Addition of a Glycophosphatidylinositol to Acetylcholinesterase

PROCESSING, DEGRADATION, AND SECRETION*

Received for publication, November 30, 2000, and in revised form, May 1, 2001
Published, JBC Papers in Press, May 3, 2001, DOI 10.1074/jbc.M010817200

Françoise Coussen, Annick Ayon, Anne Le Goff, Jacqueline Leroy, Jean Massoulié, and Suzanne Bon‡
From the Laboratoire de Neurobiologie Moléculaire et Cellulaire, CNRS UMR 8544, Ecole Normale Supérieure, 46 rue d’Ulm, 75005 Paris, France

We introduced various mutations and modifications in the GPI anchoring signal of rat acetylcholinesterase (AChE). 1) The resulting mutants, expressed in transiently transfected COS cells, were initially produced at the same rate, in an active form, but the fraction of GPI-anchored AChE and the steady state level of AChE activity varied over a wide range. 2) Productive interaction with the GPI addition machinery led to GPI anchoring, secretion of uncleaved protein, and secretion of a cleaved protein, in variable proportions. Unproductive interaction led to degradation; poorly processed molecules were degraded rather than retained intracellularly or secreted. 3) An efficient glypiation appeared necessary but not sufficient for a high level of secretion; the cleaved, secreted protein was possibly generated as a by-product of transamination. 4) Glypiation was influenced by a wider context than the triplet ωωω + 1/ωω + 2, particularly ωωω + 1. 5) Glypiation was not affected by the closeness of the ω site to the ααα helix of the catalytic domain. 6) A cysteine could simultaneously form a disulfide bond and serve as an ω site; however, there was a mutual interference between glypiation and the formation of an intercatalytic disulfide bond, at a short distance upstream of ω. 7) Glypiation was not affected by the presence of an N-glycosylation site at ω or in its vicinity or by the addition of a short hydrophilic, highly charged peptide (FLAG; DYKDDDDK) at the C terminus of the hydrophobic region.

Many proteins are anchored at the cell surface through a glycoposphatidylinositol (GPI) that is covalently attached to their C terminus (1–3). GPI-anchored proteins are recruited to glycosphingolipid/cholesterol-rich membrane microdomains (4–6), where they may interact functionally with molecules involved in intracellular signal transduction; this is, for example, the case of the APP protein, the precursor of the β-amyloid peptide that forms amyloid deposits in Alzheimer’s disease (7).

GPI anchoring also seems to be directly related with the pathological misfolding of the PrP prion protein (8).

Glypiation, the process of GPI addition, implies the cleavage of a C-terminal peptide and the concerted linkage of a preassembled GPI anchor, forming an amide bond between an ethanolamine moiety and the carboxylic group of the ω residue, at the C terminus of the mature protein. The structural requirements of the C-terminal signal peptides that induce GPI addition have been investigated extensively by the groups of Caras and Udenfriend, by mutagenesis of the “decay-accelerating factor” (9–12) and of placental alkaline phosphatase (13–17). They showed that glypiation requires a C-terminal hydrophobic sequence and an upstream cleavage/addition site (2, 13). The two groups found that positions ω and ω + 1 (according to the group of Caras) or ω and ω + 2 (according to the group of Udenfriend) must be occupied by residues with small side chains. In addition, Caras and colleagues found that, for optimal processing, the ω site should be located between 10 and 12 residues upstream of the C-terminal hydrophobic sequence (10). A systematic analysis of all reported GPI-anchored proteins and of the effects of mutations in their C-terminal region has led Eisenhaber et al. (18, 19) to formulate a prediction algorithm, which confirmed that the volumes of the side chains located near the cleavage site exert a major influence, probably because they must be accommodated within the catalytic pocket of a transamidase (15). Several components of the transamidase complex have recently been cloned (20, 21).

In mammals, acetylcholinesterase (AChE; EC 3.1.1.7) subunits containing the alternative C-terminal peptide H (AChEH) produce GPI-anchored dimers (22–25); this peptide is sufficient to induce the addition of a GPI anchor when added to a foreign protein (26). The sequences encoded by the alternative H exons of Torpedo and rat AChEs contain one or two cysteines, which form intersubunit disulfide bonds in AChE dimers, and hydrophobic C-terminal regions of 15 or 19 residues. Otherwise, the sequences of Torpedo and mammalian H peptides do not appear homologous and may have arisen independently in the AChE genes (23). By introducing threonines at different positions in Torpedo AChE, Bucht and Hjalmarsson found that the last two of a group of three consecutive serines (Ser¹–Ser³) could function as ω sites (27). In the case of mammalian AChE, biochemical analyses of C-terminal peptides from the human erythrocyte enzyme showed that the GPI anchor is linked to glycine 14 in the H peptide (28–30).

In the present work, the H peptide of rat AChE was mutated or replaced by Torpedo or composite C-terminal regions, and we report the effects of these modifications on the production of GPI-anchored AChE, on the level of active AChE in cells, and on its secretion in the culture medium.

* This work was supported by grants from CNRS, the Association Française contre les Myopathies (AFM), the Direction des Systèmes de Forces et de la Prospective (DGA/DSP/STTC 99 CO (29), and the European Community (QLK3-CT-2000-00650). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed: Dr. Suzanne Bon, Laboratoire de Neurobiologie Moléculaire et Cellulaire, CNRS UMR 8544, Ecole Normale Supérieure, 46 rue d’Ulm, 75005 Paris, France. Tel.: 33 1 44 32 38 91; Fax: 33 1 44 32 38 87; E-mail: jean.massoulie@biologie.ens.fr.
† The abbreviations used are: GPI, glycosphatidylinositol; AChE, acetylcholinesterase; PI-PLC, phosphatidylinositol-specific phospholipase C.
MATERIALS AND METHODS

Site-directed Mutagenesis—The cDNA encoding the rat AChE subunit was inserted in the pEF-BOS vector, under the control of the human EF-1α promoter (31). Site-directed mutagenesis was performed as described previously (32). In the case of rat AChE with chimeric Torpedo/rat C-terminal peptides, we removed the noncoding regions, so that all constructs were identical, except for the 3′ sequence, encoding the C-terminal peptides.

Expression in COS-7 Cells—For transfections, DNA was purified on Nucleobond AX columns (Macherey-Nagel). COS-7 cells were transfected by the diethylaminoethyl-dextran method, as described previously (33). For cultures of transfected cells, the fetal calf serum and the Nu-serum were treated with soman (10μM) to block irreversibly any cholinesterase activity, this treatment was performed at least 1 week before, so that excess soman was hydrolyzed during storage at 4°C. The cells were usually extracted 2 or 3 days after transfection.

Preparation of Extracts, Digestion with PI-PLC, and AChE Assays—

The cells were extracted with TMg buffer (1% Triton X-100, 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂) at 20°C, because the sphingolipid/cholesterol microdomains are partially insoluble in Triton X-100 in the cold. For PI-PLC digestion, detergent extracts were incubated in TMg buffer for 1 h at 30°C with 3 units/ml of PI-PLC from Bacillus thuringiensis (Glyko Europe, Upper Heyford, United Kingdom). To ensure that digestion was complete, some samples were incubated a second time, after the addition of the same quantity of fresh PI-PLC. Control extracts were incubated in the same conditions without PI-PLC. Solubilization of cell surface GPI-anchored AChE was performed by treating intact cells with the same concentration of PI-PLC for 20 min at 37°C, the released AChE was assayed in the PI-PLC from after centrifugation at 17,000 × g for 15 min to remove cell debris.

The AChE activity was assayed by the colorimetric method of Ellman (34). Enzyme samples (10μl) were added to 0.2 ml of Ellman assay medium, and the reaction kinetics was monitored at 414 nm, at 15-s intervals for 3 min, using a Multiskan RC microplate reader (Lab-systems, Helsinki, Finland).

AChE assays were expressed at least five times and most were expressed more than 10 times in independent transfections that included different sets of mutants used for various comparisons; experimental variations in the relative levels of activity and the fraction of PI-PLC-sensitive AChE did not exceed 15%. The indicated values were obtained from a representative experiment that included all mutants shown in a given table or figure.

Sucrose Gradient—Aliquots of extracts were equilibrated with 1% Brij-96, loaded on 5–20% sucrose gradients in 1% Brij-96, 10 mM MgCl₂, 25 mM Tris-HCl, pH 7. Escherichia coli β-galactosidase (16 S) and alkaline phosphatase (6.1 S) were included as internal sedimentation standards. The gradients were centrifuged for 18 h at 36,000 rpm in a SW-41 rotor, at 5°C. Fractions of 300 μl were collected and assayed for AChE, β-galactosidase and alkaline phosphatase activities.

Nondenaturing Electrophoresis—Electrophoresis of active AChE was performed in nondenaturing conditions, in 7.5% horizontal polyacrylamide gels, in the presence of detergent, as described by Bon et al. (35). AChE activity was revealed after electrophoresis by the method of Karnovsky and Roots (36). The gels were scanned and quantified with the TINA software (version 2.07d, Raytest Isotopenmessgeräte GmbH).

RESULTS AND DISCUSSION

Constructs Used in This Study—

The H peptides correspond to amino acids 536–577 of rat AChE and 536–566 of Torpedo AChE (numbering of Torpedo AChE), but for simplicity we use numbering from their first residue (Tables I and II). The rat H peptide contains two cysteines (Cys6 and Cys8), each of which is sufficient for the formation of disulfide-linked GPI-anchored dimers. The structure of the mutants is shown in Tables I, II, and III. Mutations that were restricted to the C-terminal peptide did not modify the catalytic activity of rat AChE. Mutants containing a GPI signal derived from the rat H peptide are designated rm2 to rm44 (for “rat mutant,” rm1 being the wild type). Mutants rm2–rm40 contain mutations around the ω site and sometimes minor insertions or deletions. Mutants rm41–rm43 were deleted to place the ω site at the position of Cys8. The rm44 mutant contains an internal FLAG peptidic epitope. The FLAG peptide was also added at the C terminus of some mutants (rm1-f, rm5-f, rm13-f, and rm18-f). The Torpedo H peptide is totally different from that of rat; mutagenesis showed that each of its first three consecutive serines (residues 542–544) can serve as an ω site. The catalytic domain of rat AChE was associated with Torpedo and with chimeric rat/Torpedo GPI addition signals (rr, rt, tr, and tt), which were made in long (L) and short (S) versions, by insertion or deletion of five residues (Table II, top). Composite constructs (cc) contained modified Torpedo GPI-addition signals (Table II, bottom). Finally, we analyzed mutants in which a few residues of the catalytic domain were deleted, to reduce the distance between the α10 helix and the ω site (Table III). Throughout, we indicate the immediate peptidic environment of the putative ω site (ω – 1/ω + 3), underlining the ω site itself or underlining its position when it is inactivated.

Relationships between GPI Anchoring, Cell Activity, and Secretion—By immunofluorescence of nonpermeabilized COS cells, we found that AChE was totally removed from the cell surface by PI-PLC, in the case of both poorly and efficiently processed mutants (not shown). We also verified that the level of PI-PLC-sensitive AChE in cell extracts was proportional to the PI-PLC-solubilized activity, which represents the mature, externally exposed enzyme.

For each mutant, we determined the total AChE activity in cell extracts and in the medium (Fig. 1). In the wild type, 2 and a half days after transfection, the cell extract and the medium contained about 75 and 25%, respectively, of the total AChE activity. In the mutants, the level of cellular activity varied from less than 10% to nearly 150% of the wild type activity. This shows that the biosynthetic capacity of the COS cells was not saturated. The released AChE activity in the culture medium was low when the cellular activity was low and also for

2 S. Bon, unpublished result.
The C-terminal peptides of mutants rm1 to rm44 are derived from the rat H peptide, except that the third residue before the C terminus is a proline rather than an arginine, as in mice (48). The sequence of the wild type H peptide of rat AChE (the cysteines are doubly underlined, the ω site is underlined, and the hydrophobic region is shown in boldface type) is as follows: 

\[
\text{ATEVPCTCPSPAHGAEARPPGPALESSLFFLHLHGGLWL}
\]

Numbering of the mature AChE is indicated on the line (Torpedo numbering), and numbering of the H peptide is indicated below. In the second column, the predicted ω site position is underlined. In the top of Table I, mutants rm1 to rm12 contain point mutations in the wild type GPI-addition signal. The columns on the right indicate the level of cellular activity (cell act.), as a percentage of the wild type, the proportion of GPI-anchored AChE in the cell extracts (Glyp.), the level of secreted activity as a percentage of the wild type (Secr. act.), and the score given by the big-PI algorithm. In the middle part, mutants rm13 to rm34 differ only in the 15–19 interval, the rest of the C-terminal peptide being identical: ATEVPCTCPSPAH [...]. GPI ADDITION SIGNAL. The bottom of the table shows mutants rm35 to rm44 which contain deletions (dashes) or insertions (doubly underlined). All of these mutants share the same N- and C-terminal segments: ATEVPCTCPSPAHGAEARPPGPALESSLFFLHLHGGLWL. In mutants rm1–f, rm5–f and rm13–f, the FLAG epitope was added at the C terminus (not illustrated).

### Table I

| Name | Mutations | Comments | Cell. act. | Glyp. | Secr. act. | Score |
|------|-----------|----------|------------|-------|------------|-------|
| rm1  | HGEAA     | Wild type | Proline at 40, as in mouse | 100 75 | 100 67  |
| rm1' | HGEAA     | C8S      | Wild type with only the first cysteine | 100 75 | 100 62 |
| rm1+ | S25P      |          | Suppression of a potential ω site at Ser<sup>25</sup> | 100 75 | 100 52  |
| rm2  | HGEAA     | G14P     | Suppression of the natural ω site Gly<sup>14</sup> | 31 21 | 11 3  |
| rm3  | HGEAA     | G14S/S25P | Suppression of the two preceding sites | 24 22 | 11 4 7 |
| rm4  | HGEPA     | A16P     | Proline at ω + 2 | 19 32 | 7 3 9 |
| rm5  | HGEAA     | A12T     | Threonine at ω - 2 | 96 61 | 53 6 7 |
| rm6  | HGEPA     | TGP14PS25P | Prolines at 7, 14, and 25 | 20 9 | 11 4 3 |
| rm7  | HGEPA     | G14PA16PS25P | Prolines at 14, 16, and 25 | 15 2 | 7 5 2 |
| rm8  | HGNAS     | E15N/A17S | Potential N-glycosylation site at ω + 1 | 91 70 | 113 11 6 |
| rm9  | HGEAA     | E15N     | Non glycosylable control of rm8 | 109 65 | 111 14 |
| rm10 | HGEAS     | A17S     | Non glycosylable control of rm9 | 86 62 | 127 7 3 |
| rm11 | HGEAA     | G14N     | Replacement of Gly by Asn at the ω site | 112 68 | 104 6 7 |
| rm12 | HGEESG    | NENGT at 14–18, S25P | Potential N-glycosylation site at ω + 1 | 127 66 | 173 6 8 |

| Name | Mutations | Comments | Cell. act. | Glyp. | Secr. act. | Score |
|------|-----------|----------|------------|-------|------------|-------|
| rm13 | PEGPTR    | Mutant showing essentially no glypliation | 25 0.4 | 11 3.4 |
| rm14 | EESGR     | SGS at ω+1/ω+2 | 75 65 | 22 6 5 |
| rm15 | EESGR     | SGT at ω+1/ω+2 | 50 54 | 20 6 9  |
| rm16 | EESGR     | SEG at ω+1/ω+2 | 30 21 | 16 4 6 |
| rm17 | EESGR     | SGE at ω+1/ω+2 | 30 29 | 16 4 2  |
| rm18 | EESGR     | GGT at ω+1/ω+2 | 31 25 | 16 4 1 |
| rm19 | EESGR     | GEA at ω+1/ω+2 | 19 7.5 | 9 1 6 |
| rm20 | EESGR     | CEA at ω+1/ω+2 | 25 0.9 | 9 2 4  |
| rm21 | PESGR     | Like rm15, but with proline at ω - 1 | 53 54 | 18 3 6 |
| rm22 | EESGTP    | Effect of a proline at ω + 2 (compare with rm15) | 115 75 | 222 7 3 |
| rm23 | HNGGR     | Effect of an histidine at ω - 1 (compare with rm28) | 137 67 | 102 5 8 |
| rm24 | HNGGR     | Effect of a cysteine at ω (compare with rm41) | 25 12 | 16 6 0 |
| rm25 | HNGGR     | SGS at ω+1/ω+2 (nonglycosylable control of rm26) | 123 75 | 22 8 1 3 |
| rm26 | HNGGR     | Potential N-glycosylation at the engineered ω site | 87 62 | 22 4 9 |
| rm27 | HNGGR     | Nonglycosylable control of rm26 | 30 30 | 18 4 4 |
| rm28 | HNGGR     | Nonglycosylable control of rm26 | 87 62 | 22 7 0 |
| rm29 | HNGGR     | Suppression of the preceding ω site by a threonine | 11 16 | 9 1 2 |
| rm30 | HNGGR     | Effect of an histidine at ω - 1 (compare with rm27) | 76 56 | 42 4 4 |
| rm31 | HNGGR     | Effect of an histidine at ω - 1 (compare with rm15) | 142 72 | 116 5 0 |
| rm32 | HNGGR     | Effect of an histidine at ω - 1 (compare with rm19) | 40 40 | 36 0 4 9 |
| rm33 | HNGGR     | Effect of a cysteine at ω - 1 (compare with rm19) | 36 34 | 18 2 0 9 |
| rm34 | HNGGR     | Effect of a lysine at ω - 1 (compare with rm19) | 88 56 | 49 1 3 |

| Name | Mutations | Comments | Cell. act. | Glyp. | Secr. act. | Score |
|------|-----------|----------|------------|-------|------------|-------|
| rm35 | HNGGR     | PCTCPSPAHH--NGGRPGPALESSLFFLHLHGGLWL | Residues preceding ω as in wild type | 112 80 | 107 6 7 |
| rm36 | HGEAA     | PCTCPSPAHHGAEARPPGPALESSLFFLHLHGGLWL | Reduced spacer after ω | 84 66 | 111 0 5 |
| rm37 | HGEAA     | PCTCPSPAHHGAEARPPGPALESSLFFLHLHGGLWL | ω and upstream as in wild type | 56 66 | 71 6 2 |
| rm38 | HGEAA     | PCTCPSPAHHGAEARPPGPALESSLFFLHLHGGLWL | ω and upstream as in wild type | 93 65 | 113 3 4 |
| rm39 | HNGGR     | PCTCPSPAHHGAEARPPGPALESSLFFLHLHGGLWL | Wild type sequence upstream of ω | 67 66 | 36 7 2 |
| rm40 | HNGGR     | PCTCPSPAHHGAEARPPGPALESSLFFLHLHGGLWL | Suppression of preceding ω site | 37 32 | 18 1 4 |
| rm41 | HNGGR     | PCTCPSPAHHGAEARPPGPALESSLFFLHLHGGLWL | Disulfide bond by ω site cysteine? | 25 75 | 40 6 5 |
| rm42 | HNGGR     | PCTCPSPAHHGAEARPPGPALESSLFFLHLHGGLWL | Control of preceding: no cysteine | 97 90 | 111 6 3 |
| rm43 | HNGGR     | PCTCPSPAHHGAEARPPGPALESSLFFLHLHGGLWL | Control of rm14, suppression of ω | 31 65 | 43 0 6 9 |
| rm44 | HNGGR     | PCTCPSPAHHGAEARPPGPALESSLFFLHLHGGLWL | Insertion of FLAG peptide upstream of the hydrophobic region | 34 60 | 28 19 5 9 |
Chimeric constructs are shown at the top. The GPI addition signal is composed of intervening regions (located between the cysteine and the hydrophobic region) and hydrophobic regions from rat (r) or Torpedo (t). They are called rr, rt, tr, and tt, either long (L) or short (S), depending on the deletion of five residues (ALSLS) at the boundary between the rat spacer and hydrophobic region or insertion of the same residues in Torpedo (doubly underlined), thus elongating the hydrophobic region. These chimeric peptides were added downstream of Cys\(^{6}\) from the rat H peptide, so that the formation of an intersubunit disulfide bond should be identical in all cases. The Torpedo regions are shown in italics. The sequence of the wild type H peptide of Torpedo AChE, with numbering of Torpedo AChE (the possible natural \(\omega\) sites are underlined) is as follows:

\[ \text{AGELSSGTTSSKGIFVYLFSLYY} \]

Composite constructs are shown at the bottom. The long and short composite constructs (ccL, ccS) were partly derived from the QN/HC protein, in which the N-terminal region of ColQ was fused to the Torpedo wild type H peptide of Torpedo AChE, that the formation of an intersubunit disulfide bond should be identical in all cases. The Torpedo CATEVP[TETNIL]\[.....\]PSPTPSPKG[IIFYVLFSILYLIFY], thus elongating the hydrophobic region. These chimeric peptides were added downstream of Cys\(^{6}\) from the rat H peptide, so

\[ \text{LSPSP} \]
\[ \text{LTPSP} \]

**TABLE II**

| Name | \(\omega - 1/\omega + 3\) | C-terminal sequence |
|------|-----------------|-----------------|
| rrL  | HGEAA           | PSAPHEGAARPPGPALSLSLFLFILHSLGPLW |%
| rtL  | HGEAA           | SPAHEAARPPGPALSLSLFLFILHSLGPLW |%
| trL  | LSSGT           | DGELELSSGTTSSKGAALSLSLFLFILHSLGPLW |%
| ttL  | LSSGT           | DGELELSSGTTSSKGAALSLSLFLFILHSLGPLW |%
| rm35 | HGGGR           | LPKLLSATATEVPTCPSPAHEGRRGPAALSLSLFLFILHSLGPLW |%
| rm42 | HGGGR           | LPKLLSATATEV---HNGRRGPAALSLSLFLFILHSLGPLW |%
| D1   | HGGGR           | LPKLLSA---------HNGRRGPAALSLSLFLFILHSLGPLW |%
| D2   | HGGGR           | LPKLL---------HNGRRGPAALSLSLFLFILHSLGPLW |%
| D3   | HGGGR           | LPK---------HNGRRGPAALSLSLFLFILHSLGPLW |%
| S1   | .. LPKLLHN-stop | Same mature protein as for D2, but without a GPI | 100
| S2   | .. LPKLL-stop   | Same as preceding, with leucines 536–537 but without HN | 87
| S3   | .. LPKHN-stop   | Same mature protein as for D3, but without a GPI | 78
| S4   | .. LPK-stop     | Mature protein without leucines 536–537 | 70

**TABLE III**

Deletions between helix \(\alpha_{10}\) and the \(\omega\) site and corresponding truncated constructs

The sequence shown at the top starts at Leu\(^{528}\) (Torpedo numbering) and thus includes the end of helix \(\alpha_{10}\), which is shown in italics (LPKL). The mutants contain an \(\omega\) site asparagine with the same immediate environment, at various distances from the catalytic domain. The bottom of the table shows truncated constructs, expressed as controls for the effect of deletions at the C terminus of the catalytic domain. The cellular and secreted activities produced by the truncated constructs are expressed as percentage of S1, which corresponds to the mature D2 enzyme, but without a GPI anchor. For all truncated mutants, the cellular activity represented less than 5% of the secreted activity, 2 days after transfection.

The \(\omega\) site asparagine with the same immediate environment, at various distances from the catalytic domain. The bottom of the table shows truncated constructs, expressed as controls for the effect of deletions at the C terminus of the catalytic domain. The cellular and secreted activities produced by the truncated constructs are expressed as percentage of S1, which corresponds to the mature D2 enzyme, but without a GPI anchor. For all truncated mutants, the cellular activity represented less than 5% of the secreted activity, 2 days after transfection.

**The Peptidic Environment of the \(\omega\) Site**—A number of mutants were designed to analyze the effect of residues around the \(\omega\) site on the efficiency of glypiation. We particularly wished to test the proposed rules that

\[ \text{AGELSSGTTSSKGIFVYLFSLYY} \]

components: 1) amphiphilic and PI-PLC-insensitive; 2) amphiphilic and PI-PLC-sensitive; and 3) nonamphiphilic. The first component contains uncleaved molecules, which retain their C-terminal hydrophobic region, as shown in the case of "flagged" constructs (see below). In cellular extracts, this component consisted of one or several electrophoretic bands; we did not explore this complexity in the present study, but preliminary experiments suggest that it reflects a heterogeneity in the glycosylation of AChE. The PI-PLC-sensitive component represents the GPI-anchored species. The nonamphiphilic component is generally minor and probably represents a lytic form, produced either in the cells or after homogenization. In the medium, we also found amphiphilic and nonamphiphilic components, but no PI-PLC-sensitive AChE.

Fig. 3 shows that the total cellular activity was strongly correlated with the proportion of GPI-anchored enzyme, as determined by an entirely independent experimental method.

Fig. 4, A and B, show that the amphiphilic, PI-PLC-resistant component was relatively constant in the cell extracts and varied little in the medium, except that it was very low for the poorest mutants. Thus, the variations in total cellular and secreted activities mostly resulted from variations in the levels of GPI-anchored and nonamphiphilic components, respectively.
Fig. 1. Analysis of rat mutants. Histograms show the relative activities obtained with the rat rm1–rm40 mutants, relative to the wild type (rm1), in cell extracts (left) and in the media (middle), 2 and a half days after transfection. The proportions of the various components were evaluated from electrophoretic patterns such as those shown in Fig. 2. In the cell extracts, the dark grey zone corresponds to the PI-PLC insensitive amphiphilic component (uncleaved precursors), the densely striped zone to the PI-PLC-sensitive, GPI-anchored enzyme, and the lightly striped zone to the nonamphiphilic fraction, observed before PI-PLC treatment. In the culture media, the dark grey zone corresponds to the slow, amphiphilic component, and the striped zone corresponds to the fast, nonamphiphilic component. Note that secretion is not systematically correlated with cellular activity. The right panel represents the prediction scores obtained for each mutant, according to the big-PI predictor algorithm (19); the theoretical score obtained with the rat AChE catalytic domain followed by a consensus sequence defined by the algorithm was 31.5.
the production of GPI-anchored AChE by mutation of this site in mutant rm2 (G14P) or by any other single mutation, revealing the presence of alternative, less efficient sites (Table I, top). A multiplicity of possible sites has also been reported in the case of other proteins, such as the folate receptor (38–40).

We particularly examined the effect of prolines at various positions. A proline at \( \omega_1 \) appears to suppress glypiation, since rm4 (HGEPA) was not better than rm2 (HPEAA). Comparison of mutants rm15 (ESGTR), rm21 (PSGTR), and rm22 (ESGTP) shows that prolines exert no strongly adverse influence at either \( \omega_1 \) or \( \omega_2 \). Similarly, a proline at \( \omega_2 \) did not reduce GPI addition or secretion in mutants rm23 (PAH-PHNGGR) and rm35 (PAHNGGR). Prolines at \( \omega_1 \) did not block glypiation of the composite constructs ccL and ccS (LSPSP), which were very well processed. The identity of the \( \omega_1 \) site in these mutants was clearly established by the fact that its replacement by a threonine (LTPSP) abolished glypiation completely. This is consistent with the report of Bucht et al. (41) that, in human AChE, the triplet GPG, with a proline at \( \omega_1 \), produced about 20% of the wild type level of GPI-anchored AChE. These authors found a similar efficiency with the GSP triplet, but our data and the big-PI predictor algorithm (19) suggest that in that case the \( \omega_1 \) residue was probably Ser, instead of Gly. Thus, prolines probably block processing at \( \omega_1 \) but not at \( \omega_2 \), \( \omega_3 \), or \( \omega_4 \).

We took advantage of the fact that glypiation was essentially abolished by multiple mutations in mutant rm13 to investigate the efficiency of \( \omega_1 \) sites in peptide sequences that we introduced in the interval 15–19, bracketed by prolines at 14 and 20 (Table I, middle). A comparison of mutants rm14 (ESGSR), rm15 (ESGTR), rm16 (ESEGR), and rm17 (ESGER) shows that processing was reduced by the presence of bulky residues in the \( \omega_1/\omega_2 \) interval. However, the efficiency of processing was influenced by a wider context than the triplet \( \omega_1 + 1/\omega_2 + 2 \).

We took advantage of the fact that glypiation was essentially abolished by multiple mutations in mutant rm13 to investigate the efficiency of \( \omega_1 \) sites in peptide sequences that we introduced in the interval 15–19, bracketed by prolines at 14 and 20 (Table I, middle). A comparison of mutants rm14 (ESGSR), rm15 (ESGTR), rm16 (ESEGR), and rm17 (ESGER) confirms that processing is reduced by the presence of bulky residues in the \( \omega_1/\omega_2 + 1 \) interval. However, the efficiency of processing was influenced by a wider context than the triplet \( \omega_1 + 1/\omega_2 + 2 \). For example, GEA is a good triplet in the context of the wild type but not in mutant rm19.

The \( \omega_1 \) residue had a strong influence on processing, as shown by analysis of mutants that differed only at this position (Fig. 5). The production of GPI-anchored AChE and the secretion of AChE were both increased when Glu was replaced by

![Electrophoretic patterns of representative mutants](http://www.jbc.org/)

**FIG. 2.** Electrophoretic patterns of representative mutants. Mutants producing similar patterns are indicated below each lane, in smaller type. After nondenaturing electrophoresis, the gels were histochemically stained for AChE activity. Cellular extracts were analyzed after treatment with PI-PLC; the digestion was complete, since a second treatment with fresh PI-PLC had no further effect. We used identical volumes of 50-fold diluted cell extracts and of undiluted culture media, so that the staining intensities reflect the relative AChE activities of the mutants. The slow components were accelerated by Na\(^+\) deoxycholate and are therefore amphiphilic, while the fast components migrated at the same rate, showing that they are nonamphiphilic (not shown).

**FIG. 3.** Relationship between the cellular activity and the nonamphiphilic component after PI-PLC treatment. The level of cellular AChE activity and the proportion of nonamphiphilic component in cell extracts after treatment with PI-PLC were determined 2 days after transfection; the two coordinates correspond to totally independent analyses. The **numbered circles** correspond to the rat mutants rm1 to rm44 (Table I), the **squares** correspond to the chimeric constructs (Table II, top), and the **diamonds** correspond to the composite constructs (Table II, bottom). All mutants produce GPI-anchored dimers of rat AChE.
His, as in the wild type. A comparison with mutants containing Lys or Cys suggests that the basic character of \( v \) is more important than its nucleophilicity. An effect of the \( v \) residue is illustrated in the case of rm5, which only differs from the wild type by the replacement of an alanine by a threonine at position 12. This mutant produced a similar level of activity, including GPI-anchored AChE, but was significantly less well secreted.

Glypiation and Degradation—Since mutants rm2 to rm40 only differed from the wild type by a few residues in the central region of their C-terminal peptide, which is entirely distinct from the catalytic domain, we expected all of these proteins to be synthesized at the same rate. Metabolic labeling of steady state cultures (2 days after transfection) showed that incorporation of radioactive amino acids into the AChE protein during a period of 5 min was actually equivalent in well processed and poorly processed mutants (see below; Fig. 9B).

We followed the production of active enzyme over a period of 1 h after irreversible inhibition of the cellular enzyme by an organophosphate inhibitor (not shown). The synthesis of active AChE was similar in the wild type and in the poorly processed mutants rm13 (PSPTR) and rm18 (EGGTR), despite the large differences observed in the steady state levels.

In poorly processed mutants, the level of uncleaved molecules was reduced rather than increased. Since these mutants were correctly synthesized and folded but produced low levels of activity, an unproductive interaction with the transamidase targets them toward degradation rather than storage within subcellular compartments, membrane-anchoring, or release. These observations are consistent with previous reports that mutations around the \( v \) site of placental alkaline phosphatase reduced both the amount of enzyme exposed at the cell surface and the level of activity in total cell extracts, although the mutants could become catalytically active (13). The presence of a defective GPI addition signal has previously been shown to induce retention and degradation in the endoplasmic reticulum or in a postendoplasmic reticulum compartment (11, 42).

Possible Interference between Glypiation and N-Glycosylation at or Near the \( v \) Site—It is well established that the addition of a preformed glycan to an N-glycosylation site takes

---

**Fig. 4. Correlations between cellular and secreted components and prediction scores.**

A, relationships of the GPI-anchored component and of the PI-PLC-resistant amphiphilic component with the total cellular AChE activity. Activities are expressed as percentages of the total wild type activities, in the cells and in the media, respectively. B, relationships of the amphiphilic and nonamphiphilic secreted components with the total secreted activity. C, relationship between the level of released lytic nonamphiphilic AChE, 2 days after transfection, and the level of GPI-anchored AChE in COS cells. The numbers identify the mutants, rm1–rm40, as indicated in Table I. Some mutants present a comparatively high cellular activity but a low level of released activity. D, relationship between the level of cellular GPI-anchored AChE and the scores obtained with the big-PI predictor.
place in the endoplasmic reticulum on the nascent polypeptide chain and that glypiation occurs rapidly after completion of the polypeptide chain in the same compartment (43). We wondered whether the addition of an N-linked glycan could interfere with glypiation. We found that mutants that possessed an N-glycosylable asparagine at or near ω, such as rm8 (HGNAs), rm12 (HNESG) and rm26 (ENGSR), produced the same level of GPI-anchored AChE as nonglycosylable controls, respectively rm9 (HGNAA), rm11 (HNEAA), and rm28 (ENGGR). These results suggest that glypiation takes precedence over N-glycosylation.

\[ \text{Glypiation and Secretion: Origin of the Secreted Lytic Component—By comparing various mutants, we tried to determine the origin of the lytic, nonamphiphilic component that is predominant in highly secreted mutants. Its level did not systematically reflect that of the GPI-anchored form (Fig. 4C). However, all GPI-anchored molecules carry the same glycolipid; even mutants such as rm22 and rm25, which were well glypilated but poorly secreted, could be released from the cell surface by PI-PLC with the same efficiency as the wild type. It is therefore unlikely that the lytic secreted form would be produced by the action of a lipase on the cell surface enzyme.} \]

We tested the possibility of a proteolytic cleavage of the GPI-anchored enzyme by comparing the wild type with mutants rm37 and rm38, which possess the same peptide sequence preceding the ω site, and mutant rm39, which contains three additional residues (PEN) (Table I, bottom). The three mutants present similar electrophoretic patterns, but the ratio of secreted G2\textsuperscript{\text{rm37}} to cellular GPI-anchored AChE was somewhat higher in rm38 than in the wild type and markedly lower in rm39. This suggests that the secreted enzyme was not produced in the same manner and therefore probably not by proteolysis at a sensitive bond upstream of the ω site. Thus, the action of a lipase or of a protease on the mature GPI-anchored enzyme seems to be excluded.

A third possibility is that the lytic secreted molecules may be generated by an aborted transamidation, in which cleavage of the polypeptide chain would be followed by reaction with a water molecule instead of the GPI anchor (17, 44). Such a reaction was observed with the nucleophilic reagent hydrazine: by reacting with an intermediate of the transamidation reaction, hydrazine prevents the attachment of the GPI anchor and releases a cleaved, soluble protein. In fact, the transamidase catalytic subunit, Gpi8p, contains an active cysteine and is related to caspases (45).

\[ \text{Influence of the Hydrophobic Region: Rat/Torpedo Chimeric and Composite GPI Addition Signals—Assuming that the C-terminal hydrophobic domain of rat AChE starts at Leu\textsuperscript{36}, it contains 19 residues, in contrast with 15 in Torpedo. In mutant rm36, deletion of the first two residues from the rat hydrophobic region, LS, reduced the yield of cellular activity to about 80% of the wild type but slightly increased secretion.} \]

As shown in Table II (top), we analyzed constructs in which the rat catalytic domain was associated with the first 6 residues of the rat H peptide, including Cys\textsuperscript{8}, which allows the formation of disulfide-linked dimers, followed by a GPI addition signal in which the upstream region (around the ω site) and the hydrophobic region were derived either from rat or Torpedo. The resulting constructs (rr, rt, tr, and tt) are called “long” (L) and “short” (S), depending on the presence of residues 23–27 of the rat sequence (ALSLS).

Chimeric constructs containing either the complete rat hydrophobic region or the Torpedo hydrophobic region were processed like the wild type. Those containing the “short” rat hydrophobic region, combined with either rat or Torpedo ω regions, produced lower levels of cellular and secreted activity (Fig. 6), suggesting that the beginning of the rat hydrophobic region is important, despite the fact that this region is longer than that of Torpedo.

\[ \text{Distance between the ω Site and the Catalytic Domain—It has been reported that GPI addition requires a separation of at least 11 residues, between the ω site and an organized protein structure (19). By analogy with Torpedo AChE (46), we assume that the α\textsubscript{10} helix, which constitutes the last secondary structure element of the catalytic domain, terminates at residue} \]
FIG. 6. Analysis of mutants containing chimeric GPI addition signals. Histograms show relative activities and predicted scores. The levels of cellular activity were similar for all constructs, except those with shortened rat hydrophobic regions, but the levels of secreted activity varied more widely.

FIG. 7. Electrophoretic analysis of mutants differing in the distance between the ω site and the α10 helix of the catalytic domain (Table II, bottom). The lanes correspond to cell extracts, untreated and treated with PI-PLC, and to the medium, as indicated. The residues belonging to the α10 helix are shown in italic type, and the ω site is underlined.

FIG. 8. Intercatenary disulfide bond at an ω site cysteine; sedimentation and electrophoretic analyses of rm41 (HCGGR) and control mutants. Top, sedimentation profiles of cell extracts, untreated (○) and after PI-PLC digestion (□). The rm1' mutant, containing a single cysteine at position 6, like rm41 (HCGGR), is shown as a control. Bottom, electrophoretic patterns. In each case, the three lanes correspond to control and PI-PLC-treated cell extract and medium. Both types of analyses show that mutants lacking a cysteine produce only monomers and that rm41 produces PI-PLC-sensitive dimers.
Leu531 (46). It is separated by 9 residues from the $v$ site in mutant rm41 or rm42.

To find out whether this distance could be reduced further, we introduced deletions in mutant rm42, which produces the same level of activity as the wild type and is even better processed (Table III). Knowing its influence on processing, we maintained the $v_2$ histidine residue of rm42. The spacer between the $a_{10}$ helix and the $v$ site N residue was reduced to 4 residues in mutant D1 and 2 residues in mutant D2; in mutant D3, we removed 2 more residues, so that the $v$ site immediately follows the $a$ helix. All three mutants produced GPI-anchored, monomeric AChE (Fig. 7). Mutants D1 and D2 produced as much activity as mutant rm42; in the case of D3, the cellular and secreted activities were reduced by 25–50%.

Deletion of two leucines at the C terminus of helix $a_{10}$ also reduced the production of activity in truncated constructs, S3 and S4. This deletion probably destabilizes the helix and prevents the correct folding of a fraction of the protein, explaining the fact that D3 produced less activity than rm42, D1, or D2. In any case, the fraction of GPI-anchored active AChE was not reduced by the proximity of the $v$ site to an organized secondary structure in the protein.

Interference between the Formation of an Intercatenary Disulfide Bond and the Glypiation Process—The proximity of the disulfide bond that links two subunits might prevent a correct interaction of the $v$ site with the transamidase complex. In our various rat mutants, $v$ was separated from the cysteines involved in intercatenary disulfides by 5–7 residues. In the composite constructs (Table II, bottom) this distance was larger (23 residues) in ccL but only 6 residues in cc S; nevertheless, processing was equally efficient in both cases.

Can a cysteine at the $v$ site form an intercatenary disulfide bond? In mutant rm41 (HCGGR), the predicted $v$ site corresponds to Cys 6, which forms an intercatenary disulfide bond. We found that this mutant produced a low level of activity, including 42% GPI-anchored monomers and 33% GPI-anchored dimers (Fig. 8). Replacement of this cysteine by an asparagine in rm42 (HNGGR) increased the AChE activity to the wild type level and only produced GPI-anchored monomers. In mutant rm43, with a threonine at the same position (HTGGR), we obtained a similarly low level of activity as for rm41, but with a slightly reduced proportion of GPI-anchored AChE, corresponding to monomers. Cys$^6$ is therefore necessary for the production of dimers; glypiation could occur with an asparagine, a cysteine, or a threonine at this position, which is predicted to represent the only possible $v$ site, but with efficiencies varying in the order Asn $\gg$ Cys $>$ Thr, as also observed in mutants rm35, rm24, and rm29, in which the $v$ site is located.
GPI Addition, Secretion, and Degradation of AChE 7 residues downstream of the disulfide bridge. Note that a threonine can act as a poor but functional ω site in this case.

In the rm41 mutant, the cysteine residue serves as the ω site and also forms an intercatenary disulfide bond. This mutant produced more GPI-anchored monomers than dimers, in contrast to the wild type and to mutants in which the cysteine was distinct from the ω site. Thus, the positioning of the cysteine at the ω site reduced its capacity for disulfide linkage.

Conversely, the formation of a disulfide bond may interfere with glypiation, since the proportion of PI-PLC-sensitive AChE in the cell extracts was systematically and markedly higher in mutants rm42, D1, D2, and D3, which produce only GPI-anchored monomers, than in mutant rm35 (Table III, top). The formation of a disulfide bond at a short distance upstream of the ω site therefore interferes with glypiation, possibly because of a steric hindrance with the transamidase. This suggests that disulfide bonding may occur before glypiation or that the two processes can be simultaneous.

Influence of the Spacer between the ω Site and the Hydrophobic Region—We introduced the FLAG peptide immediately upstream of the hydrophobic sequence (rm44); this peptide appears unlikely to contain a new ω site itself, considering its short distance to the hydrophobic domain and its amino acid sequence. It should therefore increase the length of the spacer by 8 residues. In the resulting mutant, the total activity was reduced by a factor of 3, and the level of GPI-anchored enzyme was about 20% of the wild type (Table I, bottom). Thus, glypiation was reduced but could still occur with a 21-residue spacer, although this was not predicted by the big-PI algorithm.

Addition of a Hydrophilic FLAG Peptide at the C Terminus: Characterization of Uncleaved Molecules in Cell Extracts and in the Medium—We found that the addition of the highly charged FLAG peptide (DYKDDDDK) at the C terminus of the hydrophobic region did not significantly modify the efficiency of glypiation, the total yield of active AChE, or the level of AChE release; this was true for the wild type (rm1-f), for an intermediate mutant (rm5-f), for a poorly processed mutant (rm18-f), and for an essentially unprocessed mutant (rm13-f). This observation confirms a previous report that the GPI addition signal can be internal in the primary sequence (9).

In non-denaturing electrophoresis, the anti-FLAG monoclonal antibody M2 retarded the migration of at least a fraction of the amphiphilic, PI-PLC-resistant component of all mutants containing a C-terminal or an internal FLAG epitope, both in cell extracts and in the medium (Fig. 9A). This component therefore contains uncleaved precursors. In the case of the wild type rm1-f construct, these precursors represent about half of the PI-PLC-resistant component in the cell extract but were barely detectable in the medium. In the less efficiently processed mutants, we observed higher levels of uncleaved, flagged molecules in the medium.

In metabolic labeling experiments, newly synthesized polypeptides were immunoprecipitated by the anti-rat AChE antiserum or by M2, after a 5-min incorporation period. The labeling intensities obtained with both antibodies were similar, indicating that most AChE molecules carried the FLAG epitope (Fig. 9B). Therefore, the cells contained a large fraction of uncleaved precursors at this stage. After labeling for 3 h and a chase period of 20 h, the rm1-f protein was still present in the cells, as shown by immunoprecipitation with anti-rat AChE, but it was very weakly recognized by M2, and its apparent molecular mass was increased during the chase period; this increase probably reflects the maturation of N-glycans, in agreement with the fact that this wild type enzyme mostly consists of mature, GPI-anchored protein. At that time, labeling correspondingly to the poorly processed mutant rm13-f was considerably lower in the cells, indicating degradation, since it was not significantly secreted. The rm13-f protein remaining in the cell extracts was also recognized by M2 at a similar level, suggesting that the C-terminal peptide was not cleaved before degradation of the catalytic domain.

Prediction and Assessment of Processing Efficiency—Fig. 4D shows the relationship between the scores obtained from the big-PI predictor and the level of cellular GPI-anchored AChE. The efficiency of GPI addition can be appreciated in two ways, either by the fraction of cellular amphiphilic molecules that were sensitive to PI-PLC, or by the cellular content of GPI-anchored AChE. These two parameters are strongly correlated; however, the fraction of PI-PLC-sensitive amphiphilic AChE plateaus around 85% for GPI-anchored dimers, as in the wild type, while the level of GPI-anchored activity presents a wider range of variation. The latter parameter therefore appears to be a better indicator of processing, in the case of rat AChE. It reflects the level of cellular activity (Fig. 4A).

In general, we find that the big-PI predictor (18, 19) produces scores that are reasonably well correlated with processing efficiency, although there are exceptions in both directions: some of our poorly processed mutants obtained a score superior to 4, e.g. rm24 and rm41 (HCGG), rm16 (ESEGER), and rm27 (EGGSR), while some mutants that obtained less than 1.5 were in fact comparable with the wild type, e.g. rm34 (KGEAR) and rm36 (HGEAA). In contrast with our results, Glu at ω − 1 was predicted to be better than His, and this certainly results from a bias in the learning set. Although the presence of a FLAG epitope at the C terminus resulted in very negative scores, it did not actually influence processing.

Conclusion—In the present study, the rat AChE catalytic domain was associated with its own GPI addition signal (H peptide), carrying various mutations, and with composite signals that were partially derived from the totally different Torpedo AChE H peptide. These constructs covered a wide range of processing efficiencies, from essentially no GPI-anchored AChE to about 150% of the wild type. Quantitative and qualitative analyses of the mutants showed that uncleaved molecules were initially synthesized in an active form.

The process of glypiation was accompanied by a significant release of both uncleaved and lytic molecules in the medium. The secretion of uncleaved molecules was not expected, because precursors have been reported to be retained and degraded within the endoplasmic reticulum (42, 47). The lytic molecules did not seem to be derived from GPI-anchored molecules, by cleavage of the GPI anchor or of the peptide preceding the ω site; they may be produced by an aborted transamidation. In any case, secretion was clearly not a defect mechanism; glypiation itself, because these two processes are partially independent.
Glypiation could accommodate considerable variations in the spacer distances between an organized protein domain and ω and between ω and the hydrophobic region; we found no inferior limit to the distance between the secondary structure of the protein and the ω site. We showed that a cysteine could serve as an ω site and simultaneously form an intrasubunit disulfide bond; however, the formation of an intercatenary degradation, anchoring at the cell surface, or secretion will be useful to examine the mechanisms of trafficking toward their processing, both quantitatively and qualitatively. They calculated or secreted, suggesting a direct relationship between the derivatives, and GPI anchoring. When processing was inefficient depending on the quality of the signal, the interaction with the transamidation complex in the endoplasmic reticulum. De- pending on the quality of the signal, the interaction with the transamidation complex in the endoplasmic reticulum. Degradation of AChE resulted in four different outcomes: degradation, anchoring at the cell surface, or secretion. Thus, glypiation appeared to be a very flexible and robust process.

The presence of a GPI addition signal targets the protein to the transamidation complex in the endoplasmic reticulum. Depending on the quality of the signal, the interaction with the transamidase complex resulted in four different outcomes: degradation, secretion of uncleaved precursors, secretion of lytic derivatives, and GPI anchoring. When processing was inefficient, uncleaved molecules were degraded rather than accumulated or secreted, suggesting a direct relationship between the transamidase complex and the degradation machinery. The AChE mutants described in this study differ widely in their processing, both quantitatively and qualitatively. They will be useful to examine the mechanisms of trafficking toward degradation, anchoring at the cell surface, or secretion.

Acknowledgment—We thank Dr. Birgit Eisenhaber for analyzing the various C-terminal peptides according to her algorithm and for helpful discussions.

REFERENCES
1. Low, M. G. (1987) Biochem. J. 244, 1–13
2. Ferguson, M. A. J., and Williams, A. F. (1988) Annu. Rev. Biochem. 57, 285–320
3. Ferguson, M. A. (1999) J. Cell Sci. 112, 2789–2809
4. Friedrichson, T., and Kurrechal, T. V. (1998) Nature 394, 802–805
5. Kenworthy, A. K., Petranova, N., and Ededin, M. (2000) Mol. Biol. Cell 11, 1645–1655
6. Palle, A., Keller, P., Florin, E. L., Simons, K., and Horber, J. K. (2000) J. Cell Biol. 148, 997–1008
7. Mouillet-Richard, S., Ermonval, M., Chebassier, C., Laplanche, J. L., Lehmann, S., Launay, J. M., and Kellermann, O. (2000) Science 289, 1925–1928
8. Lehmann, S., and Harris, D. A. (1995) J. Biol. Chem. 270, 24589–24597
9. Caras, I. W. (1991) J. Cell Biol. 113, 77–85
10. Moran, P., and Caras, I. W. (1991) J. Cell Biol. 115, 1595–1600
11. Moran, P., and Caras, I. W. (1992) J. Cell Biol. 119, 763–772
12. Moran, P., and Caras, I. W. (1994) J. Cell Biol. 123, 333–343
13. Micanovic, R., Gerber, L. D., Berger, J., Kodukula, K., and Udenfriend, S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 157–161
14. Kodukula, K., Gerber, L. D., Anthauser, R., Brink, L., and Udenfriend, S. (1995) J. Cell Biol. 120, 657–664
15. Maxwell, S. E., Ramalingam, S., Gerber, L. D., and Udenfriend, S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1550–1554
16. Udenfriend, S., and Kodukula, K. (1995) Annu. Rev. Biochem. 64, 563–591
17. Ramalingam, S., Maxwell, S. E., Medof, M. E., Chen, R., Gerber, L. D., and Udenfriend, S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7528–7533
18. Eisenhaber, B., Bork, P., and Eisenhaber, F. (1998) Protein Eng. 11, 1155–1161
19. Eisenhaber, B., Bork, P., and Eisenhaber, F. (1999) J. Mol. Biol. 292, 741–758
20. Riezman, H., and Conzelmann, A. (1998) in Handbook of Protolytic Enzymes (Barrett, A. J., Rawlings, N. D., and Woessner, J. F. ed) pp. 756–759, Academic Press, London
21. Ohishi, K., Inoue, N., Maeda, Y., Takeda, J., Riezman, H., and Kinosita, T. (2000) Mol. Biol. Cell 11, 1525–1533
22. Massoulié, J., Pezzementi, L., Bon, S., Krejci, E., and Vallette, F. M. (1993) Proc. Neurobiol. 41, 31–91
23. Massoulié, J., Anselmet, A., Bon, S., Krejci, E., Legay, C., Mayat, E., Morel, N., and Simons, S. (1998) Structure and Function of Cholinesterases and Related Proteins (Doctor, B. P., Quinn, D. M., Rotundo, R. L., and Taylor, P., eds) pp. 3–24, Plenum Press, New York
24. Futerman, A. H., Low, M. G., and Silman, I. (1983) Neurosci. Lett. 40, 85–89
25. Futerman, A. H., Low, M. G., Ackermann, K. E., Sherman, W. R., and Silman, I. (1985) Biochem. Biophys. Res. Commun. 129, 312–317
26. Durov, N., Krejci, E., Grasal, J., Coussen, F., Massoulié, J., and Bon, S. (1992) EMBO J. 11, 3255–3262
27. Bucht, G., and Hjalmarsson, (1996) Biochim. Biophys. Acta 1292, 223–232
28. Roberts, W. L., and Rosenberg, T. L. (1986) Biochemistry 25, 3091–3098
29. Haas, R., Brandt, P. T., Knight, J., and Rosenberg, T. L. (1986) Biochemistry 25, 3098–3105
30. Haas, R., Jackson, B. C., Reinhold, B., Foster, J. D., and Rosenberg, T. L. (1996) Biochem. J. 314, 817–825
31. Mizushima, S., and Nagata, S. (1990) Nucleic Acids Res. 18, 5322
32. Bon, S., and Massoulié, J. (1997) J. Biol. Chem. 272, 3007–3015
33. Selden, R. F., Howe, K. B., Rowe, M. E., Goodman, H. M., and Moore, D. D. (1986) Mol. Cell. Biol. 6, 3173–3179
34. Ellman, G. L., Courtney, K. D., Andres, V., and Featherstone, R. M. (1961) Biochem. Pharmacol. 7, 88–95
35. Bon, S., Toutjani, J. P., Mellah, K., and Massoulié, J. (1988) J. Neurochem. 51, 786–794
36. Karnovsky, M. J., and Roots, L. (1964) J. Histochem. Cytochem. 12, 219–222
37. Marsh, B., Grassi, J., Vigny, M., and Massoulié, J. (1984) J. Neurochem. 43, 204–213
38. Furukawa, Y., Tsukamoto, K., and Ikezawa, H. (1997) Biochim. Biophys. Acta 1328, 185–196
39. Yan, W., and Ratnam, M. (1995) Biochemistry 34, 14594–14600
40. Tomassetti, A., Bottero, F., Mazi, M., Mott, S., Colnaghi, M. L., and Canevari, S. (1999) J. Cell. Biochem. 72, 111–118
41. Bucht, G., Wikstrom, P., and Hjalmarsson, K. (1999) Biochim. Biophys. Acta 1431, 471–482
42. Delabaysy, M. D., Stafford, F. J., Yuan, L. C., Shaz, D., and Bonifaci, J. S. (1993) J. Biol. Chem. 268, 12017–12027
43. Ferguson, M. A., Dasenbrock, M., Lamont, G. S., Overath, P., and Cross, G. A. (2000) J. Biol. Chem. 275, 356–362
44. Sharma, D. K., Vidugiriene, J., Bangs, J. D., and Menon, A. (1999) J. Biol. Chem. 274, 16479–16486
45. Meyer, U., Benghelzal, M., Imhof, I., and Conzelmann, A. (2000) Biochimie 82, 3461–3471
46. Sussman, J. L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L., and Silman, I. (1991) Science 253, 872–879
47. Doering, T. L., and Schekman, R. (1996) EMBO J. 15, 182–191
48. Li, Y., Camp, S., Rabinow, T. L., Getman, D., and Taylor, P. (1991) J. Biol. Chem. 266, 23083–23090
Addition of a Glycophosphatidylinositol to Acetylcholinesterase: PROCESSING, DEGRADATION, AND SECRETION
Françoise Coussen, Annick Ayon, Anne Le Goff, Jacqueline Leroy, Jean Massoulié and Suzanne Bon

J. Biol. Chem. 2001, 276:27881-27892.
doi: 10.1074/jbc.M010817200 originally published online May 3, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M010817200

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 46 references, 23 of which can be accessed free at http://www.jbc.org/content/276/30/27881.full.html#ref-list-1