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GANGLIOSIDE GM1 SENSITIZES TUMOR CELLS TO GROWTH INHIBITORY GLYCOPEPTIDES

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We have purified and characterized a glycopeptide from surfaces of brain cortex cells that inhibits cellular protein synthesis in normal but not transformed cells. Data are presented that correlate the ability of C3H fibrosarcoma 1316 cells to avoid the inhibitor's activity with a reduced cellular level of the ganglioside GM1. When 1316 cells were incubated with GM1 under conditions in which the ganglioside is incorporated into the cells, the cells became sensitive to the inhibitor. Similar experiments with ceramide resulted in no change in 1316 sensitivity. In addition, when 1316 cells were incubated with GM1 for 18 h, they apparently metabolized GM1 to simpler gangliosides and lost their sensitivity to the glycopeptide inhibitor.

INTRODUCTION

We have described the isolation, purification and characterization of glycopeptides, released from the surfaces of brain cortical cells by mild proteolysis, which are potent inhibitors of cell growth and protein synthesis (1,2). These glycopeptides may be naturally occurring growth regulators that mediate their effects by cell-cell contact in a manner similar to that proposed by Dulbecco and Stoker (3). These macromolecules inhibit protein synthesis in a reversible and non-lethal manner, at the level of elongation of nascent peptide chains, requiring membrane mediation and/or an intra-cellular modification (4). The glycopeptides can inhibit protein synthesis by 50% at a concentration of 2×10^5 molecules per target cell, which is in the concentration range associated with hormone action (2). The inhibitor is most active against primary cells in culture, somewhat less active against established tissue culture cell lines, and relatively or completely ineffective against transformed or

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tumor-derived cell lines (1,5). Polyoma virus transformed baby hamster kidney cells (pyBHK) were able to bind the biological activity of the glycopeptides in a saturable fashion but escaped inhibition because they inactivated the glycopeptides (5). We now report that mouse fibrosarcoma 1316 cells escape inhibition by a different mechanism. Sensitivity to the glycopeptide inhibitors only occurs when the intact ganglioside GM1 remains in their cell membranes.

MATERIALS AND METHODS

Isolation and Purification of Glycopeptides. The glycopeptides were prepared from cerebral cortex cells of 6 week-old Swiss albino outbred mice by mild proteolysis as described previously (2). The macromolecules released by proteolysis were purified by ethanol precipitation and chloroform/methanol extraction, followed by gel filtration on BioGel P-100 and the active material eluting at apparent MW 30,000 was concentrated by ultrafiltration before use. Some experiments were performed with material purified by affinity chromatography with agarose bound Ulex europaeus agglutinin I (UEA-I), eluted with 0.1 M fucose and dialyzed against HKM buffer (20 mM Hepes; 120 mM KCl; 5 mM $MgCl_2$; pH 7.1) (2).

Antisera. Antisera to the inhibitor were prepared from hyperimmunized Balb/c mice. Blood was obtained from tail bleedings, clotted, the sera were decomplemented, and then clarified by centrifugation at 10,000 x g for 1 hr. The clarified sera were diluted in an equal volume of borate saline buffer (BSB) (162 mM sodium borate, 133 mM sodium chloride pH 8) and salt fractionated with $(NH_4)_2SO_4$ as described by Heide and Schwick (6), dialysed, then fractionated on DEAE-Sephadex and Sephadex G-200 (7). The recovered IgG fraction was diluted to a final concentration of 6 mg/ml in BSB and characterized by quantitative precipitation assays with anti-mouse IgG, and double antibody radioimmunoassays (RIA) with UEA-I purified inhibitor that had been labelled with ^{125}I using the method of Wood et al. (8).

Cell Culture. BHK-21 cells were grown as described previously (5). Fibrosarcoma 1316 cells, induced by UV irradiation of C3H mice (10), were provided by Dr. G. Wm. Fortner, Division of Biology, Kansas State University. Cells were routinely grown as monolayers at 37°C in a 5% CO_2; 95% air atmosphere in humidified incubators.

Ganglioside Analysis. Cells which had been incubated for 2 hours at 2-4°C, with or without added GM1, were washed three times with phosphate-buffered saline (0.1 M sodium phosphate, 0.09 M NaCl), scraped with a rubber policeman, and centrifuged for 5 minutes at 1000 x g. Cell pellets were extracted by the method of Irwin and Irwin (9), and gangliosides were purified by silicic acid column chromatography (9). Gangliosides and appropriate standards were resuspended in $CHCl_3/CH_3OH$ (2/1, v/v), and spotted on 250 cm silica gel G thin layer chromatography (TLC) plates. Chromatograms were developed at room temperature with $CHCl_3/CH_3OH/12$ mM $MgCl_2$/ 15 N NH_4OH (60/35/7.5/3,v/v) (12), air-dried, sprayed with 50% H_2SO_4, and charred overnight at 120°.

Protein Synthesis Assays. Cellular protein synthesis was measured in 100 µl DMEM containing 25 mM hepes plus 25 µl HKM buffer (controls) or 25 µl HKM containing specified concentrations of glycopeptides as described previously (1,5).

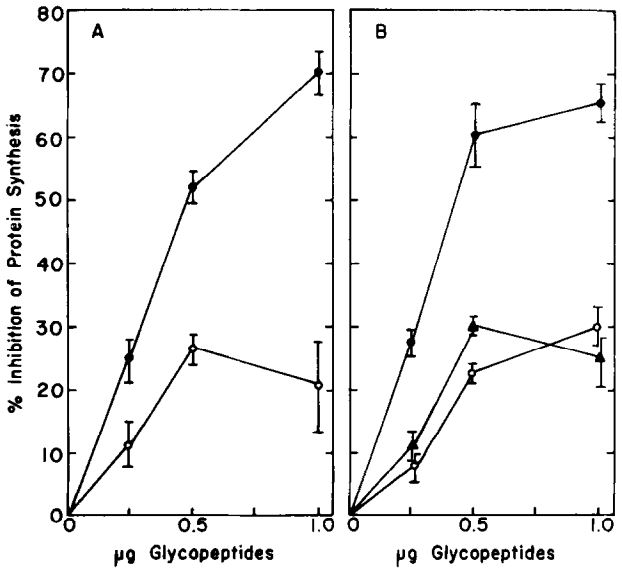

Fig. 1. Inhibition of Cellular Protein Synthesis by Brain Cell Surface Glycopeptides. 2 x 10^6 BHK-21 or fibrosarcoma 1316 cells were incubated with 0.25 to 1.0 µg Ulex europaeus affinity purified glycopeptides for 30 min then radiolabelled with ^{35}S-methionine. Panel A, (●) BHK-21 cells, (o) 1316 cells; Panel B, (●) 1316 cells incubated with GM1 2 h at 0°C prior to protein synthesis assay, (o) 1316 cells incubated with ceramide 2 h at 0°C prior to assay, (▲) 1316 cells incubated with GM1 2 h at 0°C then 18 h at 37°C prior to assay. All results are means and standard deviations of 2 experiments, each performed in triplicate.

Inactivation of Inhibitor by Conditioned Media. Medium that had been exposed to exponentially growing 1316 cells for 16 h was collected and clarified by centrifugation at 2,000 g then frozen at -20°C until use. Inactivation assays were performed as described previously (5).

RESULTS

Treatment of 1316 cells with the growth inhibitory glycopeptides resulted in only a limited degree of inhibition of cellular protein synthesis compared to BHK-21 cells where protein synthesis could be inhibited by 70% (Fig. 1A). Unlike medium from pyBHK-21 cells (5), conditioned medium from the 1316 cells did not inactivate the biological activity of the inhibitory glycopeptides (data not shown).

The possible role of ganglioside GM1 as a receptor for a number of glycoproteins with physiological activity (11,12) suggested to us that the insensitivity of 1316 cells to the glycopeptide growth inhibitor might be due to insufficient levels of GM1 in the cell surface membrane. Monolayers of 5 x 10^6 1316 cells were incubated with 0.7 µM GM1 for 2 h in an ice bath, conditions which were selected to allow incorporation of

the ganglioside into the cell surface membrane (13,14), while limiting endocytosis or enzymatic degradation of the gangliosides. The cells were then washed three times with ice cold HKM buffer, harvested by scraping with a rubber policeman, and pelleted by centrifugation. Affinity purified glycopeptides, at 0.25 to 1.0 μg per 10^6 1316 cells, were then reacted with the cells in suspension for 10 min in an ice bath to allow the glycopeptides to bind to the cells under conditions that would restrict degradation of surface membrane incorporated GM1. The cells were then incubated at 37°C for 30 min, followed by a 30 min radiolabelling period. The incorporation of ^{35}S-methionine into cellular protein was measured and compared to 1316 cells that received no GM1, or that received 2.5 μM ceramide/10^6 cells. The results indicated that preincubation of the 1316 cells with GM1 increased the sensitivity of 1316 cells to the inhibitory glycopeptides while ceramide did not influence subsequent protein synthesis in the presence of the inhibitors (Fig. 1B).

TLC analysis of 1316 cells treated in this manner indicated that GM1 had been incorporated into the cells (Fig. 2, lane B). The intensity of the band migrating with the Rf value of GM1 indicated that there was approximately 0.1 μg of the ganglioside present. When the 1316 cells not preincubated with GM1 were tested, little, if any, material migrating at the Rf of GM1 was evident (Fig. 2, lane C).

Since fibrosarcoma 1316 cells are cultured in medium supplemented with 5% fetal calf serum, and serum usually contains GM1 and other gangliosides, it was somewhat surprising to us that the cells had such low levels of GM1 and that a short incubation with GM1 could produce such a marked change in the cell's sensitivity to the inhibitory glycopeptide. We tested the possibility that 1316 cells could metabolize GM1 by incubating 1316 cells with 0.7 μM GM1 for 2 h at 0°C and then transferring them to a 37°C incubator for 2, 8 or 18 h and measuring the remaining GM1 by TLC. By 18 h after the addition of the ganglioside, none was detectable in the cells by either TLC (Fig. 2, lane E) or cell sensi-

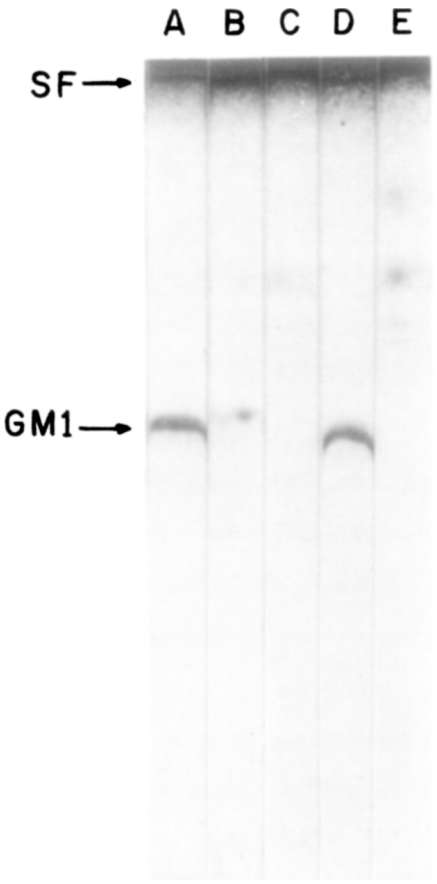

Fig. 2. Thin-layer Chromatographic Analysis of 1316 Cell Gangliosides. 3×10^6 cells were incubated on ice for two hours in minimal Eagle's medium (without serum) containing 0.7 μM of ganglioside GM1. Control cells were incubated in medium without ganglioside. After rinsing the monolayers three times with phosphate-buffered saline, the cells were removed with a rubber policeman, washed twice with 5.0 ml phosphate-buffered saline, extracted, and chromatographed as described in Materials and Methods. Lanes A and D = ganglioside GM1 standard, Lane B = cells incubated with GM1, Lane C = cells incubated without GM1, Lane E = cells incubated with GM1 and subsequently incubated for 18 hours at 37°C in medium + 5% calf serum. SF = solvent front. Results shown are typical results from one of four experiments.

tivity to the glycopeptide inhibitor (Fig. 1B). The loss of GM1 was accompanied by the appearance of other gangliosides, presumably metabolic products, of greater Rf that were not detected in 1316 cells not treated with GM1 (compare lanes C and E, Fig. 2).

DISCUSSION

Our previous data suggested that the growth inhibitory glycopeptides require a membrane mediation of their inhibitory activity. Both BHK-21

cells, which are sensitive to the glycopeptides, and pyBHK cells, which are not, bind the inhibitor in a saturable fashion. The virally transformed cells apparently possessed more binding sites for the inhibitor (5), which is consistent with the observation that polyoma virus transformed BHK cells have more GM1 on their surfaces than do untransformed BHK cells (16).

It has been previously demonstrated that incubation of cultured cells with gangliosides results in uptake (11) and subsequent incorporation of the glycolipid into the plasma membrane (12). This phenomenon has been observed using a variety of cell types including transformed mouse fibroblasts (11, 13), normal human fibroblasts (12), mouse neuroblastoma cells (14), and chick erythrocytes (15). The data presented in Fig. 1 demonstrate that the presence of GM1 in 1316 cell membranes substantially increases their sensitivity to the glycopeptides. The increased sensitivity is not observed when cells are preincubated with ceramide or if the cells are allowed to metabolize the GM1 to simpler gangliosides.

The observation that 1316 cells, incubated with GM1 at 37°C, actually contain little or no GM1, suggests that these fibrosarcoma cells fit the pattern of ganglioside simplification upon malignant transformation as proposed by Hakomori (12). If this is the case, the reduced level of growth regulation observed in these cells, the removal of GM1 from their surface membranes, and their reduced sensitivity to the growth regulatory glycopeptide, may reflect one underlying transformation event associated with the expression of gangliosides on the cell surface. Further experiments in our laboratory will test whether GM1 is directly acting as a receptor for the inhibitory glycopeptides, or whether its presence in the cell surface membrane, as a structural element, indirectly confers sensitivity to the 1316 fibrosarcoma cells.

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