Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M., & Sporn, M. B. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5339–5343.
Roberts, A. B., Anzano, M. A., Wakefield, L. M., Roche, N. S., Stern, D. F., & Sporn, M. B. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 119–123.
Rose, S. P., Pruss, R. M., & Herschman, H. R. (1975) *J. Cell. Physiol. 86*, 593–598.
Ross, E. M., & Gilman, A. G. (1980) *Annu. Rev. Biochem. 49*, 533–564.
Rubin, R. A., & Earp, H. S. (1983) *Science (Washington, D.C.) 219*, 60–63.
Rubin, R. A., O'Keefe, E. J., & Earp, H. S. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 776–780.
Scatchard, G. (1949) *Ann. N.Y. Acad. Sci. 51*, 660–672.
Schreiber, A. B., Lax, I., Yarden, Y., Eshhar, Z., & Schlessinger, J. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 7535–7539.
Schreiber, A. B., Libermann, T. A., Lax, I., Yarden, Y., & Schlessinger, J. (1983) *J. Biol. Chem. 258*, 846–853.
Segrest, J. P., Jackson, R. L., Andrews, E. P., & Marchesi, V. T. (1971) *Biochem. Biophys. Res. Commun. 44*, 390–395.
Simonds, W. F., Koski, G., Streaty, R. A., Hjelmeland, L. M., & Klee, W. A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4623–4627.
Smith, J. M., Sporn, M. B., Roberts, A. B., Derynck, R., Winkler, M. E., & Gregory, H. (1985) *Nature (London) 315*, 515–516.
Sporn, M. B., & Todaro, G. J. (1980) *N. Engl. J. Med. 303*, 878–880.
Staros, J. V. (1982) *Biochemistry 21*, 3950–3955.
Taylor, J. M., Mitchell, W. M., & Cohen, S. (1972) *J. Biol. Chem. 247*, 5928–5934.
Thom, D., Powell, A. J., Lloyd, C. W., & Rees, D. A. (1977) *Biochem. J. 168*, 187–194.
Todaro, G. J., De Larco, J. E., Fryling, C., Johnson, P. A., & Sporn, M. B. (1981) *J. Supramol. Struct. Cell Biochem. 15*, 287–301.
Tucker, R. F., Branum, E. L., Shipley, G. D., Ryan, R. J., & Moses, H. L. (1984a) *Proc. Natl. Acad. Sci. U.S.A. 81*, 6757–6761.
Tucker, R. F., Shipley, G. D., Moses, H. L., & Holley, R. W. (1984b) *Science (Washington, D.C.) 226*, 705–707.
Yamamoto, T., Noshida, T., Miyajima, N., Kawai, S., Ooi, T., & Toyoshima, K. (1983) *Cell (Cambridge, Mass.) 35*, 71–78.

Selective Radiolabeling and Isolation of the Hydrophobic Membrane-Binding Domain of Human Erythrocyte Acetylcholinesterase†

William L. Roberts and Terrone L. Rosenberry*
Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106
Received October 25, 1985; Revised Manuscript Received January 14, 1986

ABSTRACT: The hydrophobic, membrane-binding domain of purified human erythrocyte acetylcholinesterase was labeled with the photoactivated reagent 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine. The radiolabel was incorporated when the enzyme was prepared in detergent-free aggregates, in detergent micelles, or in phospholipid liposomes, but the highest percentage of labeling occurred in the detergent-free aggregates. Papain digestion of the enzyme released the hydrophobic domain, and polyacrylamide gel electrophoresis in sodium dodecyl sulfate or gel exclusion chromatography demonstrated that the label was localized exclusively in the cleaved hydrophobic domain fragment. This fragment was purified in a three-step procedure. Digestion was conducted with papain attached to Sepharose CL-4B, and the supernatant was adsorbed to acridinium affinity resin to remove the hydrophilic enzyme fragment. The nonretained fragment associated with Triton X-100 micelles was then chromatographed on Sepharose CL-6B, and finally detergent was removed by chromatography on Sephadex LH-60 in an ethanol–formic acid solvent. The fragment exhibited an apparent molecular weight of 3100 on the Sephadex LH-60 column when compared with peptide standards. However, amino acid analysis of the purified fragment revealed only 1 mol each of histidine and glycine per mole of fragment in contrast to the 25–30 mol of amino acids expected on the basis of the molecular weight estimate. This result suggests a novel non-amino acid structure for the hydrophobic domain of human erythrocyte acetylcholinesterase.

Acetylcholinesterase (AChE,[1] EC 3.1.1.7) occurs in a variety of forms in vertebrate tissues. These forms can be classified as either asymmetric if they contain a collagen-like tail or globular if they lack such a tail structure [see Massoulié & Bon (1982) and Rosenberry (1985)]. The globular AChEs include a class of membrane-bound forms which are the predominant AChEs in mammalian brain and in muscle cells outside of the neuromuscular junction. These forms possess a hydrophobic domain that anchors the enzyme in the cell

†This investigation was supported by Grant NS-16577 from the National Institutes of Health and by grants from the Muscular Dystrophy Association. W.L.R. is a Medical Scientist Predoctoral Trainee supported by Grant T32 GM07250 from the National Institutes of Health.

[1] Abbreviations: AChE, acetylcholinesterase; RBC AChE, human erythrocyte acetylcholinesterase; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; [^{125}I]TID, 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; G_n, a globular AChE form with *n* catalytic subunits; PIPLC, phosphatidylinositol-specific phospholipase C from *Staphylococcus aureus*; kDa, kilodalton(s); HPLC, high-pressure liquid chromatography.

0006-2960/86/0425-3091$01.50/0

plasma membrane, and they require detergents for extraction. A dimeric form (G_2) in this class is the only AChE present on human erythrocytes, and this enzyme (denoted RBC AChE) provides a general model for the study of integral membrane AChE forms. Highly purified RBC AChE associates with detergent micelles (Wiedmer et al., 1979; Rosenberry & Scoggin, 1984) but forms soluble aggregates on removal of detergent (Ott & Brodbeck, 1978). RBC AChE is an amphiphatic protein whose small hydrophobic domain can be cleaved from the remaining hydrophilic enzyme by papain digestion of soluble enzyme aggregates, enzyme in detergent micelles, or enzyme in small phospholipid liposomes (Dutta-Choudhury & Rosenberry, 1984; Kim & Rosenberry, 1985). In these reports, the existence of the hydrophobic domain was inferred exclusively from the alteration in RBC AChE properties following papain digestion. While the data indicated that the hydrophobic domain was removed by papain digestion, no attempt was made to isolate the hydrophobic domain fragment from residual intact enzyme or from other fragments produced by papain.

To pursue the structure of the hydrophobic domain in RBC AChE, we investigated a specific radiolabel that could identify the hydrophobic domain fragment and permit its isolation following papain cleavage. The hydrophobic domains of other integral membrane proteins have been selectively labeled with photoactivatable reagents (Klip & Gitler, 1974; Bayley & Knowles, 1980; Frielle et al., 1982). One of these reagents, [^{125}I]TID, partitions nearly quantitatively into the hydrophobic phase of membranes or detergent micelles and, on photoactivation, reacts covalently with protein sequences in this phase (Brunner & Semenza, 1981; Spiess et al., 1982). We have reported preliminary observations of the selective labeling of the RBC AChE hydrophobic domain with [^{125}I]TID [see Dutta-Choudhury & Rosenberry (1984)], and this reagent also selectively labeled the hydrophobic domain of a torpedo G_2 AChE (Stieger et al., 1984). In this paper, we document that [^{125}I]TID specifically labels the hydrophobic domain of RBC AChE and isolate the labeled hydrophobic domain fragment generated by papain. The amino acid composition of the isolated fragment indicates the presence of non-amino acid components. This observation is extended by concurrent work in our laboratory which indicates that the isolated hydrophobic domain fragment includes 2 mol of covalently attached fatty acids (Roberts & Rosenberry, 1985) as well as 2 mol of ethanolamine and 1 mol of glucosamine (Haas et al., 1986).

Materials and Methods

Proteins. RBC AChE was extracted from outdated erythrocyte membranes with Triton X-100, purified by affinity chromatography on acridinium resin to a specific activity of 5000–5800 units/mg of protein, and depleted of Triton X-100 by chromatography on hydroxyapatite as described by Rosenberry and Scoggin (1984). The same acridinium resin stock was used for all repurifications of radiolabeled enzyme.

Bovine catalase, chicken egg white lysozyme (M_r 14 300), bovine aprotinin (M_r 6500), a mixture of bovine and porcine glucagon (M_r 3490), *Bacillus brevis* gramicidin D (M_r 1850), and papain in suspension (25–28 mg/mL) were obtained from Sigma. Reference molecular weight values were from Frielle et al. (1982). Papain resin (papain-linked Sepharose CL-4B, 2.5 mg of papain/mL of packed resin) was prepared as previously described (Dutta-Choudhury & Rosenberry, 1984).

Photolabeling of RBC AChE. The photolabeling reagent [^{125}I]TID was obtained either from Amersham (10 Ci/mmol) or by direct synthesis according to the last step of the procedure in Brunner and Semenza (1981) (approximately 5 Ci/mmol). The precursor 3-(trifluoromethyl)-3-[*m*-(formylamino)-phenyl]diazirine for the synthesis was kindly provided by Dr. Josef Brunner, Eidgenossischen Technischen Hochschule, Zurich, Switzerland. The labeling results obtained with reagent from both sources appeared equivalent. The reagent is volatile, and precautions were taken to ensure that reaction vessels were sealed.

RBC AChE was labeled with [^{125}I]TID in three solution conditions: detergent-free enzyme aggregates, Triton X-100 micelles, and reconstituted liposomes. A 10–30-μL aliquot of [^{125}I]TID in ethanol (3–200 μCi) was added to 0.5–2.0 mL of buffered RBC AChE in a 12 × 75 mm rubber-stoppered borosilicate glass test tube and agitated gently to ensure complete mixing. Photolysis was performed for 15–30 min at 350 nm in a Farrand Mark I spectrofluorometer with the slits removed. The sample was applied to a 2-mL column of acridinium resin and washed extensively with 0.1% Triton X-100 in 20 mM sodium phosphate buffer, pH 7. If radiolabeled RBC AChE in Triton X-100 micelles was desired, the enzyme was eluted from the resin with 10 mM decamethonium bromide in buffered 0.1% Triton X-100. If the radiolabeled enzyme was to be reconstituted into liposomes, the resin was further washed with buffered 0.5 M NaCl to remove residual detergent, and the enzyme was eluted with 10 mM decamethonium bromide in buffered 0.5 M NaCl. Detergent depletion by this elution procedure was more efficient than hydroxyapatite chromatography for small samples of concentrated RBC AChE. Decamethonium bromide was removed from the samples by dialysis.

Liposome Reconstitution. Reconstitution of unlabeled RBC AChE or of [^{125}I]TID-labeled RBC AChE into small phospholipid liposomes was conducted as described in Kim and Rosenberry (1985). Reconstitution mixtures contained the enzyme (10 units/mg of phospholipid), crude egg yolk phospholipid (Sigma Chemical Co., 2–50 mg/mL), 1 mM edrophonium chloride, and 10 mM Tris-HCl, pH 7.4. Stock phospholipid solutions were prepared by probe sonication before addition to the reconstitution mixture.

Papain Digestion of Radiolabeled RBC AChE. [^{125}I]-TID-labeled RBC AChE that had been labeled in protein aggregates was used in all digestion experiments. Radiolabeled enzyme that had been reconstituted into liposomes was digested with soluble papain (0.1 mg/mL) that had been activated with cysteine (Kim & Rosenberry, 1985). RBC AChE associated with Triton X-100 micelles was digested with activated papain resin in 0.1% Triton X-100–20 mM sodium phosphate (pH 7) at 4 °C for 3–6 h as outlined in Dutta-Choudhury and Rosenberry (1984). Resin digestion was terminated by brief centrifugation and removal of the supernatant. The sedimented resin (1 volume) was washed 3 times with 0.1% Triton X-100–20 mM sodium phosphate (0.3 volume of each), and the washings were combined with the original supernatant. Edrophonium chloride (0.1 mM) was included in all digests to minimize degradation of the RBC AChE subunits (Dutta-Choudhury & Rosenberry, 1984).

Affinity Chromatography of Radiolabeled RBC AChE following Digestion with Papain Resin. Supernatants containing 0.1% Triton X-100 from the papain resin digest were applied to a 20-mL acridinium affinity column (1.5 × 12 cm; Rosenberry & Scoggin, 1984) at 4 °C with a flow rate of 0.2-0.3 column volume/h. The column was washed with 1–3 volumes of buffered (20 mM sodium phosphate, pH 7) 0.1% Triton X-100, 1–3 volumes of detergent-free buffer (20 mM sodium phosphate, pH 7) at 1–2 volumes/h, and 1 volume of buffered 0.5 M NaCl at 0.5 volume/h, and retained enzyme

Table I: Efficiency of Photolabeling RBC AChE with [^{125}I]TID under Three Solution Conditions[a]

RBC AChE condn[b]	n	% label incorpn
detergent-free aggregates	2	6.4 ± 1.8
Triton X-100 micelles	3	1.6 ± 0.6
reconstituted liposomes	2	0.032 ± 0.018

[a] Following addition of [^{125}I]TID to each sample, a 2-μL aliquot was removed for determination of the initial total radioactivity. The samples were photolyzed for equivalent periods, and radiolabeled RBC AChE was isolated by affinity chromatography as outlined under Materials and Methods. The efficiency of radiolabel incorporation was the percent of initial total radioactivity incorporated into the repurified enzyme. The standard errors listed were based on n determinations for each condition. [b] Detergent-free aggregates of RBC AChE (200–940 μg/mL) were labeled (50–100 μCi/mL) in 200 mM sodium phosphate buffer, pH 7. Triton X-100 micelles associated with RBC AChE (70–1900 μg/mL) were labeled (25–100 μCi/mL) in 20 mM buffer containing 0.1% Triton X-100, and liposomes reconstituted with RBC AChE (10–70 μg/mL) were labeled (3–40 μCi/mL) in 20 mM buffer.

was eluted with 10 mM decamethonium bromide in buffered 0.5 M NaCl at 0.2–0.3 volume/h. The column was regenerated by exposure to 1% SDS at 25 °C for 1 h followed by overnight reequilibration in buffered 0.1% Triton X-100.

Analytical Procedures. Assay of AChE activity, AChE protein determination, PAGE in SDS, and PAGE gel slicing were conducted as indicated in Rosenberry and Scoggin (1984).

Results

Covalent Incorporation of [^{125}I]TID into RBC AChE. To establish optimum solvent conditions for radiolabeling, [^{125}I]TID was introduced to RBC AChE in Triton X-100 micelles, in reconstituted phospholipid liposomes, or in detergent-free solutions in which the enzyme forms small soluble aggregates. The percent of radiolabel incorporation into the different preparations is shown in Table I. Enzyme labeled in detergent-free aggregates incorporated about 4 times as much radioactivity as that labeled in detergent micelles and about 200 times as much radioactivity as that which had been reconstituted into liposomes. Covalent incorporation of the label into the RBC AChE subunit polypeptides was confirmed by SDS–PAGE and gel slicing. The radioactivity profile (Figure 1A) of the ^{125}I-labeled enzyme in Figure 1A showed a sharp peak at the same relative electrophoretic migration of 0.52 observed previously for RBC AChE (Scoggin & Rosenberry, 1984). Because of the higher relative incorporation of label into the detergent-free aggregates, the further studies reported here were conducted with RBC AChE labeled under this condition and subsequently incorporated into Triton X-100 micelles or reconstituted into liposomes.

Selectivity of Label Incorporation into the RBC AChE Hydrophobic Domain. Previous work in our laboratory indicated that papain cleaves a small hydrophobic domain of RBC AChE that is responsible for membrane association and detergent binding. To investigate the extent to which [^{125}I]TID labeling is restricted to this domain, labeled enzyme in Triton X-100 micelles was digested with papain resin and analyzed by SDS–PAGE. The attachment of papain to resin permits more complete removal of papain following digestion and minimizes nonspecific proteolysis of the sample in SDS (Dutta-Choudhury & Rosenberry, 1984). The radioactivity profile of the digested sample on SDS–PAGE (Figure 1B) indicated that about 20% of the radioactivity remained associated with the RBC AChE catalytic subunits while 75% ran near the gel dye front. The mobility of most of the radioactivity near the dye front is consistent with its release in a small hydrophobic domain, but 20% of the radioactivity could remain

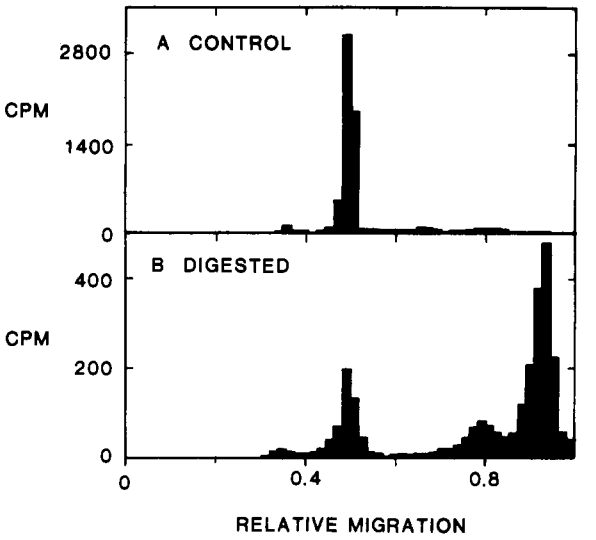

FIGURE 1: SDS–PAGE analysis of label distribution in intact and papain-cleaved [^{125}I]TID-labeled RBC AChE. Enzyme in detergent-free aggregates was labeled and purified by affinity chromatography on acridinium resin, and a portion (2700 units, 5×10^6 cpm in 6.6 mL) was dialyzed and digested with papain resin in 0.1% Triton X-100 as outlined under Materials and Methods. Samples (10 μL) of the purified stock (7400 cpm) and the digestion supernatant (4000 cpm) were mixed with SDS sample buffer containing dithiothreitol and run on cylindrical 3.5% PAGE gels. The gels were immediately sliced into 2-mm sections (Rosenberry & Scoggin, 1984), and radioactivity was determined in a γ radiation counter. (A) Control intact enzyme. (B) Digestion supernatant. Recovery of onput radioactivity was 80% in panels A and B.

with the catalytic subunits for one of two reasons. Either the papain digestion did not go to completion, leaving residual labeled intact enzyme, or the radiolabeling was not specific to the hydrophobic domain and some label remained associated with the hydrophilic enzyme fragment following papain cleavage of the hydrophobic domain.

To distinguish between these two possibilities, [^{125}I]TID-labeled RBC AChE was reconstituted into liposomes and isolated by chromatography on Sepharose CL-4B (Figure 2A). Elution of the labeled RBC AChE liposomes was centered at about 60 mL, the same column volume previously observed for unlabeled small RBC AChE liposomes (Kim & Rosenberry, 1985). A sample of the reconstituted RBC AChE liposomes from the column was digested with soluble papain and rechromatographed on Sepharose CL-4B (Figure 2B). The digestion released 66% of the enzyme activity from the liposomes, and this activity was eluted at the column volume characteristic of RBC AChE from which the hydrophobic domain had been cleaved (75% of the elution volume of $K_2Cr_2O_7$; Dutta-Choudhury & Rosenberry, 1984). Of particular importance, however, was the fact that the ratio of radioactivity to enzyme activity in this peak was less than 4% of that of the intact enzyme. Most of the radiolabel following digestion (62%) appeared to remain with the small liposomes and was eluted at the same 60-mL column volume as the undigested liposome pool in Figure 2A. The digestion chromatographed in Figure 2B was somewhat atypical in that some large liposomes had formed from the small liposome pool. These large liposomes were eluted at the 40-mL column void volume and contained both radiolabel and enzyme activity (27% and 11% of the respective totals). RBC AChE liposome size, even following papain digestion, usually remains constant during subsequent Sepharose CL-4B rechromatography (Kim & Rosenberry, 1985; see Figure 5A). The significance of the digestion displayed in Figure 2B is that virtually complete release of the enzyme activity from the liposomes was achieved

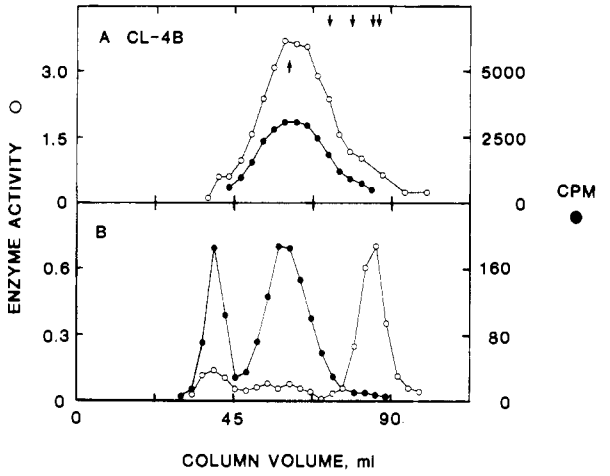

FIGURE 2: Analysis of liposome reconstitution and papain digestion of [^{125}I]TID-labeled RBC AChE by chromatography on Sepharose CL-4B. Samples (2–3 mL) were mixed with 10 μL of saturated $K_2Cr_2O_7$ to mark the solvent elution volume and applied to a 120-mL column (1.5 × 70 cm) equilibrated in 20 mM Tris-HCl (pH 7.4) at 4 °C. (A) [^{125}I]TID-labeled AChE (40 units, 35 000 cpm) reconstituted with phospholipid (4 mg in 2 mL) as outlined under Materials and Methods. (B) Digest of a 3-mL aliquot of the two-fraction peak of liposomal RBC AChE fractions (upward arrow in panel A) with papain at 25 °C for 1 h conducted as outlined under Materials and Methods. (●) ^{125}I radioactivity (cpm); (○) enzyme activity per fraction (units). Recoveries of onput radioactivity and enzyme activity were 80% in panel A and >90% in panel B. Elution positions of standard proteins are indicated by downward arrows (from left, intact RBC AChE aggregates, β-galactosidase, papain-disaggregated RBC AChE, and catalase). The elution position of $K_2Cr_2O_7$ corresponds to the right axis.

without an accompanying loss of radiolabel. Thus, we conclude that virtually all of the radiolabel was confined to the hydrophobic domain fragment cleaved by papain digestion which remained anchored to liposomes. In Figure 1B, the retention of 20% of the radioactivity with the catalytic subunit band represented residual intact enzyme.

Purification of the [^{125}I]TID-Labeled Hydrophobic Domain Fragment Produced by Papain Digestion. To isolate and characterize the hydrophobic domain fragment, it was necessary to prepare much larger quantities than those in Figure 2. A large-scale digest of RBC AChE in Triton X-100 micelles to which a small amount of [^{125}I]TID-labeled RBC AChE had been added was prepared with papain resin and applied to an acridinium affinity column (Figure 3A). More than 75% of the ^{125}I radioactivity passed through the column without being retained. This pool presumably corresponded to the cleaved hydrophobic domain fragment in Triton X-100 micelles because only 0.1% of the enzyme activity was associated with this radioactivity. The retained enzyme was eluted from the column with a decamethonium bromide solution that contained 0.5 M NaCl. Dialysis and rechromatography of this detergent-free mixture of intact and papain-cleaved RBC AChE gave better resolution of the two enzyme species on Sepharose CL-4B (Figure 3B) and permitted more accurate estimates of the extent of digestion and the specificity of radiolabel incorporation. About 70% of the enzyme activity was eluted at the column volume characteristic of papain-cleaved RBC AChE (see Figure 2B), while the remainder of the enzyme activity and virtually all of the radioactivity appeared in an earlier peak corresponding to intact RBC AChE aggregates (62% of the elution volume of $K_2Cr_2O_7$; Rosenberry & Scoggin, 1984). Despite the partial overlap of the papain-cleaved and the aggregated enzyme peaks in Figure 3B, ratios of radioactivity to enzyme activity in the papain-cleaved peak were less than 1% of that of the initial radiolabeled enzyme.

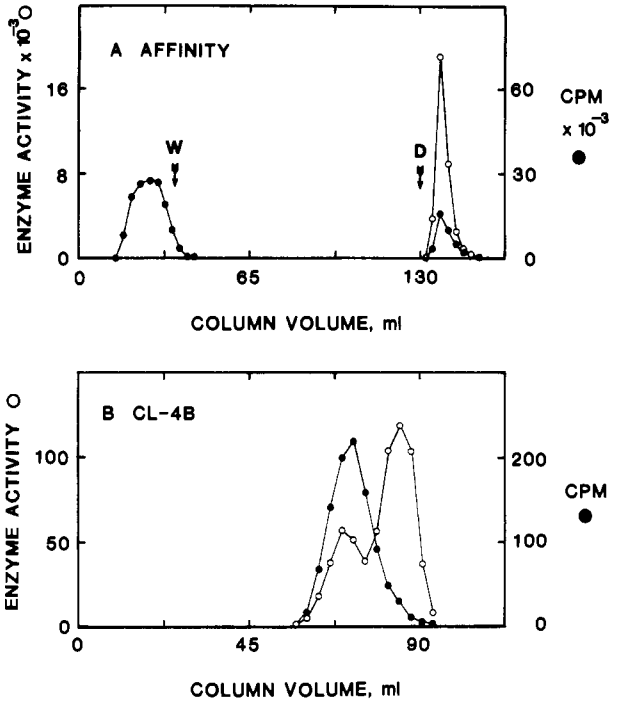

FIGURE 3: Large-scale fractionation of a papain digest of RBC AChE. [^{125}I]TID-labeled RBC AChE (600 units, 275 000 cpm) was combined with ^{14}C-radiomethylated RBC AChE (Haas et al., 1985; 50 000 units, 4 × 10^6 cpm), repurified by affinity chromatography on acridinium resin, and digested with papain resin (5 mL) in 15-mL total mixture as outlined under Materials and Methods. The recovery of enzyme activity and radioactivity from both isotopes in the digestion supernatant was 80%. (A) Affinity chromatography of the digestion supernatant (15 mL) on a 20-mL acridinium affinity column was conducted as outlined under Materials and Methods. Initiation of the buffered wash (W) and decamethonium bromide elution (D) are indicated. (B) Chromatography of the enzyme eluted with decamethonium bromide in panel A on Sepharose CL-4B. The six peak fractions eluting after D in panel A were pooled, dialyzed against 1 mM sodium phosphate (pH 7), and concentrated on a Speedvac concentrator. A small aliquot of the concentrate (600 units, 1000 ^{125}I cpm) was mixed with 2 mL of 20 mM Tris-HCl (pH 7.4) and applied to the Sepharose CL-4B column equilibrated as in Figure 2. The $K_2Cr_2O_7$ elution position is at the right axis. (●) ^{125}I radioactivity (cpm); (○) enzyme activity per fraction (units). Recoveries of onput radioactivity and enzyme activity were 90–100% in panel A and 80–100% in panel B.

Thus, prior removal of the radiolabeled hydrophobic domain fragment by affinity chromatography reduced contamination of the papain-cleaved RBC AChE peak and confirmed the previous conclusion that the radiolabel is incorporated exclusively into the hydrophobic domain.

The nonretained pool from the affinity column in Figure 3A was concentrated and rechromatographed on Sepharose CL-6B to verify that the radiolabel had been cleaved from the intact RBC AChE. The elution profile in Figure 4A showed a sharp radioactive peak at about 90 mL, clearly distinct from the tiny amount of enzyme activity (0.04% of the affinity column onput) which corresponded to a trace of residual intact micellar RBC AChE. The elution volume of the radioactivity was much smaller than the column solvent volume even though the size of the hydrophobic domain fragment estimated in the following section is about 3 kDa. This observation indicated that the hydrophobic domain fragment remained associated with Triton X-100 micelles during the Sepharose CL-6B chromatography. This chromatographic step thus provides purification of the labeled fragment from any small hydrophilic peptide fragments arising from nonspecific papain digestion of RBC AChE.

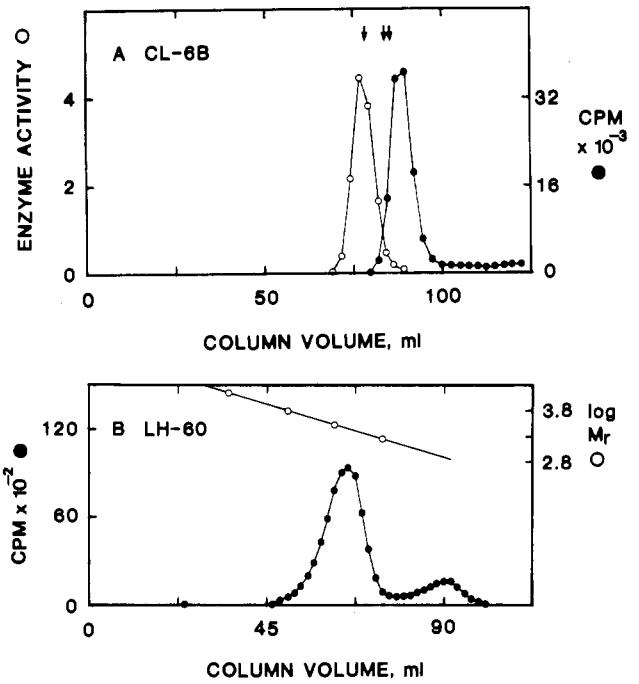

FIGURE 4: Purification of the [^{125}I]TID-labeled hydrophobic domain fragment by column chromatography. (A) The six nonretained fractions (140000 ^{125}I cpm) eluting in the peak just before W in Figure 3A were pooled, concentrated 7-fold on a Speedvac concentrator, and applied to a 130-mL Sepharose CL-6B column (1.5 × 75 cm) equilibrated in 0.1% Triton X-100–20 mM sodium phosphate (pH 7) at 4 °C. (●) ^{125}I radioactivity (cpm); (○) enzyme activity per fraction (units). Recoveries of onput radioactivity and enzyme activity were 95–100%. Elution positions of standard proteins are indicated by arrows (from left, intact RBC AChE aggregates, papain-disaggregated RBC AChE, and catalase). The elution position of $K_2Cr_2O_7$ is at the right axis. (B) The six fractions corresponding to the ^{125}I radioactivity peak in panel A were concentrated to 0.2–0.3 mL on the Speedvac, mixed with 1 mL of 88% formic acid, and applied to a 120-mL Sephadex LH-60 column (1.5 × 70 cm) equilibrated in ethanol–88% formic acid at 25 °C (Gerber et al., 1979). Onput ^{125}I radioactivity was 92000 cpm, and recovery was 88%. The elution positions indicated by (○) corresponded to a polypeptide standards (from left, lysozyme, M_r 14300; aprotinin, M_r 6500; glucagon, M_r 3490; and gramicidin D, M_r 1850).

Size Estimates of the [^{125}I]TID-Labeled Hydrophobic Domain Fragment Produced by Papain Digestion. Further purification of the radiolabeled hydrophobic domain fragment was achieved by chromatography on Sephadex LH-60 in ethanol–formic acid (Gerber, 1979). Peak radioactive fractions from the Sepharose CL-6B column in Figure 4A were concentrated,[2] suspended in formic acid, and chromatographed on LH-60 as indicated in Figure 4B. Two radioactive peaks were observed, a major peak that was eluted at about 60 mL and a minor peak near the column solvent volume at about 90 mL. The elution position of the major peak was compared with a molecular weight calibration curve obtained with peptides of known molecular weight (Figure 4B). The calibration curve was linear, and the major peak of radioactivity corresponded to an apparent molecular weight of 3100.

To compare the size of the [^{125}I]TID-labeled hydrophobic domain fragment produced by papain digestion of RBC AChE in Triton X-100 micelles with that generated by papain digestion of RBC AChE liposomes, a liposomal digest was chromatographed on Sepharose CL-4B (Figure 5A), and the

[2] Care was taken to ensure that the Speedvac concentrations did not go to dryness or involve heating of the sample, since both of these conditions severely reduce the amount of radiolabeled hydrophobic domain that can be recovered on resuspension.

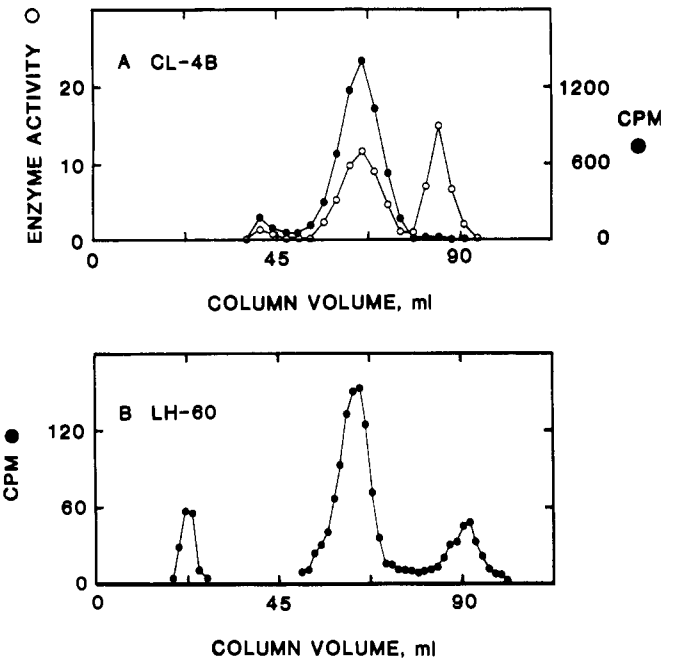

FIGURE 5: Column chromatographic analysis of the [^{125}I]TID-labeled hydrophobic domain fragment generated by papain digestion of small RBC AChE liposomes. A mixture of [^{125}I]TID-labeled and unlabeled RBC AChE (2000 units, 100000 cpm) was reconstituted (500 units/mL), and small RBC AChE liposomes were isolated by Sepharose CL-4B chromatography as in Figure 2A. (A) An aliquot of liposomal enzyme (150 units, 8000 cpm, 2.5 mL) was digested with papain at 4 °C for 20 h and applied to Sepharose CL-4B equilibrated as in Figure 2. The elution volume of $K_2Cr_2O_7$ corresponds to the right axis. Recoveries of ^{125}I cpm and enzyme activity from the combination of digestion and chromatography were 60%. (B) The fraction with peak radioactivity from the column in panel A was concentrated and chromatographed on Sephadex LH-60 as in Figure 4B. Onput radioactivity was 1550 cpm, and recovery was 81%.

peak radioactive fraction was rechromatographed on Sephadex LH-60 (Figure 5B). Only a partial digestion was achieved, as about 40% of the enzyme activity was recovered in the papain-cleaved RBC AChE peak at about 85 mL in Figure 5A. The peak liposomal fraction at about 65 mL in Figure 5A contained radiolabel in both intact RBC AChE and cleaved hydrophobic domain fragment. Chromatography of this fraction on Sephadex LH-60 in ethanol–formic acid revealed the same major and minor peaks seen in Figure 4B together with a small peak near the column void volume at about 20 mL. This radioactivity near the void volume corresponded to intact enzyme and was smaller in amount than expected, since Figure 5A indicated slightly more intact than cleaved enzyme. However, recoveries of control intact radiolabeled RBC AChE from chromatography on Sephadex LH-60 were low under these conditions, probably because of the low solubility of intact enzyme in the organic solvent. The elution position of the major radioactive peak corresponded precisely to that observed in Figure 4B, and thus, the hydrophobic domain fragments obtained from papain digests of Triton X-100 micellar or liposomal RBC AChE appear identical.

Amino Acid Analysis of the Purified Hydrophobic Domain Fragment Obtained from Sephadex LH-60 Chromatography. The amino acid composition of the pooled peak fractions from the Sephadex LH-60 column in Figure 4B is shown in Table II. Only 1 mol of histidine and 1 mol of glycine were found per mole of hydrophobic domain fragment, and no other amino acid exceeded 0.15 mol/mol of fragment. These stoichiometries were calculated from the radioactive content of the samples taken for amino acid analysis and assumed that the radioactivity per mole of fragment was identical with the

Table II: Amino Acid Composition of Hydrophobic Domain Fragment[a]

amino acid	residues per hydrophobic domain[b]	amino acid	residues per hydrophobic domain[b]
aspartic acid	0.11	methionine	0.00
threonine	0.14	isoleucine	0.00
serine	0.11	leucine	0.04
glutamic acid	0.08	tyrosine	0.00
proline	0.05	phenylalanine	0.05
glycine	1.06	lysine	0.01
alanine	0.06	histidine	1.06
valine	0.00	arginine	0.00

[a] A 2.2-mL aliquot (7.9 nmol of RBC AChE hydrophobic domain fragment) from the 12.8-mL pool of fragment obtained from the Sephadex LH-60 column in Figure 4B was dried, hydrolyzed, and subjected to amino acid analysis as described for the three-buffer system in Haas and Rosenberry (1985). [b] The moles of hydrophobic domain fragment was calculated from the ^{14}C radiolabel specific activity in the intact enzyme (cpm per gram of protein where the protein was determined by amino acid content) assuming 70 000 g of protein/mol of catalytic subunit and one hydrophobic domain per subunit (Rosenberry & Scoggin, 1984). While the specific activity associated either with the [^{125}I]TID label or with the ^{14}C-reductive radiomethylation could in principle be used, the latter was more accurate because radiolabel of known specific activity attached to identified non-amino acid components in the hydrophobic domain was measured simultaneously during the amino acid analysis [see Haas et al. (1985)]. Tabulated amino acid residues are uncorrected for background amino acid contaminants. Average values for glycine and histidine for this sample and two smaller (1.4 and 2.1 nmol) independent hydrophobic domain fragment preparations were 1.11 ± 0.03 (SEM) and 1.15 ± 0.11, respectively.

radioactivity per mole of catalytic subunit prior to papain digestion. Thus, the error in the stoichiometries was limited primarily by the accuracy of the subunit molecular weight estimate (Rosenberry & Scoggin, 1984) and is probably not larger than 10–15%. The discrepancy between the molecular weight estimate of 3100 for the hydrophobic domain fragment from the Sephadex LH-60 column and the observation of only two amino acids in this fragment is considered further under Discussion.

Discussion

Evaluation of the Photolabeling Procedure. The efficiency of [^{125}I]TID incorporation into membrane proteins is dependent on the labeling conditions employed. Since the carbene generated by photoactivation of a diazirine is highly reactive and can insert covalently into any component in the hydrophobic phase (Brunner & Semenza, 1981), one would expect detergent or phospholipid simply to dilute the membrane protein available for labeling. In agreement with this expectation, incorporation of [^{125}I]TID into sucrase–isomaltase is 1 order of magnitude higher in Triton X-100 micelles than in phospholipid liposomes presumably because the protein mole fraction is larger in detergent than in liposomes (Spiess et al., 1982). The observation in Table I that the photolabeling of detergent-free RBC AChE aggregates is 4-fold higher than that of the enzyme in detergent micelles and 200-fold higher than that of the enzyme in RBC AChE liposomes also is consistent with this notion. Although all the papain digestion studies reported under Results involved RBC AChE labeled in detergent-free aggregates, RBC AChE labeled in Triton X-100 micelles and digested with papain in Triton X-100 micelles gave SDS–PAGE profiles identical with those in Figure 1 and hydrophobic domain fragment elution volumes identical with those in Figure 4 (data not shown). Thus, the selectivity of radiolabel incorporation into the RBC AChE hydrophobic domain fragment appears the same with either the aggregated or the micellar enzyme, and RBC AChE in detergent-free aggregates must form a distinct hydrophobic phase composed of the membrane-binding domain of the enzyme into which [^{125}I]TID can partition. No studies were conducted of RBC AChE labeled in reconstituted liposomes.

Isolation and Characterization of the RBC AChE Hydrophobic Domain Fragment. The presence of detergent or incorporation into liposomes was essential to good recovery of the cleaved fragment in aqueous solutions. The ^{125}I-labeled fragment produced by papain digestion of detergent-free radiolabeled enzyme aggregates eluted as a smear of radioactivity from the column void to the column solvent volume when applied to Sepharose CL-4B equilibrated in the absence of detergent (data not shown). While liposomal preparations of fragment described here arose from papain digestion of reconstituted RBC AChE liposomes, preliminary data indicate that the apparent 3.1-kDa cleaved fragment can be reconstituted into liposomes by the same detergent dialysis procedure.[3] Liposomal fragment fractions were well resolved from nonspecific papain digestion products on the Sepharose CL-4B columns, but liposomes were rejected as a purification vehicle because of the phospholipid and slight protein contamination that they introduced. Large-scale preparations of the hydrophobic domain fragment were initiated by papain digestion of RBC AChE in Triton X-100. Virtually all the nonspecific digestion products could be separated from the hydrophobic domain fragment associated with Triton X-100 micelles by chromatography on Sepharose CL-6B, and any residual peptide contaminants as well as more than 90% of the Triton X-100 were removed from the radiolabeled fragment pool by the subsequent Sephadex LH-60 chromatography. Preparations of as much as 50 nmol of hydrophobic domain fragment have been obtained with this procedure.

Hydrophobic peptides have been separated by reverse-phase HPLC on C_{18} bonded phase columns with water–acetonitrile or water–propanol gradients in 0.1% trifluoroacetic acid (Mahoney & Hermodson, 1980; Aitken et al., 1982; Carr et al., 1982). Effects to purify the RBC AChE hydrophobic domain fragment by these procedures were unsuccessful as less than 5% of the [^{125}I]TID-labeled fragment typically was recovered.[4] We therefore investigated the hydrophobic domain fragment preparation directly from the Sephadex LH-60 column. Protein content determinations by amino acid analysis of pooled fractions across the Sephadex LH-60 column elution profile indicated background amino acid contamination low enough to permit an estimate of the amino acid content of the hydrophobic domain fragment. On the basis of the molecular weight estimate of 3100 for this fragment from its elution position on Sephadex LH-60 relative to peptide standards, we expected some 25–30 largely hydrophobic amino acids per mole of fragment. However, Table II clearly demonstrated only 1 mol of histidine and 1 mol of glycine per mole of fragment. This observation invalidated the molecular weight estimate because the standards were inappropriate, but of more importance, it indicated that the hydrophobic domain contained additional non-amino components. This has been confirmed by our identification of 1 mol of saturated and 1 mol of unsaturated fatty acids per mole of hydrophobic domain fragment following methanolysis either of intact RBC AChE or of the hydrophobic domain fragment (Roberts & Rosenberry, 1985). Furthermore, radiomethylation experiments have revealed 2 mol of ethanolamine and 1 mol of glucosamine in

[3] W. L. Roberts, B. Kim, and T. L. Rosenberry, unpublished observations.

[4] W. L. Roberts, unpublished observations.

the hydrophobic domain fragment (Haas et al., 1986).

Comparison of the Hydrophobic Domain of RBC AChE with That of Torpedo AChE. Human RBC AChE is similar in several respects to a membrane-bound form of AChE isolated from the electric organs of *Torpedo californica* and *Torpedo marmorata*. This torpedo AChE is also an amphipathic G_2 form which requires detergent for extraction and aggregates in the absence of detergent (Viratelle & Bernhard, 1980; Bon & Massoulié, 1980; Lee et al., 1982). The torpedo G_2 AChE can be digested with proteinase K or Pronase to yield a catalytically active hydrophilic G_2 enzyme which no longer interacts with detergents or reconstitutes into liposomes and is only slightly, if at all, smaller than undigested enzyme according to SDS–PAGE (Bon & Massoulié, 1980; Stieger & Brodbeck, 1985; Futerman et al., 1985). The hydrophobic domain of torpedo G_2 AChE has been labeled with [^{125}I]TID, and the radiolabeled hydrophobic domain fragment generated by proteinase K was chromatographed on Sephadex LH-60 under conditions similar to those in Figure 4 (Stieger et al., 1984). The apparent molecular weight of 3100 estimated from the elution position of the labeled fragment was identical with the value we estimated here for the RBC AChE hydrophobic domain fragment. Finally, the indication that the hydrophobic domain of the torpedo AChE also contains non-amino acid components is discussed below. Despite these striking similarities, small differences involving the hydrophobic domains of the RBC and torpedo AChEs are apparent. Papain was relatively ineffective in cleaving the hydrophobic domain from the torpedo enzyme (Stieger & Brodbeck, 1985). Furthermore, the susceptibility of the hydrophobic domains in the two AChEs to bacterial phosphatidylinositol-specific phospholipase C (PIPLC) appears to differ. Incubation of torpedo membrane fragments with PIPLC released the hydrophilic G_2 AChE fragment (Futerman et al., 1983), and digestion of purified amphipathic torpedo enzyme with PIPLC abolished its interaction with detergents and liposomes without changing its apparent molecular weight determined from SDS–PAGE (Futerman et al., 1984, 1985a). Although the PIPLC cleavage of torpedo G_2 AChE closely parallels that produced by proteinase K, the PIPLC activity does not appear to result from a protease contaminant[5] (Futerman et al., 1983) and thus suggests an inositol phosphatide structure in the torpedo AChE hydrophobic domain. This suggestion is supported by the measurement of about 1 mol of *myo*-inositol per mole of torpedo catalytic subunit (Futerman et al., 1985b). Our observation of 2 mol of fatty acids per mole of hydrophobic domain distributed as equimolar amounts of saturated and unsaturated fatty acids as in diacylglycerols suggests that diacylglycerol could be present in the RBC AChE hydrophobic domain (Roberts & Rosenberry, 1985), and we have recently confirmed that RBC AChE contains about 1 mol of *myo*-inositol per mole of catalytic subunit (Roberts & Rosenberry, 1986). However, treatment of human erythrocytes with PIPLC released only 5–12% of the RBC AChE activity (Low & Finean, 1977; Futerman et al., 1984), and PIPLC released only about 5% of the RBC AChE reconstituted into small liposomes and disaggregated about 5% of the enzyme in detergent-free aggregates.[6] Whether this implies that an inositol phosphatide is not a component of the RBC AChE hydrophobic domain or that such a phosphatide is modified to render it unsusceptible to PIPLC remains to be resolved.

ACKNOWLEDGMENTS

We thank Michael Hodges, Benedict Kim, and Patricia Brandt for their excellent technical assistance.

Registry No. AChE, 9000-81-1.

REFERENCES

Aitken, A., Cohen, P., Santikarn, S., Williams, D. H., Calder, A. G., Smith, A., & Klee, C. B. (1982) *FEBS Lett. 150*, 314–318.

Bayley, H., & Knowles, J. R. (1980) *Biochemistry 19*, 3883–3892.

Bon, S., & Massoulié, J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4464–4468.

Brunner, J., & Semenza, G. (1981) *Biochemistry 20*, 7174–7182.

Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C., & Titani, K. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6128–6131.

Dutta-Choudhury, T. A., & Rosenberry, T. L. (1984) *J. Biol. Chem. 259*, 5653–5660.

Frielle, T., Brunner, J., & Curthoys, N. P. (1982) *J. Biol. Chem. 257*, 14979–14982.

Futerman, A. H., Low, M. G., & Silman, I. (1983) *Neurosci. Lett. 40*, 85–89.

Futerman, A. H., Fiorini, R. M., Roth, E., Michaelson, D. M., Low, M. G., & Silman, I. (1984) in *Proceedings of the 2nd International Meeting on Cholinesterases* (Brzin, M., Barnard, E. A., & Sket, D., Eds.) pp 99–113, de Gruyter, West Berlin.

Futerman, A. H., Fiorini, R. M., Roth, E., Low, M. G., & Silman, I. (1985a) *Biochem. J. 226*, 369–377.

Futerman, A. H., Low, M. G., Ackermann, K. E., Sherman, W. R., & Silman, I. (1985b) *Biochem. Biophys. Res. Commun. 129*, 312–317.

Gerber, G. E., Anderegg, R. J., Herlihy, W. C., Gray, C. P., Biemann, K., & Khorana, H. G. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 227–231.

Haas, R., & Rosenberry, T. L. (1985) *Anal. Biochem. 148*, 154–162.

Haas, R., Brandt, P. T., Knight, J., & Rosenberry, T. L. (1986) *Biochemistry* (following paper in this issue).

Kim, B. H., & Rosenberry, T. L. (1985) *Biochemistry 24*, 3586–3592.

Klip, A., & Gitler, C. (1974) *Biochem. Biophys. Res. Commun. 60*, 1115–1162.

Lee, S. L., Heinemann, S., & Taylor, P. (1982) *J. Biol. Chem. 257*, 12283–12291.

Low, M. G., & Finean, J. B. (1977) *FEBS Lett. 82*, 143–146.

Mahoney, W. C., & Hermodson, M. A. (1980) *J. Biol. Chem. 255*, 11199–11203.

Massoulié, J., & Bon, S. (1982) *Annu. Rev. Neurosci. 5*, 57–106.

Ott, P., & Brodbeck, U. (1978) *Eur. J. Biochem. 88*, 119–125.

Roberts, W. L., & Rosenberry, T. L. (1985) *Biochem. Biophys. Res. Commun. 133*, 621–627.

Roberts, W. L., & Rosenberry, T. L. (1986) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 45*, 1816.

[5] The specific activity of cleavage by PIPLC is about an order of magnitude greater than the specific activity of cleavage by proteinase K (I. Silman, personal communication).

[6] Purified PIPLC was kindly provided by Dr. Martin G. Low, Oklahoma Medical Research Foundation, Oklahoma City, OK, and used at concentrations up to 120 units/mL (W. L. Roberts, B. Kim, and T. L. Rosenberry, unpublished observations).

Rosenberry, T. L. (1985) *Enzymes Biol. Membr. 4*, 403-429.
Rosenberry, T. L., & Scoggin, D. M. (1984) *J. Biol. Chem. 259*, 5643-5652.
Spiess, M., Brunner, J., & Semenza, G. (1982) *J. Biol. Chem. 257*, 2370-2377.
Steiger, S., & Brodbeck, U. (1985) *J. Neurochem. 44*, 48-56.
Steiger, S., Brodbeck, U., Reber, B., & Brunner, J. (1984) *FEBS Lett. 168*, 231-234.
Viratelle, O. M., & Bernhard, S. A. (1980) *Biochemistry 19*, 4999-5007.
Weidmer, T., Di Francesco, C., & Brodbeck, U. (1979) *Eur. J. Biochem. 102*, 59-64.

Identification of Amine Components in a Glycolipid Membrane-Binding Domain at the C-Terminus of Human Erythrocyte Acetylcholinesterase†

Robert Haas, Patricia T. Brandt, Jonathan Knight, and Terrone L. Rosenberry*
Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106
Received October 25, 1985; Revised Manuscript Received January 14, 1986

ABSTRACT: Purified human erythrocyte acetylcholinesterase was labeled by reductive radiomethylation with saturating amounts of [^{14}C]formaldehyde and sodium cyanoborohydride. Acid hydrolysis and automated amino acid analysis permitted both identification of radiomethylated components by their coelution with radiomethylated standards and quantitation of these components. The methylated N-terminal amino acids glutamate and arginine were observed at levels of 0.66 and 0.34 residues, respectively, per 70-kilodalton subunit, and lysine residues were methylated on their ϵ-amino groups to a level of 7.40 residues per subunit [Haas, R., & Rosenberry, T. L. (1985) *Anal. Biochem. 148*, 154-162]. In addition, each subunit contained 1.35 residues of methylated ethanolamine and 0.98 residue of methylated glucosamine. Papain digestion cleaved the intact enzyme into two fragments, an enzymatically active hydrophilic fragment and a small hydrophobic fragment that represented the membrane-binding domain. The radiomethylated amino acids were quantitatively retained in the hydrophilic fragment, while the methylated ethanolamine and glucosamine were confined exclusively to the hydrophobic domain fragment. This fragment included the C-terminal dipeptide of the subunit. Peptide sequencing by manual Edman methods was combined with radiomethylation to demonstrate the sequence His-Gly-ethanolamine-Z for the hydrophobic domain fragment. The ethanolamine residue in this sequence is in amide linkage to the C-terminal Gly and is clearly distinct from the ethanolamine residues in Z which are susceptible to radiomethylation in the intact enzyme. Since Z also includes glucosamine and 2 mol of fatty acids [Roberts, W. L., & Rosenberry, T. L. (1985) *Biochem. Biophys. Res. Commun. 133*, 621-627], we conclude that the membrane-binding domain of human erythrocyte acetylcholinesterase is a covalently linked glycolipid at the C-termini of the subunits. Analogies to the membrane-binding domains of murine Thy-1 glycoprotein and trypanosome variant surface glycoproteins are discussed.

Several forms of acetylcholinesterase (AChE,[1] EC 3.1.1.7) are found in vertebrate tissues [see Massoulié & Bon (1982) and Rosenberry (1985)]. One major class of AChE forms corresponds to integral membrane proteins in brain and muscle, and this class is also represented by a dimeric (G_2) form (RBC AChE) that is the only AChE present in human erythrocyte membranes. We have purified RBC AChE to homogeneity (Rosenberry & Scoggin, 1984) and demonstrated that it is an amphipathic protein with a small hydrophobic domain that can be cleaved from the remaining enzymatically active hydrophilic fragment by papain (Dutta-Choudhury & Rosenberry, 1984). Papain cleavage dissaggregates detergent-free RBC AChE, abolishes the interaction of RBC AChE with Triton X-100 micelles, and releases RBC AChE from the membranes of reconstituted small liposomes (Dutta-Choudhury & Rosenberry, 1984; Kim & Rosenberry, 1985). In the preceding paper (Roberts & Rosenberry, 1986a), we showed that [^{125}I]TID is a selective radiolabel of the RBC AChE hydrophobic domain and that this label can be used to monitor the isolation of the hydrophobic domain fragment produced by papain. Amino acid compositions of the isolated fragment revealed only small amounts of amino acids inconsistent with a hydrophobic peptide domain (Roberts & Rosenberry, 1986a), but methanolysis of either intact RBC AChE or the isolated hydrophobic domain fragment released 2 mol of fatty acid esters per mole of domain (Roberts & Rosenberry, 1985).

Reductive radiomethylation, another useful procedure for labeling RBC AChE (Haas & Rosenberry, 1985), also provides important information about the hydrophobic domain

† This investigation was supported by postdoctoral fellowships to R.H. from the National Institutes of Health (Grant NS-07118) and the American Heart Association, Northeast Ohio Affiliate, by Grant NS-16577 from the National Institutes of Health, and by grants from the Muscular Dystrophy Association.

[1] Abbreviations: AChE, acetylcholinesterase; RBC AChE, human erythrocyte acetylcholinesterase; G_n, a globular AChE form with n catalytic subunits; [^{125}I]TID, 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)-diazirine; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; ATZ, 2-anilino-5-thiazolinone; PIPLC, phosphatidylinositol-specific phospholipase C from *Staphylococcus aureus*; mfVSG, membrane-bound form of trypanosome variant surface glycoprotein; kDa, kilodalton(s).

0006-2960/86/0425-3098$01.50/0