Release of carbohydrates from sphingoglycolipid by osmium-catalyzed periodate oxidation followed by treatment with mild alkali

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SUMMARY The carbohydrate moiety of sphingoglycolipid, after preliminary acetylation, can be released by periodate oxidation catalyzed by a trace amount of osmium tetroxide, followed by alkaline treatment.

Cerebroside, lactosyl ceramide, hematoside, globoside, and gangliosides were degraded to yield, respectively, galactose, lactose, sialyl lactose, a tetrasaccharide, and various oligosaccharides containing sialic acid. Oligosaccharides were separated by paper chromatography and paper electrophoresis. The procedure is useful for characterizing micromolar amounts of sphingoglycolipids.

KEY WORDS osmium-catalyzed · periodate · oxidation · acetylated · sphingoglycolipid · cleavage · sphingosine–carbohydrate bond · separation · oligosaccharides

THE LIBERATION of oligosaccharides, for the determination of the glycolipid's homogeneity and of the structure of the carbohydrate moiety, has been previously reported (1, 2). The method described by Wiegandt and Baschang (2) was based on ozonolysis of the double bond of sphingosine followed by hydrolysis in aqueous sodium carbonate; the degradation was performed on amounts varying from twenty to several hundred milligrams of gangliosides.

The present communication describes another method for liberating oligosaccharides from micromolar amounts of glycolipids. The method is based on a selective oxidation of the double bond of sphingosine by osmium-catalyzed periodate oxidation (3), followed by an unknown sequence of reactions catalyzed by sodium methoxide, which results in a cleavage of glycoside–lipid bond.

Materials. The lactosyl ceramide and globoside were prepared from human erythrocytes according to the methods of Yamakawa, Irie, and Iwanaga (4), and Makita and Yamakawa (5). The gangliosides of human brain were prepared from the upper phase of the extract prepared by the Folch procedure (6), and the cerebroside was isolated from the lower phase of the same extract and purified by chromatography on Florisil (7). Some of the lactosyl ceramide and all the hematoside were gifts of Professor T. Yamakawa.

Degradation Procedure. About 1–2 μmoles (1–3 mg) of glycolipid was dried in vacuo and dissolved in 0.2 ml of pyridine–acetic anhydride 3:2. After several hours, 5 ml of toluene was added and the solvents were evaporated off under a vigorous flow of nitrogen. The residue was dissolved in 0.2–0.4 ml of dioxane. To this solution were added 0.05–0.1 ml of 0.2 M sodium metaperiodate (10–20 μmoles) in methanol–water 7:3 and 10 μl of a 1% solution of osmium tetroxide in ether (0.1–0.2 μmole). The reaction mixture was kept at 4°C for 10 hr. During the reaction, sodium iodate precipitated; the excess of periodate was precipitated by addition of 1,2-ethanediol. Several milliliters of chloroform were added and the suspension was mixed and centrifuged. The supernatant solution was transferred to a conical centrifuge tube, shaken with water, and centrifuged; washing with water was repeated ten times.

The chloroform layer, which contained the oxidized glycolipid acetate, was evaporated under a flow of nitrogen and dried in vacuo. Osmic acid sublimed off during the evaporation. The dried residue was dissolved in 0.2 ml of methanol, and 0.05 ml of 0.5% sodium methoxide was added. After 30–60 min, the pH of the reaction mixture was about 9.5, and it was neutralized with 0.05 ml of 0.5% aqueous acetic acid. The precipitate that appeared during the reaction was centrifuged off.

Separation and Quantitative Determination of Carbohydrates Liberated by Degradation. Aliquots of the solution were applied to Whatman 3MM filter paper that had been previously washed with water. The chromatographic analysis was performed in the following solvent systems: ethyl acetate–pyridine–water 12:5:4, ethyl acetate–pyridine–acetic acid–water 5:5:1:3, and butanol–acetic acid–water 2:1:1. Electrophoresis took place in 0.025 M sodium tetraborate under 10 v/cm or in butanol–pyridine–acetic acid–water 20:10:2:968 under 25 v/cm. A two-dimensional separation fractionated well the sialyl oligosaccharides from gangliosides; it was performed by electrophoresis in butanol–pyridine–acetic acid–water, followed by chromatography in butanol–acetic acid–water (8). The oligosaccharides were detected by treatment with silver nitrate in acetone and sodium hydroxide in ethanol (9), the latter reagent being applied at least five times. Benzidine–trichloroacetic acid reagent (10) was also useful. In addition, the oligosaccharides containing sialic acid and acetamidodeoxysugars were detected by treatment with chlorine in carbon tetrachloride and iodine–starch (11). Quantitative determination of galactose was made directly on paper by the phthalic acid–aniline test (12). Determination of lactose and sialyl lactose was made by the cysteine–sulfuric acid (13) and by the thiobarbituric acid (14) tests, after elution of the spot areas.

This is paper No. 48 of the series dealing with amino sugars and publication No. 416 of the Robert W. Lovett Memorial Laboratories for the Study of Diseases Causing Deformities at the Massachusetts General Hospital, and Harvard Medical School.

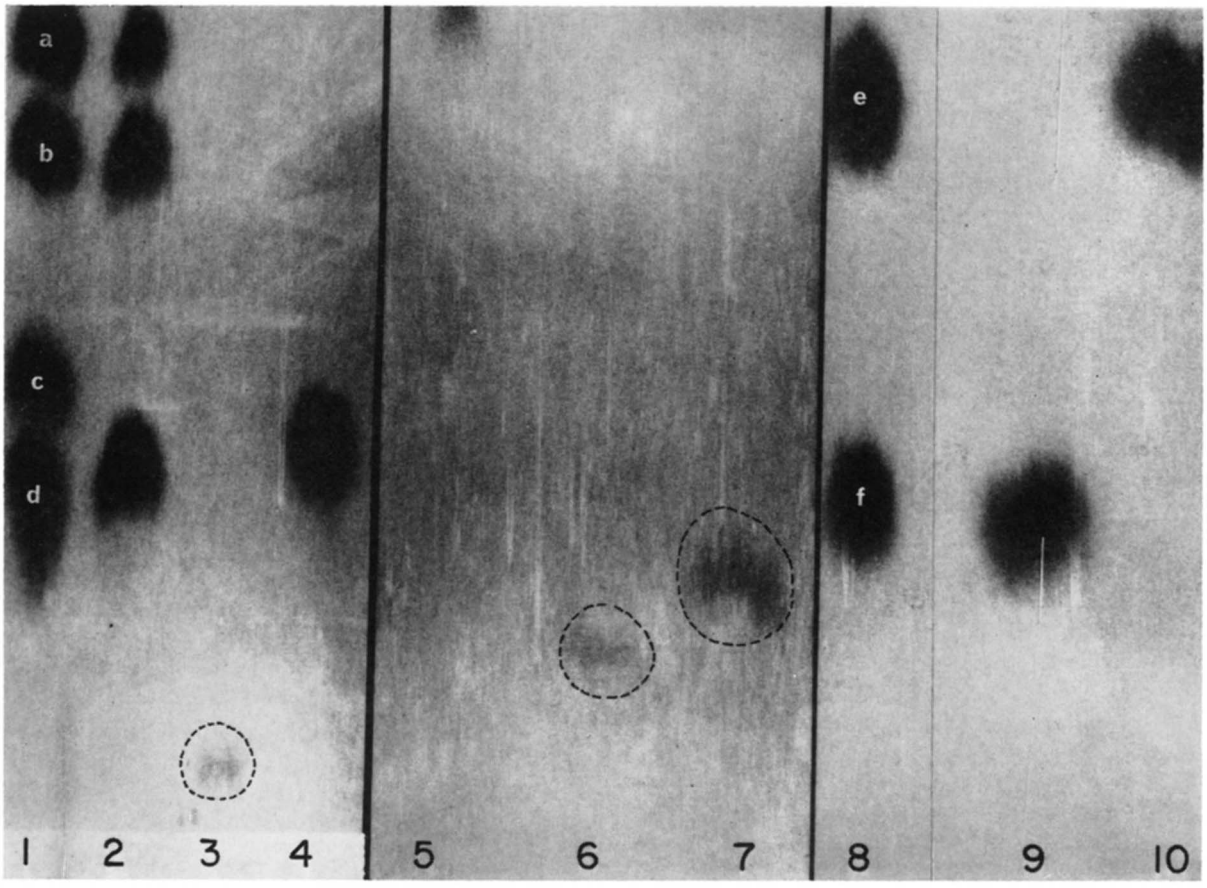

FIG. 1. Paper chromatography of the carbohydrates liberated from globoside, hematoside, lactosyl ceramide, and cerebroside.

Run *1*, reference sugars: *a*, *b*, *c*, and *d* are glucose, galactose, 2-amino-2-deoxyglucose, and 2-amino-2-deoxygalactose, respectively; *2*, an acid hydrolysate of 100 μg of a tetrasaccharide liberated from globoside; *3*, a tetrasaccharide liberated from about 100 μg of globoside; *4* and *5*, lactose; *6*, about 200 μg of *N*-glycolyl neuraminyl lactose liberated from hematoside; *7*, *N*-acetyl neuraminyl lactose; *8*, spots *e* and *f*: galactose and lactose, respectively; *9*, lactose liberated from 100 μg of lactosyl ceramide; *10*, galactose liberated from 100 μg of cerebroside. These chromatograms were developed with ethyl acetate–pyridine–water 12:5:4 for 20 hr in *1–4* and *8–10*, for 48 hr in *5–7*.

Millimolar amounts of oligosaccharides were separated as follows. The degradation product was passed successively through columns of Amberlite IR-120 (H^+-form) and Amberlite IR-45 (acetate form). The effluent and washings (fraction A, neutral fraction) were evaporated in vacuo. The acidic oligosaccharides containing sialic acid were eluted from the Amberlite IR-45 column by 0.1 N sodium acetate. The eluate was treated with Amberlite IR-120 for the removal of sodium ions, neutralized with pyridine, and concentrated in vacuo (fraction B). Fractions A and B were eventually purified further by chromatography on washed Whatman 3MM paper in the ethyl acetate–pyridine–water system. The sugar bands, indicated by guide strips, were eluted with water and lyophilized.

Results. The liberation of galactose, lactose, and sialyl lactose from, respectively, cerebroside, lactosyl ceramide, and hematoside, was estimated quantitatively.

A homogeneous oligosaccharide was liberated from a globoside prepared from human erythrocytes, and consisted of glucose, galactose, and 2-acetamido-2-deoxygalactose in the molar ratio of 1:2:1. Recovery of this tetrasaccharide was determined on the basis of the weight of the purified material. These results are shown in Table 1 and Fig. 1. In all cases, the carbohydrate moieties were liberated without evident structural changes, and the recovery was 20–80%.

At least six oligosaccharides, liberated from human brain gangliosides, could be separated on paper by two-dimensional chromatography (Fig. 2). The recovery of sialyl oligosaccharides, calculated by the amount of sialic acid in fraction B was about 30% of the theoretical amount (the intact ganglioside is not present in this fraction).

Discussion. Sphingoglycolipids are difficult to resolve into homogeneous groups by conventional chromato-

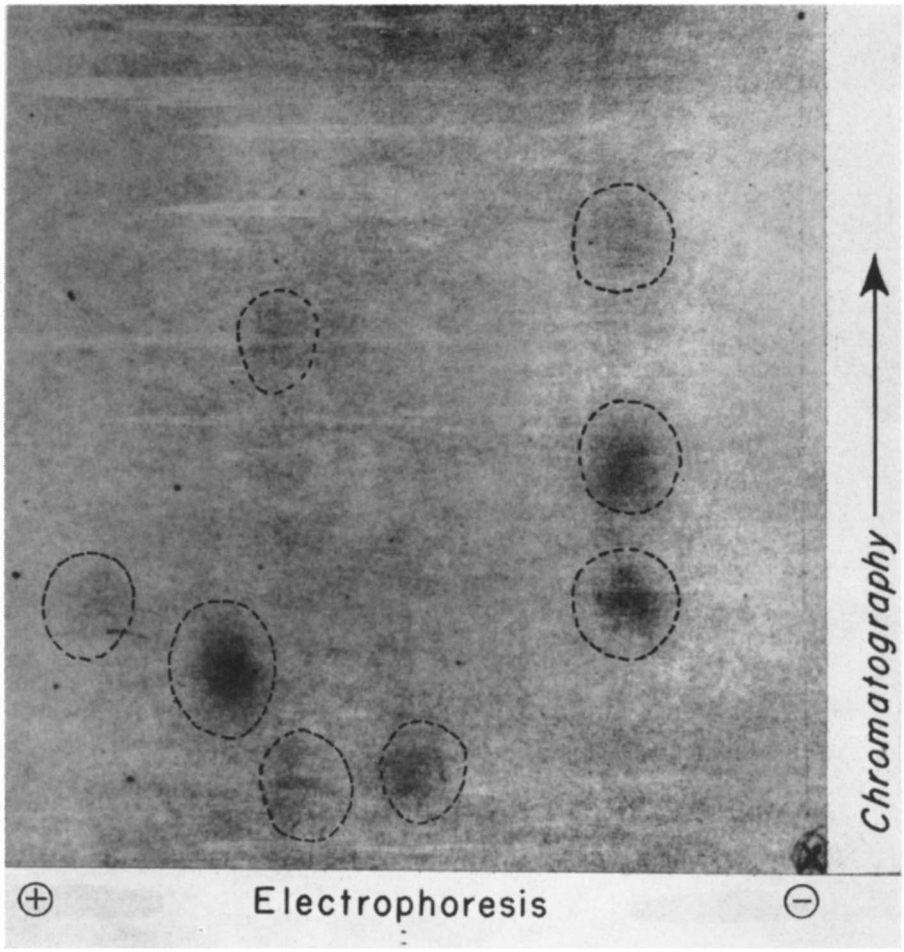

FIG. 2. Oligosaccharides liberated from 2.0 mg of human brain gangliosides and separated by electrophoresis followed by chromatography. First separation by electrophoresis in butanol–pyridine–acetic acid–water 20:10:2:968 under 25 v/cm for 2 hr; second separation by chromatography in butanol–acetic acid–water 2:1:1.

TABLE 1 YIELD OF CARBOHYDRATES LIBERATED FROM GLYCOLIPIDS BY OSMIUM-CATALYZED PERIODATE OXIDATION AND MILD ALKALINE TREATMENT

Glycolipid, Amount Used	Carbohydrate Liberated*	Recovery of Carbohydrates
μg	μg	% of theoretical
Cerebroside	*Galactose*	
790	110 (178)	61
3,000	420 (680)	62
Lactosyl ceramide	*Lactose*	
960	290 (340)	82
1,900	410 (670)	61
Hematoside	*Sialyl lactose*†	
1,200	210 (620)	33
1,200	260 (620)	41
Globoside	*Tetrasaccharide*‡	
40,000	4,100 (21,000)	20

* Theoretical value in parentheses.
† *N*-glycolyl neuraminyl lactose.
‡ Purified tetrasaccharide, containing 2 moles of galactose, 1 mole of 2-acetamido-2-deoxygalactose, and 1 mole of glucose.

graphic or electrophoretic procedures because of the various chain lengths of fatty acids and fatty bases (15, 16) that are linked to a variety of oligosaccharides. The lack of resolution may also be due to the strong tendency to form micelles in aqueous (17), as well as in organic, solution (18). The release of intact oligosaccharides from glycolipids, therefore, provides a useful tool for the study of homogeneity and the structure of complex glycolipid molecules.

The method described in the present report is suitable for degradation of a few milligrams of glycolipids. Thus the structure of the carbohydrate moiety of a glycolipid can be elucidated by comparison of the migration rate of the liberated oligosaccharide, on paper chromatography or paper electrophoresis, with the migration rate of known oligosaccharides. Recently, this procedure has been used for the structural elucidation of a glycolipid isolated from human cancerous tissue (19, and unpublished results).

Osmium-catalyzed periodate oxidation selectively split the olefinic double bond to yield an aldehyde group at the C_4 position of sphingosine (3). The mechanism of subsequent cleavage of the glycoside–lipid bond is unknown. It could be assumed, however, that shifting of the carbonyl bond from the C_4 to C_3 position of sphingosine is induced by mild alkali, which is analogous to the Lobry de Bruyn-van Ekenstein transformation (20). Hydrolysis of the resulting 3-ketoglycoside may readily be induced by mild alkali via a reaction analogous to the β-elimination of 3-keto-*n*-butyl glycoside (21).

Neither the present procedure nor that of Wiegandt and Baschang is applicable to the degradation of glycolipids that contain sphingosine bases with a saturated hydrocarbon chain (C_{18}- and C_{20}-dihydrosphingosine, and phytosphingosine).

The low recovery of oligosaccharides after degradation of gangliosides, as compared to lactosyl ceramide and cerebrosides, is probably due to the negatively charged, bulky carbohydrate groupings in gangliosides which may hamper the access of methoxide anion.

Another limitation of this degradation is a further breakdown of oligosaccharide chains that contain an alkali-sensitive linkage at the reducing end. Fortunately, most glycolipids isolated from mammalian tissue have an oligosaccharide moiety composed of a lactosyl unit linked to the lipid moiety, thus providing an oligosaccharide having a lactosyl reducing end. Much more drastic alkaline conditions than were used in this method are required for degradation of oligosaccharides having a disaccharide unit without an amino sugar at the reducing end (22).

The author thanks Professor R. W. Jeanloz for valuable advice, and Professor T. Yamakawa for supplying some of the specimens used in this investigation.

This investigation was supported by a grant from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, No. CA 08418-01.

Manuscript received 19 May 1966; accepted 27 July 1966.

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