BLOOD GROUP H ACTIVE GLYCOLIPID FROM RAT ASCITES HEPATOMA AH 7974F

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Received May 24,1976

SUMMARY : A new type of fucose-containing glycolipid exhibiting blood group H activity was isolated from rat ascites hepatoma cell AH 7974F. As a result of studying its structure by partial acid hydrolysis, enzymatic degradation and immuno-precipitation reaction, the structure was tentatively proposed as Fuc(1 → 2)Gal(1 → 3)GalNAc(1 → 4)Gal(1 → 4)Glc(1 → 1)Cer.

INTRODUCTION

Glycolipids exhibiting blood group H activity, which have been found in dog (1) and porcine intestine (2), stomach mucosa (3), human erythrocyte (4, 5) and bovine liver (6), characteristically possess L-fucose as the terminal sugar moiety. Recently, we found the presence of a novel L-fucose-containing lipid exhibiting blood group H activity in rat ascites hepatoma cell line AH 7974F (7). The present paper describes the separation and structure of the fucose-containing ceramide pentasaccharide.

MATERIALS AND METHODS

Isolation and purification of fucolipid. About 50 ml of packed ascites hepatoma AH 7974F cells from donor rats of the Moriyama strain, which were contaminated with red cells, were suspended in 0.3 % NaCl solution. The red cells were hemolyzed and removed by centrifugation of the suspension. The cells thus obtained as pellet were then lyophilized. Lipid extraction was carried out by the method reported previously (8), and the glycolipid obtained by the acetylation method described by Saito and Hakomori (9) were further fractionated by silicic acid column chromatography. Since the fucose-containing glycolipid eluted from the column by a mixture of $CHCl_3$-CH_3OH (2 : 3, v/v) was still contaminated with a small amount of other glycolipids, further purification was attempted on preparative thin layer plates of Silica Gel H (Merck), which had previously been refluxed with 3 % HCl in CH_3OH for 5 - 10 hours and washed with distilled water to remove the sugar contaminants. The glycolipid areas on the thin layer plates were scraped and extracted with $CHCl_3$-CH_3OH-H_2O (10 : 10 : 1, v/v/v).

Analytical methods. Carbohydrate and fatty acid composition was determined by gas-liquid chromatography according to the method of Vance and Sweeley (10). Quantitative analysis of hexosamine was performed by the method of Gatt and Berman (11), protein by the method of Lowry et al. (12).

Degradation of fucoside linkage with mild acid and fucosidase. The

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Abbreviations : Cer, ceramide; Fuc, L-fucose; Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine; Glc, D-glucose; asialo-GM_1, Gal(1 → 3)GalNAc(1 → 4)Gal(1 → 4)Glc(1 → 1)Cer; asialo-GM_2, GalNAc(1 → 4)Gal(1 → 4)Glc(1 → 1)Cer.

fucosyl residue of the fucolipid was liberated specifically by hydrolysis with 1 N trichloroacetic acid at 100°C for 2 hours (5). Degradation of the fucolipid with fucosidase from Cl. perfringens, which is specific for α-L-fucosyl(1 → 2) linkage, was carried out by the method of Aminoff and Furukawa (13).

Immunological analysis. Blood group H activity was estimated by the inhibition of hemagglutination caused by Ulex europeus lectin. Lectin dilutions were incubated with a series of decreasing concentration of the glycolipid for 10 min at room temperature. Then, equal volumes of 2 % suspension of red cells were added to the incubation mixtures. After 30 min at 37°C, the degree of agglutination was estimated.

Immuno-diffusion test was carried out on 0.6 % agarose gel using anti-asialo-GM_1 serum which was a kind gift from Dr. Naiki, M. (Medical Science Institute, Tokyo University).

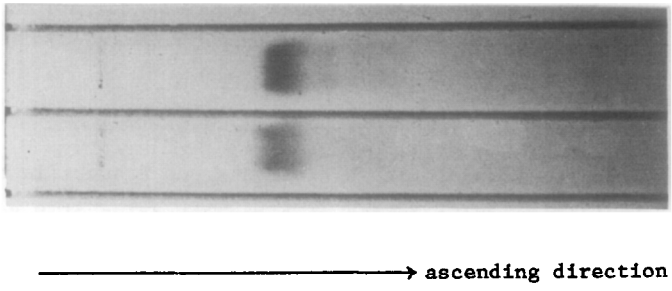

Fig. 1. Thin layer chromatogram of fucolipid from AH 7974F cells. Chromatography was carried out on a plate of Silica Gel H in ascending way using $CHCl_3$-CH_3OH-H_2O (60 : 35 : 8, v/v/v) as developing solvent.
1. Crude fucolipid eluted from the silicic acid column with $CHCl_3$-CH_3OH (2 : 3, v/v).
2. Purified fucolipid obtained by preparative thin layer chromatography on Silica Gel H plates.

RESULTS

Fucolipid eluted from the silicic acid column by a mixture of $CHCl_3$-CH_3OH (2 : 3, v/v) was further purified by preparative thin layer chromatography. The purified glycolipid gave a single spot on the thin layer plate (Fig. 1).

Molar ratios of fucose, galactose, N-acetylgalactosamine and glucose present in the glycolipid were estimated to be 1.01 : 1.92 : 0.88 : 1 by gas-liquid chromatographic analysis of the trimethylsilyl derivatives of those carbohydrates (Fig. 2) and chemical analysis of hexosamine (11).

Fatty acids of the glycolipid were composed of long chain acids such as $C_{20:0}$ (13 %), $C_{22:0}$ (12.7 %), $C_{24:0}$ (28.6 %) and $C_{24:1}$ (24.2 %). Hydroxy fatty acid could not be detected.

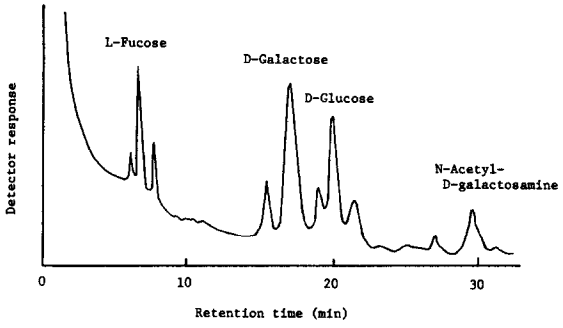

Fig. 2. Gas-liquid chromatogram of trimethylsilyl derivatives of carbohydrates prepared from the purified fucolipid. Analysis was performed by the method of Vance and Sweeley (10).
Operating parameters of the chromatography : 200 cm x 0.3 cm glass column packed with 5 % SE-30 on 80 - 100 mesh Shimalite; column temperature, 140°C to 200°C (at the rate of 2°C/min); and carrier gas (N_2 gas), 35 ml/min.

This glycolipid was found to inhibit blood group H specific hemagglutination caused by Ulex europeus lectin at a concentration of 6 μg/0.4 ml of incubation mixture. This finding suggested that non-reducing terminal sugar of this glycolipid was fucose. Therefore, attempts were made to liberate fucosyl residue from the glycolipid by either fucosidase (A) or mild acid hydrolysis (B). (A) : About 500 μg of the fucolipid was incubated with 1.0 mg of fucosidase preparation from Cl. perfringens (13). Incubation for 30 min resulted in hydrolysis of approximately 70 % of the fucolipid and produced a glycolipid in the organic phase with the mobility of asialo-GM_1 on a thin layer chromatogram. This finding shows that the terminal fucose linkage of the glycolipid is α-L-fucosyl(1 → 2), for which the enzyme is specific (13). (B) : Heating the fucolipid with 1 N trichloroacetic acid at 100°C for 2 hours liberated fucose, forming the defucosylated lipid with the mobility of asialo-GM_1 on a thin layer plate. The asialo-GM_1-like glycolipid thus obtained was also examined for immunological double diffusion test on agarose gel. Precipitation line formed with the defucosylated glycolipid and anti-asialo-GM_1 was found to fuse with that of asialo-GM_1 and the antiserum (Fig. 3). These result suggest that the structure of the defucosylated glycolipid is Gal(1 → 3)GalNAc(1 → 4)Gal(1 → 4)Glc(1 → 1)Cer.

DISCUSSION

The sugar moiety of the fucose-containing glycolipid purified by silicic acid column and successive thin layer chromatography was composed of

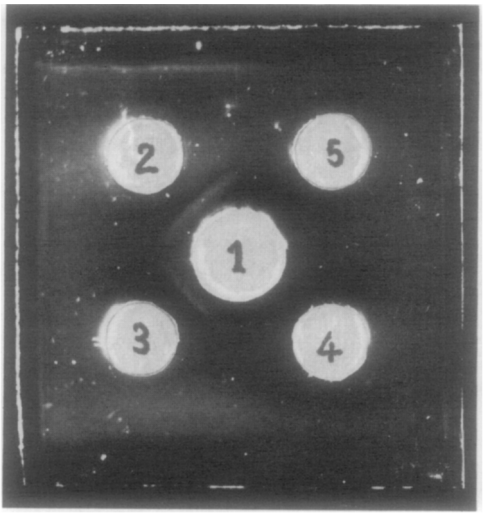

Fig. 3. Immuno-precipitation reaction on agarose gel. 1. Anti-asialo-GM_1 serum; 2. Asialo-GM_1; 3. Glycolipid obtained by hydrolysis of the fucolipid with 1 N trichloroacetic acid; 4. Purified fucolipid; 5. Ganglioside (GM_1).

L-fucose, D-galactose, N-acetyl-D-galactosamine and D-glucose in molar ratios of 1 : 2 : 1 : 1. It was shown that the fucolipid possessed blood group H activity by inhibition of the hemagglutination caused by Ulex europeus lectin. As the result of studying the sugar composition, partial acid hydrolysis products, immunological reaction and hydrolysis with specific fucosidase, the structure of the glycolipid is tentatively proposed to be Fuc(1 → 2)Gal(1 → 3)GalNAc(1 → 4)Gal(1 → 4)Glc(1 → 1)Cer.

In 1973, Wiegandt demonstrated the presence of blood group H active fucoganglioside in bovine liver (6). The fucolipid described in the present paper seems to be an asialo type of the fucoganglioside. Recently, we detected the presence of asialo-GM_1 as well as asialo-GM_2 in AH 7974F cells (7), which might metabolically be in connection with the fucolipid.

ACKNOWLEDGEMENT : The authors are much obliged to Dr. Kojima, K. (Cancer Institute, Nara Medical College) for his kind gift of AH 7974F cells.

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