Aminopropyl silica gel as a solid support for preparation of glycolipid immunoadsorbent and purification of antibodies

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Abstract Aminopropyl silica gel was prepared from porous silica gel and was used as a solid support for immunoadsorbent in the purification of anti-glycolipid antibodies. For neutral glycosphingolipids, a carboxyl function was generated by oxidation of the olefinic double bond of the sphingosine moiety, whereas for gangliosides the carboxyl group of sialic acid was used to couple with aminopropyl silica gel in the presence of a carbodiimide. These compounds were used for purifying anti-glycolipid antibodies from serum of immunized rabbits. The antibodies bound to the substrate were released by 2 M potassium thiocyanate and their immunological properties were studied. Aminopropyl silica gel may be preferred over the conventional organic solid supports for the following reasons: *1*) faster flow rate; *2*) higher capacity; *3*) easier handling; *4*) more economical; and *5*) lower susceptibility to microbial attack.—**Kundu, S. K., and S. K. Roy.** Aminopropyl silica gel as a solid support for preparation of glycolipid immunoadsorbent and purification of antibodies. *J. Lipid Res.* 1979. **20:** 825–833.

Supplementary key words affinity chromatography · aminoalkyl-agarose · polyacrylamide

The recent recognition of the antigenic (1, 2) and receptor properties of glycolipids (3–10) and of the striking changes in the glycolipid content of malignant cells (11) has stimulated much interest in the functional properties of these molecules and their possible role in cellular interactions. Although there are considerable amounts of data on the structure and content of glycolipids of mammalian organs (12–15), little knowledge of their distribution in individual types of cells is known. Antibodies directed against a single glycolipid with definite carbohydrate structure could provide a means of defining the localization of specific glycolipid on cell membranes and in individual cells and organelles in tissue sections (16–19). The purified antibodies would also be useful to study various phases of surface function related to specified carbohydrate structure and also in detection and quantitation of glycolipids in patients with abnormal glycolipid composition for clinical diagnosis.

For the purification of anti-glycolipid antibodies, two types of glycolipid antigen immunoadsorbents have been used. One involves incorporation of glycolipids into a polyacrylamide gel (16) and the other involves the covalent attachment of glycolipid to aminoalkyl-agarose (20). The major problems associated with organic supports are due to low capacity and slow flow rate. Recently, Hakomori and co-workers (21, 22) described a method in which the olefinic double bond of the sphingosine moiety of glycolipid was cleaved by oxidation and the resulting "glycolipid-acid" was then coupled to the amino groups of the solid supports.

We have previously prepared an ion exchanger, DEAE-silica gel, and have demonstrated that it is an improvement over the conventional DEAE-Sephadex and DEAE-controlled porous glass matrices for the quantitative isolation of gangliosides and neutral glycosphingolipids from animal cells and tissues (23–25). The use of chemically modified porous silica gel, prepared from inexpensive and readily available commercial materials, has not yet been fully explored. In the present report, we describe the preparation of aminopropyl silica gel and its application in the preparation of glycolipid immunoadsorbents which were utilized in purifying anti-glycolipid antibodies. A comparative study between aminopropyl silica gel and other conventional organic supports was made; the results indicated that the former was preferable.

MATERIALS AND METHODS

Porous silica gel (pore diameter, 500 Å; 120–230 mesh; surface area, 35–65 m^2/g; pore volume, 0.78 m^3/g) was obtained from E. Merck Laboratories, Inc.,

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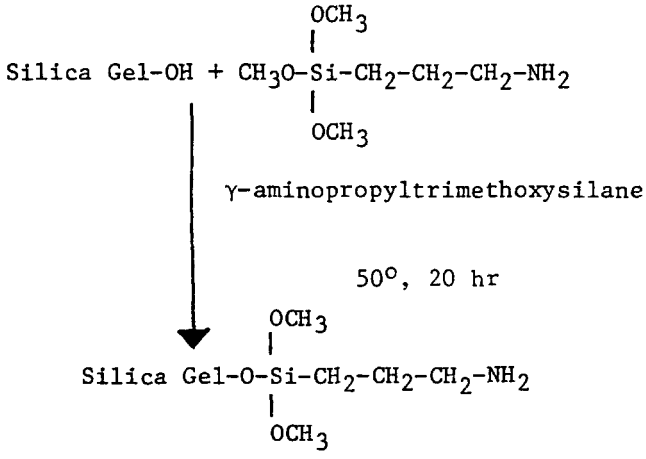
Germany. ν-Aminopropyltrimethoxysilane was purchased from Polyscience, Inc., Warrington, PA; dicyclohexyl-18-crown-6, *N*-hydroxysuccinimide, and egg lecithin were from Aldrich Chemical Company, Milwaukee, WI; dicyclohexylcarbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, methylated bovine serum albumin, human serum albumin, and Sephadex LH-20-100 were from Sigma Chemical Company, St. Louis, MO; aminoalkyl-agarose (Affigel 102) and Dowex 50 WX8 (200–400 mesh, hydrogen form) were from Bio-Rad Laboratories, Richmond, CA. Beef brain ganglioside mixture was prepared by the procedure of Ledeen, Yu, and Eng (26) and the individual gangliosides, viz. G_{M2}, G_{M1}, G_{D1a}, G_{D1b}, and G_{T1b} were then purified by successive chromatographies on DEAE-Sephadex (27) and iatrobead silica gel (28). Hematoside (G_{M3}) was isolated from beef adrenal medulla (29); disialohematoside (G_{D3}) was from human spleen (30), and sialosylparagloboside was from human erythrocytes (31). Ceramide monohexoside, ceramide dihexoside, ceramide trihexoside, and globoside were purified from the neutral glycosphingolipid mixture of human erythrocytes (23) by chromatography on iatrobead silica gel (28). Forssman glycolipid was purified from sheep erythrocytes (32). Paragloboside was prepared from sialosylparagloboside after treatment with *Vibrio cholerae* neuraminidase (Behring Diagnostics, Sommerville, NJ) in dilute acetic acid (pH ~4) for 4 hr followed by chromatography on DEAE-Sephadex (29). ^{3}H-Labeled G_{M1} ganglioside and Forssman glycolipid were prepared by oxidation with galactose oxidase (Worthington Biochemicals, Freehold, NJ) followed by reduction with ^{3}H-labeled sodium borohydride (New England Nuclear Corporation, Boston, MA) according to the procedure of Suzuki and Suzuki (33). The lipid-bound sialic acid was determined by gas–liquid chromatography (34) and total hexose by phenol–sulfuric acid reaction (35).



Scheme 1. Preparation of aminopropyl silica gel.

Preparation of aminopropyl silica gel (Scheme 1)

Silica gel (10 g) was deaerated under vacuum for 30 min and then shaken at 50°C for 20 hr with ν-aminopropyltrimethoxysilane (10% in toluene, 60 ml). After cooling to room temperature, the reaction mixture was filtered and successively washed with 200 ml each of toluene, methanol, and water. The gel was then converted to the chloride form with dilute hydrochloric acid (pH 4) and stored at room temperature after drying under vacuum. Total capacity of aminopropyl silica gel was found to be 50 μmol/g dry weight as determined by titration of the basic form of the gel with 0.1 N perchloric acid in a nonaqueous solvent (36).

Preparation of "glycolipid-acid"

Neutral glycosphingolipid (30 mg) was acetylated with acetic anhydride–pyridine (37) and then oxidized with potassium permanganate which was solubilized in benzene by dicyclohexyl-18-crown-6 as described by Young, Laine, and Hakomori (22). The acetylated glycolipid-acid was then separated from the liberated free fatty acid by column chromatography on a Sephadex LH-20-100 column (2 × 60 cm, prepared in chloroform–methanol 1:1) using chloroform–methanol 1:1 as an eluant. Better separation of glycolipid-acids was achieved using chloroform–methanol 1:1 as an eluant rather than benzene–acetone 1:1 as was employed earlier (22). The purified acetylated glycolipid-acid was deacetylated by treatment with 0.2 M potassium hydroxide in methanol at room temperature for 3 hr and then dialyzed against water (15, 38). The yields were in the range of 70–80%.

Coupling of glycolipid-acid or intact gangliosides with aminopropyl silica gel

Glycolipid-acid (10 mg) or native ganglioside (10 mg) was dissolved in dioxane–water 1:1 (10 ml). An activated glycolipid-ester was then formed with *N*-hydroxysuccinimide (15 mg) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (200 mg) according to Young et al. (22). Aminopropyl silica gel (2 g) suspended in dioxane–water 1:1 (5 ml) was then added and the reaction mixture was gently mixed at room temperature for 48 hr. To block any residual amino group, the reaction mixture was treated with 4 drops of glacial acetic acid and the mixing was continued at room temperature for an additional 15 hr. The gel was filtered and successively washed

with 50 ml each of dioxane–water 1:1, chloroform–methanol 1:1, and methanol. The combined filtrate was evaporated, dialyzed against water, and quantitatively assayed. Utilizing the above procedure, the glycolipid-acids from ceramide trihexoside, globoside, paragloboside, Forssman glycolipid, G_{M1}, and native gangliosides, viz. G_{M3}, G_{D3}, G_{M1}, G_{D1a}, G_{D1b}, and G_{T1b}, were coupled to aminopropyl silica gel. The results are shown in **Table 1.** The gel was then successively washed with 100 ml each of water and 0.02 M phosphate-buffered saline, pH 7.3, and stored in the refrigerator. The gel did not give blue color with 1% ninhydrin, indicating the absence of free amino group.

Using the above procedure, G_{M1} ganglioside (15 mg) was coupled with aminoalkyl-agarose (Affigel, 15 ml).

Preparation of glycolipid–polyacrylamide gel immunoadsorbent

Glycolipid–polyacrylamide gel immunoadsorbent columns were prepared as described previously (16). These included ceramide trihexoside, globoside, paragloboside, Forssman glycolipid, and G_{M1} ganglioside.

Preparation of antisera

Rabbit antisera against G_{M1}, ceramide trihexoside, globoside, paragloboside, and Forssman glycolipid were produced by immunization with the respective glycolipid mixed with an equal amount of methylated bovine serum albumin as described previously (39). Antisera to gangliosides G_{D3} and G_{M1} were prepared by immunizing rabbits with the respective ganglioside–human serum albumin conjugate (30).

Purification of antibodies

Rabbit antibodies to glycolipids were purified on appropriate glycolipid–immunoadsorbent columns as follows. Antiserum (30–50 ml) was diluted with an equal volume of 0.1 M Tris-HCl buffer, pH 7.3, containing 0.1 M sodium chloride–0.01 M EDTA-Na_4 (starting buffer) and the mixture was passed through the column at 4°C at a flow rate of 0.5–0.7 ml per min. The column was washed with the starting buffer until the effluent was free of protein ($OD_{280} < 0.002$). The antibodies were then eluted with 2 M potassium thiocyanate in phosphate-buffered saline, pH 7.3 (100 ml). After concentration by ultrafiltration in a Diaflo cell (Amicon Corporation, Lexington, MA) to about 10 ml, the antibodies were dialyzed exhaustively against 0.02 M phosphate-buffered saline, pH 7.3. The column was washed with 4 M guanidine hydrochloride to remove impurities, followed by washing with 0.02 M phosphate-buffered saline, pH 7.3. The column is stored in this buffer containing 0.02% sodium azide and can be used repeatedly without obvious loss of activity. The purified antibodies were separated into IgG and IgM fractions by chromatography on a 5 × 100-cm column of Sephadex G-200 as described previously (40). The immunoglobulin content of these fractions was analyzed by immunoelectrophoresis with antisera to rabbit serum, rabbit IgG and IgM (17) (Behring Diagnostics, Sommerville, NJ).

TABLE 1. Amount of glycolipid coupled to 2 g of aminopropyl silica gel[a]

Glycolipid (10 mg)	Glycolipid Coupled
	%
G_{M3}	75
G_{D3}	80
G_{M1}	79
G_{D1a}	74
G_{D1b}	72
G_{T1b}	76
Globosidic acid	46
Forssman glycolipid acid[b]	40
Ceramide trihexoside acid	41
Paraglobosidic acid	48

[a] The percentage of coupling was determined by measurement of uncoupled glycolipid acid or ganglioside. For glycolipid-acid, the values were determined from the hexose analysis by phenol–sulfuric acid reaction (35); for gangliosides, the measurement was carried out from the lipid-bound sialic acid by gas–liquid chromatography (34).

[b] For Forssman glycolipid and G_{M1} ganglioside, the reaction mixtures were mixed with the respective ^3H-labeled glycolipids and the amounts coupled were also ascertained by measuring the radioactivity.

Specificity test of the glycolipid–aminopropyl silica gel immunoadsorbent columns

The specificity test of the glycolipid–aminopropyl silica gel immunoadsorbent columns was carried out by passing non-cross-reactive antiserum (30 ml) through an immunoadsorbent column (2 g) and elution with 2 M potassium thiocyanate in phosphate-buffered saline, pH 7.3, as described above. The amount of protein obtained in the thiocyanate eluate after passing antiserum to Forssman glycolipid through G_{M1}– or G_{D3}–aminopropyl silica gel column was found to be less than 0.5 mg (total $OD_{280} \sim 0.5$); similarly, when passing antiserum to ganglioside G_{M1} through a Forssman–, globoside–, or G_{D3}–aminopropyl silica gel column, the amount of protein eluted in each case was less than 0.5 mg (total $OD_{280} \sim 0.4$). In another set of experiments, preimmune serum (10 ml) was passed through each glycolipid–aminopropyl silica gel column (2 g) and

TABLE 2. Structures of neutral glycosphingolipids[a]

Trivial Name	Abbreviation According to IUPAC-IUB Commission[b]	Structure
Ceramide dihexoside	$LcOSe_2Cer$	Gal(β,1→4)Glc-Cer
Ceramide trihexoside	$GbOSe_3Cer$	Gal(α,1→4)Gal(β,1→4)Glc-Cer
Asialo G_{M2}	$GgOSe_3Cer$	GalNAc(β,1→4)Gal(β,1→4)Glc-Cer
Asialo G_{M1}	$GgOSe_4Cer$	Gal(β,1→3)GalNAc(β,1→4)Gal(β,1→4)Glc-Cer
Globoside	$GbOSe_4Cer$	GalNAc(β,1→3)Gal(α,1→4)Gal(β,1→4)Glc-Cer
Paragloboside	$nLcOSe_4Cer$	Gal(β,1→4)GlcNAc(β,1→3)Gal(β,1→4)Glc-Cer
Forssman glycolipid		GalNAc(α,1→3)GalNAc(β,1→3)Gal(α,1→4)Gal(β,1→4)Glc-Cer

[a] Abbreviations: Lc, lacto-; Gb, globo-; Gg, ganglio-; Cer, Ceramide; Gal, D-galactose; Glc, D-glucose; GalNAc, *N*-acetyl-D-galactosamine; GlcNAc, *N*-acetyl-D-glucosamine.

[b] IUPAC-IUB Commission on Biochemical Nomenclature: The nomenclature of lipids. 1978. *J. Lipid Res.* **19:** 114–128.

eluted with 2 M thiocyanate in phosphate-buffered saline, pH 7.3. In all cases, the amount of protein eluted was less than 0.5 mg (total $OD_{280} \sim 0.4$). Finally, to confirm the specific adsorption of antibodies on the specific immunoadsorbent column, 5 mg each of purified antibodies (total $OD_{280} = 7.0$) to G_{M1}, G_{D3}, Forssman glycolipid, and globoside was passed through the respective glycolipid–aminopropyl silica gel columns (2 g). In all cases the amount of antibodies recovered from the thiocyanate eluate was approximately 4.8 mg (total $OD_{280} \sim 6.7$).

Immunologic techniques

Double diffusion in 0.5–0.75% agarose gel and immunoelectrophoresis were performed as described previously (41). The antigen solutions used in gel diffusion studies contained glycolipid and egg lecithin in a weight ratio of 1:1. All antibodies were titrated by a semiquantitative complement fixation assay, performed with a microtiter apparatus (42). The antibodies to gangliosides G_{M1} and G_{D3} were also titrated by the microcomplement fixation technique of Wasserman and Levine (43). The antigens for complement fixation assays were prepared by mixing glycolipid, egg lecithin, and cholesterol in a weight ratio of 1:2:10 as described previously (39).

RESULTS AND DISCUSSION

In the purification of anti-glycolipid antibodies by affinity adsorption methods, organic and inorganic supports have both been used (16, 21–22). In spite of a number of advantages, the major problems associated with inorganic supports are due to nonspecific adsorption and denaturation of many sensitive materials (44). These problems can be minimized by a variety of coating techniques (45, 46). Recently, we have demonstrated that porous silica gel can be coated with DEAE groups (23). Our studies have also shown that the DEAE-silica gel system is an improvement over the conventional DEAE-Sephadex and DEAE-controlled porous glass matrices for the quantitative isolation of acidic and neural lipids (23–25).

The present report describes the preparation of aminopropyl silica gel from readily available commercial materials and its application as an alternative and more economical support material for the preparation of glycolipid immunoadsorbents. These were prepared by covalent attachment of glycolipids to silica gel matrix. The reaction involves coupling of the amino group of aminopropyl silica gel with the carboxyl function of glycolipids. For neutral glycolipids (ceramide dihexoside, ceramide trihexoside, globoside, paragloboside, and Forssman glycolipid) (**Table 2**), a carboxyl function was generated by oxidation of the olefinic double bond of the sphingosine moiety by the procedure described by Young et al. (22). For gangliosides (G_{M3}, G_{D3}, G_{M1}, G_{D1a}, G_{D1b}, and G_{T1b}) (**Table 3**), the carboxyl function(s) of sialic acid was used directly to couple with aminopropyl silica gel. The amount of glycolipid coupled to aminopropyl silica gel is presented in Table 1. For native gangliosides, approximately 7–8 mg was coupled to 2 g of silica gel matrix, whereas for neutral glycolipid-acids approximately 4–5 mg was attached. The polyacrylamide gel immunoadsorbent column that contained about 20 mg of trapped glycolipid (G_{M1}, ceramide trihexoside, globoside, paragloboside, or Forssman glycolipid) was prepared as described previously (16).

The antibodies directed against glycolipid (G_{M1}, ceramide trihexoside, globoside, paragloboside, or Forssman glycolipid) were purified on appropriate glycolipid–aminopropyl silica gel columns as well as by glycolipid–polyacrylamide gel column chromatog-

TABLE 3. Structures of gangliosides[a]

Designation According to IUPAC-IUB Commission	Designation According to Svennerholm[b]	Structure
II^3NeuNAc-LacCer	G_{M3}	Gal(β,1→4)Glc-Cer 3 ↑α 2 NeuNAc
II^3(NeuNAc)$_2$-LacCer	G_{D3}	Gal(β,1→4)Glc-Cer 3 ↑α 2 NeuNAc(α,8←2)NeuNAc
II^3NeuNAc-GgOSe$_3$Cer	G_{M2}	GalNAc(β,1→4)Gal(β,1→4)Glc-Cer 3 ↑α 2 NeuNAc
II^3NeuNAc-GgOSe$_4$Cer	G_{M1}	Gal(β,1→3)GalNAc(β,1→4)Gal(β,1→4)Glc-Cer 3 ↑α 2 NeuNAc
IV^3NeuNAc,II^3NeuNAc-GgOSe$_4$Cer	G_{D1a}	Gal(β,1→4)GalNAc(β,1→4)Gal(β,1→4)Glc-Cer 3 3 ↑α ↑α 2 2 NeuNAc NeuNAc
II^3(NeuNAc)$_2$-GgOSe$_4$Cer	G_{D1b}	Gal(β,1→3)GalNAc(β,1→4)Gal(β,1→4)Glc-Cer 3 ↑α 2 NeuNAc(α,8←2)NeuNAc
IV^3NeuNAc,II^3(NeuNAc)$_2$-GgOSe$_4$Cer	G_{T1b}	Gal(β,1→4)GalNAc(β,1→4)Gal(β,1→4)Glc-Cer 3 3 ↑α ↑α 2 2 NeuNAc NeuNAc(α,8←2)NeuNAc
IV^3NeuNAc-nLcOSe$_4$Cer[c]		Gal(β,1→4)GlcNAc(β,1→3)Gal(β,1→4)Glc-Cer 3 ↑α 2 NeuNAc

[a] Abbreviations: NeuNAc, *N*-acetylneuraminic acid; all other symbols are the same as in Table 2.
[b] The nomenclature most commonly used for ganglioside designation is that of Svennerholm (51).
[c] Commonly known as sialosylparagloboside.

raphy for direct comparisons of yield and also to determine the specificities of the purified antibodies. Due to scarcity of antisera, only two of the antibodies, viz. anti-G_{M1} and anti-Forssman glycolipid antibodies, were studied in detail. The yields of antibodies against these two glycolipids by different immunoadsorbent columns are presented in **Table 4.** The yields of the purified antibodies by glycolipid–aminopropyl silica gel columns were approximately three times higher compared to glycolipid–polyacrylamide or amino-alkyl-agarose gel columns, even though the glycolipid incorporated in silica gel matrix was much lower (<½). A number of other antibodies against G_{M1}, asialo G_{M1}, ceramide trihexoside, globoside, paragloboside, and Forssman glycolipid were purified routinely in our laboratory via glycolipid–polyacrylamide affinity columns but, in no cases, could more than 4 mg of purified antibodies be obtained from the same sized column as described in this report for the Forssman-polyacrylamide gel column. The lower capacities of these organic supports are possibly due to the fact that the immobilized

TABLE 4. Yield of antibodies against glycolipids from 30–50 ml of rabbit antisera[a]

Column	Amount of Glycolipid Incorporated	Volume of Gel	Yield of Antibody
	mg	*ml*	*mg*
Forssman–aminopropyl silica gel	4	3.5	12.5
Forssman–polyacrylamide gel	20	30	4.0
G_{M1}–aminopropyl silica gel	7.5	3.5	10.4
G_{M1}–polyacrylamide gel	20	30	3.5
G_{M1}–aminoalkyl-agarose (affigel)	15	15	3.0

[a] All experiments were performed from pools of anti-Forssman glycolipid (R 602) and anti-G_{M1} (R 596) sera, each made from several bleedings. The yields presented in this table are the means of two or three experiments.

glycolipids in these gels are not accessible to the antibodies, whereas for the silica gel matrix, because the glycolipids are coated on the surface of the gel, most of them are easily available to the antibodies thereby giving higher yield.

The antibodies purified on the immunoadsorbent columns were tested by agarose gel diffusion (Ouchterlony plate) against native glycolipids mixed with an equal amount of egg lecithin. Precipitation bands were visible after keeping the plate at 4°C for 2 days. Immunoelectrophoresis of purified antibodies indicated that they were mostly IgG; a smaller proportion of IgM was also present. The purified antibodies (8–12 mg) were separated into IgG and IgM fractions by gel filtration on a Sephadex G-200 column. As an illustration, the elution profile of antibodies to G_{M1} is shown in **Fig. 1.** All other anti-glycolipid antibodies also showed similar elution profiles.

The immunoelectrophoresis of the purified antibodies to G_{M1} is shown in **Fig. 2.** All other purified antiglycolipid antibodies also showed exactly similar patterns. The IgG antibodies to ceramide trihexoside, globoside, paragloboside, and Forssman glycolipid were titrated by semiquantitative complement fixation assays using a micro plate (42) and for antibodies against G_{M1} and G_{D3} gangliosides, titration of antibodies were carried out using microcomplement fixation technique (43). The specificity of purified IgG antibody to G_{M1} is presented in **Table 5.** The results indicate that the IgG antibodies purified via three different immunoadsorbent columns have similar antibody titer and cross-reacted extensively with

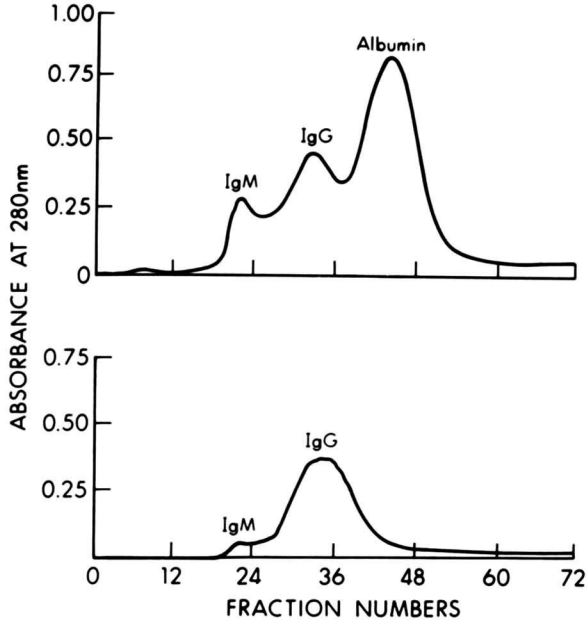


Fig. 1. Elution profile of antibodies to G_{M1} on Sephadex G-200 column. Bed dimensions: 44 × 3.1 cm (Pharmacia column). Flow rate, 10 ml/hr. Upper picture, sample applied, 2 ml of rabbit serum. Lower picture, 8 mg of anti-G_{M1} antibodies purified via G_{M1}–aminopropyl silica gel column. Eluant, 0.1 M Tris-HCl buffer, pH 7.3, containing sodium chloride, 0.01 M ethylenediaminetetraacetic acid, tetrasodium salt. Fractions of 2.5 ml of effluent were collected.

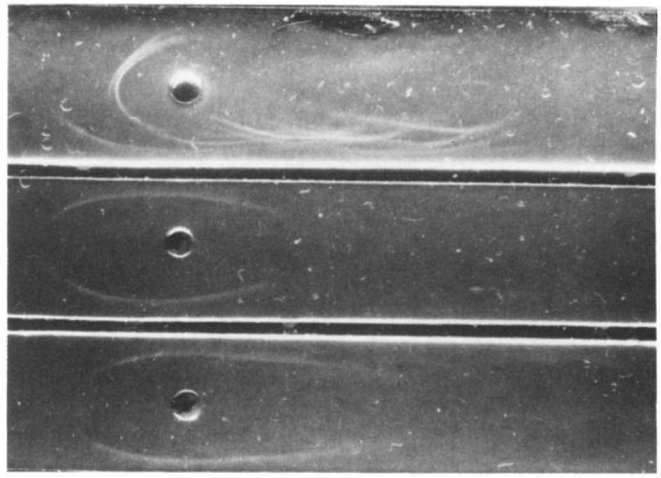


Fig. 2. Immunoelectrophoresis of purified antibodies to G_{M1}. Upper well, rabbit serum. Middle well, anti-G_{M1} IgG antibody (concentration, 2.5 mg/ml) purified on G_{M1}–aminopropyl silica gel column followed by Sephadex G-200 column chromatography. Lower well, anti-G_{M1} antibodies (concentration, 3 mg/ml) after purification on G_{M1}–aminopropyl silica gel column. Trough, goat anti-rabbit serum.

TABLE 5. Specificity of purified IgG antibody to G_{M1}

Antigen[a]	Titer of Complement-Fixing IgG Antibody[b]		
	Purified via G_{M1}–Polyacrylamide Gel Column	Purified via G_{M1}–Aminoalkyl-agarose Column	Purified via G_{M1}–Aminopropyl–Silica Gel Column
G_{M1}	51	50	53
Asialo G_{M1}	75	62	55
G_{D1b}	154	196	132

[a] Each antigen preparation contained 20 ng of glycolipid, 40 ng of lecithin, and 200 ng of cholesterol.

[b] The titer represents minimum amount of antibody in ng that fixed 50% of the complement in a quantitative microcomplement fixation assay (43). All experiments were performed from a pool of anti-G_{M1} serum made from several bleedings of a single rabbit (R 596).

asialo G_{M1} and G_{D1b} having the same disaccharide [Gal(β,1→3)GalNAc] immunodominant residue; no cross-reaction with other gangliosides, viz. G_{D1a}, G_{T1b}, G_{M3}, sialosylparagloboside, and neutral glycosphingolipids, viz. ceramide dihexoside, ceramide trihexoside, globoside, paragloboside and Forssman glycolipid was observed. The specificity of the IgG antibody to G_{M1} thus appeared to be very similar to those prepared earlier in our laboratory by immunizing rabbits with G_{M1} mixed with an equal amount of methylated bovine serum albumin and purification on a G_{M1}–polyacrylamide immunoadsorbent column (39, 47). The IgG antibody to Forssman glycolipid purified via Forssman–polyacrylamide gel and Forssman–aminopropyl silica gel columns behave similarly in complement fixation assays.

The specificity of the purified IgG antibody to Forssman glycolipid is presented in **Table 6.** The results show that IgG antibody purified via both immunoadsorbent columns has exactly the same antibody titer with Forssman glycolipid and, as expected, weak cross-reactivity with globoside was observed (48). Similarly, the specificity of the purified IgG antibody to globoside, purified via globoside–polyacrylamide gel and globoside–aminopropyl silica gel columns, was examined by complement fixation assays. The results, presented in **Table 7** indicate exactly the same antibody titer with globoside and an extensive cross-reactivity with Forssman glycolipid, as was observed earlier in our laboratory (48). The IgG antibodies to Forssman glycolipid and globoside did not cross-react with ceramide dehexoside, ceramide trihexoside, asialo G_{M2}, asialo G_{M1}, and paragloboside, even at much higher antibody concentrations (20 times antibody titers). The IgG antibody to paragloboside purified via paragloboside–polyacrylamide gel and paragloboside–aminopropyl silica gel columns showed the same specificities in complement fixation assays as described by Schwarting and Marcus (49); the purified IgG antibody did not cross-react with globoside, ceramide trihexoside, and sialosylparagloboside, but weak cross-reactivities with asialo G_{M1} and ceramide dihexoside were observed. Similarly, the purified IgG antibody to ceramide trihexoside, purified on ceramide trihexoside–polyacrylamide gel and ceramide trihexoside–aminopropyl silica gel columns, behave similarly in fixing the complement. No cross-reactivity with ceramide dihexoside, asialo

TABLE 6. Specificity of purified IgG antibody to Forssman glycolipid

Antigen[a]	Titer of Complement-Fixing IgG Antibody[b]	
	Purified via Forssman–Polyacrylamide Gel Column	Purified via Forssman–Aminopropyl Silica Gel Column
Forssman glycolipid	1024	1024
Globoside	8	4
Ceramide trihexoside	0	0

[a] Each antigen preparation contained 125 ng of glycolipid, 250 ng of lecithin, and 1250 ng of cholesterol.

[b] The titer represents the highest dilution of antibody that fixed the complement in a semiquantitative complement fixation assay (42). The concentrations of the purified IgG fractions from both preparations were approximately 500 μg/ml. All experiments were performed from a pool of anti-Forssman glycolipid serum made from several bleedings of a single rabbit (R 602).

TABLE 7. Specificity of purified IgG antibody to globoside

Antigen[a]	Titer of Complement-Fixing IgG Antibody[b]	
	Purified via Globoside–Polyacrylamide Gel Column	Purified via Globoside–Aminopropyl Silica Gel Column
Globoside	512	512
Forssman glycolipid	256	256
Ceramide trihexoside	0	0

[a] The concentrations of the purified IgG fractions from both preparations were approximately 300 μg/ml. All experiments were performed from a pool of anti-globoside serum made from several bleedings of a single rabbit (R 48). Other details are exactly the same as in Table 6.

[b] See Table 6.

G_{M2}, asialo G_{M1}, globoside, paragloboside, and Forssman glycolipid was observed[3] due to its specificity for a terminal disaccharide [Gal(α1→4)Gal] immunodominant residue (50). The antibodies to G_{D3} ganglioside, prepared for the first time in our laboratory (30), were purified on a G_{D3}–aminopropyl silica gel immunoadsorbent column. The purified IgG antibody was highly specific for native G_{D3}. The specificity of the purified IgG antibody to G_{D3} was determined by microcomplement fixation assays (43). Fifty percent complement fixation (antibody titer) was obtained with 17 ng of IgG antibody, whereas no complement fixation was observed with 500 ng of antibody and G_{M3}, G_{D1b}, ceramide dihexoside, and other glycolipids (Tables 2 and 3). The details of this interesting antibody will be published elsewhere. We have also utilized aminopropyl silica gel for immobilizing trypsin, chymotrypsin, and human serum albumin via glutaraldehyde and used these immobilized matrices for specific purposes, the details of which will be forthcoming.

The above results have demonstrated that aminopropyl silica gel can be used as a potential support material in the preparation of glycolipid immunoadsorbents and may be preferred over the conventional glycolipid–organic support materials for the following reasons: *1*) faster flow rate; *2*) higher capacity; *3*) more economical; *4*) easier handling; and *5*) lower susceptibility to microbial attack.

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