Synaptic Membrane Glycoproteins gp65 and gp55 Are New Members of the Immunoglobulin Superfamily*

(Received for publication, September 4, 1996, and in revised form, October 23, 1996)

Kristina Langenaese, Philip W. Beesley‡, and Eckart D. Gundelfinger§

From the Department of Neurochemistry and Molecular Biology, Federal Institute for Neurobiology, D-39008 Magdeburg, Germany and the Division of Biochemistry, School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, United Kingdom

Glycoproteins gp65 and gp55 are major components of synaptic membranes prepared from rat forebrain. Both are recognized by the monoclonal antibody SMgp65. We have used SMgp65 to screen a rat brain cDNA expression library. Two sets of overlapping cDNAs that contain open reading frames of 397 and 281 amino acids were isolated. The deduced proteins are members of the immunoglobulin (Ig) superfamily containing three and two Ig domains, respectively. The common part has ~40% sequence identity with neurothelin/basigin. The identity of the proteins as gp65 and gp55 was confirmed by production of new antisera against a common recombinant protein fragment. These antisera immunoprecipitate gp65 and gp55. Furthermore, expression of gp65 and gp55 cDNAs in human 293 cells treated with tunicamycin results in the production of unglycosylated core proteins of identical size to deglycosylated gp65 and gp55. Northern analysis revealed that gp65 transcripts are brain-specific, whereas gp55 is expressed in most tissues and cell lines examined. The tissue distribution was confirmed at the protein level though the pattern of glycosylation of gp55 varies between tissues. In situ hybridization experiments with a common and a gp65-specific probe suggest differential expression of gp65 and gp55 transcripts in the rat brain.

Synaptic junctions are highly specialized areas of contact and communication between neurons. They comprise the membranes and the underlying cytoskeleton of the pre- and postsynaptic neurons. In particular, the postsynaptic nerve ending is characterized by the presence of an electron-dense cytoskeletal structure, the postsynaptic density (PSD), which underlies the postsynaptic membrane. It is well established that synaptic structures and biochemically isolated synaptic subfractions, particularly synaptic membrane (SM) and PSD fractions, are enriched in distinct sets of glycoproteins that bind the lectin concanavalin A (1, 2). Some of these glycoproteins have been identified and their functions established. These include the major 180-kDa PSD glycoprotein that corresponds to the NR2B subunit of the N-methyl-D-aspartate receptor (3) and the SM-enriched glycoprotein, gp50, that has been identified as the β2 subunit of the Na⁺/K⁺-ATPase (4, 5).

SM and PSD glycoproteins, which are oriented with their oligosaccharide-containing domains facing into the synaptic cleft, are ideally placed to mediate adhesive interactions between the pre- and postsynaptic nerve endings. Indeed, the PSD-enriched PAC 1 glycoproteins (6, 7) have been identified as members of the cadherin family (8), providing evidence for such a role. Furthermore, integrin-type adhesion molecules (9) and NCAM (10), a member of the immunoglobulin (Ig) superfamily, have been recognized as SM components. Considering the complex interplay between stabilization and plasticity at synaptic connections, it is likely that more synaptic glycoproteins with adhesive functions are to be identified.

Here we describe the molecular cloning of cDNAs encoding two synaptic glycoproteins, gp65 and gp55, and the use of these cDNAs to characterize the proteins at the molecular level. GP65 and gp55 are two closely related glycoproteins with apparent molecular masses of 65 and 55 kDa, respectively, that were originally identified and biochemically characterized using the monoclonal antibody (mAb) SMgp65 (11, 12). Both molecules behave as integral membrane proteins and are enriched in the SM fraction. Gp65 is further enriched in the PSD fraction, whereas gp55 is conspicuously absent from this fraction. Immunocytochemical studies indicate that the antibody recognizes gp65, but not gp55 in tissue sections (11). The gp65-like immunoreactivity is specifically localized to the processes and nerve terminals of subsets of forebrain neurons. Biochemical studies suggest that the two molecules contain similar amounts of N-linked oligosaccharides and differ by a single extracellular 10–12-kDa peptide sequence that contains little or no carbohydrate (12). The present data demonstrate that gp65 and gp55 are novel members of the Ig superfamily containing three and two Ig domains, respectively. Thus, a plausible function for these proteins is to mediate adhesive interactions, particularly at the synapse.

EXPERIMENTAL PROCEDURES

Isolation of cDNA Clones—The cDNA clones gp55/K4 and gp55/K7 were isolated from a random primed rat brain Agt11 expression library (13) with the SMgp65 monoclonal antibody (11) following standard protocols for expression screening (14). Immunopositive clones were detected with a goat anti-mouse secondary antibody (Sigma) using the ECL system (Amersham) according to manufacturer instructions.

A radiolabeled fragment of cDNA clone gp55/K4 was used to screen various rat brain cDNA libraries for overlapping clones. Hybridization was carried out for 4 h at 65 °C in Rapid-hyb buffer (Amersham Buchler). The cDNA inserts were subcloned into plBluescript vectors (Stratagene) and sequenced from both strands using the fluorescent dye dideoxy termination method in combination with an automated DNA sequencer (Applied Biosystems). Sequences were analyzed using the...
New Glycoproteins of the Immunoglobulin Superfamily

Northern Analysis and in Situ Hybridization—Total RNA was prepared from various cell lines, i.e. C6 glioma cells, PC12 pheochromocytoma cells, and human embryonic kidney (HEK 293) cells, and tissues, i.e. liver, heart, skeletal muscle, kidney, spleen, and thymus, and various immortalized hybridoma lines according to Chirgwin et al. (14). RNA was isolated by guanidine isothiocyanate followed by centrifugation and washing with two steps of ethanol precipitation. Hybridization signals were detected using Kodak X-OMAT x-ray films.

In situ hybridizations were performed essentially as described (16) using the following hybridization buffer: 10 mM HEPES, pH 7.5, 600 mM NaCl, 100 mM diethiothreitol, 1 mM EDTA, 50% formamide, 10× denaturant solution, 1× Denhardt’s solution, and 100 μg/ml each of sonicated salmon sperm DNA and yeast tRNA. Hybridization signals were visualized using a Fuji BAS3000 Bio Image.

Construction of Recombinant Protein and Production of Antiserum—A segment of the cDNA insert of clone gp55/K4 encoding amino acid (aa) residues 2–192 of gp55 (see Fig. 1) was amplified by polymerase chain reaction using appropriate oligonucleotide primers and cloned into the bacterial expression vector pQE30 (Qiagen Inc.). The accuracy of the construct was verified by DNA sequence analysis. From this construct, a recombinant protein of 26 kDa containing 6 amino-terminal His residues was produced and affinity purified on nickel-nitrilotriacetic acid resin under denaturing conditions as described by the manufacturer (Qiagen). The fusion protein was dialyzed against PBS and used to produce polyclonal antibodies in rabbits. Two independent antisera were obtained that reacted with both gp55 and gp65.

Subcellular Fractionation and Western Blotting—SM were prepared as described previously (12). Detergent-soluble membrane proteins of 30-day-old rats were isolated from brain and liver by homogenizing in 25 mM Tris·HCl, pH 7.4, 500 mM NaCl, 1% Triton X-100, containing protease inhibitors as above, and centrifuged at 100,000 × g for 1.5 h on ice; then 40 μl of protease inhibitors were added as controls. TBST (20 mM Tris·HCl, pH 8.0, 150 mM NaCl containing 1% Triton X-100) was used to adjust the volume to 1 ml. Protease inhibitors were added as above. The mixture was incubated at 1.5 h on ice; then 40 μl of GammaBind Plus Sepharose (Pharmacia) were added and incubated for another hour. Sepharose was pelleted by centrifugation and washed twice with TBST, twice with Tris-buffered saline and once with 50 mM Tris·HCl, pH 8.8. Protein was eluted from the Sepharose with gel-loading buffer (18) and subjected to SDS-PAGE.

Stable Expression of gp55 and gp65 in HEK293 Cells—Using an appropriate polynucleotide chain reaction strategy, the open reading frame for mature gp55 and gp65 was fused in frame to the BM40 signal peptide (20). The constructs were introduced into the pRC/CMV vector (Invitrogen, Carlsbad) and their accuracy was verified by DNA sequence analysis. Transfection was performed using the calcium phosphate transfection method following the protocol of the mammalian transfection system (Stratagene). Stably transfected cell lines were selected under G418, and resistant colonies were propagated. Tunicamycin treatment of gp55 and gp65 transfected cell lines was performed by adding tunicamycin to the culture medium to a final concentration of 10 μg/ml for 20 h before harvesting the cells. For immunoblotting, cells were solubilized in 25 mM Tris·HCl, pH 7.4, 500 mM NaCl, 1% Triton X-100, containing protease inhibitors as above, centrifuged at 15,000 × g, and the supernatant was applied to SDS-PAGE.

For immunocytochemistry, cells were grown on glass chamber slides. Expression of gp65 on the cell surface was visualized by confocal laser scan microscopy.

RESULTS

Cloning of gp55 and gp65 cDNAs—Expression screening of a rat brain cDNA library with the monoclonal antibody SMgp65, which recognizes the peptide moieties of both gp55 and gp65, resulted in the isolation of six independent cDNA clones. The two longest ones, gp55/K4 and gp55/K7, were sequenced (Fig. 1). As deduced from the nucleotide sequence, they contained a single open reading frame for 281 aa. As cDNAs for two polyepitopes were expected, a fragment of clone gp55/K4 (Fig. 1A, probe 1) was used to screen additional rat brain cDNA libraries. The isolated cDNAs corresponded to the known ones, whereas others harbored an insertion of 348 nucleotides within the open reading frame. In total, three cDNA clones for either open reading frame were analyzed in detail (Fig. 1).

The proteins deduced from the open reading frames of the two cDNAs differ by an insertion of 116 aa residues after Asn-30. They are thought to correspond to gp55 and gp65. A computer analysis of the deduced aa sequences revealed interesting structural features for the two proteins. Both contain an amino-terminal hydrophobic sequence. According to the Y–3 rule (21), the first 28 amino acids may represent a cleavable signal peptide. Thus, putative mature gp55 and gp65 consist of 253 and 365 aa residues and have calculated molecular masses of 28.8 and 41.4 kDa, respectively. These molecular masses are in good agreement with apparent molecular masses of 28 and 40 kDa determined for deglycosylated gp55 and gp65 by SDS-PAGE (12).

Both proteins contain a single potential membrane-spanning region that extends from Leu-192 to Tyr-215 in gp55 and corresponding to residues 308 to 331 in gp65. This transmembrane domain is unusual in that it contains a charged aa residue (Glu-200/316). Analysis of the extracellular sequences reveals that gp55 has two and gp65 has three Ig domains. The sequence data show that the gp65-specific and the most C-terminal domain are C2-type Ig domains (22), whereas the middle Ig domain cannot be definitely assigned to one of the known Ig domain types. Gp55 as well as gp65 have six potential N-glycosylation sites. Since both molecules contain high mannose and complex oligosaccharide residues, several of these sites are expected to be glycosylated in vivo (11, 12). The intracellular 34-aa long C-terminal domain of gp55 and gp65 is highly hydrophilic and contains several charged aa residues. Part of the gp55 cDNA clones had a small insertion in the open reading frame that encodes four additional aa residues after Glu-309/320. The putative mature gp55 and gp65 consist of 253 and 365 aa residues and have calculated molecular masses of 28.8 and 41.4 kDa, respectively. The molecular masses are in good agreement with apparent molecular masses of 28 and 40 kDa determined for deglycosylated gp55 and gp65 by SDS-PAGE (12).

Identity of gp55 and gp65 with the Polypeptides Encoded by the Cloned cDNAs—To test if the cDNAs isolated with SMgp65 actually encode gp55 and gp65, new antibodies were produced against the recombinant proteins covering the common extracellular part of both molecules (Fig. 1). Two different rabbit antisera were obtained that gave identical results. The polyclonal antibodies recognized the same protein bands of 55 and 65 kDa on Western blots of brain membrane proteins as SMgp65 (Fig. 2A, lanes 1 and 3). Consistent with previous data (12), protein cores of 28 and 40 kDa were detected by both antibodies after.
The deglycosylation of the membrane protein fraction with endo-F (Fig. 2A, lanes 2 and 4). Furthermore, the 55- and 65-kDa antigens immunoprecipitated from detergent soluble membrane proteins by the rabbit antisera are detected by SMgp65 on Western blots (Fig. 2B, lane 4). No gp55 or gp65 immunoreactive bands were detected in control experiments using the rabbit antisera.
were treated with tunicamycin to determine the apparent mo-

lanes 5, 65 kDa in gp65-transfected cells (Fig. 2). Analysis (Fig. 2) was established for both proteins as revealed by Western blots, immunoreactive protein bands of 45 and 65 kDa were detected in detergent extracts of gp55-transfected cells (Fig. 2C, panel a, lane 2) and of 55 and 65 kDa in gp65-transfected cells (Fig. 2C, panel a, lane 5). As the sizes of the expressed proteins did not correspond to the expected sizes of 55 and 65 kDa, transfected cells were treated with tunicamycin to determine the apparent mol-

FIG. 2. Confirmation that gp55 and gp65 are the polypeptides encoded by the cloned cDNAs. A, immunoblots containing 20 µg lane of an untreated SM protein fraction (lanes 1 and 3) and endo-F-digested SM (lanes 2 and 4) were developed using polyclonal rabbit anti-gp55/65 antisera (lanes 1 and 2) or mAb SMgp65 (lanes 3 and 4). B, gp55 and gp65 were immunoprecipitated from a detergent-soluble fraction of rat brain proteins with polyclonal rabbit anti-gp55/65 anti-serum. Immunoblots were developed with the monoclonal SMgp65 antibody. Lanes 1 and 3 show supernatants of extracts immunoprecipitated with pre-immune and immune serum, respectively. Lanes 2 and 4 show precipitates from extracts incubated with pre-immune and immune serum, respectively. Lane 5 shows detergent-soluble protein fraction (20 µg) used as starting material (St mat). Identical results were obtained with a second anti-gp55/65 antisera (not shown). C, expression of gp55 and gp65 in HEK 293 cells. Panel a, detection of gp55 and gp65 in transfected HEK293 cells on immunoblots with mAb SMgp65 antibody. D, Detergent-soluble protein fractions (20 µg/lane) of untransfected cells (lane 1), gp55-transfected cells (lanes 2 and 3), and gp65-transfected cells (lanes 5 and 6) were applied. Cells of lanes 3 and 6 were treated with tunicamycin (+T) for 20 h before extraction. Lane 4 contains 20 µg of detergent-soluble protein fraction (St mat). Panels b–d, immunofluorescence with rabbit polyclonal anti-gp55/65 antisera in combination with goat anti-rabbit fluorescein isothiocyanate-conju-gated secondary antibody on untransfected (b), gp55-transfected (c), and gp65-transfected (d) 293 cells.

pre-immune sera (Fig. 2B, lane 2).

Additional confirmation of the identity of gp55 and gp65 with the proteins encoded by the cloned cDNAs was obtained from studies of human embryonic kidney 293 cells stably transfected with these cDNAs. In contrast to other cell lines examined, 293 cells only weakly express gp55 transcripts and do not express gp65 (Fig. 3C). Furthermore, no immunoreactivity was detected in 293 cells either on immunoblots using mAb SMgp65 (Fig. 2C, panel a, lane 1) or by immunocytochemistry using rabbit polyclonal antisera against recombinant gp55/gp65 (Fig. 2C, panel b). Several stably expressing transfected cell lines were established for both proteins as revealed by Western analysis (Fig. 2C, panel a, lanes 2 and 5) and immunocytochemistry (Fig. 2C, panels c and d). On Western blots, immunoreactive protein bands of 65 and 45 kDa were detected in detergent extracts of gp55-transfected cells (Fig. 2C, panel a, lane 2) and of 55 and 65 kDa in gp65-transfected cells (Fig. 2C, panel a, lane 5). As the sizes of the expressed proteins did not correspond to the expected sizes of 55 and 65 kDa, transfected cells were treated with tunicamycin to determine the apparent mol-

FIG. 3. Northern analysis of the tissue distribution of gp55 and gp65 transcripts. Nylon filters containing 20 µg lane total RNA were hybridized with a radiolabeled probe derived from gp55/65 common cDNA (panels A and C, lanes 1–3, probe 1) or with a gp65-specific probe (panels B and C, lanes 4–6, probe 2) (for localization of the probes, see Fig. 1A). A, tissue distribution of gp55 and gp65 transcripts in 30-day-old rats (lane 1, kidney; lane 2, spleen; lane 3, thymus; lane 4, skeletal muscle; lane 5, heart; lane 6, liver; lane 7, cerebral cortex; lane 8, cerebellum; lane 9, hippocampus). B, tissue distribution of gp65-specific transcripts (lane 1, skeletal muscle; lane 2, heart; lane 3, liver; lane 4, cerebral cortex; lane 5, cerebellum; lane 6, hippocampus). C, gp55 and gp65 transcripts in permanent cell lines (lanes 1 and 4, C6 glioma cells; lanes 2 and 5, HEK293 cells; lanes 3 and 6, PC12 pheochromocytoma cells).

lecular mass of the non-glycosylated protein moiety expressed. This treatment resulted in the production of polypeptides corresponding to the molecular masses of deglycosylated gp55 and gp65, i.e. 28- and 40-kDa polypeptides for the cells transfected with the gp55 and gp65 cDNAs, respectively (Fig. 2C, panel a, lanes 3 and 6). These data indicate that when gp55 and gp65 are expressed in 293 cells, they are glycosylated differently compared with the brain glycoforms.

Immunocytochemical staining of gp55 and gp65-transfected 293 cells with rabbit polyclonal antibodies against recombinant gp55/65 shows prominent staining of the cell surface (Fig. 2C, panels c and d). This indicates a normal targeting to the cell membrane of gp55 and gp65 in 293 cells.

Tissue Distribution of gp55 and gp65—Northern blot analysis with a probe common for gp55 and gp65 (Fig. 1, probe 1) revealed two transcripts of 2.5 and 2.2 kb in several brain regions, namely cortex, cerebellum, and hippocampus (Fig. 3A, lanes 7–9), but only a single 2.2-kb transcript in other tissues, including kidney, spleen, thymus, skeletal muscle, heart, and liver (Fig. 3A, lanes 1–6). A probe derived from the gp65-specific cDNA insert (Fig. 1, probe 2) detected only the brain-specific 2.5-kb band (Fig. 3B). No hybridization signal was observed in other tissues or cell lines (Fig. 3, B, lanes 1–3, and C, lanes 4–6). Within the brain, gp55 transcripts appeared to be distributed differentially. They were strongly detectable in the hippocampus and the cerebral cortex, whereas much lower amounts were detected in the cerebellum and the lower brain regions. Thus the 2.5-kb transcript encodes the brain-specific gp55, whereas the 2.2-kb transcript encodes gp55. We have also tested various cell lines for their expression of gp55 and gp65. C6 rat glioma cells and PC12 rat pheochromocytoma cells strongly express gp55 transcripts (Fig. 3C), whereas only a very weak hybridization signal was obtained from HEK 293 cells. Gp65 transcripts were not detected in any of the cell lines tested (Fig. 3C, lanes 4–6).

The Northern data demonstrated that the gp65 transcript is brain-specific, whereas gp55 transcripts are ubiquitous. As this is not in agreement with our previously published immunoblot data (11) that suggested that both gp55 and gp65 were brain-specific, further experiments were carried out to clarify this
Immunoblot analysis of samples of detergent-solubilized membrane proteins (20 µg/lane) from liver and brain incubated in the absence (lanes 1 and 3) or presence of endo-F (lanes 2 and 4). One dominant band of 28 kDa was detected in the endo-F-treated samples from liver. Two prominent bands of 40 and 28 kDa were detected in the endo-F-treated brain sample. Note, other bands present in the endo-F-treated samples represent partially deglycosylated products.

Two prominent bands of 40 and 28 kDa were detected in the endo-F-treated brain sample. Note, other bands present in the endo-F-treated brain sample have been shown to correspond to partially deglycosylated products (12).

In situ hybridization experiments with a gp65-specific and a common oligonucleotide probe were performed to study the distribution of gp65 and gp55 within the brain in more detail. Sense oligonucleotide probes did not yield any hybridization signal (not shown). The oligonucleotide probe that recognizes both gp55 and gp65 transcripts gave strong, almost homogeneous labeling throughout the brain (Fig. 5, A and B). In contrast, marked regional differences in the expression of gp65 transcripts were observed in experiments using the gp65-specific oligonucleotide probe (Fig. 5, C and D). Particularly strong expression occurs in the hippocampus, striatum, and cerebral cortex. In the cerebellum, gp65 expression appears somewhat reduced as compared with the forebrain regions. In the midbrain and the brain stem, only low amounts of gp65 transcripts were detected. This indicates that, in these regions, the hybridization signal observed for the common probe derived primarily from hybridization to gp55 transcripts.

Expression of gp55 and gp65 During Postnatal Development—To study the developmental expression of gp55 and gp65 transcripts, Northern analyses of samples prepared from animals of postnatal age of 1–50 days were performed using common probe 1 and the gp65-specific probe 2 (Fig. 1). Whereas significant amounts of gp55 mRNA were detectable at all developmental stages starting from postnatal day 1 (Fig. 6), the level of gp65 transcripts is low at postnatal day 1, increases steadily until postnatal days 20–25, and then declines to an intermediate level (Fig. 6B). Thus, the transcript levels parallel the developmental profiles for expression of gp65 and gp55, as revealed by Western blot analysis using SMgp65 (23).

DISCUSSION

The present study identifies the synapse-enriched glycoproteins gp65 and gp55 as members of the Ig superfamily. This is based on cloning and sequence analysis of the cDNAs isolated using the mAb SMgp65, which was employed to identify these glycoproteins (11). Evidence that the cDNAs do indeed encode gp65 and gp55 is that 1) antisera raised against recombinant gp55/65 was employed to identify these antisera are identical to those of deglycosylated gp65 and gp55; 2) the molecular masses of the deglycosylated proteins recognized by these antisera are identical to those of deglycosylated gp65 and gp55; 3) the molecular masses calculated from the deduced aa sequences are in good agreement with those published for the deglycosylated gp65 and gp55 molecules; and 4) the molecular masses of the products expressed in cells transfected with appropriate cDNA constructs and treated with tunicamycin are identical to the molecular masses of deglycosylated gp65 and gp55. Furthermore, the developmental profiles for expression of the 2.2- and 2.5-kb transcripts hybridizing to the cDNA in brain are in good agreement with those for gp65 and gp55 (23), and the distribution of transcripts as revealed by in situ hybridization is consistent with that reported for gp65 and gp55 glycoproteins (11).

The gp65 and gp55 molecules contain three and two Ig domains, respectively. Both proteins are produced from the same gene by alternative processing of the transcripts, confirming...
the previously suggested close structural relationship between the two glycoproteins (12). The sequence data show that gp65 and gp55 differ by a single 116 aa insert that encodes an Ig domain located at the N terminus of the molecule. This is in good agreement with the published data that provided evidence that the two molecules differed by a single 10–12-kDa cell-surface peptide sequence, which contains little or no carbohydrate (12). Indeed, gp55 and gp65 both contain six potential N-glycosylation sites.

Similarity of gp55 and gp65 to the Neurothelin/Basigin Group of Ig Domain Proteins—Numerous members of the Ig superfamily have now been described (24). Comparison of the sequence data for gp55 and gp65 with those of other Ig superfamily members revealed that gp55 exhibits highest sequence homology with other species expressing two Ig domains. The highest degree of identity is shown with the HT7 antigen (25) and several species homologs of this protein (Fig. 7A). HT7 antigen (also known as neurothelin or 5A11 antigen) was originally identified as a protein expressed in the blood-brain barrier endothelium and in distinct neurons of chick central nervous system (25–30). Its species homologs include the mouse protein basigin (31, 32) (also known as gp42, see Ref. 33), the MRC OX-47 antigen from rat (34) and human M6 antigen (also named EMMPRIN) (35, 36). Sequence homologies range from 40–44%. Interestingly, the highest degree of homology occurs within and around the transmembrane domain and in the intracellular domain (Fig. 7A). All proteins contain a conserved charged aa (Glu-204 in gp55) within the transmembrane region, which might be involved in the interaction with other membrane proteins and/or in signal transduction events (22, 25). A sequence comparison between the gp65-specific Ig loop and aa sequences encoded in the first intron of the basigin gene (37) revealed about 50% identity (Fig. 7B). This raises the questions as to whether the basigin gene may have an additional, alternatively spliced exon, which has been previously overlooked, and whether three Ig loop isoforms of basigin and its species homologs may also exist.

Differential Glycosylation of gp55—Analysis of tissue distribution by Northern and Western blotting confirmed that gp65 is brain-specific, whereas gp55 is expressed in a wide range of tissues. However, gp55 exists as a number of glycoforms. The originally described gp55 glycoform is detected only in brain. In contrast, the gp55 glycoforms of apparent molecular masses of 44 and 61 kDa detected in liver are also present in other adult tissues examined (unpublished data). These results supersede previously published data (11) that suggested that gp55 was also brain-specific. This discrepancy is probably due to use of homogenates rather than detergent-solubilized membrane proteins coupled with use of a less sensitive visualization procedure in the earlier study.

The occurrence of tissue-specific glycoforms has also been reported for many glycoproteins including neurothelin, basigin (30, 38), and Thy-I (the smallest member of the Ig superfamily), which is a prime example for differential glycosylation (39). In this context, it should be noted that gp65 expressed by transfected HEK 293 cells is glycosylated differently from brain gp65.

Possible Functions of gp65 and gp55—The functions of gp65 and gp55 have yet to be established. However, members of the Ig superfamily other than antibodies fulfill two major functional roles, namely cell-cell and cell-substrate recognition and adhesion and as receptors for growth factors (22, 24, 40). Gp65 and gp55 do not possess consensus sequences characteristic of receptor kinase domains of classical growth factor receptors. It is therefore most plausible that they are involved primarily in recognition and adhesion phenomena though distinct signaling

![Fig. 7. Alignment of gp55 and gp65 protein sequences with members of the neurothelin/basigin group of Ig domain proteins. A. GenBank™/EBI data base accession numbers of the aligned sequences are: X52751 (HT7 antigen, neurothelin), D00611 (basigin), X54640 (MRC OX47 antigen), and X64364 (M6 antigen). The aa residues identical in all aligned proteins are indicated by asterisks. The transmembrane segment is underlined. B. Alignment of the gp65-specific Ig loop with the peptide sequence deduced from nucleotides 6798 to 7145 of the first intron of the basigin gene (37). Note, there are also similarities with more than 30 expressed sequence tags included in the data bases.](https://example.com/fig7.png)
in situ hybridization results are in good agreement with the biochemical and immunocytochemical data on regional localization of gp65 (11). Taken together, these data show that gp65 is localized to the neurites and nerve terminals of subsets of neurons that are predominantly localized to forebrain regions including cortex, hippocampus, and striatum. Gp65 is most enriched in the PSD fraction. Developmentally, gp65 is only expressed postnatally, and its level both in homogenates and in situ cellular functions of these glycoproteins.

It will be suggested that it interacts with other cell surface and cytoskeletal components. Disruption of cell-cell contacts induced a rapid change in neurothelium distribution, which was hampered by disruption of microfilaments (28). Along this line, it will be plausible that gp65 may also be involved in formation and/or stabilization of particular subsets of synapses. In contrast to gp65, gp55 is expressed early in brain development and reaches the adult level by postnatal day 9. Furthermore, it is expressed reasonably uniformly in all brain regions. Gp55 transcripts occur in a wide range of cell types, and gp55 may not be neuron-specific in the brain. Therefore, it seems unlikely that gp55 has a specific role in synapse formation or stabilization but may be a positive or negative effector of adhesion events between a wide range of cell types.

Although the function of neurothelin, the Ig family member most closely related to gp55, remains unclear, recent evidence suggests that it interacts with other cell surface and cytoskeletal components. Disruption of cell-cell contacts induced a rapid change in neurothelium distribution, which was hampered by disruption of microfilaments (28). Along this line, it will be interesting to identify molecules that bind to the extra- and intracellular domains of gp65 and gp55 to establish the cellular functions of these glycoproteins.

Acknowledgments—We are grateful to Kathrin Hartung, Rosemary Mummery, Kathrin Schumacher, and Kathrin Zobel for expert technical assistance, to Werner Zschatter for help with the confocal microcopy, to Reinhard Fassler for the gift of mammalian transfection vector, and to Constanze Seidenbecher and Karl-Heinz Smalla for many helpful suggestions and discussions throughout this work.

REFERENCES

1. Gurd, J. W. (1980) Can. J. Biochem. 58, 941–951
2. Gurd, J. W. (1989) in Neurobiology of Glycoconjugates (Margolis, R. U., and Margolis, R. K., eds) pp. 219–242. Plenum Press, NY
3. Moon, I. S., Apperson, M. L., and Kennedy, M. B. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 3954–3958
4. Beesley, P. W., Paladino, T., Gravel, C., Hawkes, R. A., and Gurd, J. W. (1987) Brain Res. 408, 65–78
5. Gloor, S., Nasse, K., Essen, L., and Appel, P. (1992) Gene (Amst.) 120, 307–312
6. Willott, T. G., Selkirk, C. P., Hawkes, R. B., Philippe, E., Gordon-Weeks, P. R., and Beesley, P. W. (1991) Neuroscience 44, 627–641
7. Willott, T., Williamson, T. L., Mummery, R., Hawkes, R. B., Can, A. G., Gurd, J. W., Gordon-Weeks, P. R., and Beesley, P. W. (1994) Neuroscience 58, 115–129
8. Beesley, P. W., Mummery, R., and Tihalidi, J. (1995) J. Neurochem. 64, 2288–2294
9. Bahr, B. A., and Lynch, G. (1992) Biochem. J. 281, 137–142
10. Persohn, E., Pollerberg, G. E., and Schachner, M. (1989) J. Comp. Neurol. 288, 92–100
11. Hill, I., Selkirk, C. P., Hawkes, R. B., and Beesley, P. W. (1988) Brain Res. 461, 27–43
12. Willott, T., Skitsa, I., Hill, I., Mummery, R., and Beesley, P. W. (1992) J. Neurochem. 58, 2037–2043
13. Kistner, U., Wenzel, B. M., Veh, R. W., Cases-Langhoff, C., Garner, A. M., Appeltauer, U., Voss, B., Gundelfinger, E. D., and Garner, C. C. (1993) J. Biol. Chem. 268, 4580–4583
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
15. Cheregin, J. J., Przychy, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294
16. Wissen, W., and Morris, B. J. (1994) In In Situ Hybridization Protocols for the Brain (Wissen, W., and Morris, B. J., eds) pp. 9–34, Academic Press, London
17. Burgin, K. E., Waxham, M. N., Rickling, S., Westgate, S. A., Mobley, W. C., and Kelly, P. T. (1990) J. Neurosci. 10, 1788–1798
18. Lammi, U. K. (1979) Nature 283, 696–698
19. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4350–4354
20. Mayer, U., Nischt, R., Poschl, E., Mann, K., Fukuda, K., Gerl, M., Yamada, Y., and Timpl, R. (1993) J. Cell Biol. 122, 1473–1485
21. von Heijne, G. (1986) Nucleic Acids Res. 14, 4683–4690
22. Williams, A. P., and Barclay, A. N. (1988) Annu. Rev. Immunol. 6, 381–405
23. Hill, I., Willott, T., Skitsa, I., Selkirk, C., Murphy, S., Gordon-Weeks, P., and Beesley, P. W. (1989) Biochem. Soc. Transactions 17, 770–771
24. Brummendorf, T., and Rathjen, F. G. (1995) J. Neurosci. 15, 1129–1140
25. Schlosshauer, B., and Herzog, K.-H. (1990) J. Comp. Neurol. 288, 27–43
26. Schlosshauer, B. (1991) Development 113, 129–140
27. Schlosshauer, B., Bauch, H., and Frank, R. (1995) Eur. J. Cell Biol. 68, 159–166
28. Fadool, J. M., and Linser, P. J. (1993) Biochemistry 32, 252–262
29. Fadool, J. M., and Linser, P. J. (1993) J. Neurochem. 60, 1354–1364
30. Miyazaki, T., Kanekura, T., Yamaoka, A., Ozawa, M., Miyazawa, S., and Muramatsu, T. (1990) J. Biochem. (Tokyo) 107, 316–323
31. Miyasaka, T., Matsuura, S., and Muramatsu, T. (1991) J. Biochem. (Tokyo) 110, 770–774
32. Alltruda, F., Cervella, P., Gaeta, M. L., Daniele, A., Giancotti, F., Tarone, G., Stefano, G., and Silengo, L. (1989) Gene (Amst.) 85, 445–452
33. Fossun, S., Mallett, S., and Barclay, A. N. (1991) Eur. J. Immunol. 21, 671–679
34. Kisinrerek, W., Fiebig, E., Stefanova, I., Baumruker, T., Knapp, W., and Stockinger, H. (1992) J. Immunol. 149, 847–854
35. Biswas, C., Zhang, Y., DeCastro, R., Guo, H., Nakamura, T., Kataoka, H., and Nishizawa, K. (1995) Cancer Res. 55, 434–439
36. Miyazaki, T., Kimura, F., Ishiguro, T., Yu, S., Ozawa, M., and Muramatsu, T. (1995) J. Biochem. (Tokyo) 118, 717–724
37. Kanekura, T., Miyazaki, T., Tashiro, M., and Muramatsu, T. (1991) Cell Struct. Funct. 16, 23–30
38. Rademacher, T. W., Parekh, R. B., and Dwek, R. A. (1988) Annu. Rev. Biochem. 57, 785–838
39. Ullrich, A., and Schlessinger, J. (1990) Cell 61, 203–212
40. Rosales, C., O’Brien, V., Kornberg, L., and Juliano, R. (1995) Biochemist. 12, 1–8
41. Parves, D., and Lichtman, J. W. (1985) Principles of Neural Development, Sinauer Associates Inc. Publishers, Sunderland, MA
42. Steward, O. (1987) Progr. Brain Res. 71, 267–279
43. Leclere, N., Beesley, P. W., Brown, I., Colonniier, M., Gurd, J. W., Paladino, T., and Hawkes, R. (1989) J. Comp. Neurol. 280, 197–212
44. Steward, O., and Falk, P. M. (1991) J. Comp. Neurol. 314, 545–557
45. Doherty, P., Fazzii, M. S., and Walsh, P. S. (1995) J. Neurobiol. 26, 437–446
46. Takeichi, M. (1990) Annu. Rev. Biochem. 59, 237–252

I. Hill and P. W. Beesley, unpublished results.