New Gangliosides from Human Erythrocytes*

(Received for publication, February 28, 1983)

Samar K. Kundu‡§, Bo E. Samuelsson¶, Irmin Pascher¶, and Donald M. Marcus‡

From the 2Departments of Medicine, Physiology, Microbiology, and Immunology, Baylor College of Medicine, Houston, Texas 77030, 4Department of Medical Biochemistry, University of Goteborg, S-400 33 Goteborg, Sweden, and the Regional Blood Center, Sahlgren's University Hospital, S-413 45 Goteborg, Sweden

We have identified a number of gangliosides from human erythrocytes that have not previously been detected in these cells, including two new compounds. The gangliosides were separated into monosialo- and disialoganglioside fractions by DEAE-column chromatography. Two monosialogangliosides that have not been previously detected in these cells are GM2 and GM1. Two other monosialogangliosides have the same carbohydrate structure, NeuAc(α2-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc-Cer, but they contain different fatty acids. The compound with higher chromatographic mobility (MG-5) contains a predominance of C22 and C24 fatty acids, whereas the principal fatty acid of the slower compound (MG-6) is C16. Both gangliosides are receptors for human anti-p and anti-Gd cold agglutinins. Six disialogangliosides not identified previously in human red cells include GD3, GD4, GD5, GD6, DG-3, and DG-4.

DG-4,

\[
\text{NeuAc(α2-8)NeuAc(α2-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc-Cer}
\]

DG-6,

\[
\text{NeuAc(α2-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc-Cer}
\]

The latter two are newly identified compounds and DG-4 contains a sugar sequence that has not been described previously, sialic acid residues linked to different hydroxyl groups of the same galactose.

Although the major gangliosides in brain and extraneural tissues have been well characterized (2-4), structures of many less abundant gangliosides remain to be elucidated. Many of these minor components possess interesting biological and immunological properties. Of the nine gangliosides that Watanabe et al. recently isolated from human erythrocytes, gangliosides with lactonorhexaosyl and lactoisooctaosyl structures were shown to be i and I antigens, respectively (5-7). We reported previously (8) that an anti-p cold agglutinin was inhibited by sialosyllactoneotetraosylceramide, the most abundant ganglioside of human erythrocytes. We found subsequently (9) that two less abundant gangliosides from human erythrocytes, identified as MG-5 and MG-6 in this study, are more potent inhibitors of this antibody and of anti-Gd antibodies than sialosyllactoneotetraosylceramide. In this report we describe the isolation and characterization of six disialogangliosides and two monosialogangliosides that have not been identified previously in human erythrocytes. We have also identified a unique disialoganglioside structure that contains a galactosyl residue with sialic acid substituents on two separate hydroxyl groups.

* This research was supported by Research Grant AI 17712 from the National Institutes of Health, Grant Q-832 from the Robert A. Welch Foundation, and Grants 3967 and 6521 from the Swedish Medical Research Council. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed.

13857
RESULTS

The purified monosialo- and disialogangliosides isolated from human erythrocytes are shown in Figs. 1 and 2, respectively, and their approximate relative abundance is presented in Table I.

MG-1—This ganglioside, which was not detected in the original mixture (Fig. 1), co-migrated with brain GM_2 and was similar to the latter in carbohydrate composition (Table II) and in its resistance to Vibrio cholerae neuraminidase. The structure was confirmed by methylation analysis (Table III) and partial acid hydrolysis of native ganglioside. The desialylated MG-1 was identified as gangliotriacetylsphingosine on the basis of chromatographic mobility and complement fixation with purified anti-gangliotriacetylsphingosine. Thus, the oligosaccharide structure of MG-1 appears identical with that of brain GM_2.

MG-3—This ganglioside co-migrated with brain GM_1 and was similar to the latter in carbohydrate composition (Table II) and in its resistance to V. cholerae neuraminidase. Partially O-methylated heptitol and hexosaminolactones identified in the hydrolysate of permethylated ganglioside were identical with the products obtained from brain GM_1 (Table III). On treatment with Arthrobacter ureafaciens neuraminidase, MG-3 yielded a desialylated glycolipid which was identified as gangliotetraacetylsphingosine on the basis of chromatographic mobility and complement fixation with purified anti-gangliotetraacetylsphingosine.

MG-5 and MG-6—These two gangliosides occur in approximately equal concentration and together comprise approximately 5% of the monosialoganglioside fraction (Table I). Both gangliosides contained galactose, glucose, glucosamine, and N-acetylenuraminic acid in approximate molar ratios of 2.8:1:0.1:0.8 (Table II). On treatment with V. cholerae neuraminidase, MG-5 and MG-6 yielded the asialoglycolipids. The TLC migration rates relative to lacto- and neolactotetraosylceramide (R_L,NeOT) were 0.58 and 0.49, respectively. Treatment of the asialoglycolipids with jack bean P-N-acetylhexosaminidase yielded products with R_L,NeOT 0.80 and 0.70, respectively, and hydrolysis of these products by jack bean P-N-acetylenuraminidase yielded compounds with R_R 1.0 and 0.85, respectively. Both compounds were identified as lacto- and neolactotetraosylceramide by complement fixation with purified anti-lacto- and neolactotetraosylceramide.

Both gangliosides yielded methylation products (Table III) identical with those obtained from 2,3-sialosylactoneotetraosylceramide which was kindly provided by Dr. S. Hakomori. Ganglioside MG-5 co-migrated with the standard 2,3-sialosylsphingosine (not shown), whereas MG-6 migrated more slowly (Fig. 1).

These two gangliosides were shown by direct inlet mass spectrometry to have identical carbohydrate structures and to differ in their ceramide moieties. Both fractions were analyzed as permethylated, permethylated-reduced, and permethylated-reduced-trimethylsilylated derivatives. The mass spectra of the latter derivative of MG-6 is shown in Fig. 3. Molecular ions are obtained for the major molecular species of MG-6 containing normal C16:0 fatty acids and C18:1 sphingosine base at m/z 2233. Ions containing the fatty acid (C16) plus the complete carbohydrate chain are seen at m/z 1981. A number of terminal saccharide fragments as well as rearrangement fragments containing the fatty acid plus part of the carbohydrate chain are present in the spectra of all three derivatives, confirming the structure given in Fig. 3.

Although no molecular ions were recorded for the corresponding derivatives of MG-5, the mass spectra (not shown) nevertheless give conclusive evidence for a ganglioside with a carbohydrate part identical with that of MG-6 but with a different ceramide composition. The major fatty acids of MG-5 are C22:0, C24:1, and C24:0 normal fatty acids (Table IV).

DG-1—This ganglioside displayed two bands that co-migrated with the two bands of human spleen GD_3 and that were similar to the latter in carbohydrate composition. Removal of both sialic acid residues by V. cholerae neuraminidase or mild acid hydrolysis yielded lactosylceramide. Periodate borohydride treatment followed by mild methanolysis (24) yielded equimolar amounts of intact NeuAc and NeuAc-7 indicating a NeuAc(2-8)NeuAc sequence.

The mass spectra (not shown) of permethylated, permethylated-reduced, and permethylated-reduced-trimethylsilylated DG-1 were similar to those published previously for GD_3 of bovine retina (37). The complete sugar sequence of DG-1 was thus established to be identical with that of GD_3. The mass spectra further show that DG-1 predominantly contains normal C16:0 fatty acid but also minor amounts of C16:0 and C18:0 hydroxy fatty acids (Table IV).

DG-2—This ganglioside co-migrated with brain GD_3 and was similar to the latter in carbohydrate composition (Table III). On treatment with V. cholerae neuraminidase, DG-2 yielded a ganglioside that co-migrated on TLC with brain GM_1 and that were similar to the latter in carbohydrate composition. Removal of both sialic acid residues by V. cholerae neuraminidase or mild acid hydrolysis yielded lactosylceramide. Periodate borohydride treatment of DG-2 converted both sialic acid residues to the lower homologue NeuAc-7 which

| Fatty acids | Gangliosides |
|------------|-------------|
| 14:0       | 15          | 10         |
| 15:0       | 10          | 5          |
| 16:0       | 10          | 50         | 60         | 20         | 10         | 5          |
| 17:0       | 15          | 5          | 10         |
| 18:0       | 5           | 5          | 45         |
| 18:1       | 5           | 5          | 45         |
| 19:0       | 10          | 10         | 15         |
| 20:0       | 10          | 10         | 5          |
| 20:1       |             |            |            |
| 22:0       | 15          | 5          | 5          | 20         | 20         |
| 22:1       | 5           | 5          | 5          |            |            |
| 23:0       | 10          | 10         | 5          | 10         |
| 23:1       | 5           | 5          | 5          |            |            |
| 24:0       | 30          | 45         | 35         |
| 24:1       | 20          | 10         | 20         | 15         |
| 25:0       | 5           | 5          | 5          |
| 26:0       | 5           |
| h16:0      | 10          |
| h18:0      | 10          |

<sup>a</sup> h, hydroxy.
indicated that the sialic acids were on two different sugar residues. On methylation analysis (Table III), DG-2 gave products identical with the products obtained from brain GD-2.

The sugar sequence was confirmed by mass spectra of permethylated, permethylated-reduced, and permethylated-reduced-trimethylsilylated DG-2 which were found to be similar to those published (38) for GD-2 from brain. The approximate fatty acid composition is shown in Table IV.

DG-3—This ganglioside co-migrated with DG-4 on TLC with chloroform:methanol:water solvent systems and preparative TLC with a chloroform:methanol:2.5 N NH₄OH, 60:40:9 solvent was necessary to purify it. Carbohydrate analysis revealed that DG-3 contained galactose, glucose, glucosamine, and sialic acid in an approximate molar ratio of 2:1:2:1 (Table II). On treatment with V. cholerae neuraminidase, the ganglioside lost both sialic acids and the asialoglycolipid co-migrated with lactoneotetraosylceramide. Periodate borohydride treatment followed by mild methanalysis yielded equimolar amounts of intact NeuAc and NeuAc-7 which indicated that the two sialic acid residues are linked by a 2-8 bond. The products of methylation analysis of DG-3 were identical with those (Table III) obtained from 2,3-sialosyllactoneotetraosylceramide.

The sugar sequence was confirmed by direct probe mass spectrometry of permethylated (not shown), permethylated-reduced (not shown), and permethylated-reduced-trimethylsilylated derivatives (Fig. 4). Molecular ions were recorded at m/z 2301 for the major molecular species containing C18:1 sphingosine base and C24:0 fatty acid. The peak at m/z 2049 contains the fatty acid (C24:0) plus the complete carbohydrate chain and thus gives the exact molar ratio NeuAc:hexose:hexosamine, 2:3:1; m/z 1716, 1644, 1325, 1253, 1049, and 818 are rearrangement sequence ions (for explanation see formula for interpretation). The conditions analysis were: electron energy, 40 eV; trap current, 500 μA; acceleration voltage, 4 kV; ion source temperature, 260 °C at evaporation. Peaks below m/z 80 were not reproduced.

DG-4—This ganglioside was purified by preparative TLC in an ammonia solvent system (Fig. 2) from a column fraction that also contained DG-3. Carbohydrate analysis (Table II) indicated that DG-4 contained galactose, glucose, galactosamine, and sialic acids in an approximate molar ratio of 3:1:1:2. On treatment with V. cholerae neuraminidase, DG-4 lost both sialic acids to give a neutral glycolipid which co-migrated on TLC with gangiotetraosylceramide in chloroform:methanol:water solvent system (60:30:5 or 60:35:8, v/v). The asiaglycolipid obtained from DG-4 by neuraminidase treatment fixed complement with purified anti-gangiotetraosylceramide antibody as well as gangiotetraosylceramide (0.5 μg/ml of each glycolipid was needed for fixation of complement). This suggests that the terminal disaccharide of desialylated DG-4 has the same terminal Gal(β1-3)GalNAc moiety as ganglio-N-tetraosylceramide, but the terminal galactose residue of desialylated DG-4 could not be hydrolyzed by jack bean β-galactosidase (6). However, desialylated DG-4 could be completely hydrolyzed by the use of Charonia lampas β-galactosidase (60 milliunits of enzyme/50 μg of glycolipid) to a product that co-migrated on TLC with human erythrocyte globotetraosylceramide. The hydrolyzed product was identified as globotetraosylceramide by TLC autoradiographic procedure with purified anti-globotetraosylceramide (41).

Methylation analysis (Table III) revealed the presence of a 3,6-di-substituted galactose in the native compound, and the disappearance of this compound and the appearance of an unsubstituted terminal galactose (2,3,4,6-tetra-O-methylgalactitol) in the neuraminidase-treated glycolipid (Table III). The presence of sialic acid residues linked to the 3- and 6-hydroxyl groups of the terminal galactose is also supported by absence of native NeuAc following periodate borohydride treatment.

The DG-4 fraction was analyzed as permethylated, meth-
FIG. 6. Mass spectrum of permethylated-reduced ganglioside DG-4 from human erythrocytes and a simplified formula for interpretation. The conditions of analysis were: electron energy, 50 eV; trap current, 500 μHz; acceleration voltage, 4 kV; ion source temperature, 235 °C at evaporation. Peaks below m/z 80 were not reproduced.

ylated-reduced, and methylated-reduced-trimethylsilylated derivatives. The spectra of all three derivatives are shown (Figs. 5-7) and are discussed below because DG-4 represents a new type of ganglioside.

The intense peak at m/z 2253 in Fig. 7 is indicative of an ion composed of a C24:0 fatty acid combined with the complete carbohydrate chain. The molar ratio of the constituent sugars is thus established to be NeuAc:hexose:hexosamine, 2:4:1; m/z 2225 is the corresponding peak for C22:0 fatty acids. Molecular ions are also found for molecular species containing this fatty acid and a C18:1 sphingosine base.

The peak at m/z 1920 is a secondary ion originating from m/z 2253 by the loss of one NeuAc and the rearrangement of one TMS group. The ions at m/z 1587 and 1515 are similarly produced by the loss of two NeuAc and rearrangement of two TMS groups or one TMS group and one hydrogen atom, respectively; m/z 818 is a rearrangement ion containing the C24 fatty acid plus the proximal two hexoses. The rearrangement of one hydrogen (+1) indicates a non-branched chain; m/z 1022 is the analogous ion containing three hexoses in a straight chain. The small peak at m/z 1253 is indicative for a further extension of the straight carbohydrate chain with one hexosamine. Terminal NeuAc is seen at m/z 406 and m/z 374. The mass spectral data discussed so far are consistent with a structure (presented above the spectra) with two NeuAc residues attached to the same penultimate hexose. The position of the branching point is further strengthened by information from the spectrum of the methylated-reduced derivatives in Fig. 6. The very intense peaks at m/z 1443 and 1415 are ions containing the fatty acid (C24, C22) plus the carbohydrate chain from which two terminal NeuAc are lost and replaced by two hydrogen atoms. The somewhat less intense peaks at m/z 1253 and 1225 are similar fragments, but with an additional loss of one hexose and the uptake of only one hydrogen atom. This strongly indicates that the branching point is located on this last hexose. A number of additional diagnostic fragments indicated in the spectra and fragmentation formulas of all three derivatives (Figs. 5-7) provide further support for the presented structure.

On the basis of the above evidence, the structure of DG-4 must be as follows:

\[ \text{NeuAc}(\alpha_2 \text{Gal}(\beta_1-3)\text{GalNAc}(\beta_1-3)\text{Gal}(\alpha_1-4)\text{Gal}(\beta_1-4)\text{Glc-Cer}} \]

DG-5—This minor ganglioside co-migrated with brain GDb on TLC (data not shown). Carbohydrate composition (Table II) and the appearance of a monosialoganglioside that co-migrated with brain GMa after V. cholerae neuraminidase treatment suggested its identity with GDb. This view was
further substantiated by periodate borohydride-methanolysis treatment and methylation analysis (Table III).

The sugar sequence was further confirmed by direct probe mass spectrometry of permethylated (not shown) and permethylated-reduced derivatives (Fig. 8). Molecular ions \((M - 1)\) at \(m/z 2074\) are seen in Fig. 8 for the major molecular species containing C18:0 normal fatty acid and C18:1 long chain base. The fragment at \(m/z 1821\) contains the complete carbohydrate chain (two NeuAc, three hexoses, and one hexosamine) combined with C18:0 normal fatty acid; \(m/z 1488, 1169, 951, 720,\) and 530 are secondary ions confirming the sequence given. The combined data from spectra of both derivatives are fully consistent with the structure proposed for DG-5 as \(\text{G}_{\text{DG}-5}\). The fragment pattern around the peaks at \(m/z 1488\) and 1169 in Fig. 8 shows the approximate fatty acid composition (Table IV).

**DG-6**—This ganglioside contained galactose, glucose, glucosamine, and sialic acid in an approximate molar ratio of 4:1:3:2. On treatment with *V. cholerae* neuraminidase the ganglioside lost both sialic acids, and a neutral glycolipid which co-migrated on TLC with lactoisoctanoylceramide was produced. Periodate borohydride reduction-mild methanolysis treatment of DG-6 yielded only NeuAc-7, which indicated that the two sialic acids are not linked to each other. On methylation analysis (Table III), DG-6 revealed the presence of 3-substituted galactose, 3,6-disubstituted galactose, and 4-substituted glucose in an approximate molar ratio of 3:1:1. The 4-substituted hexosamine was confirmed by the presence of 3,6-di-O-methyl-2-deoxy-(N-methylacetamido)-glucitol. The asialoglycolipid obtained from DG-6 reacted with jack bean \(\beta\)-galactosidase to give a product with \(R_\text{f} 0.47\). This product, on further reaction with jack bean \(\beta\)-N-acetylhexosaminidase, yielded lactoneotetraosylceramide \((R_\text{f} 1.00)\) (Fig. 9). The identification of this glycolipid was based on the
complement fixation results with purified anti-lacto-noe-traosylceramide antibody (33).

The DG-6 fraction was further analyzed by direct inlet mass spectrometry as permethylated (not shown) and permethylated-reduced derivatives (Fig. 10). No useful spectrum was obtained after silylation. The peak at m/z 2109 in Fig. 10 is most probably due to a rearrangement ion containing the major fatty acid (C24:0) combined with a carbohydrate chain composed of five hexoses and three hexosamines. The addition of two hydrogens indicates a loss of additional carbohydrate units in two positions. This means that the original structure is branched. The series of peaks at m/z 1905, 1701, 1674, and 1470 indicates that the two branches contain together at least two hexoses and one hexosamine. The peaks at m/z 1049 and 818 indicate that the proximal carbohydrate chain is unbranched and contains at least two hexoses and one hexosamine with the sequence given in the formula. The small primary carbohydrate fragments at m/z 1088, 900, 884, 873, and 857 are also consistent with a branched terminal carbohydrate containing at least two hexoses and one hexosamine. The peaks at m/z 436 and 452 indicate in fact that the two branches contain together two hexoses and two hexosamines.

The spectrum of a permethylated derivative (not shown) showed peaks at m/z 376 and 344 (376–32) for terminal NeuAc but no indication of a NeuAc-NeuAc peak. Ions originating from the terminal trisaccharide(s) were seen at m/z 825, and 765 (825–60). A number of additional secondary carbohydrate fragments were also present. They originated from the terminal branched structure and are all consistent with the structure given.

Although they are strongly suggestive, the mass spectra alone give no absolute proof for the branching point or the total number of constituent sugars. They do, however, provide evidence of a branched 8-sugar basic structure with two free hydroxyls. The terminal trisaccharide fragments in the spectrum of the permethylated derivatives (NeuAc-hexose-hexosamine) appears to overlap with the basic sequence, which makes the proposed structure highly probable.

The major sphingosine base was C18:1, as indicated by an intense peak at m/z 364 in the spectrum of permethylated derivative. The major fatty acids were C22:0, C24:1, and C24:0 (Table IV) as indicated in Fig. 10 by peaks at m/z 2081, 2107, and 2109.

On the basis of all the evidence described above, the structure of DG-6 can be proposed as shown in Scheme A.
DISCUSSION

Approximately 80–85% of the gangliosides of human erythrocytes contain a single sialic acid and 15–20% are disialogangliosides. The monosialogangliosides were studied by Watanabe et al. (5–7), who identified nine compounds. We have identified two additional monosialogangliosides that were not detected previously in erythrocytes. MG-1 and MG-3, which have the same oligosaccharide structures as brain gangliosides GM2 and GM1, respectively. MG-5 and MG-6 have the same oligosaccharide structure as ganglioside Gd identified by Watanabe et al. (6). MG-5, like Gd, contains mostly long chain fatty acids, C22-C24 (Table IV), whereas MG-6 contains mostly C16 fatty acids. Kannagi et al. (42) recently presented data on the relationship between the ceramide and carbohydrate structures of a number of glycolipids. They reported that human erythrocyte monosialogangliosides that contain a terminal NeuAcα2-3Gal structure contain mostly short chain fatty acids, whereas compounds with a terminal NeuAcα2-3Gal structure had a predominance of long chain fatty acids. The predominance of short chain fatty acids in MG-6, the structure of which has been confirmed by Hakomori,9 suggests that this relationship may not be as complete as suggested by this group, but it does not detract from the conceptual importance of their proposal. We previously made a similar suggestion about the role of fatty acids in determining the carbohydrate structure of glycolipids (11) based on our analysis of the erythrocytes of the rare p phenotype. These cells cannot synthesize globotriaosylceramide and they contain an excess of lactosylceramide that has long chain fatty acids, and a normal amount of lactosylceramide with short chain fatty acids (11).

The disialogangliosides of human erythrocytes have not been studied previously. Four of the six compounds that we characterized have been isolated from other tissues. DG-2 and DG-5 are identical with brain gangliosides GD2 and GD1b, respectively. DG-1 is GD1a, and DG-3 is disialoylacto-neotetraosylceramide. It is interesting that both brain gangliosides have a predominance of C16 and C18 fatty acids, and that all of the gangliosides of the lacto and globo series, except MG-6, contain mostly C22 and C24 fatty acids. DG-1 (GD1a) is the only ganglioside that contains an appreciable quantity of hydroxy fatty acids. Ganglioside DG-6, which is a new compound, has a branched lactosioctaosylceramide structure with a terminal sialic acid residue on each branch. Monosialogangliosides with the same basic lactoisoctosylceramide structure and with a terminal sialosyl lacto octacosylceramide or Ga1c1-3 substituent on one branch, have been isolated from erythrocytes by Watanabe et al. (5).

Ganglioside DG-4 has a novel structural feature, the presence of sialic acid residues attached to different hydroxyl groups of the same galactose. To the best of our knowledge, this is the first example of this structure in glycoconjugates. This compound is only the second ganglioside described that belongs to the globo series of glycolipids. A ganglioside isolated from chicken muscle (43) has the structure NeuAcα2-3Galα1–4GlcNAcβ1–3Galα1–4Glc-Cer. This compound contains a predominance of C16-C18 fatty acids, in contrast to DG-4, which contains mostly C22-C24 fatty acids. A preliminary report of a disialoganglioside of the globo series has been published (44) but definitive data have not been published.

Including the data in this report, 11 monosialogangliosides and 6 disialogangliosides have been identified in human erythrocytes, and additional compounds have been detected but not purified at present. Although definitive information about the functions of these compounds is not available, there is an increasing body of data about their potential functions as cell membrane receptors and antigens (2, 45). A number of monoclonal antibodies against human tumors are directed against gangliosides, and these compounds isolated from erythrocytes provide a valuable library of defined oligosaccharide structures for analysis of the specificity of antibodies and lectins.

Acknowledgments—We sincerely thank Drs. Y. T. and S. C. Li for their generous gifts of β-galactosidase and β-N-acetylgalactosaminidase. The gift of sialosylacto nortetraosylceramide from Dr. S. Hakomori and the assistance of Charlene Shackelford in preparation of this manuscript are gratefully acknowledged.

REFERENCES

1. Svennerholm, L. (1963) J. Neurochem. 10, 613–623
2. Hakomori, S. I. (1981) Annu. Rev. Biochem. 50, 733–764
3. Ledeen, R. W., and Yu, R. K. (1982, Methods Enzymol. 83, 139–191
4. Wiegandt, H. (1982) Adv. Neurochem. 4, 149–223
5. Watanabe, K., Powell, M., and Hakomori, S. (1978) J. Biol. Chem. 253, 8962–8967
6. Watanabe, K., Powell, M. E., and Hakomori, S. (1979) J. Biol. Chem. 254, 8223–8229
7. Watanabe, K., and Hakomori, S. (1979) Biochemistry 18, 5502–5504
8. Schwarting, G. A., Marcus, D. M., and Metaxas, M. (1977) Vox Sang. 32, 251–261
9. Kundu, S. K., Marcus, D. M., and Roelecke, D. (1982) Immunol. Lett. 4, 263–267
10. Ledeen, R. W., Yu, R. K., and Eng, L. F. (1973) J. Neurochem. 21, 829–839
11. Marcus, D. M., Naiti, W., and Kundu, S. K. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3263–3267
12. Kundu, S. K. (1981) Methods Enzymol. 72, 174–185
13. Ando, S., Isobe, M., and Nagai, Y. (1978) Biochem. Biophys. Acta 424, 36–105
14. Kundu, S. K. (1981) Methods Enzymol. 72, 185–204
15. Yang, H., and Hakomori, S. (1971) J. Biol. Chem. 246, 192–200
16. Suzuki, A., Karol, R. A., Kundu, K. S., and Marcus, D. M. (1981) Int. J. Cancer 28, 271–276
17. Hakomori, S. (1964) J. Biochem. (Tokyo) 55, 205–208
18. Kundu, S. K., Ledeen, R. W., and Gorin, P. A. J. (1975) Carbohydr. Res. 39, 179–191
19. Bjorndal, H., Hellerquist, C., and Lindberg, B., and Svensson, S. (1970) Angew. Chem. Int. Ed. Engl. 9, 610–619
20. Price, H., Kundu, S., and Ledeen, R. (1975) Biochemistry 14, 1512–1518
21. Kundu, S. K., Ledeen, R. W., and Gorin, P. A. J. (1975) Carbohydr. Res. 39, 329–334
22. Ando, S., and Yu, R. K. (1977) J. Biol. Chem. 252, 6247–6250
23. Kundu, S. K., and Suzuki, A. (1981) J. Chromatogr. 224, 249–256

3 S. I. Hakomori, personal communication.
Human Erythrocyte Gangliosides

24. Yu, R. K., and Ledeen, R. W. (1970) J. Lipid Res. 11, 506-516
25. Li, Y. T., King, M. J., and Li, S. C. (1980) in Structure and Function of Gangliosides (Svennerholm, L., Mandel, P., Drew-fus, H., and Urban P. F., eds) pp. 93-104, Plenum Press, New York
26. Saito, M., Sugano, K., and Nagai, Y. (1979) J. Biol. Chem. 254, 7845-7854
27. Li, S. C., Mazzotta, M. Y., Chien, S. F., and Li, Y. T. (1975) J. Biol. Chem. 250, 6786-6791
28. Li, S. C., and Li, Y. T. (1970) J. Biol. Chem. 245, 5153-5160
29. Li, Y. T., and Li, S. C. (1977) in The Glycoconjugates (Horowitz, M. I., and Pigman, W., eds) pp. 51-67, Academic Press, New York
30. Marcus, D. M., and Schwarting, G. A. (1976) Adv. Immunol. 23, 203-240
31. Marcus, D. M. (1976) in Glycolipid Methodology (Witting, L. A., ed) pp. 233-245, American Oil Chemists‘ Society Press, Champaign, IL
32. Kundu, S. K., and Roy, S. K. (1979) J. Lipid Res. 20, 825-833
33. Schwarting, G. A., and Marcus, D. M. (1979) Clin. Immunol. Immunopathol. 14, 121-129
34. Karlsson, K. A. (1976) in Glycolipid Methodology (Witting, L. A., ed) pp. 97-122, American Oil Chemists‘ Society Press, Champaign, IL
35. Karlsson, K. A. (1978) Prog. Chem. Fats Other Lipids 16, 207-230
36. Fredman, P., Månsson, J. E., Svennerholm, L., Karlsson, K. A., Pascher, I., and Samuelsson, B. E. (1980) FEBS Lett. 110, 80-84
37. Holm, M., Pascher, I., and Samuelsson, B. E. (1977) Biomed. Mass Spectrom. 4, 77-81
38. Karlsson, K. A. (1974) Biochemistry 13, 3643-3647
39. Rauvala, H., Krusius, T., and Finne, J. (1978) Biochim. Biophys. Acta 531, 266-274
40. Kannagi, R., Levery, S. B., Ishingama, F., Hakomori, S. I., Shevinsky, L. H., Knowles, B. B., and Solter, D. (1983) J. Biol. Chem. 258, 8834-8840
41. Kundu, S. K., Misra, L. K., and Luthra, M. G. (1982) FEBS Lett. 150, 359-364
42. Kannagi, R., Nudelman, E., and Hakomori, S. I. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3470-3474
43. Chien, J. L., and Hogan, E. L. (1980) in Cell Surface Glycolipids (Sweeley, C. C., ed) Vol. 128, pp. 135-148, American Chemical Society Symposium Series, Washington, D. C.
44. Homn, E. L., Happel, R. D., and Chien, J. L. (1982) Adv. Exp. Med. Biol. 152, 273-278
45. Yamakawa, T., and Nagai, Y. (1978) Trends Biochem. Sci. 3, 128-131
Isolation of Gangliosides

All samples of washed blood were obtained in acid-citrate-dextrose anti-coagulant. The washed packed erythrocytes were suspended in an equal volume of 0.02 M phosphate buffer saline, pH 7.4, and extracted once with chloroform-methanol (2:1 v/v) previously treated with chloroform-methanol (1:1 v/v). The extracted material was filtered through Whatman filter paper number 1, and chloroform-methanol (2:1 v/v) fraction was liquid-liquid extracted on a column of landings 468-469 (Japan Laboratory, Tokyo, Japan) using a linear gradient of chloroform-methanol 3-3 M in water (v/v) (12) or 20-2.5 M in water (v/v) (13). Some of the fractions obtained by liquid-liquid column chromatography were further purified by preparative IEC on silica gel 60 plates (E. Merck and Co., Darmstadt, FRG) using chloroform-methanol (2:1 v/v). Some of the silica gel 60 plates (E. Merck and Co., Darmstadt, FRG) using chloroform-methanol (2:1 v/v) was then subjected to silicic acid column chromatography and eluted with diethyl ether (3) and then recovered and eluted with methanol (4) and ethanol. The purified lipids were then subjected to preparative IEC on silica gel 60 plates (E. Merck and Co., Darmstadt, FRG) using chloroform-methanol (2:1 v/v) was then subjected to silicic acid column chromatography and eluted with diethyl ether (3) and then recovered and eluted with methanol (4) and ethanol.

Analytical Procedures

Carbohydrate Analysis

Carbohydrate composition was determined by two methods: (1) methylation, and (2) caption. Methylation was performed by deacylation with methanolic (-1
cm)-249-340-440-540

Fig. 1. Thin-layer chromatogram of purified monosialogangliosides of human erythrocytes. STD contains monosialoganglioside fraction, LDP contains disialoganglioside fraction, and D1 contains disialoganglioside fraction. Lanes G1 to G6: G1, G2, G3, G4, G5, and G6 contain purified disialoganglioside. Km: cyclohexane-methanol-0.5 M MgCl₂ (2:1:5 v/v). All bands were purified by detection by a recombinant spray.
Human Erythrocyte Gangliosides

Fig. 8. Mass spectrum of permethylated-reduced ganglioside GS-1 from human erythrocytes and a simplified formula for interpretation. The conditions of analysis were: electron energy 55 eV, trap current 500 nA, accelerating voltage 8.2 kV, and the source temperature 290°C at evaporation. Peaks below m/z 90 were not reproduced.

Fig. 9. Thin-layer chromatogram of GS-1 and the compounds produced by enzymatic degradation. Lane 1 contains standard gangliosides. Lane 2, GS-1 treated with neuraminidase (product 1); Lane 3, product 1 treated with β-galactosidase (product 2); Lane 4, product 2 treated with α-fucosidase (product 3). All bands were purple after detection by an α-naphthol spray. The faint band in Lane 4 is indicated by a closed rectangle.

| Table I |
|--------------------------|
| Relative abundance of gangliosides in human erythrocytes |
| | Percentage of total gangliosides (μmol/L) |
| Monosialoganglioside fraction |  |
| MG-1 | 2.0 |
| MG-3 | 3.0 |
| MG-5 | 2.5 |
| MG-6 | 2.0 |
| Distalsialoganglioside fraction |  |
| DG-1 | 7.1 |
| DG-2 | 2.0 |
| DG-3 | 5.0 |
| DG-4 | 2.5 |
| DG-5 | 1.5 |
| DG-6 | 1.8 |

* Determined by GC analysis of lipid-bound steric acid.
* The percentage of individual ganglioside components were determined after purification.
* The major monosialogangliosides are OD-2 (70%) and stialylofucosylceramide (20%), MO-2.

| Table II |
|--------------------------|
| Carbohydrate composition of purified erythrocyte gangliosides (μmol/L) |
| | Ganglioside |
| Component sugars | Monosialo | Distal |
| MG-1 | 90-1 | DG-2 | DG-4 | DG-5 | DG-6 |
| Galactose | 0.96 | 1.43 | 2.48 | 1.95 | 1.48 | 1.95 |
| Glucose | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Gluconamide | 0 | 0 | 1.77 | 1.90 | 0 | 1.06 |
| Galactosamine | 1.05 | 1.28 | 0 | 0 | 0.78 | 0.72 |
| Stialic Acid | 0.90 | 0.85 | 0.95 | 0.73 | 2.05 | 1.62 |

* Expressed as molar ratios relative to glucose as 1.00, as determined by GC as alditol acetates after acetolysis. The stialic acid was analyzed as TMA derivatives after methanolysis.

| Table III |
|--------------------------|
| Partially-permethylated hexosyl and hexosaminyl esterates in the hydrolysates of permethylated intact gangliosides (μmol/L) |
| | DM-1 | DM-2 | DM-3 | DM-4 | DM-5 | DM-6 |
| MG-1 | 0 | 0 | 0 | 0 | 0.98 | 0 | 0 | 1.05 |
| MG-3 | 0.9 | 0 | 0 | 1.00 | 0 | 0.51 | 0 | 4.0 |
| MG-5 | 0 | 0 | 2.9 | 1.00 | 0 | 0 | 0.24 | 0 |
| MG-6 | 0 | 2.6 | 1.00 | 0 | 0 | 0.25 | 0 | 0 |
| SG-1 | 0 | 0 | 1.96 | 1.00 | 0 | 0 | 0 | 0.58 |
| SG-2 | 0 | 0 | 2.82 | 1.00 | 0 | 0 | 0.95 | 0 |
| SG-3 | 0 | 0 | 1.91 | 1.00 | 0 | 0 | 1.25 | 0 |
| SG-4 | 1.00 | 0.85 | 1.00 | 0.6 | 0 | 0 | 0.25 | 0 |
| SG-5 | 0.85 | 0 | 1.00 | 1.00 | 0 | 0 | 0.22 | 0 |
| SG-6 | 0 | 2.6 | 1.00 | 0.78 | 0 | 0.82 | 0 | 0 |
| Aslalo | 1.00 | 1.10 | 1.00 | 0 | 0 | 3.25 | 0 |

* Expressed as molar ratios relative to glucose as 1.00.
New gangliosides from human erythrocytes.
S K Kundu, B E Samuelsson, I Pascher and D M Marcus

J. Biol. Chem. 1983, 258:13857-13866.

Access the most updated version of this article at http://www.jbc.org/content/258/22/13857

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/22/13857.full.html#ref-list-1