# *Drosophila* Acetylcholinesterase: Demonstration of a Glycoinositol Phospholipid Anchor and an Endogenous Proteolytic Cleavage†

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ABSTRACT: The presence of a glycoinositol phospholipid anchor in *Drosophila* acetylcholinesterase (AChE) was shown by several criteria. Chemical analysis of highly purified *Drosophila* AChE demonstrated approximately one residue of inositol per enzyme subunit. Selective cleavage by *Staphylococcus aureus* phosphatidylinositol-specific phospholipase C (PI-PLC) was tested with *Drosophila* AChE radiolabeled by the photoactivatable affinity probe 3-(trifluoromethyl)-3-(*m*-[$^{125}$I]iodophenyl)diazirine ([$^{125}$I]TID), a reagent that specifically labels the lipid moiety of glycoinositol phospholipid-anchored proteins. Digestion with PI-PLC released 75% of this radiolabel from the protein. Gel electrophoresis of *Drosophila* AChE in sodium dodecyl sulfate indicated prominent 55- and 16-kDa bands and a faint 70-kDa band. The [$^{125}$I]TID label was localized on the 55-kDa fragment, suggesting that this fragment is the C-terminal portion of the protein. In support of this conclusion, a sensitive microsequencing procedure that involved manual Edman degradation combined with radiomethylation was used to determine residues 2–5 of the 16-kDa fragment. Comparison with the *Drosophila* AChE cDNA sequence [Hall, L. M. C., & Spierer, P. (1986) *EMBO J. 5*, 2949–2954] confirmed that the 16-kDa fragment includes the N-terminus of AChE. Furthermore, the position of the N-terminal amino acid of the mature *Drosophila* AChE is closely homologous to that of *Torpedo* AChE. The presence of radiomethylatable ethanolamine in both 16- and 55-kDa fragments was also confirmed. Thus, *Drosophila* AChE may include a second posttranslational modification involving ethanolamine.

Acetylcholinesterase (AChE,[1] EC 3.1.1.7) is an enzyme which occurs in both collagen-tailed "asymmetric" and non-collagenous "globular" forms (Massoulié & Bon, 1982; Rosenberry, 1985). The globular AChE forms from human and bovine erythrocytes and *Torpedo* are dimers ($G_2$) which have been shown by a variety of methods to contain a novel glycoinositol phospholipid at the C-terminus of the protein that is responsible for anchoring these AChEs in membranes (Roberts et al., 1987; Rosenberry et al., 1986; Futerman et al., 1985). Similar anchors have been found in certain other membrane proteins (Low & Saltiel, 1988; Ferguson & Williams, 1988). The central nervous system of the fruit fly *Drosophila melanogaster* contains a single type of AChE which is also a $G_2$ membrane-bound form. This enzyme is of considerable interest for genetic analysis because it is coded by a single locus *Ace*, and mutations at the *Ace* locus have dramatic impact on the insect's development and behavior [reviewed in Hall et al. (1980)]. Recently, cDNAs corresponding to the gene for *Drosophila* AChE have been sequenced (Hall & Spierer, 1986), and the AChE protein has been purified to homogeneity (Gnagey et al., 1987). Reductive radiomethylation demonstrated that the purified enzyme contained ethanolamine and glucosamine with free amino groups, components indicative of a glycoinositol phospholipid-anchored protein (Gnagey et al., 1987). Like the $G_2$ AChEs of human and bovine erythrocytes and *Torpedo*, *Drosophila* AChE is a dimer of 70-kDa subunits. In contrast to these other $G_2$ AChEs, disulfide reduction and SDS–PAGE analysis yields primarily 55- and 16-kDa bands and suggests that a single proteolytic cleavage has converted the 70-kDa chain to two fragments. The interpretation was obscured, however, by the finding that *both* 55- and 16-kDa fragments contained radiomethylatable ethanolamine and glucosamine (Gnagey et al., 1987). The goals of the present investigation were therefore to confirm the presence of a glycoinositol phospholipid anchor in *Drosophila* AChE and to determine the order of the 16- and 55-kDa fragments by independent procedures. Confirmation of the anchor utilized phosphatidylinositol-specific phospholipase C (PI-PLC) purified from *Staphylococcus aureus*, an enzyme that selectively cleaves the phospholipid in most glycoinositol phospholipid-anchored proteins [Low & Saltiel, 1988; but see Roberts et al. (1987)]. The fragment order was clarified through microsequencing the 16-kDa band by a procedure involving radiomethylation (Haas et al., 1986).

## Materials and Methods

*Enzymes.* AChE was extracted from *Drosophila* heads with Triton X-100 and purified to homogeneity by affinity chromatography on acridinium Sepharose CL-4B (Gnagey et al., 1987). Radiolabeling by reductive methylation involved 10 mM H$^{14}$CHO and $NaCNBH_3$ [Gnagey et al., 1987; cf. Haas and Rosenberry (1985)]; photoaffinity labeling utilized [$^{125}$I]TID (Roberts & Rosenberry, 1986). *S. aureus* PI-PLC was a generous gift of M. Low.

*Edman Sequencing.* Unlabeled AChE mixed with a trace amount of [$^{125}$I]TID-labeled AChE was repurified by affinity chromatography on acridinium resin to deplete it of detergent and dialyzed against 10 mM phosphate buffer, pH 7. Equal aliquots were dried on four 12 × 75 mm glass tubes in a Speedvac concentrator (Savant Instruments, Inc.). Amino

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[1] Abbreviations: AChE, acetylcholinesterase; PI-PLC, phosphatidylinositol-specific phospholipase C; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; [$^{125}$]TID, 3-(trifluoromethyl)-3-(*m*-[$^{125}$I]iodophenyl)diazirine.

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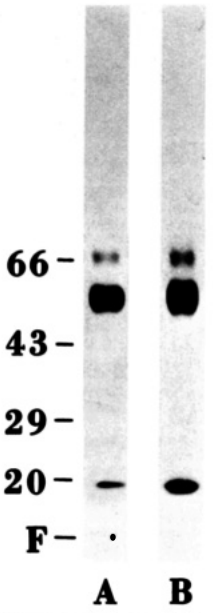


FIGURE 1: SDS–PAGE profile of *Drosophila* AChE. Samples were reduced and alkylated and electrophoresed in adjacent lanes of a 5–13% gradient slab gel which was silver-stained and then fluorographed. (A) *Drosophila* AChE (1 $\mu$g), silver-stained. (B) $^{14}$C-Radiomethylated *Drosophila* AChE (0.5 $\mu$g, 30 000 cpm), fluorographed. Numbers at left represent molecular weights ($\times 10^{-3}$) estimated from the position of the standards bovine serum albumin, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor; F marks the position of the pyronin Y dye front.

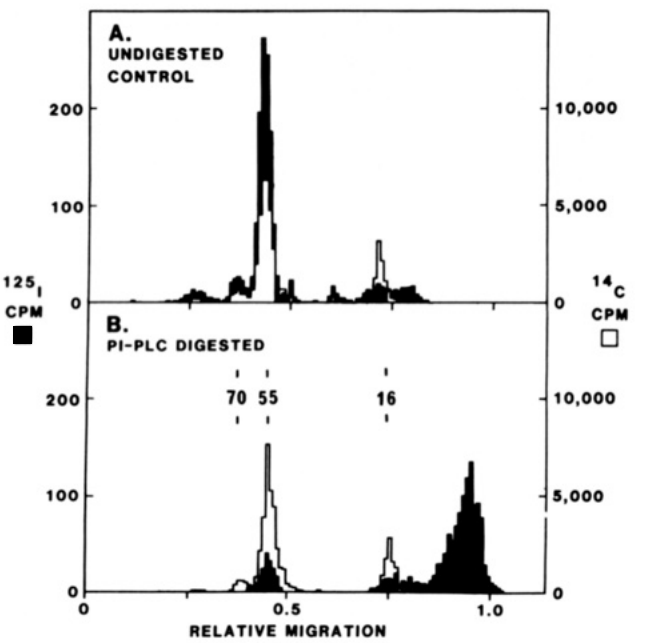


FIGURE 2: SDS–PAGE gel profiles before and after PI-PLC digestion of $^{14}$C-radiomethylated, [$^{125}$I]TID-labeled *Drosophila* AChE. Purified *Drosophila* AChE was radiomethylated with 10 mM H$^{14}$CHO and repurified by affinity chromatography, then labeled with [$^{125}$I]TID and repurified again. To the AChE (1.6 $\mu$g; 6200 cpm of $^{125}$I; 110 000 cpm of $^{14}$C) was added sodium deoxycholate (0.1% final concentration), and the sample was dialyzed for 4 h at 4 °C against 20 mM sodium phosphate, pH 7, containing 0.1% sodium deoxycholate. The sample was subsequently divided into two equal portions, one of which was left untreated at room temperature as a control while the other was digested for 1 h at 37 °C with 20 $\mu$g/mL PI-PLC. The samples were then dried to 100 $\mu$L on the Speed-Vac, reduced, alkylated, and electrophoresed on a 5–20% gradient SDS–PAGE gel. After silver staining, the gel was sliced into 1-mm slices and $\gamma$ counted 10–50 min per slice; the slices were then solubilized with NCS (Amersham) and scintillation counted. Shown are the profiles of (□) $^{14}$C radioactivity (26 cpm background subtracted) and (■) $^{125}$I radioactivity (35 cpm background subtracted, and peaks with maxima less than 10 cpm omitted) for the undigested control (A) and the digested (B) samples. No corrections were made for slight contributions arising from crossover of $^{125}$I radioactivity in the scintillation counter (approximately 80%) or from $^{14}$C radioactivity in the $\gamma$ counter (approximately 0.05%). The abscissa represents relative migration from 0 (top of the separating gel) to 1.0 (pyronin Y dye front), and migrations of the 70-, 55-, and 16-kDa bands are indicated.

acids were removed sequentially from the N-terminus by subjecting the samples to manual Edman degradation essentially as described by Black and Coon (1982). After each sequencing cycle, one tube was removed, and the protein was resuspended by vortexing with four 10-$\mu$L portions of 0.1% SDS in 20 mM phosphate, pH 7, and transferred to a 1.5-mL Eppendorf tube for 15-min incubation at 37 °C with 10 mM H$^{14}$CHO (ICN) and 50 mM $NaCNBH_3$ (Haas & Rosenberry, 1985; Haas et al., 1986). Sodium phosphate (pH 7, 20 mM, 1 mL) was added, and unincorporated radioactivity was removed by dialysis against the same buffer. The sample was subjected to gradient SDS–PAGE under reducing conditions (Gnagey et al., 1987), and following silver staining, the 16-kDa band was cut from the gel, minced, dried, and hydrolyzed for amino acid analysis (Gnagey et al., 1987).

*Other Methods.* SDS–PAGE was performed on 5–13% or 5–20% slab gels prepared and silver-stained as in Gnagey et al. (1987). After disulfide reduction, samples were alkylated with 120 mM iodoacetamide. Radiolabeled bands were detected by fluorography (Bonner & Laskey, 1974) and quantitated by gel slicing (Barnett & Rosenberry, 1979). Inositol was determined by gas chromatographic analysis as in Roberts et al. (1987).

## Results

*Drosophila AChE Bears a Glycoinositol Phospholipid Anchor on Its 55-kDa Fragment.* Although AChE from *Drosophila* is a disulfide-linked dimer of 70-kDa subunits, the SDS–PAGE profile of the protein purified by affinity chromatography typically shows only a small proportion of intact 70-kDa subunits (Gnagey et al., 1987). The majority of the protein is cleaved to 55- and 16-kDa fragments, as demonstrated in Figure 1. Both unlabeled samples stained with silver (lane A) and samples radiomethylated on amine groups and subjected to fluorography (lane B) showed predominant 55- and 16-kDa bands and a faint 70-kDa band. This fragmentation of the protein permitted localization of its glycolipid anchor as outlined below.

While previous analysis of radiomethylated *Drosophila* AChE had revealed ethanolamine and glucosamine with free amino groups (Gnagey et al., 1987), additional criteria were examined to confirm the presence of a glycoinositol phospholipid anchor. A sample of *Drosophila* AChE (23 $\mu$g) was analyzed for inositol (Roberts et al., 1987) and yielded 374 pmol of myoinositol, a content of 1.13 residues of inositol per enzyme subunit. To examine the susceptibility of *Drosophila* AChE to PI-PLC, the AChE was photolabeled with the hydrophobic probe [$^{125}$I]TID, a reagent that specifically radiolabels the hydrophobic anchor domain of glycolipid-anchored proteins (Roberts & Rosenberry, 1986; Medof et al., 1986). About 90% of the total [$^{125}$I]TID label incorporated into bovine erythrocyte AChE is linked to the anchor alkylacylglycerol moiety and can be released by PI-PLC (Roberts et al., 1987). Figure 2 shows a similar experiment in which *Drosophila* AChE labeled with [$^{125}$I]TID was analyzed by SDS–PAGE before and after PI-PLC digestion. Much (75%) of the $^{125}$I radiolabel (■) was removed from the protein and shifted to a position near the dye front following the digestion, demon-

strating that the hydrophobic anchor labeled by [$^{125}$I]TID is indeed susceptible to PI-PLC.

While glycoinositol phospholipid anchors so far have been found only at protein C-termini [see Haas et al. (1986)], radiomethylation analysis of *Drosophila* AChE surprisingly revealed glucosamine and ethanolamine in *both* the 16- and 55-kDa fragments (Gnagey et al., 1987). [$^{125}$I]TID labeling and PI-PLC digestion therefore were combined with radiomethylation in Figure 2 to address the question of which fragment of the intact subunit bears the anchor. In addition to [$^{125}$I]TID labeling, the AChE sample in Figure 2 also had been radiomethylated with $^{14}$C (□). Radiomethylation was expected to label only enzyme residues proximal to the PI-PLC cleavage site, and indeed no $^{14}$C label was released to the dye front following PI-PLC cleavage (Figure 2B). Analysis of the ratio of the $^{125}$I to $^{14}$C radioactivity permitted precise identification of the bands susceptible to PI-PLC. The undigested control (panel A) showed coincident $^{125}$I and $^{14}$C peaks for the 70-, 55-, and 16-kDa bands. The absolute level of [$^{125}$I]TID labeling of the 55-kDa band, more than 7-fold higher than that of the 16-kDa band, immediately suggested that the 55-kDa fragment but not the 16-kDa fragment contained the glycolipid anchor. This conclusion is confirmed by the results of the PI-PLC digestion (panel B). The PI-PLC had no effect on the level or distribution of $^{14}$C in the SDS–PAGE bands but released most of the [$^{125}$I]TID label from both the 70- and 55-kDa bands. Following PI-PLC digestion, the average ratio of $^{125}$I to $^{14}$C across the 70-kDa band decreased by at least 77% and across the 55-kDa band by 86%. In contrast, the $^{125}$I/$^{14}$C ratio across the 16-kDa band was unchanged by PI-PLC and remained at about 0.007. This ratio was comparable to the 0.0059 ratio observed for the 55-kDa band *after* digestion, suggesting that the $^{125}$I radioactivity of the 16-kDa band represents only nonspecific background labeling of the polypeptides themselves.

*The 16-kDa Fragment of Drosophila AChE Contains the Protein N-Terminus and Non-Glycolipid Ethanolamine.* Since the PI-PLC digestion data gave no evidence of a glycoinositol phospholipid anchor in the 16-kDa fragment, the radiomethylation data were reexamined. Gnagey et al. (1987) had found that the 16-kDa fragment contained ethanolamine and a small amount of glucosamine, as well as an $N^{\alpha}$-amino group, "X", which presumably represented an unblocked N-terminus that did not correspond to a known amino acid. This experiment was repeated in Table I, and it again illustrated the considerable enrichment of both N-terminal "X" and ethanolamine in the 16-kDa fragment, although little glucosamine was observed in this fragment. Possible heterogeneity in the 16-kDa region was also indicated by radiomethylation analysis of an area of the gel directly below the 16-kDa band which contained about 40% as much $^{14}$C radioactivity (Table I, "<16 kDa"). This area had a $^{14}$C distribution similar to that of the 16-kDa band but with considerably less ethanolamine.

Reductive radiomethylation analysis as a means of N-terminal determination can be combined with Edman degradation to yield a sensitive and powerful microsequencing procedure (Haas et al., 1986). The method was extended here by the use of SDS–PAGE to resolve the 16-kDa band after the Edman degradation and radiomethylation reactions. The 16-kDa bands were cut from the gel, hydrolyzed, and subjected to amino acid analysis to yield the profiles of Figure 3. The process indicated a polypeptide sequence for residues 2–5 of Ile-Asx-Arg-Leu. The identification in all cases was confirmed by co-chromatography with $^{3}$H-radiomethylated standards (data not shown). Although formation of $N^{\alpha}$-dimethylated

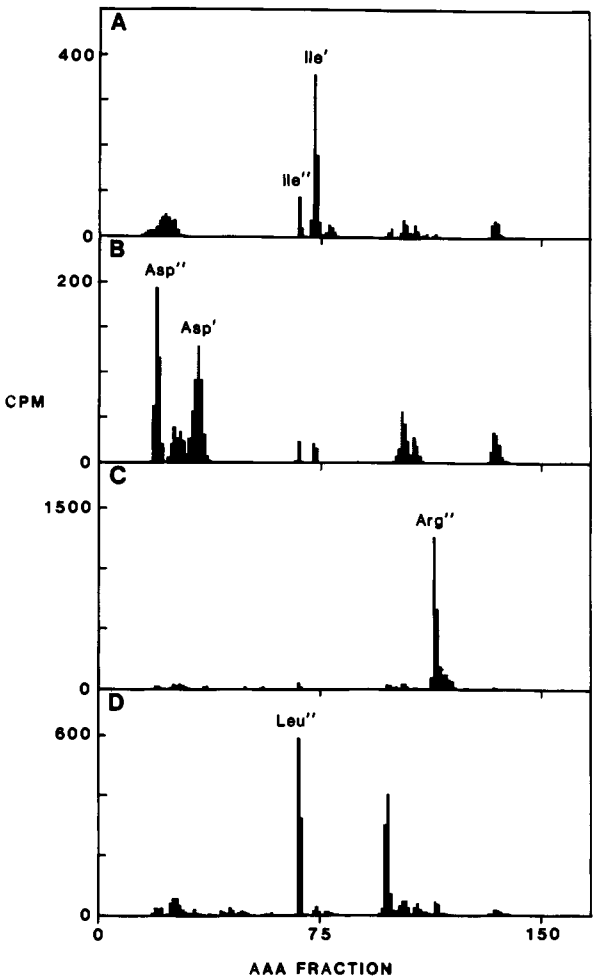


FIGURE 3: Sequencing of *Drosophila* AChE 16-kDa fragment by sequential Edman degradation and radiomethylation analysis. Samples of *Drosophila* AChE were subjected to one to four cycles of Edman degradation, radiomethylated to label the newly exposed N-terminal amino acid, and isolated by SDS–PAGE as described under Materials and Methods. The 16-kDa band was cut from the gel, hydrolyzed, and then subjected to amino acid analysis (AAA) to identify the radiomethylated N-terminus as in Haas and Rosenberry (1985). Panels A–D show the profiles of radioactivity eluted from the analyzer for samples after 1–4 cycles, respectively. A background of 25–30 cpm has been subtracted, and labeled peaks with maxima less than 10 cpm have been deleted. Fraction size was 28 drops (approximately 0.9 mL). Each initial sample of *Drosophila* AChE contained 350 pmol, as determined by enzymatic activity, and 400 cpm of tracer [$^{125}$I]TID-labeled AChE. As estimated from the $^{125}$I radioactivity, 100–250 pmol was recovered from the tubes after Edman degradation, and final gel loads following radiomethylation, dialysis, and drying to low volume were 15–40 pmol. The recovery of the labeled amino acids was determined by comparing the gel onput to the observed peak areas in the figure [normalized by the known specific activity of the radioformaldehyde; cf. Haas and Rosenberry (1985)] and was 0.42–0.77 mol of N-terminus/mol of subunit. Peaks in panels B and D near fraction 100 represent contaminants which appeared irregularly but did not correspond to any of the standard amino acids. The elution protocol used here will not resolve dimethyl-Leu and -Ile. However, the peak in panel D was confirmed as dimethyl-Leu by comigration with standards under a different elution protocol using only three buffers (Haas & Rosenberry, 1985) that distinguishes these two methylated amino acids (data not shown).

amino acids during the radiomethylation reaction was incomplete following the first two Edman cycles [perhaps because of interference by SDS (Jentoft & Dearborn, 1979)], the assignments were clear because the additional peaks comigrated with the appropriate *mono*-methylated amino acid standards. This sequence corresponded uniquely to residues 40–43, Ile-Asp-Arg-Leu, deduced from the full-length *Drosophila* AChE cDNA coding sequence (Hall & Spierer, 1986).

Table I: Radiomethylation Analysis of Amines in SDS-PAGE Bands of *Drosophila* AChE[a]

| amine | % of residues in band | | | |
|---|---|---|---|---|
| | 70 kDa | 55 kDa | 16 kDa | <16 kDa |
| glucosamine | 3 | 79 | 13 | 5 |
| ethanolamine | 3 | 40 | 50 | 6 |
| lysine | 4 | 75 | 14 | 7 |
| "X" | 2 | <10 | 64 | 24 |

[a] *Drosophila* AChE, affinity repurified after reductive methylation with 10 mM $H^{14}CHO$ and dialyzed 5.5 h in the presence of 0.2% SDS (2 mg, 140000 cpm), was reduced, alkylated, and electrophoresed on a 5–20% gradient gel. The gel was silver-stained, and the 70-, 55-, and 16-kDa bands and an area "<16 kDa" directly below the 16-kDa band were cut from the gel. The gel pieces were minced, dried, and hydrolyzed for amino acid analysis as in Gnagey et al. (1987), except that hydrolysis time was restricted to 12 h rather than 16 h to minimize breakdown of glucosamine [cf. Haas et al. (1986)]. The samples were subjected to amino acid analysis (Haas & Rosenberry, 1985), and the radioactivity corresponding to methylated glucosamine, ethanolamine, lysine, and N-terminal "X" was determined (Haas & Rosenberry, 1985; Gnagey et al., 1987). Percentages were calculated from the distribution of total radioactivity among the bands and the distribution of label in each band. The distribution of total radioactivity had been determined in a separate gel slicing experiment as follows: 70 kDa, 4%; 55 kDa, 68%; 16 kDa and <16 kDa combined, 28%. The latter quantity was allocated as 16 kDa, 21%, and <16 kDa, 8%, in proportion to the recovery of radioactivity following hydrolysis of the samples in the present experiment.

The dipeptide Asx-Arg in fact occurs only this once in the protein. Hence, a major component if not all of the 16-kDa band is unambiguously identified as the N-terminal portion of the AChE.

## Discussion

A new class of proteins has been discovered within the past few years which are bound to membranes exclusively by a novel glycoinositol phospholipid anchor (Low & Saltiel, 1988; Ferguson & Williams, 1988). The study of Gnagey et al. (1987) suggested that AChE from *Drosophila*, like AChE from human erythrocytes (Haas et al., 1986; Roberts & Rosenberry, 1986), bovine erythrocytes (Roberts et al., 1987), and *Torpedo* (Futerman et al., 1985), has such an anchor by demonstrating the presence of ethanolamine and glucosamine. In this paper, we confirm the presence of this glycolipid anchor in *Drosophila* AChE by two more criteria: detection of covalently bound inositol and specific removal of the hydrophobic affinity label [$^{125}$I]TID by PI-PLC (Figure 2). PI-PLC susceptibility is characteristic of many of these glycolipid anchors (Low & Saltiel, 1988), including that from bovine AChE (Roberts et al., 1987). However, the human erythrocyte proteins decay accelerating factor (Davitz et al., 1986) and AChE (Roberts et al., 1987) are important exceptions and display PI-PLC resistance, apparently because the inositol is further modified by palmitoylation (Roberts et al., 1988). Glycolipid-anchored proteins studied to date are primarily from mammals and their protozoan parasites (Low & Saltiel, 1988; Ferguson & Williams, 1988). The presence of the anchor in the insect protein *Drosophila* AChE thus is interesting evidence for the more general occurrence of this novel type of post-translational modification.

In a concurrent study, Fournier et al. (1988) showed that *Drosophila* AChE is susceptible to PI-PLC by the criteria of Triton X-114 partitioning and electrophoresis in nondenaturing gels. Our PI-PLC digestion experiment extends this observation by defining the quantitative extent of cleavage and localizing the anchor to the 55-kDa fragment of the protein. At least 86% of the anchor on the 55-kDa fragment was cleaved by PI-PLC, as measured by release of [$^{125}$I]TID label, and actual release was higher because of some [$^{125}$I]TID labeling outside of the anchor lipid. The glycoinositol phospholipid anchor is attached to the protein C-terminus in human erythrocyte acetylcholinesterase (Haas et al., 1986) and indeed in all such proteins currently known (Ferguson & Williams, 1988). Thus, by analogy the 55-kDa fragment represents the C-terminus of *Drosophila* AChE.

To confirm this assignment, we analyzed the amine composition (Table I) and amino acid sequence of the 16-kDa fragment (Figure 3). The earlier methylation analysis by Gnagey et al. (1987) showed a single methylatable $\alpha$-amino acid "X" in both the 70-kDa and the 16-kDa SDS–PAGE bands, suggesting that the N-terminus of the protein is "X" and lies in the 16-kDa fragment. Sequencing of the 16-kDa fragment (Figure 3) and comparison with the cDNA sequence of *Drosophila* AChE (Hall & Spierer, 1986) here confirmed this assignment. The versatility of combined Edman degradation and radiomethylation (Haas et al., 1986) was further enhanced in this experiment by the inclusion of a gel purification step which allowed the polypeptide mixture to be separated *after* the degradation and radiomethylation reactions. The correspondence of residues 2–5 of the 16-kDa fragment sequence to residues 40–43 of the coding region predicted by *Drosophila* AChE cDNAs (Hall & Spierer, 1986) indicates that the N-terminus of the mature subunit following removal of a 38 amino acid leader sequence should be Val$^{39}$. Because only a small proportion of the *Drosophila* AChE remained in the intact 70-kDa form, it was not possible to determine its N-terminus directly by the same methods. Theoretically, it could lie further upstream, for example, if exoproteolytic trimming occurred at the same time as the fragmentation of the 70-kDa chain to 55- and 16-kDa chains. It is noteworthy, however, that when the amino acid sequence of *Torpedo* AChE is aligned with the highly homologous one of *Drosophila* AChE, the observed N-terminus of *Torpedo* AChE is only one residue away at the position corresponding to *Drosophila* AChE residue 38 (Hall & Spierer, 1986; Schumacher et al., 1986).

Three questions remain unresolved concerning *Drosophila* AChE. The first is the failure of the predicted *Drosophila* AChE N-terminus Val$^{39}$ to agree with the N-terminal amino acid "X" observed by radiomethylation. Radiomethylated "X" does not correspond to a standard amino acid and elutes from the amino acid analyzer at a position near radiomethylated lysine. The data could be reconciled with the predicted N-terminus if Val$^{39}$ were modified posttranslationally to a more basic amino acid *without* loss of its $N^{\alpha}$-amino group, but the nature of such a modification is unknown. The second question concerns the nature of the proteolytic reaction that converts the mature 70-kDa chain of *Drosophila* AChE to the 16-kDa + 55-kDa fragments. This conversion could not be blocked by the addition of protease inhibitors to fly head extraction buffers (Gnagey et al., 1987). Furthermore, radiomethylation and sequencing failed to reveal an N-terminal amino acid sequence on the 55-kDa fragment (data not shown). These observations suggest that the N-terminus of the 55-kDa fragment may be blocked and, therefore, that the proteolytic cleavage of the 70-kDa polypeptide to the 55- and 16-kDa fragments may occur in vivo.

The third open issue is the nature of the ethanolamine persistently found in the 16kDa fragment (Gnagey et al., 1987; Table I). There was some heterogeneity in the 16-kDa band, as shown by the differing compositions of the 16-kDa and "<16-kDa" bands in Table I. Thus, the ethanolamine could reflect a contaminant species derived from the C-terminal

anchor of *Drosophila* AChE that fortuitously coelectrophoresed with the N-terminal fragment detected by sequencing, as suggested by Gnagey et al. (1987). However, attempts to identify discrete 16-kDa fragments by radiomethylation and [$^{125}$I]TID labeling followed by fluorography and autoradiography have failed.[2] It is possible that ethanolamine is covalently linked to the 16-kDa fragment in a posttranslational modification unrelated to glycoinositol phospholipid anchors. Evidence that one 45–55-kDa protein in a variety of cells can undergo such a modification has recently been obtained (Fatemi et al., 1987; Tisdale & Tartakoff, 1988). *Drosophila* AChE may include a second ethanolamine-containing domain of this type in its 16-kDa fragment.

## ACKNOWLEDGMENTS

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**Registry No.** AChE, 9000-81-1; ethanolamine, 141-43-5; D-glucosamine, 3416-24-8; L-lysine, 56-87-1.

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