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Detection of Sialic Acid and Glycosphingolipids in *Euglena gracilis* (Euglenozoa)

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Summary: A sialic acid (Neu5Ac) and glycosphingolipids have been detected in the flagellate *Euglena gracilis*. These findings indicate a possible relationship to the kinetoplastida, the only protistan group known so far to have these molecules.
Sialic acid and the sugar residues galactose, N-acetylgalactosamine, glucose, and mannose were first revealed by fluorescence microscopy using specific fluoresceinylated lectins which gave weak but significant cell labelling. For further investigations the glycosphingolipids were isolated by standard procedures. After alkaline hydrolysis of contaminating phospholipids, anion exchange chromatography and Iatrobeads adsorption chromatography, a positive resorcinol stain on HPTLC plates was found indicating lipid bound sialic acid. Neu5Ac was identified by HPLC and low amounts were detected. An HPTLC immunostaining assay (overlay technique) on gangliosides using specific antibodies and G_{M1}-specific choleragenoid revealed members of the ganglio series in trace quantities. The aminoalcohols were identified as sphingosine and its derivative dehydrosphingosine.

Key Words: *Euglena*; Kinetoplastida; Glycosphingolipids; Gangliosides; Sialic acid.

Introduction

The euglenids, and within this group especially the photoautotrophic members of the genus *Euglena,* display some interesting features in regard to their phylogenetic origin.
Some characteristic structural features, for example the cell envelope or one giant mitochondrion, are similar to those in kinetoplastids, suggesting that a common ancestor for both groups may be found in the primitive kinetoplastida. The envelope of the cell with its regular pattern of microtubules also hints at this relationship. The lack of a cell wall enables the flagellate to react very promptly to changes in the environment, giving the cell membrane an important role in evolutionary aspects. Among various carbohydrate residues which stick out of the membrane and extend into the extracellular space, the sialic acids are well known "communicating" molecules. Sialic acids are often bound to certain lipids of the outer monolayer. They are, among others, responsible for cell to cell- and cell to substrate-recognition. In animal tissue they are components of an interesting group of glycolipids: the glycosphingolipids. These are lipids built of a lipophilic carbohydrate chain and a lipophilic ceramide part. The ceramide consists of the aminoalcohol sphingosine linked to one fatty acid. If the carbohydrate chain is substituted by one or more sialic acid, the molecule is called ganglioside (HAKOMORI 1981; SCHAUER 1985, 1988). A common molecule is the ganglioside G_{M1}, the head group of which consists of glucose, N-acetylgalactosamine, two galactose molecules and the sialic acid N-acetylneuraminic acid (Neu5Ac).
Glycosphingolipids have been detected previously in protists, especially in kinetoplastids. After CARTER et al. (1966) described a sphingolipid-base in *Crithidia* species, BARRETO-BERGTER et al. (1985) and VERMELHO et al. (1986) characterized glycosphingolipids in the epimastigote form of two *Trypanosoma* species. The exometabolite of a *Leishmania* species was characterized to

be a glycopeptidophosphoglycan with trace amounts of sphingosine by SEMPREVIVO & MACLEOD (1981), and lipopeptidophosphoglycan, linked to an inositol phosphorylceramide was found by DE LEDERKREMER et al. (1990) and PREVIATO et al. (1990) in the epimastigote of *Trypanosoma cruzi.* Another group of sphingolipids, i.e. glycophosphosphingolipids, was discovered in *Leptomonas samueli* by PREVIATO et al. (1992). A sulfoglycophospholipid was partially characterized in the infective form of *Trypanosoma cruzi* by UHRIG et al. (1992). The only reports from non-kinetoplastidean protists concerning glycosphingolipids are early investigations by TAKETOMI (1961) on *Tetrahymena pyriformis* (Ciliophora) and a description of a glycophosphosphingolipid in *Tritrichomonas foetus* by SINGH et al. (1991). When investigating the negative surface charge of another kinetoplastid, *Bodo* sp., VOMMARO et al. (1993) found sialic acids, which might have been part of negatively charged glycosphingolipids, the gangliosides.
It was our interest to examine whether *E. gracilis,* a flagellate with animal- and plant-like attributes, also contained glycosphingolipids and especially gangliosides.
We hoped to gain more information about the phylogenetic origin of the genus *Euglena,* particularly its relationship to the kinetoplastids.

Materials and Methods

Cultivation of *Euglena gracilis*

Euglena gracilis strain Z (Klebs 1224-5/25 from Algensammlung Göttingen, Germany) was grown under axenic conditions at 30 °C in the dark. Cell suspensions were bubbled with air containing 3% CO_2. The culture was diluted every two days 1:10 with fresh Hutner's medium in analytical grade (HUTNER et al. 1966). For further investigations cells were washed 5 times with PBS and diluted to 10^6 cells/ml PBS.

Fluorescence microscopy with FITC-conjugated lectins

For the detection of glycoresidues at the surface of *Euglena gracilis* the following lectins were used (Sigma, Deisenhofen, Germany):

Lectin	**Specificity**
Triticum vulgare (WGA)	Neu5Ac
Limulus polyphemus (LPH)	Neu5Ac, GalNAc and GlcNAc
Bandeiraea simplicifolia(BS-I)	Gal and GalNAc
Ricinus communis (RCA_{120})	Gal
Helix pomatia (HPA)	GalNAc
Canavalia ensiformis (ConA)	Glc and Man
Tetragonolobus Agglutinin (TGA)	Fuc

All lectins were conjugated to FITC (fluorescein isothiocyanate, obtained from Sigma). Lectins were added to 200 µl of washed cellsuspension (10^6 native cells/ml PBS) in a final concentration of 200 µl lectin/ml cellsuspension. Cells were incubated for 20 min at 25 °C, then washed 3 times in PBS and finally resuspended in 200 µl PBS. To an aliquot of the solution the appropriate competing sugars (Sigma) were added to verify the specificity of the lectins. Alternatively, the cells were incubated prior to lectin labelling with trypsine (1%, by volume in PBS) 30 min at 30 °C to eliminate the protein bound sugar residues. Additionally, the cells were incubated for 3 h at 37 °C with *Vibrio cholerae* neuraminidase (Behring, Marburg, Germany; 1%, by volume in PBS) before the lectin assay was performed.
Fluorescence of the conjugated lectins was observed with an Orthoplan/Leitz Fluorescence Microscope, BP 450 to 490 nm, LP 515 mm. Images were recorded on a Kodak safety film.
The cells treated with the lectins WGA and LPH were rinsed briefly after incubation with chloroform/methanol (1:1, by volume) to verify lipid bound sialic acid.

Immunofluorescence observations with anti G_{M1}- and anti G_{M3}-antibodies

After incubation with PBS containing 1% BSA (PBS BSA) to block unspecific binding of the antibodies, native cells were incubated with rabbit anti-G_{M1}- and chicken anti-G_{M3}-antibody (obtained from J. MÜTHING, Bielefeld) 1:500 diluted in PBS BSA. After washing three times with PBS-BSA cells were incubated with goat anti-rabbit IgG or with goat anti-chicken IgG, respectively, conjugated to DTAF (1:500 in PBS BSA, purchased from Dianova). All incubation took place for 1 hour at room temperature. Fluorescence was observed as described above. Control cells were examined without primary antibody (MÜTHING et al. 1994).

Isolation of glycosphingolipids from *Euglena gracilis*

Glycosphingolipids (GSL) of 10^{10} cells were isolated by subsequent sonication with chloroform/methanol (2:1, 1:1, and 1:2, by volume, Merck, Darmstadt, Germany). The combined extracts were evaporated to dryness. Extracts were incubated with 1 N NaOH for 1 h at 37 °C to hydrolyse contaminating phospholipids. After neutralization with acetic acid samples were dialysed against 25 l bidestilled water for three days at 4 °C.
GSL were isolated by anion exchange chromatography on DEAE-Sepharose (Pharmacia Fine Chemicals, Freiburg, Germany) in the acetate form. The 0.45 M ammonium-acetate/methanol eluate contained the gangliosides. Samples were evaporated to dryness and dialysed as described above.
For further purification of the gangliosides the sample was chromatographed on Iatrobeads 6RS-8060 (Macherey-Nagel, Düren, Germany) as described by UENO et al.

(1978) with chloroform/methanol (85:15 – 3:1 – 2:1 – 1:2). Gangliosides remaining in chloroform/methanol (2:1) and (1:2) were pooled.
Final purification was achieved by Florisil chromatography of the peracetylated derivatives of the gangliosides (SAITO & HAKOMORI 1971).

High performance thin layer chromatography

High performance thin layer chromatography plates (HPTLC Silica gel 60, 10×10, Merck, Darmstadt, Germany) were used for separation of gangliosides in chloroform/ methanol/water (120:85:20, by volume) containing 2 mM $CaCl_2$. Gangliosides were visualized with resorcinol (SVENNERHOLM 1957).

HPTLC immunostaining (Overlay Technique)

The immunostaining procedure was carried out according to MAGNANI et al. (1980) modified slightly. After chromatography the plates were dried over P_2O_5 for 0.5 h in a desiccator. The silica gel was fixed by chromatography with polyisobutylmethacrylate (Plexigum P28, Röhm, Darmstadt, Germany) saturated with hexane (MÜTHING & MÜHLRADT 1988). The plates were blocked for 15 min in PBS and 1% BSA (solution A).
Incubation with choleragenoid: The plates were incubated with choleragenoid (250 µl/ml in solution A) for 1 h at room temperature (Sigma, Deisenhofen, Germany). Choleragenoid, the β-subunit of the Cholera toxin, uses G_{M1} as receptor molecule in membranes. Goat anticholeragenoid antiserum (Calbiochem, Frankfurt, Germany), and rabbit anti-goat IgG, alkaline phosphatase conjugated (Dianova, Hamburg, Germany) each diluted 1:1,000 in solution A were used for immunological staining. In between incubations, plates were rinsed three times with PBS and 0.05% Tween 21 (solution B). Alkaline phosphatase activity was detected with 5-bromo-4-chloro-3 indolyl phosphate [BCIP] (MÜTHING & MÜHLRADT 1988).
Detection of G_{M1}: The plates were overlayed for 1 h at room temperature with rabbit anti-G_{M1} antiserum 1:1,000 diluted in solution A. After rinsing in solution B plates were incubated with goat anti-rabbit IgG labeled with alkaline phosphatase (Dianova, Hamburg, Germany) 1:1,000 diluted in solution A. Bound antibodies were visualized with BCIP.
Neuraminidase treatment: Prior to treatment with anti G_{M1}-antibody plates were incubated with 50 milliunits/ml *Vibrio cholerae* neuraminidase (Behring, Marburg, Germany) for 18 h at 37 °C in 0.05 M sodium acetate/9 mM $CaCl_2$, pH 5.5. Neuraminidase converts the members of the gangliotetraose family (G_{D1a}, G_{D1b}, G_{T1b} and G_{Q1b}) to G_{M1a}). After this conversion all members can be revealed with anti G_{M1}-antibody or with the choleragenoid (WU & LEEDEN 1988). Subsequent immunoassay was performed as described above.

Determination of sialic acid

Neu5Ac of isolated gangliosides was identified and quantified as their fluorescent derivatives by HPLC as described by MÜTHING et al. (1994). Reference Neu5Ac was purchased from Biomol, Hamburg, Germany. After releasing the sialic acid from the gangliosides by incubation with 25 mM H_2SO_4 for 2.5 h at 80 °C, gangliosides were converted with 1,2-diamino-4,5-methylenedioxybenzene (DMB, Sigma) into their fluorescent derivatives.

Determination of sphingosine

After glycosphingolipid isolation of 10^9 cells the ganglioside fraction was refluxed with 15 ml 2 N HCl in methanol for 5 h after Florisil chromatography. Samples were put on ice and 5 drops of sulfuric acid were added. Samples were extracted 3 times with 2 volumes of hexane to remove fatty acid methyl esters. The solution was adjusted to pH 12 by aqueous sodium hydroxide. Solution was extracted 3 times with $^1/_{10}$ volume of water and then dehydrated by sodium sulfate. Samples were evaporated to dryness, resuspended with chloroform/methanol (2:1) and chromatographed in chloroform/methanol/2 N NH_4OH (40:10:1, by volume). Spots were visualized with ninhydrin spray (0.2 g ninhydrin in 95 ml n-butanol and 5 ml pyridine) after incubation for 5 min at 100 °C (SWEELEY & MOSCATELLI 1959; VERMELHO et al. 1986). Sphingosine was obtained from brain cerebroside (Sigma) by the same procedure.

Abbreviations

BCIP	5-bromo-4-chloro-3 indolyl phosphate
BS-I	*Bandeiraea simplicifolia* agglutinin
BSA	bovine serum albumin
ConA	Concanavalin A
DMB	1,2-diamino-4,5-methylenedioxybenzene
DTAF	5-([4,6-dichlorotriazin-2-yl]-amino) fluorescein
FITC	fluorescein isothiocyanate
Fuc	fucose
Gal	galactose
GalNAc	N-acetylgalactosamine
Glc	glucose
GlcNAc	N-acetylglucosamine
G_{M1}	monosialoganglioside 1 (SVENNERHOLM 1957)
G_{M3}(Neu5Ac)	monosialoganglioside 3 (SVENNERHOLM 1957)
GSL	glycosphingolipid
IgG	immunoglobulin
HBG	human brain ganglioside
HPA	*Helix pomatia* agglutinin
HPLC	high performance liquid chromatography
HPTLC	high performance thin layer chromatography
LPH	*Limulus polyphemus* hemagglutinin
Man	mannose
Neu5Ac	N-acetylneuraminic acid
PBS	phosphate buffered saline
RCA_{120}	*Ricinus communis* agglutinin
TGA	*Tetragonolobus* agglutinin
WGA	Wheat germ agglutinin

Results

Fluorescence observations using lectins: After incubation of *Euglena gracilis* with the FITC-conjugated lectins WGA and LPH cells show a weak but significant FITC-fluorescence. Fig. 1 shows the results of the lectin binding assay with WGA bound to the cells. The cells were photographed with a mixture of white light and fluorescence illumination to show stained and unstained cells in one figure. Cells treated with WGA showed a strong fluorescence (Fig. 1a), whereas control cells did not show any fluorescence (Fig. 1b). Incubating the cells prior to the lectin binding assay with trypsine influenced the binding of the lectin, as a small increase in fluorescence could be observed (Fig. 1c). Previous treatment of cells with neuraminidase, however, led to a strong decrease of the FITC-fluorescence, as most of the bound sialic acids of the carbohydrate chain are enzymatically eliminated through this procedure (Fig. 1d). Incubation with the competing sialic acid Neu5Ac also led to a loss of fluorescence (Fig. 1e). When cells treated with WGA were rinsed briefly with chloroform/methanol (1:1, by volume), fluorescence vanished almost completely (not shown). Experiments using LPH gave the same results, although to a lesser degree (Table 1). WGA and LPH both have an affinity to the sialic acid Neu5Ac.

The lectins BS-I, RCA_{120} and HPA, which recognize galactose and/or its derivative N-acetylgalactosamine show a stronger fluorescence (Table 1). In Fig. 2 the results of the lectin binding assay using BS-I are shown, revealing a strong fluorescence after incubation with BS-I (Fig. 2a). Additional treatment with trypsine (Fig. 2b) or neuraminidase (Fig. 2c) did not influence the fluorescence. Incubation with the competing sugars Gal and GalNAc led to a decrease of fluorescence in both cases (Fig. 2d, e) but not to a loss, as this lectin recognizes both sugars.

TGA led to a similar fluorescence, suggesting that fucose residues are present on the cell envelope (Table 1). The fluorescence of ConA, which marks glucose and mannose residues showed less intensity (Table 1).
Summarizing, we obtained evidence for the following glycoresidues by using FITC conjugated lectins: Neu5Ac, Gal, GalNAc, Glc, GlcNAc, Fuc, and Man.

Immunofluorescence observations: As sugar residues known from the glycosphingolipid ganglioside G_{M1} (LEDEEN & YU 1982) were among those sugar residues described above, we incubated the cells with a monospecific antibody raised against G_{M1} (Fig. 3a). Additionally we incubated the cells with anti-G_{M3}(Neu5Ac), and in both cases a weak but significant label was observed (Fig. 3b). A brief rinse of the cells with chloroform/methanol caused the fluorescence to vanish extracting the lipids and thus setting free the lipid-bound antibodies. Prior treatment of cells with trypsine caused no difference in labelling intensity, indicating a non-protein character of the labeled structure. Control cells treated only with the second DTAF-conjugated antibody did not show any fluorescence.

Sialic acid profile: A sialic acid profile of whole gangliosides isolated from *E. gracilis* was identified and quantified by HPLC as the fluorescent DMB derivative (Fig. 4). An isolate of 10^6 cells contained of 0.55 ng Neu5Ac. The other common sialic acid Neu5Gc was not detected.

HPTLC-analysis: After purification of the glycosphingolipids the samples were chromatographed on HPTLC plates, and stained with resorcinol, resulting in positive spots (Fig. 5). These chromatographed at nearly the same level as the reference (human brain gangliosides). An immunostaining procedure (overlay technique) was considered to be an efficient and easy method for further clarification.

Overlay technique: We used a monospecific anti G_{M1}-antibody and the β-subunit of the cholera toxin (choleragenoid) recognizing G_{M1} as receptor molecule for this ELISA on the HPTLC plates.

Table 1. Labelling of *E. gracilis* cells with FITC-conjugated lectins. 1: Cells incubated with lectins without additional treatment. 2: Prior treatment with neuraminidase. 3: Prior treatment with trypsin. 4: Treatment with competing sugar(s). –: no labelling, +: labelling of up to 45% cells, ++: labelling of 45–90% cells.

Lectin	Specificity	Fluorescence			
		1	2	3	4
Triticum vulgare (WGA)	Neu5Ac	+	–	++	–
Limulus polyphemus (LPH)	Neu5Ac/GalNAc	+	–	++	–/–
Bandeiraea simplicifolia (BS-I)	Gal/GalNAc	++	++	++	+/+
Ricinus communis (RCA_{120})	Gal	++	++	+	–
Helix pomatia (HPA)	GalNAc	++	++	++	–
Canavalia ensiformis (ConA)	Glc/Man	+	+	+	+/+
Tetragonolobus Agglutinin (TGA)	Fuc	++	++	+	–

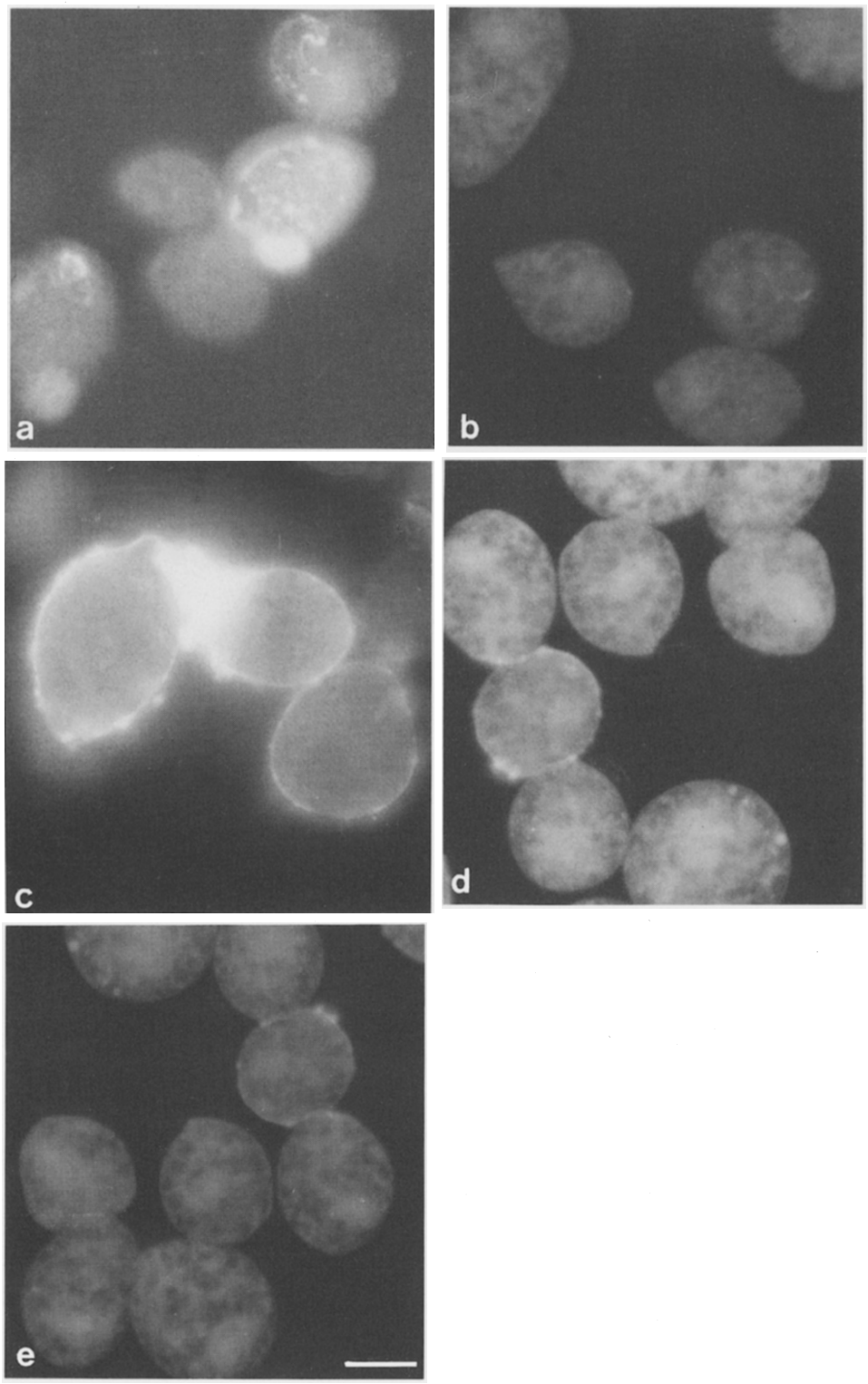

Fig. 1. Fluoresceinyl-WGA binding on cells of *E. gracilis*. Observation under fluorescence illumination with small amounts or white light. WGA binding (a), control cells (b), prior treatment with trypsine (c), prior treatment with neuraminidase (d), incubation with Neu5Ac (e). Bar: 15 μm.

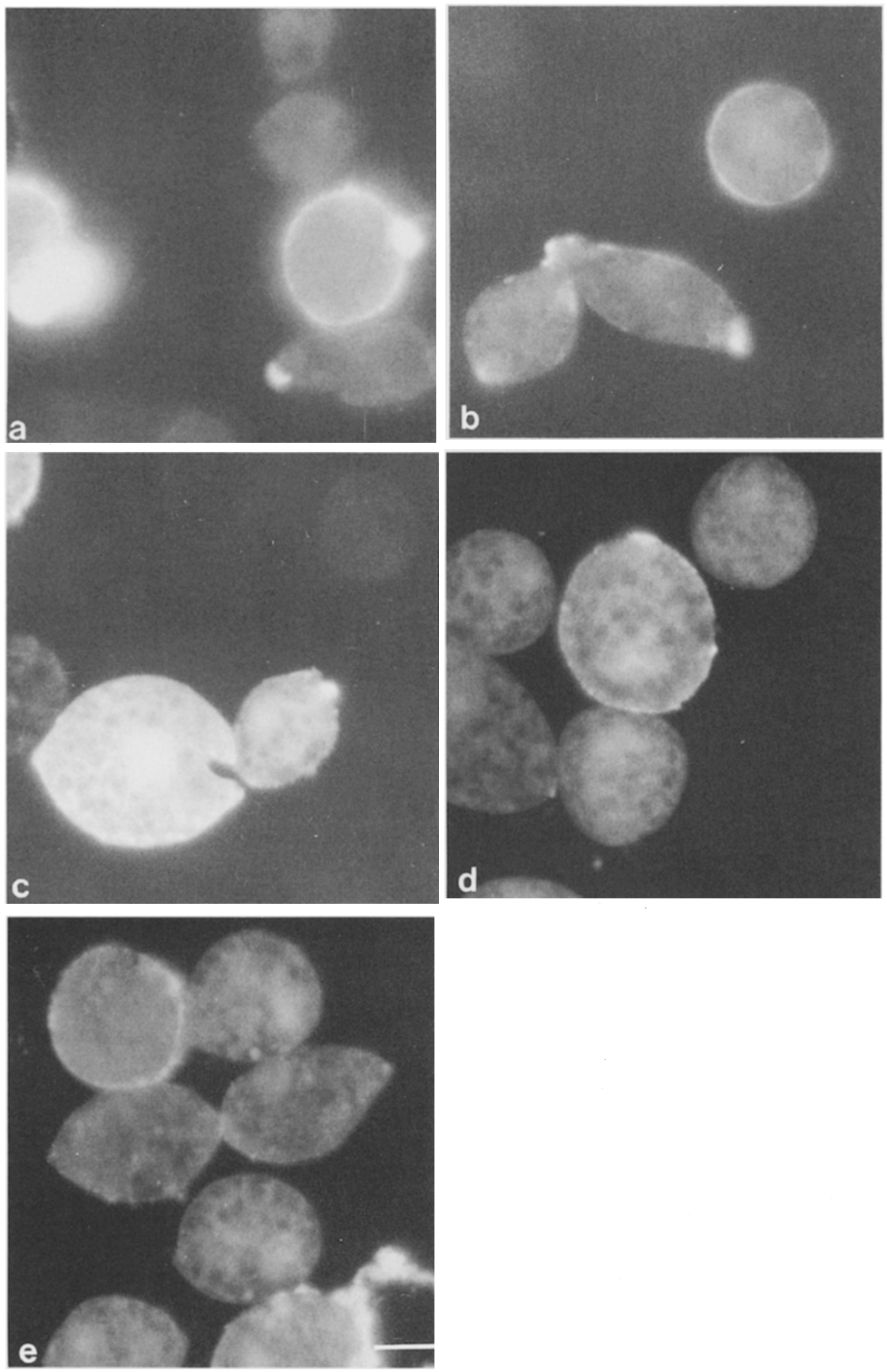

Fig. 2. Fluoresceinyl-BS-I binding on cells of *E. gracilis.* Observations like described above. BS-I-binding (a), prior trypsine treatment (b), prior neuraminidase treatment (c), competing sugar Gal (d), competing sugar GalNAc (e). Bar: 15 µm.

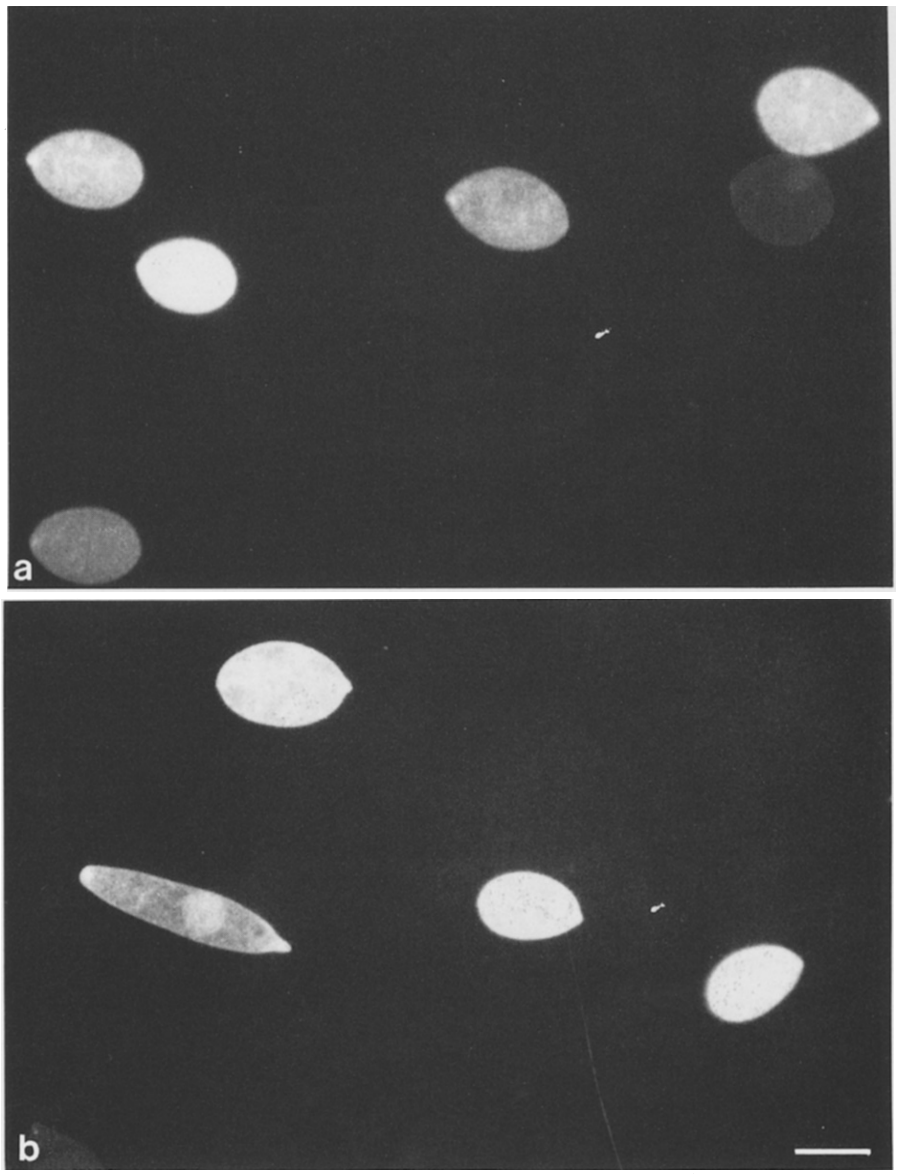

Fig. 3. Immunofluorescence staining of *E. gracilis* with a) rabbit anti G_{M1}-antibody, revealed by anti rabbit IgG-antibody conjugated to DTAF, b) chicken anti G_{M3} (Neu5Ac)-antibody, revealed by rabbit anti-chicken IgG conjugated to DTAF. Percentage of labelling: 75–90%. Bar: 20 µm.

Purified samples were chromatographed and the immunostaining was carried out after fixation of the silica gel. The anti-G_{M1} antibody (Fig. 6, lane b) recognized a spot at the same level as the reference gangliosides (Fig. 6, lane c), visualized by BCIP after incubation with the second antibody. The immunostaining with the cholera-genoid (Fig. 6, lane a) also revealed a lipid structure at the same level, indicating the presence of G_{M1} or a very similar structure.

Neuraminidase treatment: After incubation of the HPTLC-plates with neuraminidase the higher sialylated gangliosides of the G_{M1} core of *Euglena* gangliosides could be detected as G_{M1a} and G_{T1b} (Fig. 7) like in the reference.

Determination of sphingosine: The determination of the aminoalcohol sphingosine by HPTLC after methanolysis in the isolated glycolipids reveals a major spot of sphingosine and a smaller one of its derivative dehydrosphingosine as a part of the glycolipid. A very small spot indicates that, if at all, only trace amounts of o-methyl-sphingosine were present (Fig. 8c, d). The chromatographic mobilities of the samples (lane c: isolated from 2×10^7 cells; lane d: isolated from 3×10^7 cells) correspond to the sphingosine reference which showed a mixture of o-methyl-sphingosine, sphingosine and dehydrosphingosine (Fig. 8, lanes a and b).

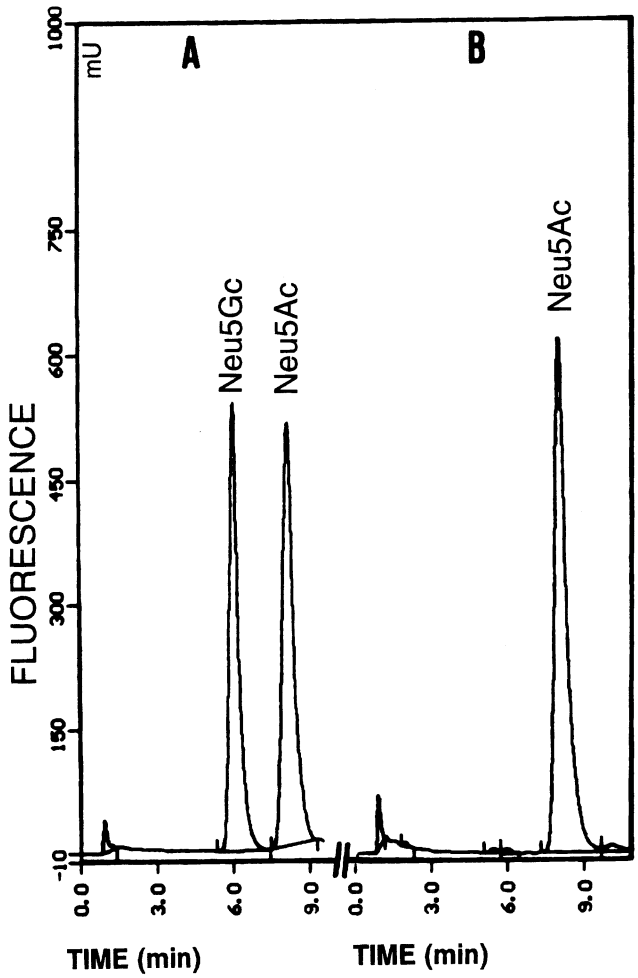

Fig. 4. HPLC elution profiles of fluorescent DMB derivatives of the sialic acid Neu5Ac of *E. gracilis*. A: Neu5Gc and Neu5Ac reference; B: sialic acid isolated from *E. gracilis*.

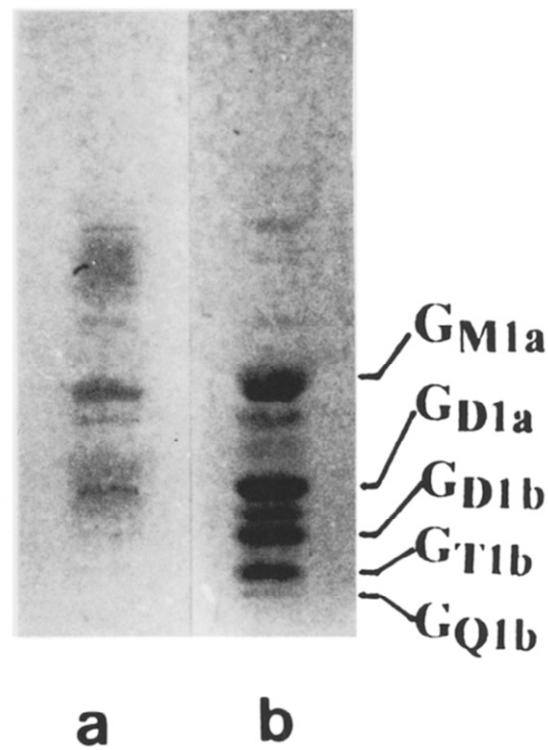

Fig. 5. HPTLC plate of isolated and purified glycosphingolipids of *E. gracilis* (lane a) detected with resorcinol. Lane b: Reference of human brain ganglioside (HBG) mixture.

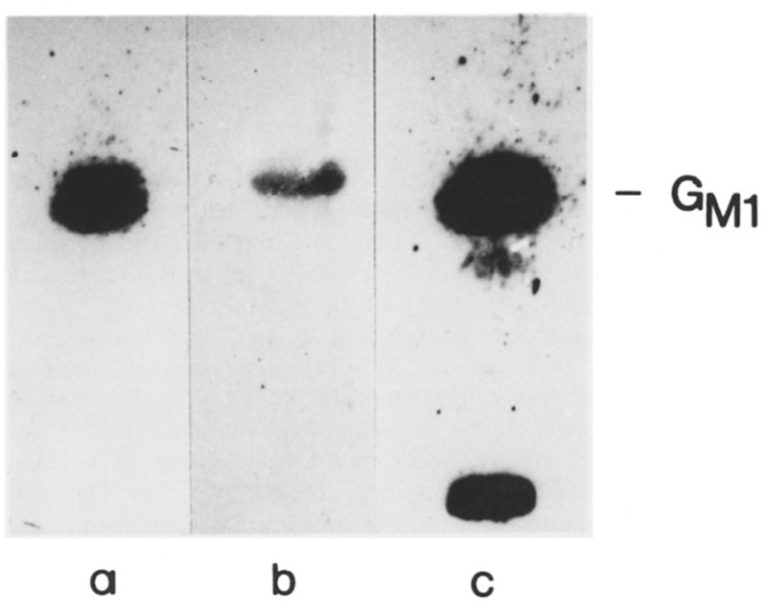

Fig. 6. HPTLC immunostaining (overlay technique) of G_{M1} in isolated GSL of *E. gracilis* by choleragenoid (lane a) and rabbit anti G_{M1}-antibody (lane b). Lane c: Reference of HBG mixture.

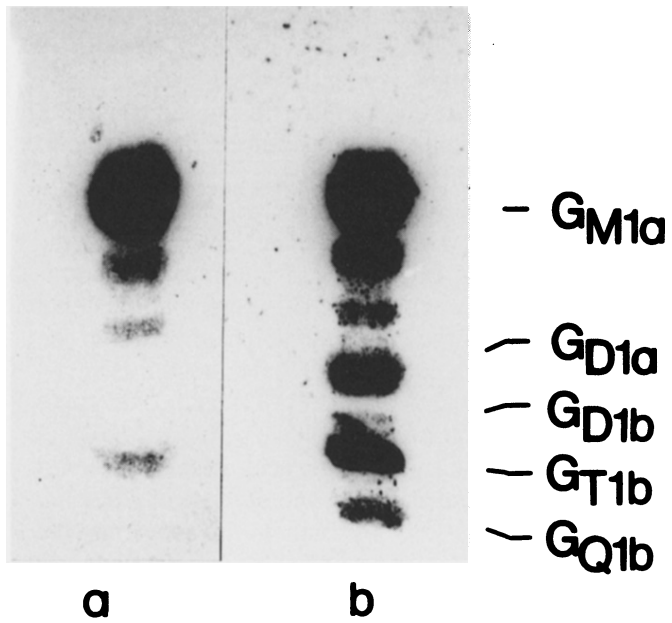

Fig. 7. HPTLC immunostaining (overlay technique) of the G_{M1} core after *V. cholerae* neuraminidase treatment in isolated GSL of *E. gracilis* with rabbit anti G_{M1}-antibody (a). Lane b: Reference of HBG mixture.

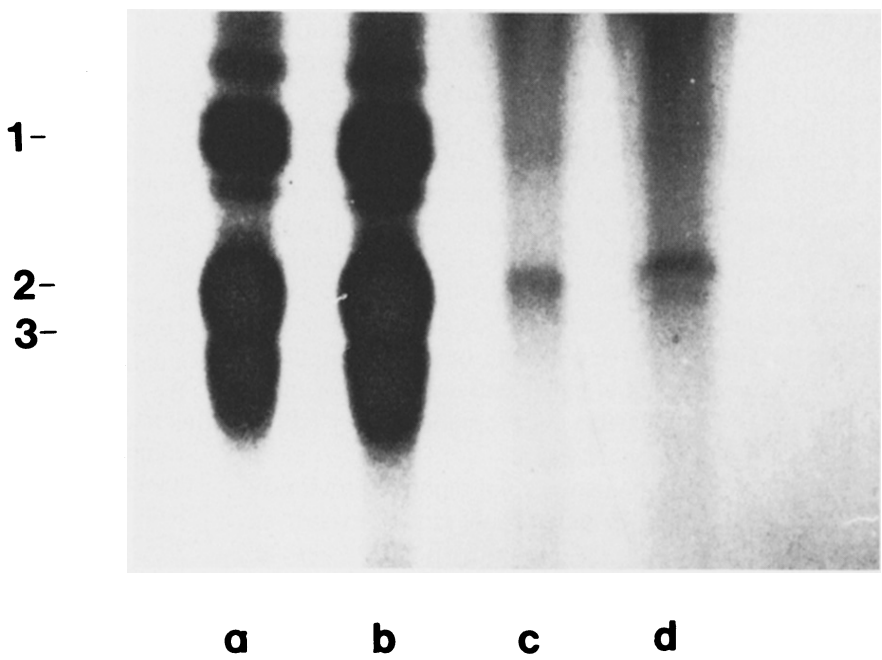

Fig. 8. HPTLC plate of isolated o-methylsphingosine (1), sphingosine (2) and dehydrosphingosine (3). Lanes a and b: reference of isolated sphingosines of brain cerebrosides. Isolated samples of *E. gracilis*: lanes c (2×10^7 cells) and d (3×10^7 cells). Detection with ninhydrin spray.

Discussion

Although some authors did work on the pellicle composition of *Euglena gracilis* (Lefort-Tran et al. 1980; Bricheux & Brugerolle 1987; Nakano et al. 1987; Dubreuil & Bouck 1988), glycosphingolipids were not among the constituents investigated. In our studies we found evidence for the presence of a certain group of glycosphingolipids, the gangliosides, in the surface of this flagellate. Sugar residues known from and described for gangliosides as well as sialic acid (Cheryl et al. 1989) were detected by lectin binding studies on the native cells. The fluorescence was inhibited after incubating the cells with the appropriate sugar or sialic acid indicating the specificity of the lectins.

Sialic acid was detected using WGA and LPH. After prior treatment with trypsine to remove protein bound carbohydrates, the fluorescence became stronger. This increase might be due to the removal of protein bound sugar residues usually masking the lipid bound residues

and thus limiting the fluorescence. This result also suggests that the sialic acid which was labelled with WGA and LPH is lipid bound – an interpretation supported by the observation that after briefly rinsing the cells with chloroform/methanol the fluorescence vanished due to the extraction of the lipids.

The sugar residues and the sialic acid identified by these studies were Gal, GalNAc, Glc, GlcNAc, Man, and the sialic acid Neu5Ac, respectively. Some of these molecules also occur in the ganglioside G_{M1}, common for animal tissue, as parts of the carbohydrate chain bound to the ceramide (FISHMAN & BRADY 1976; HAKOMORI 1986).

VON SENGBUSCH & MÜLLER (1983) and BRÉ et al. (1986) found Gal and GalNAc at the pellicle of *Euglena gracilis* but no Glc and no sialic acid, whereas VANNINI et al. (1981) revealed Glc at the surface of *Euglena.* These different results by authors investigating the carbohydrates at the surface of *E. gracilis* may find their reason in varying culture conditions such as light/dark change or continuous light, organic or inorganic media or such.

In order to quantify the sialic acid (sugars are not quantified yet) an HPLC analysis using the fluorescent DMB derivatives of sialic acid was performed. Compared to animal tissue, the amount of Neu5Ac is very low (MÜTHING et al. 1994). NAKANO et al. (1987) measured the carbohydrate moieties of the pellicular glycolipids in *E. gracilis*. They found, among others, the sugar residues Glc and Gal as part of a lipid, called "unknown lipid", which they considered to be a glycosphingolipid, as methanolysis resulted in ninhydrin-positive compounds. However, sialic acid was not detected. The evidence for sialic acid (Neu5Ac) in our study might be due to the very specific and sensitive method of measuring the DMB derivatives of the sialic acid by HPLC after glycosphingolipid isolation.

While glycosphingolipids were not reported previously from euglenoid protists, quite a few reports exist from kinetoplastidean protists. BARRETO-BERGTER et al. (1985) isolated glycosphingolipids in the kinetoplastid *Trypanosoma cruzi,* while VERMELHO et al. (1986) characterized a neutral glycosphingolipid in *T. cruzi.* However, in neither case sialic acids bound to these lipids were detected. These results were confirmed by PREVIATO et al. (1990), SINGH et al. (1991), and PREVIATO et al. (1992), who found glycosphingolipids in several kinetoplastids without revealing sialic acids. Only in one member of the kinetoplastids, in *Bodo* sp. (Bodonida), VOMMARO et al. (1993) analysed the negative surface charge which they could attribute to the occurrence of sialic acids without further analyzing the detected sialic acids and their lipid or protein bound character.

ROUND (1980) and CORLISS (1984) among others (see also: MARGULIS et al. 1990) proposed that the photosynthetic euglenids have arisen from a phagotrophic ancestor. KIVIC & WALNE (1984) considered a primitive bodonid or *Isonema* as a common ancestor for the euglenids and the trypanosomatids.

Summarizing these investigations on glycosphingolipids, sialic acids appear to be absent in the trypanosomatids and to persist in the direction of the euglenids and bodonids. The evolutionary significance of these preliminary findings needs to be proven by further investigations of related organisms.

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