Gα is the major G protein in bovine brain, with at least three isoforms, GαA, GαB, and GαC. Whereas GαA and GαB arise from a single Gα gene as alternatively spliced mRNAs, Gαα and Gαc are thought to differ by covalent modification. To test the hypothesis that αoα and αoc have different N-terminal lipid modifications, proteolytic fragments of αo were immunoprecipitated with an N terminus-specific antibody and analyzed by matrix-assisted laser desorption ionization mass spectrometry. The major masses observed in immunoprecipitates were the same for all three Gα isoforms and corresponded to the predicted mass of a myristoylated N-terminal fragment. Structural differences between αoA and αoc were also compared before and after limited tryptic proteolysis using SDS-polyacrylamide gel electrophoresis containing 6 M urea. Based upon the αo subunit fragments produced under activating and nonactivating conditions, differences between αoA and αoc were localized to a C-terminal fragment of the protein. This region, involved in receptor and effector interactions, implies divergent signaling roles for these two Gα proteins. Finally, the structural difference between αoA and αoc is associated with a difference of at most 2 daltons based upon measurements by electrospray ionization mass spectrometry.

G proteins, composed of an α subunit bound to a βγ dimer, mediate the effects of many extracellular ligands that act on specific cell surface receptors (1). Gα is the major brain G protein, comprising up to 1% of particulate protein in bovine brain (2, 3). The exact function of Gα is still unclear, but it has been implicated in the regulation of voltage-gated calcium channels (4–6); the activation of the mitogen-activated protein kinase pathway (7); the development of neuronal growth cones (8), where it is highly concentrated (9); and the regulation of vesicle trafficking (10–13).

The role of the αo subunit on the level of the whole animal has also been studied. In Caenorhabditis elegans, the αo protein influences behaviors such as locomotion and reproduction (14, 15). Mice lacking the αo gene are afflicted with tremors and seizures (16, 17) and show loss of motor control and a propensity to run continuously in circles in a counterclockwise direction (17). In both studies with mice deficient in αo, life span was significantly reduced. Although the precise function of αo in neuronal tissue remains to be defined, in the knockout mice, regulation of Ca2+ channels by opioid receptors in dorsal root ganglion cells is altered (17), as is regulation of L-type calcium channels in heart (16). Thus, the expression of αo is clearly required for normal neuronal function.

One gene codes for the αo subunit, which gives rise in brain to multiple splice variants with two different coding sequences contained in mRNAs αoA and αoc (18–20). Based upon protein characterization, however, there are at least four αo isoforms in bovine brain, αoA, αoB, αoC, and αoD (21–23). Three of the isoforms, αoA, αoB, and αoC, are purified associated with βγ dimers as GαA, GαB, and GαC, while αoD, an inconsistently observed αo isoform, is not. Immunological evidence and peptide mapping suggest that αoB and αoD are protein products of the αo mRNA, whereas αoA and αoC are translated from the αo1 mRNA (23–26). The functional significance of these different αo isoforms is not yet clear, but the GαA, GαB, and GαC heterotrimers each contain a different assortment of βγ dimers (23).

An αoC-like protein has been observed by a number of groups (21–23, 27–30). Recently, we have found that it is a relatively abundant protein in brain, constituting about a third of the Gα of cerebral cortex.1 Several studies have tried to identify the way in which αoC differs from its immunologically related αoA protein (23–26) but without success. One suggestion is that αoC differs from αoA at the N terminus and that it might be an unmyristoylated form of the protein (29, 30). Such speculations are more plausible by the fact that the related αo protein is heterogeneously modified at the N terminus (31, 32). In this paper, mass spectrometry of intact α subunits and limited tryptic proteolysis studies, in conjunction with urea/SDS-PAGE2 and matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) mass spectrometry, were used to determine the region of the structural differences between αoA and αoC. Surprisingly, the masses of the αoA and αoC proteins do not differ significantly despite their different mobilities on urea/SDS-PAGE. The N termini of αoA, αoB, and αoC are identical, but a region located at the C terminus was different between αoA and αoC, analogous to the region differing between αoA and αoB. This structural difference is localized to a part of the protein suggesting differential interactions of GαA and GαC with receptors or effectors.

1 W. E. McIntire, J. Dingus, M. D. Wilcox, and J. D. Hildebrandt, unpublished observations.

2 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; ESI, electrospray ionization; GTPγS, guanyl-5′-imidodiphosphate; MALDI, matrix-assisted laser desorption ionization; MS, mass spectrometry; MS/MS, tandem mass spectrometry; DTT, dithiothreitol; HPLC, high pressure liquid chromatography.

This paper is available on line at http://www.jbc.org
**EXPERIMENTAL PROCEDURES**

**Purification of G Protein Isoforms**—G proteins were purified from bovine brain using a modification (33, 34) of the method of Sternweis and Robishaw (2). Isoforms of G proteins were purified using a Mono Q anion exchange column with a 0–300 mM NaCl gradient (23). G protein subunits were separated in the presence of aluminum, magnesium, and fluoride as described previously (33, 34).

**Production of Site-specific Antibodies**—Synthetic peptides corresponding to the αN terminus, GCTLSAEERAALERSKAIEKNLKE (antiserum AON, residues 2–25) and the C terminus, AKNLRCGGLY (antiserum AOC, residues 345–354) were coupled to keyhole limpet hemocyanin with glutaraldehyde and injected into rabbits, using the method described in Green et al. (35). The A01 antibody, specific for the protein products of the α1 splice variant, was described previously (23).

**Digestion**—G proteins were digested with L-1-tosylamide-2-phenylethyl chloromethyl ketone-treated trypsin (Sigma) using a method modified from Winslow et al. (36). One to two μg of purified Gα or αi isoform was incubated with 20 μm Tris, pH 8.0, 0.1% Thesit, 1 mM EDTA, 1 mM DTT, 150 mM NaCl, 25 mM MgCl₂, either 1 μM GTP-S (Boehringer Mannheim) or 100 μM GDP (Sigma), and trypsin (1:25 (w/w) for GTP-S and 1:50 (w/w) for GDP). Samples with GTP-S were incubated for 30 min at 32 °C; those with GDP were incubated for 45 min at 32 °C. Prior to gel electrophoresis, reactions were stopped by boiling with SDS sample buffer for 5 min. G proteins digested with AspN (Boehringer Mannheim; 1:50 (w/w)) were incubated with 20 μm Tris, pH 8.0, 0.125% cholate, 1 mM EDTA, 1 mM DTT, 25 mM MgCl₂, 10 mM NaF, and 50 μM AlCl₃ overnight at 32 °C. Reactions were stopped by boiling for 5 min.

**SDS Polyacrylamide Gel Electrophoresis and Immunoblotting**—Mobility of proteins and proteolytic fragments was determined using SDS-polyacrylamide gel electrophoresis according to the methods of Laemmli (37). Gels contained 11% acrylamide, 0.29% bisacrylamide without or with 6 μl urea (28). Protein bands were stained with Coomassie Blue or silver (39). Immunoblots were performed by the method described in Towbin et al. (40); proteins were transferred onto nitrocellulose or membranes using semidy transfer apparatus using methanol-free buffer. Immunoreactivity was visualized using ECL reagents (NEN Life Science Products).

**Immunoprecipitation**—Approximately 5 μl of Protein A immobilized on Sepharose 6MB (Protein A-Sepharose) was washed twice with 500 μl of 50 mM Tris, pH 8.0, 150 mM NaCl (TBS), blocked in the above buffer with 2.5 mg/ml bovine serum albumin for 30 min, and washed four more times with TBS. Antiserum (1:25) in 300 μl of 20 mM Tris, pH 8.0, 1 mM EDTA, 1 mM DTT, 20 mM NaCl, and 0.01% Thesit (TEDNT) was incubated with 5 μl of beads at room temperature for 1 h. Excess antibody was removed by washing five times with 500 μl of TEDNT. Proteolytic digests were diluted to approximately 150 μl with TEDNT and incubated with prepared beads for 1 h at room temperature. Unbound peptides were removed by washing twice with 500 μl of TEDNT and once with 500 μl of water. Bound peptide was removed by washing twice with 500 μl of TEDNT.

**MALDI Mass Spectrometry**—Analysis was performed using a Voyager-DE MALDI mass spectrometer (PerSeptive Biosystems). One μl of the n-propanol/water/trifluoroacetic acid mixture containing the peptide was combined with 1 μl of matrix, 50 μM α-cyan-4-hydroxy-cinnamic acid (Aldrich) in 70% acetonitrile, 0.1% trifluoroacetic acid. Samples were spotted on a sample plate and allowed to crystallize at room temperature. Masses were internally calibrated by including peptides with known masses. Typically, 256 laser shots were averaged to produce a mass spectrum.

**HPLC and Mass Spectrometry of intact α Isoforms**—A 46 × 30 mm Aqueapore phenyl column (Brownlee) was used to purify 0.25 nmol (10 μg) of αA and αC. Buffer A was 90% aqueous, 10% acetonitrile with 0.1% trifluoroacetic acid, and buffer B was 90% n-propanol, 10% acetonitrile with 0.1% trifluoroacetic acid. Proteins eluted with a linear gradient of 2–98% B over 20 min at 0.5 ml/min, and absorbance at 214 nm was monitored. Approximately 5% of the eluate was analyzed by a Finnigan LCQ ion trap with an ESI source, while the other 95% was collected as fractions at 1-min intervals.

**HPLC and MS/MS of Limited Tryptic Digests**—Proteolytic fragments of αA and αC were separated on a 4.6 × 30-mm Aqueapore phenyl column (Brownlee). Buffer A was 90% aqueous, 10% acetonitrile with 0.1% trifluoroacetic acid, and buffer B was 75% acetonitrile, 25% isopropyl alcohol with 0.005% trifluoroacetic acid. A linear gradient of 2–98% B over 40 min at 0.5 ml/min was used, and absorbance at 214 nm was measured during the gradient. Five percent of the eluate was analyzed by a Finnigan LCQ ion trap with an ESI source, while the other 95% was collected as fractions at 1-min intervals. Tandem MS was performed by selection and fragmentation of the m/z corresponding to the [M + 2H]⁺ ion of the α isoforms of interest at a collision energy of 20–50%.

**Characterization of α Variants Using Site-specific Antibodies**—Fig. 1A shows a map of the αm protein, its known sites of modification, and the approximate sites of epitopes for the antibodies used here. Three Gα isoforms were isolated from bovine brain G proteins by their differential elution on FPLC (23). The Coomassie Blue-stained proteins are shown in Fig. 1B and immunoblots with site-specific antibodies in Fig. 1C. All three proteins reacted with antisera to the N and C termini of GαA and GαC (Fig. 1B). The AO1 antisera (23) was raised to the most variable region of the α2 splice variant, was described previously (23). The AO1 antibody, specific for the protein products of the α1 splice variant, was described previously (23).

**Analysis of Modified Proteins**—Molar quantities of the α isoforms were separated on a 4.6 × 30-mm Aqueapore phenyl column (Brownlee). Buffer A was 90% aqueous, 10% acetonitrile with 0.1% trifluoroacetic acid, and buffer B was 75% acetonitrile, 25% isopropyl alcohol with 0.005% trifluoroacetic acid. A linear gradient of 2–98% B over 40 min at 0.5 ml/min was used, and absorbance at 214 nm was measured during the gradient. Five percent of the eluate was analyzed by a Finnigan LCQ ion trap with an ESI source, while the other 95% was collected as fractions at 1-min intervals. Tandem MS was performed by selection and fragmentation of the m/z corresponding to the [M + 2H]⁺ ion of the α isoforms of interest at a collision energy of 20–50%.

**Characterization of α Variants Using Site-specific Antibodies**—Fig. 1A shows a map of the αm protein, its known sites of modification, and the approximate sites of epitopes for the antibodies used here. Three Gα isoforms were isolated from bovine brain G proteins by their differential elution on FPLC (23). The Coomassie Blue-stained proteins are shown in Fig. 1B and immunoblots with site-specific antibodies in Fig. 1C. All three proteins reacted with antisera to the N and C termini of GαA and GαC (Fig. 1B). The AO1 antisera (23) was raised to the most variable region of the α2 splice variant, one indicative of the α31 mRNA. These data indicate that αA and αC are similar at a site that is variable among known α splice variants and supports the conclusion (23, 24, 27) that these two proteins used in a common mRNA and differ by some post-translational modification.

In the absence of urea, the α isoforms have the same electrophoretic mobility by SDS-PAGE, traveling at 39 kDa (Fig. 1, B and C). In the presence of 6 M urea, each of the α isoforms has a unique electrophoretic mobility (28) (Fig. 1D). This differential behavior on different kinds of gels is often indicative
of subtle structural differences (28, 41). This phenomenon was exploited here to identify the location of the structural difference in the proteins.

Analysis of Intact $G_o \alpha$ Subunits by ESI-MS—ESI-MS is potentially useful for the analysis of large proteins, capable of generating very precise and accurate mass measurements. Therefore, the $\alpha$ subunits of $G_oA$ and $G_oC$ were passed over an HPLC phenyl column in-line with a Finnigan LCQ ion trap mass spectrometer. The HPLC elution profile and co-migration of immunoreactive protein with the major UV peak observed in the separation are shown in Fig. 2, along with ESI mass spectra for the $\alpha$ subunits. These proteins are known to contain some kind of structural difference (22, 23, 27–30). Nevertheless, the two proteins had very similar masses by ESI-MS (Fig. 2B), and are, in fact, indistinguishable from each other within the accuracy of the instrument. The immunoblots in Fig. 2 (A and C) substantiate that the $G_oA$ and $G_oC$ proteins retained their inherent structural differences after HPLC separation. Thus, the $G_oA$ and $G_oC$ proteins do not differ significantly in mass but are nonetheless structurally different from one another.

The data in Fig. 2D allow us to assign a possible structure to the $G_oA$ protein. The observed mass was not easily reconcilable with the predicted sequence of the reported bovine brain $G_oA$ cDNA (P08239). It was, however, compatible with an alternative sequence reported by Ovchinnikov et al. (42), in which a Thr replaces Ile$^{262}$ in the protein. The 40,147.4-Da observed mass is then compatible with a protein myristoylated at Gly$^2$ after removal of Met$^1$, with no other modifications (predicted mass of 40,145.9 Da). This substitution was verified by both MS/MS and Edman sequencing. Since this mass was found repeatedly in our $G_o$ protein preparations, this alternative sequence represents the major one observed in the cattle used in our studies.

Analysis of N-terminal Fragments of the $G_o \alpha$ Proteins—The N termini of $G_o$ subunits are known sites of variable modification involved in several important interactions. Since N-terminal variability could explain the origin of $G_oA$ and $G_oC$, we immunoprecipitated N-terminal fragments of $G_oA$ and $G_oC$ with the AON antisera to evaluate their modifications by MALDI-MS (Fig. 3). Digestion of all three $G_o \alpha$ proteins with endoproteinase AspN is predicted to produce N-terminal fragments of residues 2–25 and 2–32 of the proteins. In the MALDI mass spectrum of the digest of $G_oA$ before immunoprecipitation, we observed numerous proteolytic fragments (Fig. 3B). Similar results were seen with $G_oB$ and $G_oC$ (data not shown). Immunoprecipitation of $G_oA$ digest with preimmune serum yielded no peaks in the low mass region. In contrast, the spectra of immunoprecipitates of $G_oA$, $G_oB$, and $G_oC$ digests with the AON antibody all showed precipitation of a fragment of about $m/z$ 2859 (Fig. 3B). These fragments were all very close in mass to that predicted (2858.4 Da) for an N-terminal AspN fragment of $G_oA$ and $G_oB$, 2–32 (Fig. 3B). Although the peak at $m/z$ 3501 could suggest possible conformational differences in the N termini of $G_oA$ and the other $G_o \alpha$ isoforms, it was also variably observed in other immunoprecipitation experiments with $G_oB$ and $G_oC$.

The data in Fig. 3B suggest that the fatty acyl group at the N terminus of $G_oA$ does not account for the difference between $G_oA$ and $G_oC$. Close examination of the MALDI spectra (Fig. 3C) did show a low level of heterogeneity in the $m/z$ range of the myristoylated N terminus. One mass peak at 2829, for example, was about 28 Da less than the major peak and could be

---

3 W. E. McIntire, K. L. Schey, and J. D. Hildebrandt, unpublished observations.
explained by a lauroylated N terminus. The other minor peaks were not easily assigned, and none of these gave large enough signals to consider characterization. These results suggest the possibility that there is minor heterogeneity at the N terminus of αo. This does not explain the difference between αoA and αoC, however, since similar heterogeneity was seen for all of the αoA proteins, and the heterogeneity of the N terminus of αo observed here is insignificant compared with that seen for transducin (31, 32). Thus, the major αoA, αoC, and αoC isoforms appear to be myristoylated with no other modifications.

The other component of known heterogeneity at the N terminus of G protein α subunits is degree of saturation of the fatty acid (31, 32). Such a difference could account for the negligible mass difference between αoA and αoC. There was insufficient resolution in the MALDI spectra (Fig. 3) to differentiate single sites of unsaturation in the myristoyl group. To address this issue, the two αo isoforms were digested with trypsin, and the N-terminal fragments were analyzed by HPLC coupled to a Finnigan LCQ ion trap mass spectrometer. From the data generated with this instrument, an ion chromatogram can be constructed showing the distribution of selected ions of a given m/z within ±0.5 units. This is sufficient resolution to differentiate mono- and diunsaturated fatty acid acylation of peptide fragments. Fig. 4 (A and B) shows total ion chromatograms for the separation of digests of αoA and αoC (top tracing) and analogous selected ion chromatograms for the myristoylated N termini of αoA and αoC with zero, one, and two sites of unsaturation. The total ion currents are heavily dominated by buffer constituents as well as protein fragments. Nevertheless, both proteins have a well defined peak at about 19 min for a selected mass of 859.0, corresponding to an N-terminal fragment with a fully saturated myristic acid (2–15 + C14:0). The assignment of this peak was confirmed by MS/MS sequencing of the peptide from αoA (Fig. 4, C and D).

When ions corresponding to a myristoylated fragment containing one or two double bonds were selected (Fig. 4, 2–15 + C14:1 and 2–15 + C14:2, respectively), there was relatively little signal. The minor peaks eluting at 21 min co-elute with a major peak in the total ion current chromatogram, suggesting a possible nonspecific signal, and others have found that myristoylated peptides with one and two double bonds in the acyl chain elute earlier than peptides with fully saturated myristic acid by hydrophobic interaction chromatography (31), not later as seen here (Fig. 4). These data indicate that significant amounts of mono- and diunsaturated forms of myristic acid are not incorporated into αoA and αoC, and that heterogeneity of the N-terminal lipid does not explain the difference between αoA and αoC.

Identification of the Region of αoA and αoC with Structural Differences—Trypsin preferentially cleaves an N-terminal 2-kDa fragment from G protein α subunits activated with GTPγS (36, 43) (Fig. 1A). Removal of this fragment allows direct comparison of the electrophoretic mobilities of the remaining 37-kDa fragments by urea/SDS-PAGE. AON immunoreactivity was completely lost from all isoforms after limited tryptic digestion, confirming loss of the N terminus (Fig. 5A). The AO1 antibody, however, recognized both the intact αoA and αoC isoforms, as well as the 37-kDa trypsin fragments generated by digestion of the GTPγS-activated proteins (Fig. 5A). Retention of the difference in mobilities by the 37-kDa fragments confirmed that the structural difference between αoA and αoC does not result from differences in their N-terminal modifications.

When G protein α subunits are briefly digested with trypsin in the presence of GDP, the 37-kDa C-terminal fragment is further cleaved into an N-terminal −25-kDa fragment and a C-terminal −17-kDa fragment (Fig. 1A) (36). Immunoblotting of digests after urea/SDS-PAGE with the AOC antibody showed that the 17-kDa C-terminal fragments of αoA and αoC retain the difference in mobility as do the intact α subunits (Fig. 5B), while the 25-kDa fragments have identical mobilities.

Characterization of the intact α subunits by ESI-MS (Fig. 2) indicated that the difference between αoA and αoC is associated with a negligible mass change. Our results above (Fig. 5) indicated that the difference between αoA and αoC is contained within the 17-kDa fragment. We therefore generated the 17- and 25-kDa fragments from GoA and GoC, isolated them by HPLC, and characterized them by ESI mass spectrometry (Fig. 6). ESI-MS allowed us to determine the precise cleavage sites of the 17- and 25-kDa fragments and showed that the masses were essentially identical for both fragments, as originally suggested from the analysis of the intact proteins (Fig. 2) and despite their difference in electrophoretic mobility (Fig. 5B). These studies localize the difference between the αoA and αoC proteins to the 17-kDa fragment and, considering the mass accuracy of the instrument, the difference between these fragments would be restricted to less than 2 daltons.
**Fig. 4. Analysis of the N-terminal peptides from \( \alpha \)-isoform digests separated by HPLC and analyzed on an LCQ ion trap mass spectrometer. A, analysis of \( \alpha_{\text{OA}} \) digest; B, analysis of \( \alpha_{\text{OC}} \) digest. For A and B, from top to bottom, are shown the total ion current from the HPLC separation; the selected ion chromatogram for an N-terminal fragment containing a fully saturated myristoylated (C14:0) N-terminal peptide ([M + 2H]\(^{2+} = 859.0\); range: 858.5–859.5), a selected ion chromatogram for an N-terminal fragment with a monounsaturated myristoyl (C14:1) group ([M + 2H]\(^{2+} = 858.0\); range: 857.5–858.5), and a selected ion chromatogram for an N-terminal fragment with a diunsaturated myristoyl (C14:2) group ([M + 2H]\(^{2+} = 857.0\); range: 856.5–857.5). C, MS/MS fragmentation pattern of the N-terminal peptide from \( \alpha_{\text{OC}} \) D, predicted and observed masses for b and y ions (38) generated from MS/MS of the N terminus in C. #, mass of the analogous ion after loss of water; *, mass of the analogous ion after loss of ammonia; n.d., not detected.**

**Fig. 5. Limited tryptic digest of \( \alpha_{\text{O}} \) isoforms in the presence of GTP\(_y\)S or GDP. A map of the trypsin digestion patterns of \( \alpha_{\text{OA}} \) in the presence of Mg\(^{2+}\) and GTP\(_y\)S or GDP showing the preferential sites of trypsin cleavage and the sites of antisera reactivity is illustrated in Fig. 1A. A, immunoblot of a urea/SDS-polyacrylamide gel using the AON and AO1 antibodies, before and after trypsin digestion in the presence of Mg\(^{2+}\) and GTP\(_y\)S as described under “Experimental Procedures.” B, silver-stained urea gel and immunoblot of urea gel with AOC antiserum before and after trypsin digestion in the presence of Mg\(^{2+}\) and GDP as described under “Experimental Procedures.”**

**DISCUSSION**

The \( \alpha_{\text{OC}} \) and \( \alpha_{\text{OA}} \) proteins are both major brain \( \alpha \) subunits, accounting for approximately 35 and 60%, respectively, of the \( G_{\alpha} \) protein present in brain cortex. Available evidence suggests that they both arise from the same \( \alpha_{\text{O1}} \) mRNA (21–23, 27–30) and that they differ by some unknown modification. The evidence for this is immunologic cross-reactivity at sites exhibiting marked variability between \( G \) protein \( \alpha \) subunits and extensive common sequence.

The putative modification differentiating these two proteins gives rise to marked differences in mobility of the proteins on urea/SDS-PAGE, possibly suggesting some substantial difference in their structures. Nevertheless, we have shown by ESI-MS that these two proteins differ by less than 2 Da in mass (Figs. 2 and 6). While not specifically identifying the difference between the proteins, these results set criteria to be met in proposing candidate modifications. Any modifications accounting for these proteins would have to result in only 1–2-Da mass difference between the two proteins.

The **Significance of N-terminal Modification of G Protein \( \alpha \) Subunits and the Homogeneous Modification of \( \alpha \)—**The N terminus of \( G \) protein \( \alpha \) subunits is thought to have several important functions. It is a site of interactions with \( \beta \gamma \) dimers (44–47), a site of interaction with the C terminus of the \( \gamma \) subunit (48), a possible site of receptor interactions (49), and a probable site of membrane attachment of the \( \alpha \) subunit (46, 47, 50). Probably affecting all of these activities are the multiple modifications at the N terminus of \( \alpha \) (and other \( \alpha \) subunit isoforms). The \( \alpha \) protein is predicted to be myristoylated on an N-terminal Gly after removal of the initiating Met (51), and it can be palmitoylated through a thioester linkage on a Cys located at position 3 (55). In the case of transducin \( \alpha \), the N-terminal Gly is heterogeneously acylated, with either a lauroyl (C12:0) or a myristoyl (C14:0) group, the latter having varying degrees of unsaturation (31, 32). Finally, a recent report has identified a previously unknown, and still uncharacterized, modification of \( \alpha \) also located near the N terminus of the protein (56).

Until this study, we had considered it likely that variable
modification of the N terminus of \( \alpha \) would account for the structural differences between \( \alpha_A \) and \( \alpha_C \). In fact, it has been suggested that these proteins do differ at the N terminus, as myristoylated and unmyristoylated forms of \( \alpha \) (29, 30). Palmitoylation state was another possibility that we considered. Because the N terminus of \( \alpha \) is involved in binding \( \beta \gamma \) dimers (44–47), these possibilities might have explained the different \( \beta \gamma \) composition of \( \alpha_A \) and \( \alpha_C \) (23). Despite all of these presumptive arguments, we were unable to document any difference between \( \alpha_A \) and \( \alpha_C \) at their N termini. Furthermore, and in contrast to \( \alpha_t \), we were unable to identify significant variability in the processing of the N terminus of the \( \alpha \) proteins. Other analyses of \( \alpha \) purified from bovine brain have also not observed palmitoylation (57). The lack of this modification may be due to the reducing conditions (1 mM DTT) used in the purification, which could cleave the labile thioester linkage, or there may simply be low levels of palmitoylation in vivo. These studies do not dismiss a possible role of palmitoylation in the function of these proteins, but whatever this role may be, it does not account for the differences between \( \alpha_A \) and \( \alpha_C \).

**A** predicted mass: 20838
22 - 206
25 kDa
22 - 209
predicted mass: 21210

**B** 21211.1 ± 0.2 Da
22-209
20839.0 ± 0.3 Da
22-206
\( \alpha_oA \) 16600.0 ± 0.3 Da
210-354

**C** 21211.2 ± 0.3 Da
22-209
20838.9 ± 0.2 Da
22-206
\( \alpha_oC \) 16600.7 ± 0.2 Da
210-354

**FIG. 6.** ESI mass spectra from the LCQ analyses of digests of \( \alpha_oA \) and \( \alpha_oC \). A, map of the \( \alpha \) protein showing the predicted fragments generated by trypsin digestion in the presence of GDP and the corresponding predicted masses for the 25- and 17-kDa fragments (seen by SDS-PAGE). B, analysis of the two peaks from the \( \alpha_o \) separation. On the left are the ESI mass spectra showing all charge states of the fragments; on the right are deconvolution mass spectra with predicted masses based upon trypsin cleavage. C, analysis of the two peaks from the \( \alpha_oC \) separation. On the left are the ESI spectra showing all charge states of the fragments; on the right, the deconvoluted mass spectra are shown. Average and S.E. of estimates are calculated from the multiple charge states in the ESI mass spectra.
Structural Differences in Brain \( \Gamma \) \( \alpha \) Isoforms

1. Qin, N., Platano, D., Olcese, R., Stefani, E., and Birnbaumer, L. (1997) J. Biol. Chem. 272, 840–844
2. Sternweis, P. C., and Robishaw, J. D. (1984) J. Biol. Chem. 259, 10864–10871
3. Hoffmann, V. (1996) Nature 380, 1047–1058
4. Creighton, T. E. (1980) Annu. Rev. Biochem. 49, 65–97
5. Ikeda, S. R. (1996) J. Biol. Chem. 271, 20564–20571
6. Qin, N., Platano, D., Olcese, R., Stefani, E., and Birnbaumer, L. (1997) J. Biol. Chem. 272, 840–844
7. Gasman, S., Chauner-Golai, S., Popoff, M. R., Aim, D., and Bader, M. F. (1997) J. Biol. Chem. 272, 20564–20571
8. Mandel, J. E., Karswagen, H. C., Liu, K. S., Hadi-Abin, Y. M., Simon, M. I., Plasterk, R. H. A., and Sternberg, P. W. (1995) Science 270, 1652–1655
9. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 631–638
10. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 631–638
11. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 631–638
12. Lagriffoul, A., Charpentier, N., Carrette, J., Tougard, C., Bockaert, J., and Krug for helpful discussion and criticism during the conduct of the project.

References

1. Gilman, A. G. (1995) Biosci. Rep. 15, 65–97
2. Sternweis, P. C., and Robishaw, J. D. (1984) J. Biol. Chem. 259, 10864–10813
3. Hoffmann, V. (1996) Nature 380, 1047–1058
4. Creighton, T. E. (1980) Annu. Rev. Biochem. 49, 65–97
5. Ikeda, S. R. (1996) J. Biol. Chem. 271, 20564–20571
6. Qin, N., Platano, D., Olcese, R., Stefani, E., and Birnbaumer, L. (1997) J. Biol. Chem. 272, 840–844
7. Gasman, S., Chauner-Golai, S., Popoff, M. R., Aim, D., and Bader, M. F. (1997) J. Biol. Chem. 272, 20564–20571
8. Mandel, J. E., Karswagen, H. C., Liu, K. S., Hadi-Abin, Y. M., Simon, M. I., Plasterk, R. H. A., and Sternberg, P. W. (1995) Science 270, 1652–1655
9. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Science 270, 1652–1655
10. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 631–638
11. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 631–638
12. Lagriffoul, A., Charpentier, N., Carrette, J., Tougard, C., Bockaert, J., and Krug for helpful discussion and criticism during the conduct of the project.

References

1. Gilman, A. G. (1995) Biosci. Rep. 15, 65–97
2. Sternweis, P. C., and Robishaw, J. D. (1984) J. Biol. Chem. 259, 10864–10813
3. Hoffmann, V. (1996) Nature 380, 1047–1058
4. Creighton, T. E. (1980) Annu. Rev. Biochem. 49, 65–97
5. Ikeda, S. R. (1996) J. Biol. Chem. 271, 20564–20571
6. Qin, N., Platano, D., Olcese, R., Stefani, E., and Birnbaumer, L. (1997) J. Biol. Chem. 272, 840–844
7. Gasman, S., Chauner-Golai, S., Popoff, M. R., Aim, D., and Bader, M. F. (1997) J. Biol. Chem. 272, 20564–20571
8. Mandel, J. E., Karswagen, H. C., Liu, K. S., Hadi-Abin, Y. M., Simon, M. I., Plasterk, R. H. A., and Sternberg, P. W. (1995) Science 270, 1652–1655
9. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Science 270, 1652–1655
10. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 631–638
11. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 631–638
12. Lagriffoul, A., Charpentier, N., Carrette, J., Tougard, C., Bockaert, J., and Krug for helpful discussion and criticism during the conduct of the project.

References

1. Gilman, A. G. (1995) Biosci. Rep. 15, 65–97
2. Sternweis, P. C., and Robishaw, J. D. (1984) J. Biol. Chem. 259, 10864–10813
3. Hoffmann, V. (1996) Nature 380, 1047–1058
4. Creighton, T. E. (1980) Annu. Rev. Biochem. 49, 65–97
5. Ikeda, S. R. (1996) J. Biol. Chem. 271, 20564–20571
6. Qin, N., Platano, D., Olcese, R., Stefani, E., and Birnbaumer, L. (1997) J. Biol. Chem. 272, 840–844
7. Gasman, S., Chauner-Golai, S., Popoff, M. R., Aim, D., and Bader, M. F. (1997) J. Biol. Chem. 272, 20564–20571
8. Mandel, J. E., Karswagen, H. C., Liu, K. S., Hadi-Abin, Y. M., Simon, M. I., Plasterk, R. H. A., and Sternberg, P. W. (1995) Science 270, 1652–1655
9. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Science 270, 1652–1655
10. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 631–638
11. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 631–638
12. Lagriffoul, A., Charpentier, N., Carrette, J., Tougard, C., Bockaert, J., and Krug for helpful discussion and criticism during the conduct of the project.
Characterization of the Major Bovine Brain G_{\alpha} \alpha Isoforms: MAPPING THE STRUCTURAL DIFFERENCES BETWEEN THE \alpha SUBUNIT ISOFORMS IDENTIFIES A VARIABLE REGION OF THE PROTEIN INVOLVED IN RECEPTOR INTERACTIONS

William E. McIntire, Jane Dingus, Kevin L. Schey and John D. Hildebrandt

J. Biol. Chem. 1998, 273:33135-33141.
doi: 10.1074/jbc.273.50.33135

Access the most updated version of this article at http://www.jbc.org/content/273/50/33135

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 67 references, 32 of which can be accessed free at http://www.jbc.org/content/273/50/33135.full.html#ref-list-1