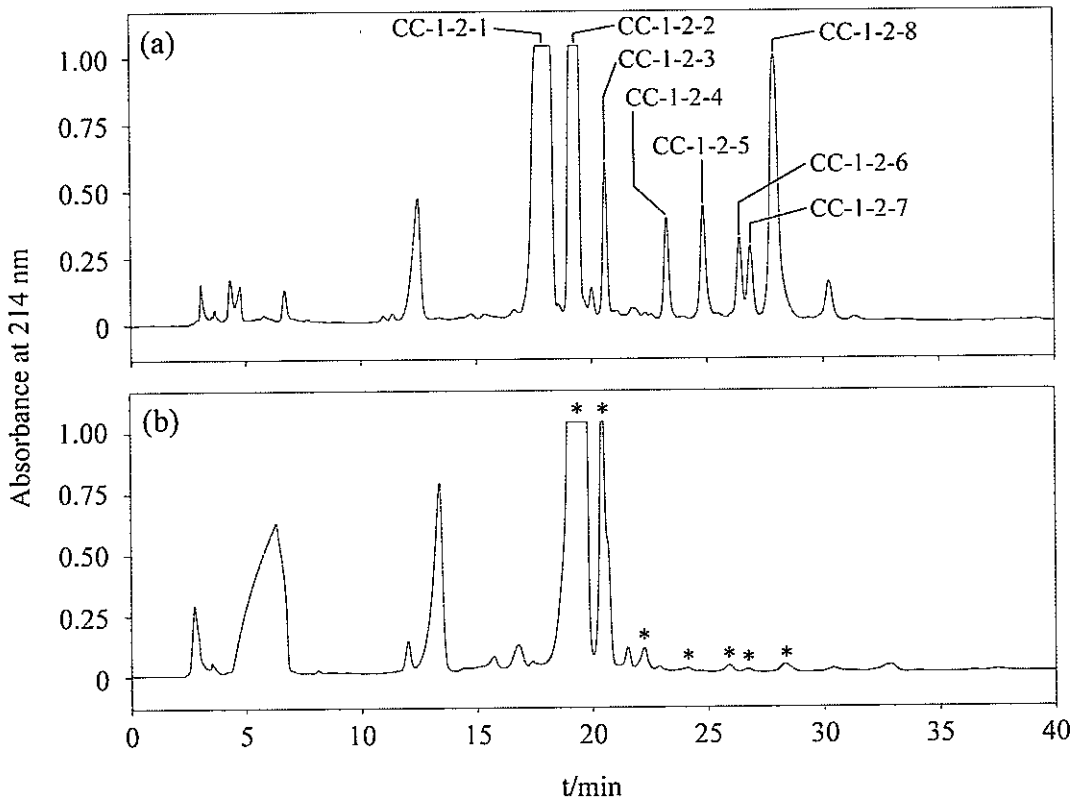

Figure 6. High-performance liquid chromatography of the fractions of (a) CC-1-2 (Figure 4a) from camel colostrum (CC) and (b) BC-1-2 (Figure 4b) from bovine colostrum. The HPLC was done using a Shimadzu LC-10 ATVP pump (Shimadzu, Tokyo, Japan) on a TSK-gel Amodo-80 column (4.6 × 250 mm, pore size 80 Å, particle size 5 μm; Tosoh, Tokyo, Japan). The mobile phase was 50% and 80% (vol/vol) acetonitrile (CH_3CN) in 15 mmol/L potassium phosphate buffer (pH 5.2). Elution was done using a linear gradient of CH_3CN from 80% to 50% at 60°C at a flow rate of 1 mL/min. The detection of peaks was done by UV absorption at 195 nm. It is assumed that the labeled peaks (*) corresponded to 3′-sialyllactose, 6′-sialyllactose, sialyl-3′-galactosyllactose, sialyllacto-*N*-tetraose c, sialyllacto-*N*-novopentaose a, sialyllacto-*N*-novopentaose b, and monosialyllacto-*N*-neohexaose, although these components were not characterized by ^{1}H-nuclear magnetic resonance spectroscopy.

3′-GL and novoLNPI. The presence of anomeric signal at δ 4.612 but not δ 4.688 showed that the β(1–3)-linked Gal residue was not substituted by Neu5Ac. The anomeric signals at δ 5.225 and 4.667 arose from reducing α-Glc and β-Glc, respectively. The NAc shift of GlcNAc at δ 2.089 and nonexistence of the shift at δ 2.061 showed the presence of a Neu5Ac(α2–6)Gal(β1–4) GlcNAc(β1–6) unit, because we found a similar NAc shift in the spectrum of DSLNnH. In the spectrum of DSLNnH, the shift at δ 2.088 was assigned to NAc of β(1–6)-linked GlcNAc of Neu5Ac(α2–6)Gal(β1–4) GlcNAc(β1–6) unit. From these interpretations of the chemical shifts, the oligosaccharide in fraction CC-1-2-6 was characterized to be Gal(β1–3)[Neu5Ac(α2–6) Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc. The ions at 1199.7 and 1237.5 of its spectrum (Figure 9c) corresponded to $[M+K]^+$ and $[M-H+2K]^+$, respectively, supporting the structural characterization.

CC-1-2-7 and CC-1-2-8. The oligosaccharide in CC-1-2-7 was not characterized in this study, because we could not assign its ^{1}H-NMR signals at this stage. The ^{1}H-NMR spectrum (chemical shifts in Table 3) of the oligosaccharide in CC-1-2-8 was identical to that of the published data for MSLNnH (Gronberg et al., 1989); it was characterized to be Neu5Ac(α2–6)Gal(β1–4) GlcNAc(β1–3)[Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc.

DISCUSSION

The hexose contents in the colostrum and mature milk were 4.99 and 5.74 g/100 mL, respectively. Because the oligosaccharide concentration was higher in colostrum than in mature milk (see Figure 1a and Figure 2a), we assumed that the lactose concentration was higher in mature milk than in colostrum. The structures of the oligosaccharides characterized in the camel colostrum and mature milk are shown in Figure 12.

Significant differences were found between camel colostrum and mature milk oligosaccharides, especially in regard to the acidic oligosaccharides. At least 8

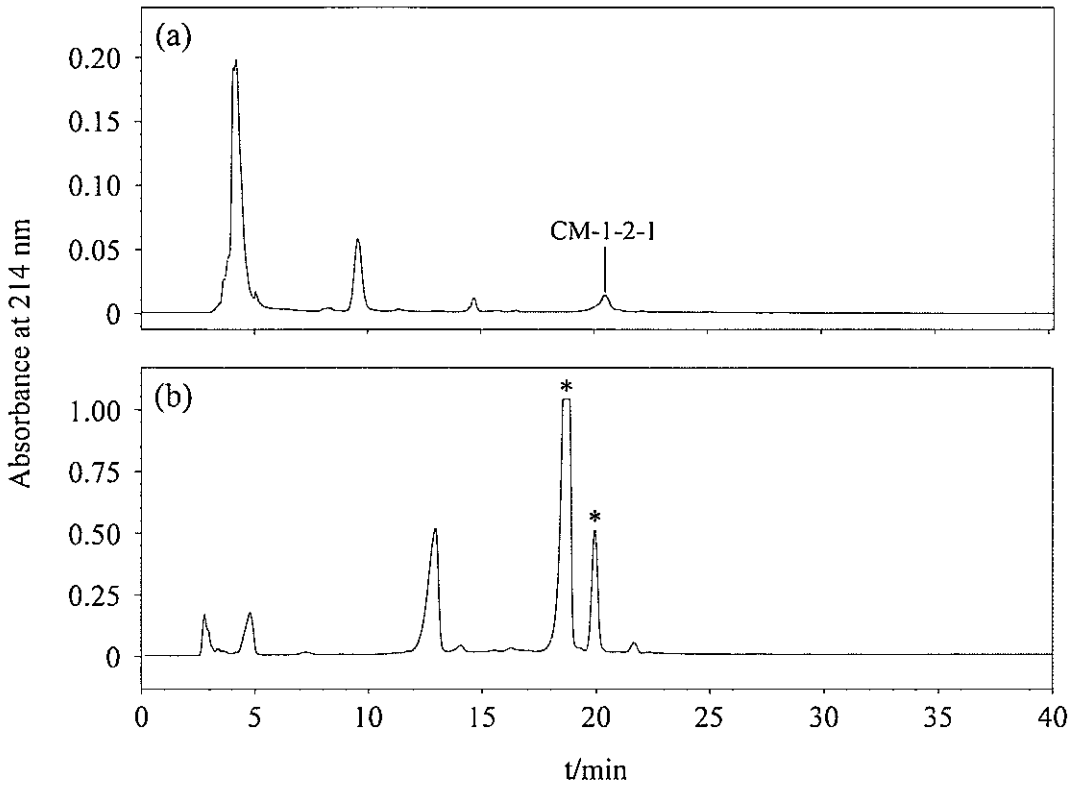

Figure 7. High-performance liquid chromatography of the fractions of (a) CM-1-2 (Figure 5a) from camel mature milk (CM) and (b) BM-1-2 (Figure 5b) from bovine mature milk. The HPLC was done using a Shimadzu LC-10 ATVP pump (Shimadzu, Tokyo, Japan) on a TSK-gel Amodo-80 column (4.6 × 250 mm, pore size 80 Å, particle size 5 μm; Tosoh, Tokyo, Japan). The mobile phase was 50% and 80% (vol/vol) acetonitrile (CH_3CN) in 15 mmol/L potassium phosphate buffer (pH 5.2). Elution was done using a linear gradient of CH_3CN from 80% to 50% at 60°C at a flow rate of 1 mL/min. The detection of peaks was done by UV absorption at 195 nm. It is assumed that the labeled peaks (*) corresponded to 3′-sialyllactose and 6′-sialyllactose, although these components were not characterized by ^{1}H-nuclear magnetic resonance spectroscopy.

acidic oligosaccharides were found in the colostrum, whereas only one, 3′-SL, was observed in mature milk (Figure 6a and Figure 7a). This suggests that most of the sialyl oligosaccharides are only synthesized by the camel mammary glands early in lactation. This finding is similar to that observed with bovine colostrum and milk: bovine colostrum contains more than 1 g/L of sialyl oligosaccharides, whereas mature milk contains only small amounts (Gopal and Gill, 2000; Martin-Sosa et al., 2003; Nakamura et al., 2003; McJarrow and Amelsport-Schoonbeek, 2004). It is generally thought that milk oligosaccharides protect neonates against infection (Urashima et al., 2009); accordingly, they may be required mostly during the newborn period in both camel and cow. However, 2 major acidic oligosaccharide peaks, the retention times of which are similar to those of 3′-SL and 6′-SL, were found in bovine colostrum and mature milk (Figure 6b and Figure 7b), suggesting no large differences in the variety of acidic oligosaccharides between bovine colostrum and mature milk, although their amounts are higher in colostrum than in mature milk.

The greatest difference between camel and cow was observed in the acidic milk oligosaccharide profiles of the colostrum, as shown in Figure 6. Camel colostrum was rich in oligosaccharides, with core units of lacto-N-neotetraose [LNnT; Gal(β1–4)GlcNAc(β1–3)Gal(β1–4)Glc], novoLNPI, and lacto-N-neohexaose [LNnH; Gal(β1–4)GlcNAc(β1–3)[Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc], as well as 3′-SL and 6′-SL.

Several oligosaccharides have been characterized in bovine colostrum to date (Saito et al., 1984, 1987; Urashima et al., 1991, 2009; Tao et al., 2008; Barile et al., 2009). In this study, significant homology and heterogeneity were observed in milk oligosaccharides between camel and cow. 3′-Galactosyllactose, 6′-GL, and novoLNPI were also found in bovine colostrum (Saito et al., 1987; Urashima et al., 1991), but 3-FL was not observed in camel colostrum and mature milk. Oligosaccharides containing N-acetyllactosamine [Gal(β1–4)GlcNAc] at the reducing end such as free N-acetyllactosamine, 3-fucosyl-N-acetyllactosamine, and 6′-N-acetylneuraminyl N-acetyllactosamine [**6′-SLN**; Neu5Ac(α2–6)Gal(β1–4)GlcNAc], have been reported

Table 1. Proton (^{1}H) chemical shifts of saccharides CC-1-2-1 to CC-1-2-3 (see Figure 6a), separated from CC-1-2 (see Figure 4a) by HPLC, and CC-7 to CC-6 (see Figure 1a) separated by gel filtration from camel colostrum

Reporter group[1]	Residue[2]	Chemical shifts, δ (coupling constants, Hz)						
		CC-1-2-3	CC-1-2-2	CC-1-2-1	CC-6-2	CC-6-1	CC-6-3	CC-7
H-1	Glcα	5.223 (3.5)	5.224 (3.4)	5.220 (4.0)	5.225 (3.4)	5.181 (4.0)	5.225 (4.0)	5.222 (3.4)
	Glcβ	4.668 (7.9)	4.669 (8.0)	4.662 (8.0)	4.668 (6.9)	4.652 (6.9)	4.671 (8.0)	4.665 (7.4)
	Galβ4	4.515 (7.6)	4.428 (7.4)	4.531 (8.0)	4.511 (8.0)	4.432 (7.4)	4.463 (8.0)	4.451 (7.4)
	Galβ3	4.690 (7.9)	—	—	4.612 (8.0)	—	—	—
	Galβ6	—	—	—	—	—	4.490 (8.0)	—
	Fucα3	—	—	—	—	5.442 (4.6)	—	—
						5.385 (4.0)		
H-3	Galβ4	—	—	4.115 (2.9[a])	—	—	—	—
	Galβ3	4.116 (2.9[a])	—	—	—	—	—	—
H-3ax	Neu5Acα2–3	1.813 (12.2[b] – 12.0[c])	—	1.800 (12.6[b] – 12.0[c])	—	—	—	—
	Neu5Acα2–6	—	1.747 (12.6[b] – 12.0[c])	—	—	—	—	—
H-3eq	Neu5Acα2–3	2.763 (4.3[d])	—	2.757 (4.6[d])	—	—	—	—
	Neu5Acα2–6	—	2.714 (4.6[d])	—	—	—	—	—
H-4	Galβ4	4.195 (2.6[e])	—	—	4.198 (3.4[e])	—	—	—
H-6	Fucα3	—	—	—	—	1.189 (6.9[f])	—	—
						1.183 (6.9[f])		
NAc	Neu5Acα2–3	2.029	—	2.029	—	—	—	—
	Neu5Acα2–6	—	2.029	—	—	—	—	—

[a] $J_{3,4}$; [b] $J_{3ax,4}$; [c] $J_{3ax,3eq}$; [d] $J_{3eq,4}$; [e] $J_{4,3}$; [f] $J_{6,5}$, where $J_{x,y}$ indicates the coupling constant of H-x and H-y.

[1] H-3ax = H-3 axial; H-3eq = H-3 equatorial; NAc = N-acetyl.

[2] Glc = glucose; Gal = galactose; Fuc = fucose; Neu5Ac = N-acetylneuraminic acid.

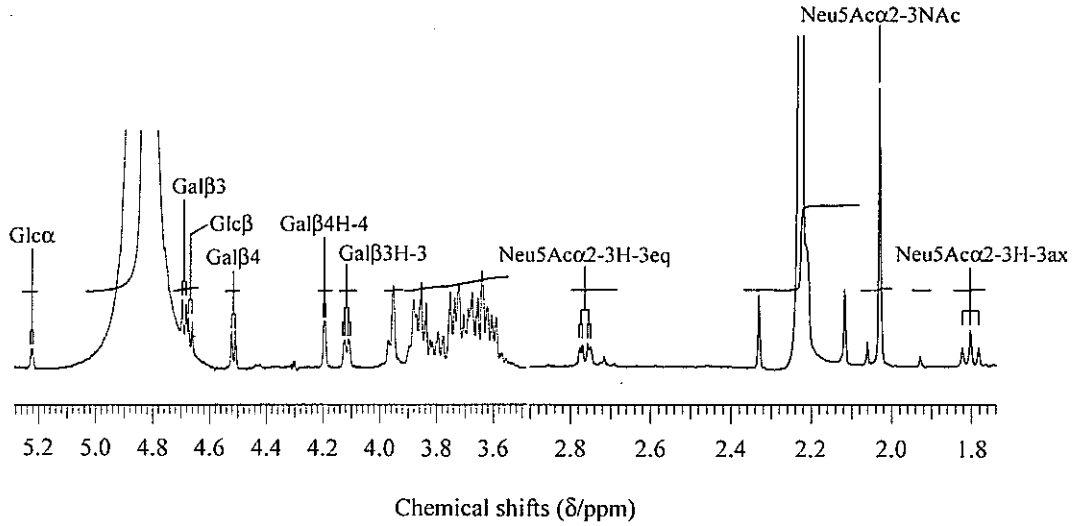

Figure 8. [1]H-Nuclear magnetic resonance spectrum of the oligosaccharide (sialyl-3′-galactosyllactose) in CC-1-2-3 isolated from camel colostrum by HPLC (Figure 6a). The spectrum was obtained in D_2O at 600 MHz with a Varian INOVA spectrometer operated at 293.1 K (Varian, Palo Alto, CA). Chemical shifts are expressed relative to internal 3-(trimethylsilyl)-1-propane sulfuric acid, sodium salt. Gal = galactose, Fuc = fucose, Glc = glucose, Neu5Ac = *N*-acetylneuraminic acid, and GlcNAc = *N*-acetylglucosamine.

in bovine colostrum (Kuhn and Gauhe, 1965; Saito et al., 1984, 1987), but this type of oligosaccharide was not detected in camel colostrum. Isoglobotriose [Gal(α1–3)Gal(β1–4)Glc] and GalNAc(α1–3)Ga(β1–4) Glc were also not found in camel colostrum, whereas these saccharides have been found in bovine colostrum (Urashima et al., 1991).

In bovine colostrum, 3′-SL is the most predominant oligosaccharide, and 6′-SL and 6′-SLN are the second most dominant ones (Martin-Sosa et al., 2003; Nakamura et al., 2003; McJarrow and Amelsport-Schoonbeek, 2004). Because the most predominant acidic oligosaccharide in camel colostrum was also 3′-SL in this study, the camel resembles cow in this respect.

The minor oligosaccharides of bovine colostrum, obtained on d 6, and of mature milk obtained on d 120 were studied using HPLC–chip/MS (Tao et al., 2008). Although, with this method, the monosaccharide composition of each oligosaccharide can be determined, the linkage positions and glycoside conformations are not clarified. In this respect, NMR analysis is more powerful, because it provides this additional

Table 2. Proton ([1]H) chemical shifts of saccharides CM-1-2-1 (see Figure 6a), separated from CC-1-2 (see Figure 4a) by HPLC, CM-2, CM-3, and CM-4 (see Figure 1a) separated by gel filtration from camel mature milk

Reporter group[1]	Residue[2]	Chemical shifts, δ (coupling constants, Hz)			
		CM-1-2-1	CM-2	CM-3	CM-4
H-1	Glcα	5.220 (4.0)	5.220 (4.0)	5.224 (3.4)	5.223 (4.0)
	Glcβ	4.662 (8.0)	4.670 (8.0)	4.667 (8.0)	4.665 (7.4)
	Galβ4	4.530 (7.4)	4.450 (8.0) 4.500 (8.0)	4.511 (8.0)	4.450 (7.4)
	Galβ3	—	4.610 (8.0)	4.612 (8.0)	—
	GlcNAcβ6	—	4.650 (7.5) 4.640 (7.5)	—	—
H-3	Galβ4	4.114 (2.9[a])	—	—	—
H-3ax	Neu5Acα2–3	1.800 (12.0[b]-12.6[c])	—	—	—
H-3eq	Neu5Acα2–3	2.755 (4.6[d])	—	—	—
H-4	Galβ4	—	4.180 (2.9[e])	4.199 (3.4[e])	—
NAc	GlcNAcβ6	—	2.062	—	—
	Neu5Acα2–3	2.029	—	—	—

[a] $J_{3,4}$; [b] $J_{3ax,4}$; [c] $J_{3ax,3eq}$; [d] $J_{3eq,4}$; [e] $J_{4,3}$, where $J_{x,y}$ indicates the coupling constant of H-x and H-y.

[1]H-3ax = H-3 axial; H-3eq = H-3 equatorial; NAc = *N*-acetyl.

[2]Glc = glucose; Gal = galactose; GlcNAc = *N*-acetylglucosamine; Neu5Ac = *N*-acetylneuraminic acid.

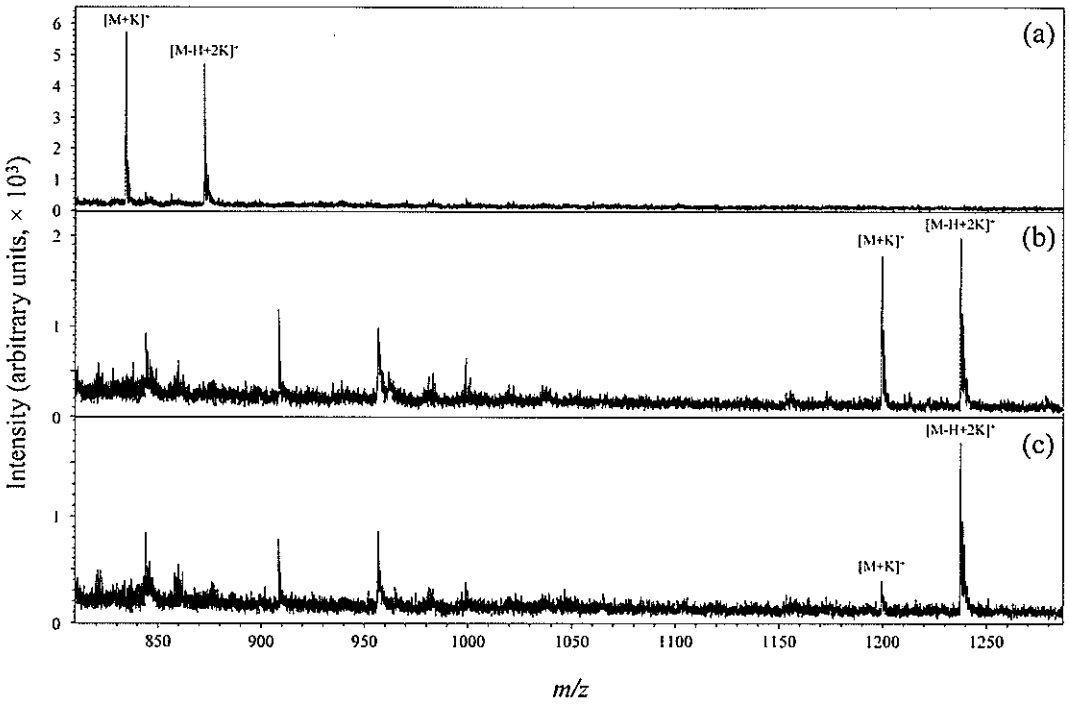

Figure 9. Matrix-assisted, laser-desorption time-of-flight (MALDI-TOF) mass spectra of (a) CC-1-2-3, (b) CC-1-2-5, and (c) CC-1-2-6 (see Figure 6a), separated from CC-1-2 (see Figure 4a) by HPLC. The sample solution (0.5 μL) and saturated DHB (2,5-dihydroxybenzoic acid) solution (0.5 μL) were mixed on the target plate. After the solvent dried, the target plate was loaded into the mass spectrometer. Mass spectra were obtained using a reflector positive ion mode optimized to the mass range of 0 to 3 kDa. Sialyl Lewis X and 3′-sialyllactose were used as external mass calibrants.

Table 3. Proton (^{1}H) chemical shifts of saccharides CC-1-2-4 to CC-1-2-8 (see Figure 6a), separated from CC-1-2 (see Figure 4a) by HPLC from camel colostrum

Reporter group[1]	Residue[2]	Chemical shifts, δ (coupling constants, Hz)			
		CC-1-2-8	CC-1-2-6	CC-1-2-5	CC-1-2-4
H-1	Glcα	5.219 (3.8)	5.225 (3.8)	5.225 (3.2)	5.220 (3.5)
	Glcβ	4.668 (7.9)	4.667 (7.9)	4.671 (8.2)	4.665 (8.2)
	Galβ4	4.432 (7.9)	4.445 (8.2)	4.471 (8.0)	4.442 (8.2)
		4.455 (7.9)	4.505 (7.9)	4.504 (7.9)	4.455 (7.9)
		4.472 (7.9)			
	Galβ3	—	4.612 (7.9)	4.688 (8.2)	—
	GlcNAcβ3	4.726 (7.3)	—	—	4.732 (8.2)
	GlcNAcβ6	4.640 (7.6)	4.657 (8.0)	4.645 (8.2)	—
		4.646 (7.6)			
H-3	Galβ3	—	—	4.117 (3.2^a)	—
H-3ax	Neu5Acα2–3	—	—	1.802 (12.3^b – 12.0^c)	
	Neu5Acα2–6	1.724 (12.3^b – 12.0^c)	1.718 (11.7^b – 12.6^c)	—	1.724 (12.0^b – 11.7^c)
H-3eq	Neu5Acα2–3	—	—	2.763 (4.0^d)	—
	Neu5Acα2–6	2.668 (4.7^d)	2.667 (4.7^d)	—	2.657 (4.7^d)
H-4	Galβ4	4.149 (2.6^e)	4.188 (3.2^e)	4.175 (3.2^e)	4.160 (2.7^e)
NAc	GlcNAcβ3	2.051	—	—	2.054
	GlcNAcβ6	2.061	2.089	2.061	—
	Neu5Acα2–3	—	—	2.029	—
	Neu5Acα2–6	2.029	2.028	—	2.027

[a] $J_{3,4}$; [b] $J_{3ax,4}$; [c] $J_{3ax,3eq}$; [d] $J_{3eq,4}$; [e] $J_{4,3}$, where $J_{x,y}$ indicates the coupling constant of H-x and H-y.

[1] H-3ax = H-3 axial; H-3eq = H-3 equatorial; NAc = *N*-acetyl.

[2] Glc = glucose; Gal = galactose; GlcNAc = *N*-acetylglucosamine; Neu5Ac = *N*-acetylneuraminic acid.

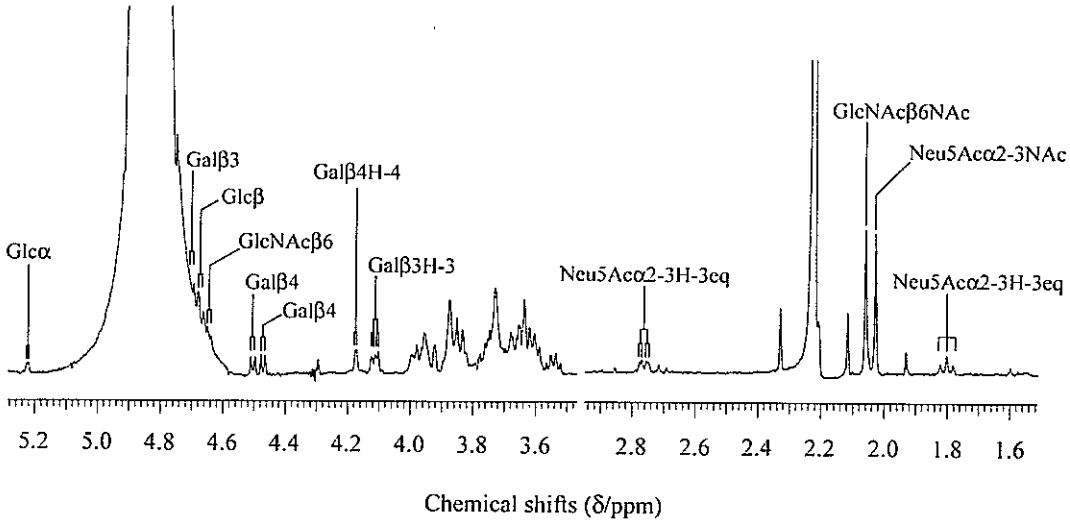

Figure 10. ^{1}H-Nuclear magnetic resonance spectrum of the oligosaccharide (sialyllacto-*N*-novopentaose a) in CC-1-2-5 isolated from camel colostrum by HPLC (Figure 6a). The spectrum was obtained in D_2O at 600 MHz with a Varian INOVA spectrometer operated at 293.1 K (Varian, Palo Alto, CA). Gal = galactose, Fuc = fucose, Glc = glucose, Neu5Ac = *N*-acetylneuraminic acid, and GlcNAc = *N*-acetylglucosamine.

information. Two tetrasaccharides (3Hex+1Neu5Ac), 2 pentasaccharides (3Hex+1HexNAc+1Neu5Ac), 2 hexasaccharides (4Hex+1HexNAc+1Neu5Ac), and 1 heptasaccharide (4Hex+2HexNAc+1Neu5Ac) were unequivocally detected in bovine milk and colostrum by using this method (Tao et al., 2008). It is possible that some of these are S-3′-GL, LST c, S-novoLNP a, S-novoLNP b, and MSLNnH, similar to those found in camel colostrum. The small peaks in the HPLC of bovine colostrum acidic oligosaccharides (Figure 6b) may correspond to these saccharides.

One of these bovine tetrasaccharides may be similar to Neu5Ac(α2–3)Gal(β1–3)Gal(β1–4)Glc of the camel. In another study, a tetrasaccharide with the sequence Neu5Ac(α2-x)Gal(β1–3)Gal(β1–4)Glc was found in bovine colostrum (Parkkinen and Finne, 1987). However, 2 other tetrasaccharides, Gal(β1–3)[Neu5Ac(α2–6)]Gal(β1–4)Glc and Gal(β1–6)[Neu5Ac(α2–3)]Gal(β1–4)Glc, were found in caprine colostrum (Viverge et al., 1997). These oligosaccharides have not been found in human milk (Urashima et al., 2009), suggesting that this nonpresence is caused by the small amounts of

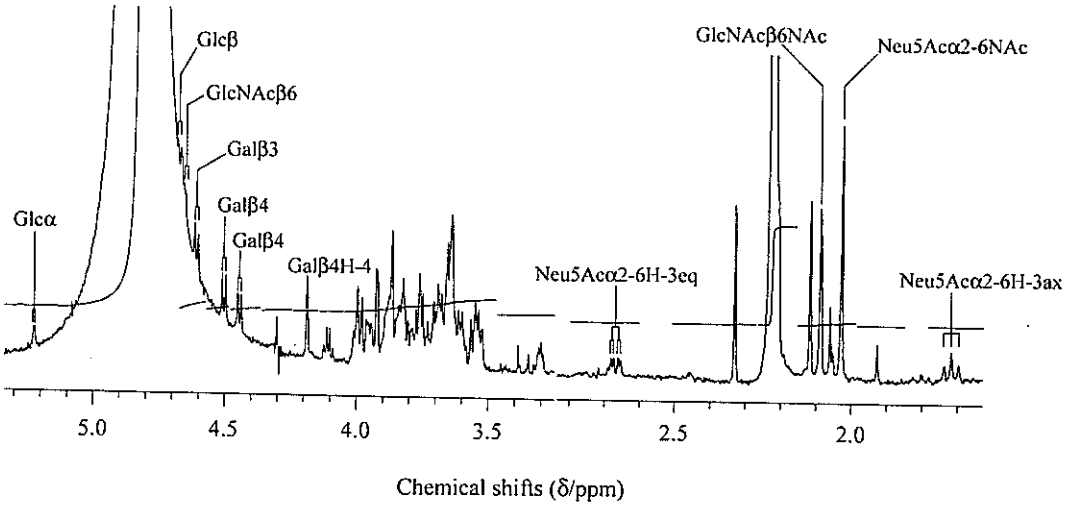

Figure 11. ^{1}H-Nuclear magnetic resonance spectrum of the oligosaccharides (sialyllacto-*N*-novopentaose b) in CC-1-2-6 isolated from camel colostrum by HPLC (Figure 6a). The spectrum was obtained in D_2O at 600 MHz with a Varian INOVA spectrometer operated at 293.1 K (Varian, Palo Alto, CA). Gal = galactose, Fuc = fucose, Glc = glucose, Neu5Ac = *N*-acetylneuraminic acid, and GlcNAc = *N*-acetylglucosamine.

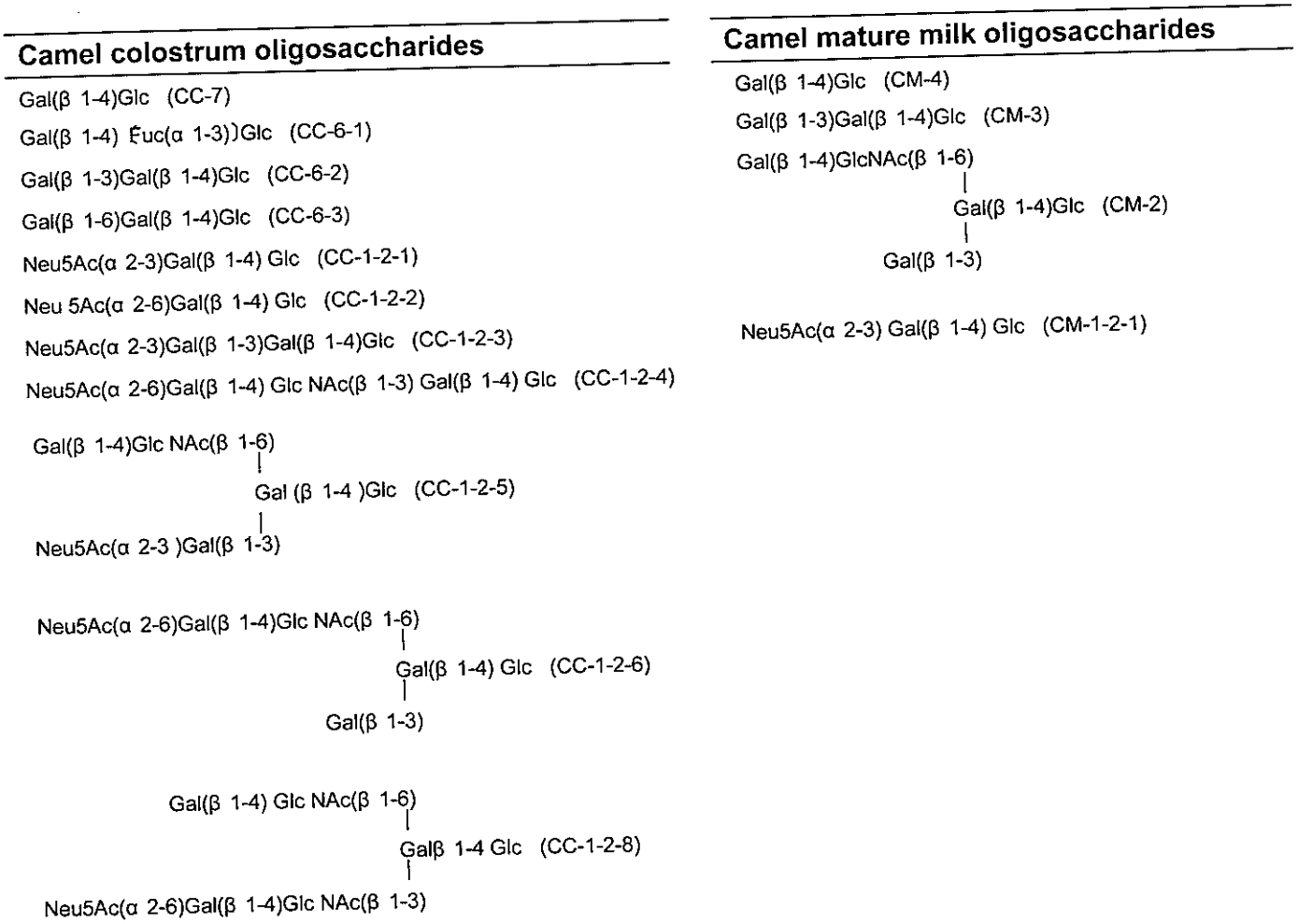

Figure 12. Structures of the oligosaccharides found in camel colostrum (CC) and mature milk (CM). Gal(β1-4)Glc = lactose (CC-7 and CM-4); Gal(β1-4)[Fuc(α1-3)]Glc = 3-fucosyllactose (CC-6-1); Gal(β1-3)Gal(β1-4)Glc = 3′-galactosyllactose (CC-6-2 and CM-3); Gal(β1-6)Gal(β1-4)Glc = 6′-galactosyllactose (CC-6-3); Gal(β1-3)[Gal(β1-4)GlcNAc(β1-6)]Gal(β1-4)Glc = lacto-N-novopentaose I (CM-2); Neu5Ac(α2-3)Gal(β1-4)Glc = 3′-sialyllactose (CC-1-2-1 and CM-1-2-1); Neu5Ac(α2-6)Gal(β1-4)Glc = 6′-sialyllactose (CC-1-2-2); Neu5Ac(α2-3)Gal(β1-3)Gal(β1-4)Glc = sialyl-3′-galactosyllactose (CC-1-2-3); Neu5Ac(α2-6)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc = sialyllacto-*N*-tetraose c (CC-1-2-4); Neu5Ac(α2–3)Gal(β1–3)[Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc = sialyllacto-*N*-novopentaose a (CC-1-2-5); Gal(β1–3)[Neu5Ac(α2–6)Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc = sialyllacto-*N*-novopentaose b (CC-1-2-6); and Neu5Ac(α2–6)Gal(β1–4)GlcNAc(β1–3)[Gal(β1–4)GlcNAc(β1–6)]Gal(β1–4)Glc = monosialyllacto-*N*-neohexaose (CC-1-2-8).

Gal-Gal–containing oligosaccharides in human milk compared with those in bovine, caprine, or camel milk or colostrum.

It was reported that 5% of the bovine sialyl oligosaccharide fraction contains Neu5Gc (*N*-glycolylneuraminic acid; Tao et al., 2008); evidently, this type of sialyl oligosaccharides is a minor constituent in bovine sialyl oligosaccharide fraction. Although oligosaccharides containing Neu5Gc were not detected in this study, it is nevertheless possible that small amounts were present in camel colostrum because we were unable to analyze all the chromatography peaks in Figure 6a.

Because 3-FL, 3′-GL, 6′-GL, 3′-SL, 6′-SL, LDT c, and MSLNnH, which were characterized in camel colostrum in this study, have also been found in human milk (Urashima et al., 2009), their presence may be advantageous for the utilization of camel colostrum as a biofunctional material. On the other hand, we believe that the most significant difference in milk oligosaccharides between human and cow or camel is the presence or absence of lacto-*N*-biose [Gal(β1–3)GlcNAc]–containing oligosaccharides (type I oligosaccharides) such as Gal(β1–3)GlcNAc(β1–3)Gal(β1–4)Glc (lacto-*N*-tetraose, LNT), Fuc(α1–2)Gal(β1–3)GlcNAc(β1–3)Gal(β1–4)Glc(lacto-*N*-fucopentaose I; **LNFPI**) or Neu5Ac(α2–3)Gal(β1–3)GlcNAc(β1–3)Gal(β1–4)Glc (sialyllacto-*N*-tetraose a; **LST a**). The type I oligosaccharides predominate over N-acetyllactosamine [Gal(β1–4)GlcNAc]–containing oligosaccharides (type II) in human milk and colostrum, whereas type I oligosaccharides including LNT have

never been found in milk or colostrum of cows (Tao et al., 2008; Urashima et al., 2009). This is disadvantageous for the utilization of the milk oligosaccharides fraction separated from cow or camel milk and colostrum for the preparation of infant formula, because it is thought that lacto-N-biose is a significant bifidobacterium growth factor (Kitaoka et al., 2005; Urashima et al., 2009). On the other hand, Nishimoto and Kitaoka (2007) have succeeded in the large-scale preparation of lacto-N-biose from sucrose.

From our results, we conclude that camel colostrum is a better source to separate sialyl oligosaccharides because it contains a higher concentration of various sialyl oligosaccharides, as shown in Figure 6a. If the biological significance of these sialyl oligosaccharides can be clarified, we expect that camel colostrum oligosaccharides will be utilized as biofunctional materials in the future.

It is noteworthy that camel colostrum contains sialyloligosaccharides whose core units are Gal(β1–3) Gal(β1–4)Glc (3′-GL) or Gal(β1–3)[Gal(β1–4) GlcNAc(β1–6)]Gal(β1–4)Glc (novoLNPI). These oligosaccharides have not previously been characterized in any eutherian milk or colostrum with the exception of the finding of Neu5Ac(α2–3)Gal(β1–3){Gal(β1–4) [Fuc(α1–3)]GlcNAc(β1–6)}Gal(β1–4)Glc in human milk (Gronberg et al., 1992), but it is possible that bovine milk/colostrum may also contain them (Tao, et al., 2008; Parkkinen and Finne, 1987). In this study, 3′-GL and novoLNPI were the only neutral oligosaccharides found in camel mature milk. These oligosaccharides have previously been found in the milk or colostrum of tammar wallaby (Messer et al., 1980; Bradbury et al., 1983), horse (Urashima et al., 1989), cow (Urashima et al., 1991), and brown capuchin (Urashima et al., 1999). On the other hand, novoLNPI has never been found in human milk (Urashima et al., 2009), suggesting that the oligosaccharides containing Gal-Gal units are minor in human milk.

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