Journal of Cellular Biochemistry 18:447-459 (1982)
Cellular Recognition 727-739

Brain Ligatin: A Membrane Lectin That Binds Acetylcholinesterase

S.M. Gaston, Richard B. Marchase, and E.R. Jakoi

Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710

Ligatin, a lectin that recognizes phosphorylated sugars, has been demonstrated in mammalian tissues to bind specific hydrolases to cell surfaces. Ligatin exists as a filament that can be released from membranes still complexed with its bound hydrolases by treatment of membrane preparations with $CaCl_2$ and/or pH 8.0. The ligatin-hydrolase complexes subsequently can be dissociated with ethyleneglycol-bis(β-amino-ethyl ether) N, N′-tetraacetic acid, resulting in a concurrent depolymerization of the ligatin filament. From membrane preparations of cerebrum, this procedure solubilized ligatin and a membrane-bound acetylcholinesterase (EC 3.1.1.7). Binding of the cosolubilized acetylcholinesterase to ligatin could be demonstrated in vitro by affinity chromatography using the immobilized lectin. Ligatin-hydrolase complexes have been shown to be dissociated by specific phosphorylated sugars (mannose 6-phosphate and glucose 1-phosphate). These sugars were also effective in eluting bound brain acetylcholinesterase from ligatin affinity columns. Analysis of labeled glycitols produced by tritiated borohydride reduction confirmed the presence of phosphorylated sugars on the ligatin-cosolubilized material from brain.

Key words: acetylcholinesterase, ligatin, membrane-bound lectin

Brain acetylcholinesterase (AChE EC 3.1.1.7) has been histochemically demonstrated to be a cell surface hydrolase, concentrated at the perikaryon membranes and along axons and dendrites of numerous groups of neurons [1]. Analysis of brain homogenates has shown that in physiological buffers, >90% of the AChE remains associated with membranes. AChE can be solubilized from brain membranes under relatively mild conditions using low ionic strength buffers containing ethylenediaminetetraacetic acid (EDTA), suggesting that this enzyme is not an "integral" membrane protein [2, 3]. The AChE solubilized from brain membranes is an acidic glycoprotein [4] exhibiting charge and mass heterogeneity, due in part to aggregation. We report here evidence that the acidic character of AChE is partially due to phosphate attached to one or more high mannose type oligosaccharides. Furthermore, the attachment of AChE to membranes in brain appears to be mediated by a

Received May 11, 1981; revised and accepted July 28, 1981.

0730-2312/82/1804-0447$04.00

lectin which recognizes the phosphorylated sugar residues on this enzyme. This lectin, ligatin, is a 10,000-dalton monomer which forms 3-nm diameter filaments (Fig. 3). Ligatin associates with membrane lipid and can, therefore, mediate membrane localization of the phosphohexosyl glycoproteins that it recognizes.

Ligatin was first isolated and identified from a specialized plasma membrane complex elaborated by the epithelial cells of the ileum in mammalian neonates [5]. There this filamentous lectin binds a large acidic hexosaminidase and constrains this enzyme into linear arrays on the cell surface. The ligatin-hexosaminidase complex from ileum is released from isolated plasma membranes by treatment of these membranes with 40 mM $CaCl_2$ and/or mildly alkaline buffers (pH 8.0). The filament is depolymerized with concurrent dissociation from the enzyme by removal of Ca^{2+} by dialysis vs ethyleneglycol-bis(β-amino-ethyl ether) N, N′-tetraacetic acid (EGTA). Using a similar procedure, ligatin has been isolated from membranes of rat cerebrum. During this isolation of ligatin, several hydrolases, including an acidic hexosaminidase [6] and acetylcholinesterase, are also solubilized. Both the ligatin-cosolubilized hexosaminidase and acetylcholinesterase bind immobilized ligatin in-vitrol. The AChE-ligatin complex is dissociated with specific phosphorylated monosaccharides, preferentially glucose 1-phosphate (GlclP) and mannose 6-phosphate (Man6P). These data suggest that the recognition signal on the bound acetylcholinesterase includes GlclP and/or Man6P residues of their steric analogs.

METHODS

Animals

Albino rat litters were purchased from Harlan/Sprague Dawley and used when the individuals were >250 gm. Animals used for intraventricular injections and for routine membrane preparations were anesthetized with sodium pentobarbitol (Nembutal, 5 mg/100 gm body weight, IP). No effects from the anesthetic were detected in control preparations.

In Vivo Labeling of Brain

For in vivo labeling of adult rat brain, 0.5 mCi of ^{32}P (dried under reduced pressure and resuspended into 10 μl of sterile saline) was injected into the right lateral ventricle using a stereotaxically guided 50μl Hamilton syringe.

Membrane Preparation

Plasma membranes were prepared from hypotonic homogenates of whole rat cerebra. Rat cerebra were dissected so as to remove the olfactory lobes and the brain stem posterior to the superior colliculus. Hypotonic homogenates were prepared in 10 mM Hepes, 1 mM NaN_3, 1 mM $MgCl_2$, pH 7.3 (Hepes-Mg buffer). Tissue (two cerebra/25 ml) was homogenized by hand using a Dounce homogenizer (8–10 strokes) and centrifuged at 14,500g for 15 min. The 14,500g pellet (P1) was retained and the supernatant (S1) was discarded. The pellet (P1) was resuspended in Hepes-Mg buffer and recentrifuged at 14,500g for 15 min. The P1 material was then resuspended in 4 vol of 20% sucrose in Hepes-Mg buffer and fractionated on discontinuous sucrose gradients containing 5, 20 (sample), 34.5, and 40% w/w sucrose in Hepes-Mg buffer by centrifugation at 110,000g for 60 min. Material at the 20/34.5 interface was collected and resuspended in 30–40 vol of Hepes-Mg buffer

and then pelleted by centrifugation at 32,500g for 15 min. Resuspension in Hepes-Mg buffer and centrifugation were repeated once. The presence and enrichment of plasma membrane in the membrane preparations were determined by enzymatic assays for 5′ nucleotidase and acid and alkaline phosphatase, and by electron microscopy. This material was enriched for plasma membranes but contained significant amounts of mitochondrial and lysosomal membranes.

Extraction of Ligatin and Associated Glycoproteins

The membrane pellets were treated with 40 mM $CaCl_2$ to release ligatin–ligand complexes and then dialyzed against 5 mM Hepes, 0.5 mM EGTA, pH 8.0, buffer overnight (4°C) to depolymerize ligatin and dissociate the ligatin–ligand complex. The dialysate was centrifuged at 110,000g for 90 min to remove the extracted membranes. The supernatant was chromatographed on a 1.5 × 20-cm Biogel P100 column equilibrated in 10 mM Hepes, 1 mM NaN_3, pH 7.6, buffer to separate monomeric ligatin from the ligand. The ligatin preparation was further purified by polymerization of the protein by dialysis against 3 mM $CaCl_2$. These polymers were then rechromatographed on a Biogel P60 column. Exclusion of the ligatin polymers from the Biogel P60 matrix was confirmed by negative stain electron microscopy. The glycoprotein hydrolases which were solubilized with ligatin were further enriched by preparative ion exchange chromatography on Whatman DEAE cellulose (1.0 × 20-cm column) equilibrated in 25 mM Na phosphate, pH 6.0, to which these hydrolases bound. Bound material was eluted from the resin by a linear gradient of 0–0.6 M NaCl (100 ml) in 25 mM Na phosphate, pH 6.0.

Biochemical Determinations

Protein was determined by the procedure of Lowry et al [7] using bovine serum albumin (Sigma Chemical Co.) as the standard. Acetylcholinesterase activity was determined by the Ellman procedure [8] or by radiometric microassay [9] using [^{3}H] acetylcholine (final specific activity of 11.82 mCi/mmol, prepared from [^{3}H] acetylcholine chloride (Amersham) at 59 mCi/mmol by dilution with nonradioactive acetylcoline (Sigma) in 10 mM Hepes, 1 mM $MgCl_2$, pH 7.⅞, buffer).

Acid phosphatase and alkaline phosphatase activities were measured using equal parts of enzyme solution and p-nitrophenyl phosphate (4 mM, 0.15 M Na acetate, pH 5.5, for acid phosphatase and 10 mM Hepes, pH 7.6, for alkaline phosphatase). Enzymatic reactions were terminated by the addition of two parts 1 M K_2CO_3. Hexosaminidase activity was measured analogously using 3 mM p-nitrophenyl-β-N-acetylglucosaminide in 0.2 N Na citrate, pH 4.5. 5′ Nucleotidase activity was determined by the procedures of Touster et al [10] and Chen et al [11].

Amino Acid Analysis

Amino acid analysis was performed on a single ion exchange column. The samples were hydrolyzed with 6 N HCl (Pierce Chemical Co.) at 100°C for 24 hr in vacuo. The analysis was performed with a Durrum Model D-500 amino acid analyzer (Durrum Instrument Corp., Sunnyvale, California).

Affinity Chromatography

From 200 to 500 μg of brain or ileal ligatin were covalently coupled to 1 to 2 ml of Affi-gel 10 resin (Biorad Laboratories). Derivatization was effected at 23°C

for 2 hr in 0.1 M Na phosphate, pH 7.0, buffer. Unreacted active esters were saturated by incubation at 23°C with 0.1 M glycine, pH 7.0, buffer for 1 hr. The affinity column was equilibrated in 10 mM Hepes, 3 mM $CaCl_2$, 50 mM NaCl, 1 mM NaN_3, pH 7.6, buffer. Phosphohexoses (K and Na salts) and nonphosphorylated sugars were purchased from Sigma Chemical Company.

Ion Exchange Chromatography

Changes in the net charge of the ligatin cosolubilized acetylcholinesterase were monitored by ion exchange chromatography using Whatman DEAE-cellulose (1 × 3-cm column) equlibrated in 25 mM Na phosphate, pH 6.0. Bound material was eluted by a linear gradient of 0–0.6 M NaCl (100 ml) in 25 mM Na phosphate, pH 6.0. The ionic strength of the elution gradient was determined by a conductivity meter.

Susceptible N-glycosidically linked oligosaccharides were removed from the ligatin cosolubilized hydrolases with 5 munits of endoglucosaminidase H (endoglycosidase H, Streptomyces griseus, Miles Chemical Co.) in 25 mM NH_4 Ac, pH 5.5, buffer for 24 hr (37°C). Phosphomonoesters were removed using 0.6 units of alkaline phosphatase (Sigma) in 10 mM Hepes, 1 mM NaN_3, pH 7.6, buffer for 12 hr (37°C). ^{32}P-labeled oligosaccharides released by endoglycosidase H were fractionated on QAE Sephadex (Sigma, Q-25-120) equilibrated in 2 mM Tris base as described by Tabas and Kornfeld [12].

Paper Electrophoresis

Ligatin cosolubilized glycoproteins were hydrolyzed with 20 mM HCl for 18 hr in the presence of Dowex 50W. The hydrolysate was subsequently reduced and tritiated with tritiated borohydride according to the procedure of Distler et al [13]. The ^{3}H-labeled monosaccharides were applied to a Dowex 50W column (0.5 × 3 cm) from which they were eluted with 15 ml of water. To enrich the acidic monosaccharides, the Dowex 50W effluent was applied in water to a DEAE-cellulose column which had been previously equilibrated in 0.5 M ammonium bicarbonate, pH 8.5, and then washed with 5–10 column vol. of water. The DEAE-cellulose bound material was subsequently eluted with 0.5 M ammonium bicarbonate, pH 8.5, and then neutralized, dried under reduced pressure, and subjected to paper electrophoresis (14 V/cm, 90 min) in pyridine acetate (1.25) M pyridine, 64 mM acetic acid, pH 6.4).

RESULTS

Isolation and Characterization of Brain Ligatin

Using a procedure which solubilizes the polymeric lectin, ligatin, and a ligatin-bound hexosaminidase from specialized epithelial plasma membranes of neonatal mammalian ileum, a protein with properties similar to ileal ligatin was released from the AChE enriched membranes from rat brain. Membranes isolated from brain were treated with 40 mM $CaCl_2$ and subsequently dialyzed against EGTA, pH 8.0, overnight. The extracted membranes were pelleted, and the solubilized material was chromatographed on a Biogel P100 column. The applied material was fractionated into two peaks with absorbance at 280 nm, one was completely included in the resin, and a second was excluded (Fig. 1). The Biogel P100 included material was

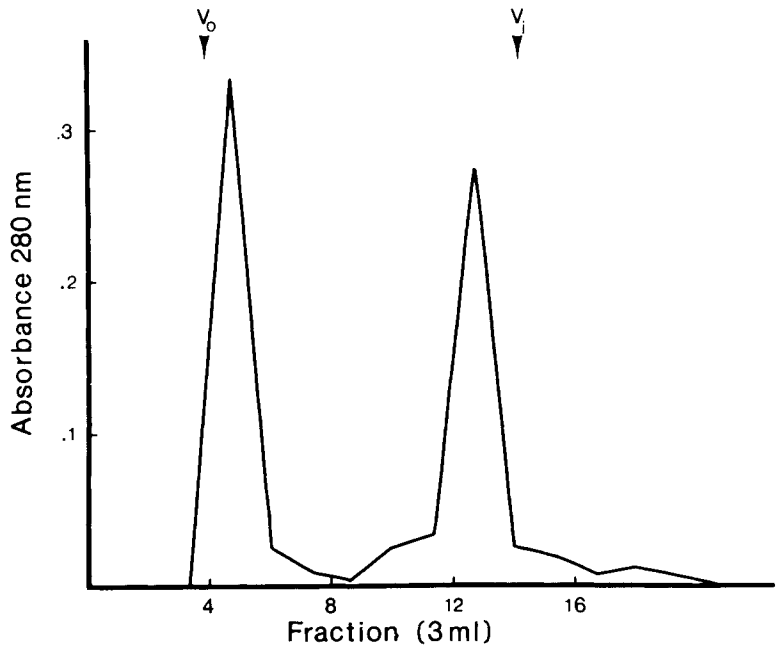

Fig. 1. Elution profile of a Biogel P100 column of material extracted from AChE enriched membranes isolated from rat cerebra. The applied material was fractionated into two protein peaks, one excluded from the matrix and the other almost totally included. All of the acetylcholinesterase activity applied to the column was found in the excluded peak. Ligatin was found in the included peak. Approximately 15–20 μg of ligatin per rat cerebrum were extracted from the AChE enriched membrane preparation. V_o denotes excluded volume. V_i denotes included volume.

further characterized to see whether it was structurally and functionally analogous to ileal ligatin. This Biogel P100 included material extracted from rat brain, like monomeric ileal ligatin, chromatographed on a Biogel P60 column as a protein with molecular weight of 10,000. When dialyzed against 3 mM $CaCl_2$, the Biogel P100 included material from brain, like ileal ligatin, formed polymers that were excluded from a Biogel P60 matrix (Fig. 2). Negative stain electron microscopy of these polymers revealed 3-nm diameter filaments of varying length, both as single filaments and as bundles, scattered over the grids. Neither monomeric ileal ligatin nor the Biogel P100 included material from brain could be visualized by negative stain prior to Ca^{2+} induced polymerization.

To further investigate their apparent homology, the Biogel P100 included material extracted from brain and the ligatin extracted from ileum were subjected to amino acid analysis (Table I). These analyses showed the ileal and brain proteins to be similar, each with 68% polar amino acid residues and 32% nonpolar residues. Because the Biogel P100 included protein extracted from brain is similar to ileal ligatin in chromatographic behavior, in polymerization properties, in electron microscopic images, and in amino acid composition this brain protein is also called "ligatin."

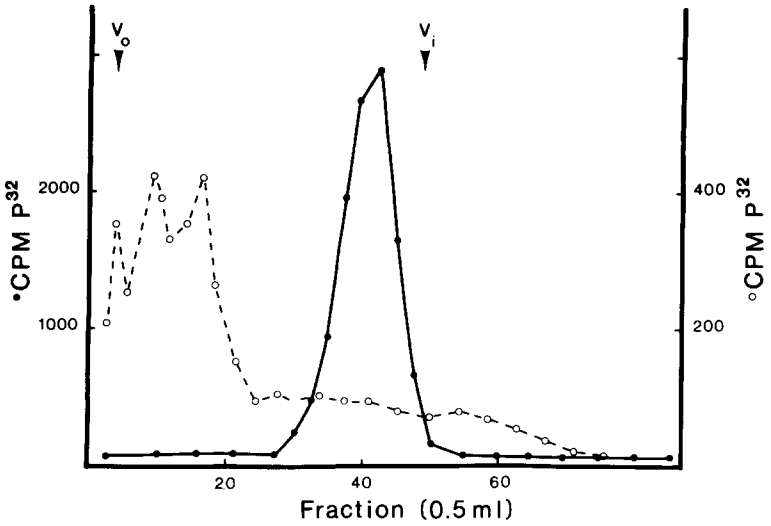

Fig. 2. Elution profile of a Biogel P60 column of endogenously ^{32}P-labeled ligatin before and after polymerization. Monomeric ligatin (○----○) was chromatographed in 10 mM Hepes 1 mM NaN_3, pH 7.6, buffer. All of the monomeric ligatin was included in the matrix and chromatographed with an apparent molecular weight of 10,000. Monomeric ligatin was subsequently dialyzed vs 10 mM hepes, 3 mM $CaCL_2$, 1 mM NaN_3 (Hepes-Ca^{2+}) and then rechromatographed in Hepes-Ca^{2+} on the same Biogel P60 column. Repolymerized ^{32}P-labeled ligatin (●——●) was now found to be partially excluded from the Biogel P60 matrix. V_o denotes excluded volume. V_i denotes included volume.

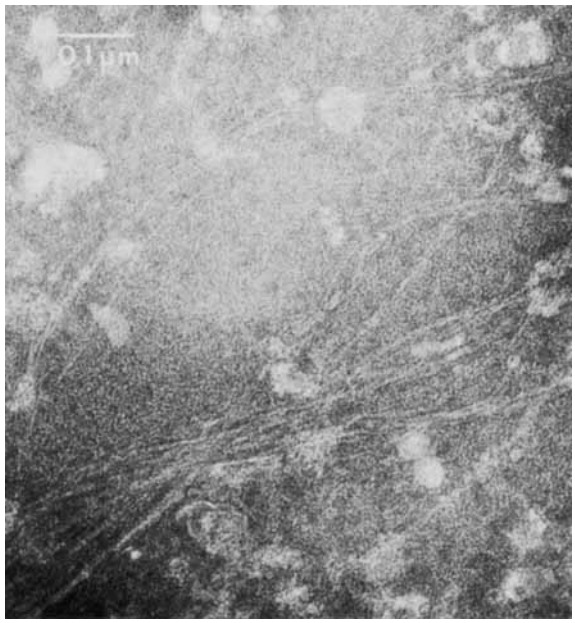

Fig.3. Negative stain preparation of brain ligatin; 3-nm diameter filaments are seen scattered over the grid surface (× 100,000).

TABLE I. Amino Acid Composition of Mammalian Ligatins

Amino acid[a]	Rat brain[b]	Rat ileum[b]
Asx	9.47	9.84
Thr	5.89	5.40
Ser	8.10	12.99
Glx	13.83	13.88
Pro	1.95	1.60
Gly	10.89	14.71
Ala	11.39	8.84
Val	5.20	2.31
Met	0.63	1.42
Ile	3.77	3.68
Leu	7.90	6.46
Tyr	2.56	2.55
Phe	4.99	3.02
His	2.87	4.27
Lys	6.66	5.87
Arg	3.87	3.14

[a]Cys and Tyr were not determined.

[b]Values are number of moles per 100 mol of amino acid.

Solubilization of Hydrolases With Ligatin

During extraction of AChE enriched membranes with $CaCl_2$ and EGTA, pH 8, to release ligatin, several hydrolase activities were also solubilized. These were separated from monomeric ligatin by gel filtration on a Biogel P100 column. This procedure resulted in the enrichment of AChE activity 40-fold over that present in the homogenate and 20-fold over that present in isolated AChE enriched membranes. After DEAE ion exchange chromatography, the AChE activity was enriched 80- to 120-fold over that present in the homogenate.

Characterization of Acetylcholinesterase

To determine how much of the ligatin associated cholinesterase activity was acetylcholine specific cholinesterase (acetylcholinesterase, EC 3.1.1.7), as compared with nonspecific cholinesterase activity, the preparation was assayed in the presence of inhibitors that discriminate between these two enzymatic activities. The cholinesterase activity in the ligatin cosolubilized extracts from brain was assayed in the presence of 1,5-bis-(4 allyldimethylammonium phenyl) pentan-3-one dibromide (BW 284c51) and tetraisopropylpyrophosphoramide (iso-OMPA). The ligatin cosolubized activity was completely inhibited by 10^{-6} M BW284c51, an inhibitor which preferentially inhibits acetylcholine specific cholinesterase. Iso-OMPA, which preferentially inhibits nonacetylcholine specific cholinesterase activity, produced no inhibition at 10^{-4} M. These data suggest that essentially all of the cholinesterase activity cosolubilized with ligatin from brain is acetylcholinesterase.

The ligatin cosolubilized AChE activity was further characterized by sucrose gradient centrifugation (Fig. 4). The sedimentation patterns of the ligatin cosolubilized acetylcholinesterase activity showed that 90–100% of this AChE behaved like the tetrameric "10 S" form of the enzyme.

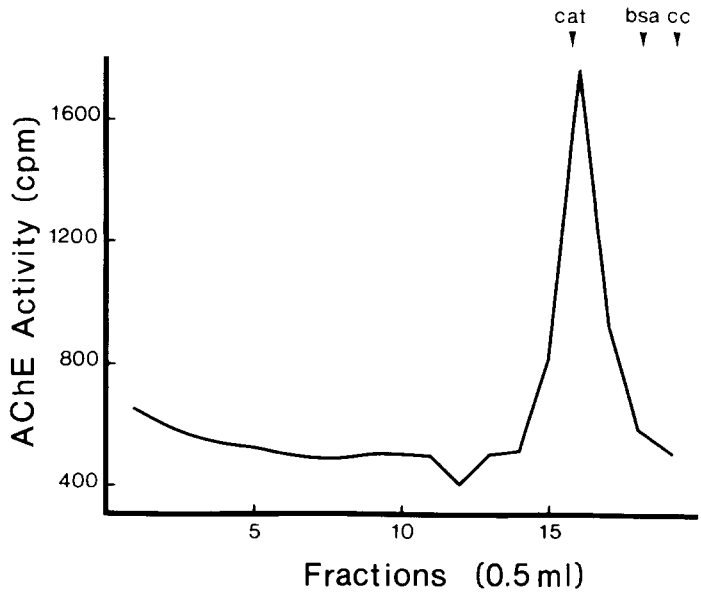

Fig. 4. Rate sedimentation profile of ligatin cosolubilized acetylcholinesterase. Material was applied to 5–20% (w/w) linear sucrose gradients and centrifuged at 110,000g for 12 hr. An apparent S value was calculated relative to the migration of standards: 11.25 S, catalase (cat); 4.55 S, bovine serum albumin (bsa); and 1.14 S, cytochrome C (cc). greater than 90% of the applied acetylchoinesterase sedimented with an apparent 10 S.

Affinity Chromatography

Affinity chromatography using a ligatin-derivatized matrix was used to investigate whether the cosolubilization of AChE with ligatin was coincidental or indicative of functional association (Table II). From 200 to 500 μg of ligatin were covalently immobilized to 1–2 ml of Affi-gel 10 resin. Application of the ligatin-cosolubilized hydrolases from brain resulted in the adsorption of AChE. AChE could be released from the affinity column by phosphohexoses (10 and 40 mM). Nonphosphorylated sugars (40 mM) had minimal or no effect, suggesting that a phosphorylated hexose present on the enzyme was recognized by ligatin. Glucose 1-phosphate and mannose 6-phosphate were the most effective of the phosphorylated sugars. Treatment of the ligatin cosolubilized hydrolases with alkaline phosphatase prior to affinity chromatography reduced binding of AChE activity to the ligatin affinity column by only 30%. However, treatment of these hydrolases with endoglycosidase H reduced binding of AChE activity to the affinity column by 60%. These results suggest that the moiety recognized by ligatin includes phosphohexose, at least some of which is resistant to phosphatase digestion.

DEAE Chromatography

Because the ligatin associated hexosaminidase from ileum exhibits a net negative charge which we have found to be characteristic of ligatin associated

TABLE II. Release of Brain Acetylcholinesterase From Ligatin-Affi Gel 10 Matrix

Treatment	Concentration (mM)	Released (%)
Galactose 1-phosphate	10	20
Galactose 6-phosphate	10	10
Mannose 1-phosphate	10	<5
Mannose 6-phosphate	10	8
Glucose 1-phosphate	10	18
Glucose 6-phosphate	10	17
Fructose 1-phosphate	10	11
Fructose 6-phosphate	10	<5
Galactose 1-phosphate	40	22
Galactose 6-phosphate	40	15
Mannose 1-phosphate	40	<5
Mannose 6-phosphate	40	46
Glucose 1-phosphate	40	48
Glucose 6-phosphate	40	31
Fructose 1-phosphate	40	19
Fructose 6-phosphate	40	23
Galactose	40	10
Mannose	40	<5
Glucose	40	20
Fructose	40	15
Glucosamine	40	13

hydrolases, the acetylcholinesterase activity cosolubilized with ligatin from brain was applied to a DEAE ion exchange column. All of the AChE activity bound to the DEAE resin, and was eluted at 0.1–0.15 M NaCl. In order to determine whether this net negative charge on the ligatin cosolubilized AChE was related to the presence of phosphohexose, as suggested by the phosphohexose elution of AChE from ligatin affinity columns, attempts were made to remove or modify the putative phosphohexose residue enzymatically. To monitor the presence of phosphate, 0.5 mCi ^{32}P in saline was injected into rats intraventricularly. The animals were sacrificed 3 days after the injection. Ligatin cosolubilized hydrolases from these animals were radiolabeled with ^{32}P. Endogenously incorporated ^{32}P cochromatographed on DEAE with acetylcholinesterase activity. Approximately 60% of the ligatin cosolubilized AChE activity was resistant to alkaline phosphatase digestion, showing no net change in charge as exhibited by its elution from DEAE. However, treatment of ligatin cosolubilized material with endoglycosidase H followed by gel filtration on Biogel P60 (see Methods) resulted in the loss of >80% of the ^{32}P label from these hydrolases. All of the ^{32}P-labeled oligosaccharides released from these hydrolases by digestion with endoglycosidase H bound to a QAE anion exchange resin. When the partially deglycosylated hydrolases were then reapplied to DEAE, 70–90% of the AChE activity eluted at 0–0.01 M NaCl, indicating a loss in negatively charged residues (Fig. 5). These data suggest that ligatin-cosolubilized AChE from brain has one or more phosphorylated residues on asparagine linked, endoglycosidase H sensitive oligosaccharides.

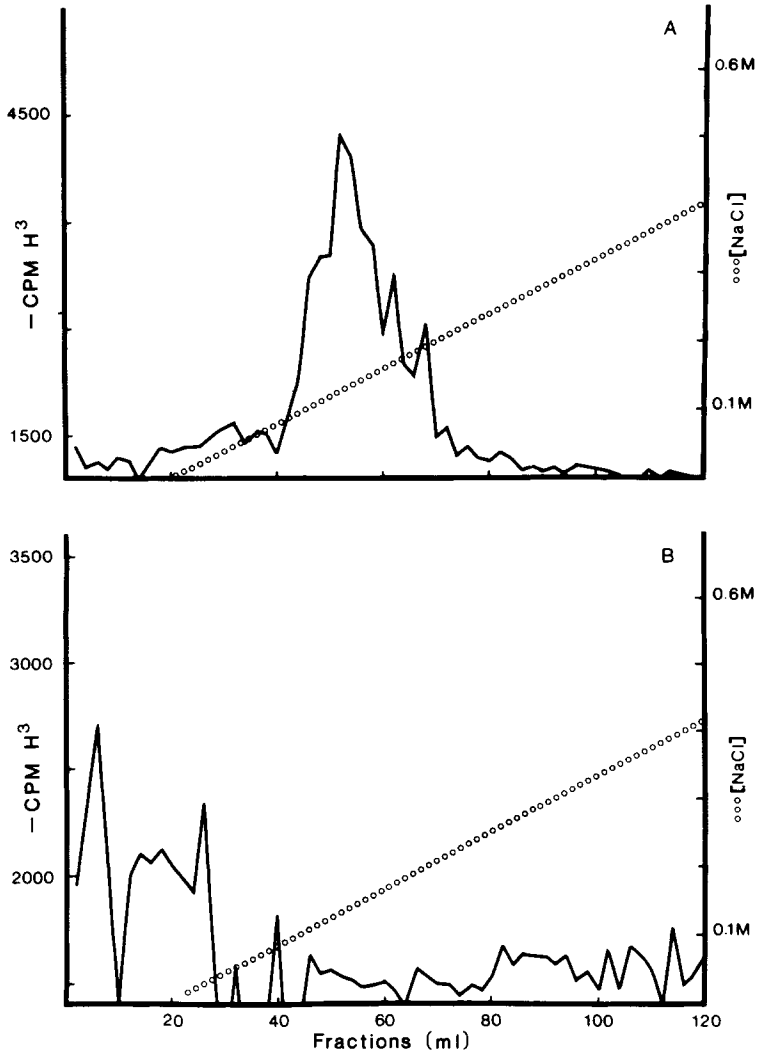

Fig. 5. Elution profile of 1 × 3-cm DEAE-cellulose columns of ligatin cosolubilized AChE activity before and after digestion with endoglycosidase H. The column was developed first with 20 ml of 25 mM Na phosphate, pH 6.0, followed by a linear gradient of 0–0.6 M NaCl in 25 mM Na phosphate, pH 6.0. The ionic strength of each 1-ml fraction collected from the elution gradient was determined by a conductivity meter. (A) Profile of untreated AChE activity is shown; (B) profile of endoglycosidase H treated AChE activity is shown.

Paper Electrophoresis

To further investigate the presence of phosphate on the oligosaccharides of ligatin associated hydrolases, including AChE, the acidic glycoproteins solubilized with ligatin from brain were enriched by DEAE chromatography and then hydrolyzed with 20 mM HCl in the presence of Dowex 50W. The hydrolysate was subse-

quently reduced and tritiated with tritiated borohydride. The ^{3}H-labeled monosaccharides were isolated from the hydrolysate and subsequently processed by ion exchange chromatography to enrich for the acidic monosaccharides, as described under Methods. This material was then subjected to paper electrophoresis in pyridine acetate. Both a neutral peak and a peak comigrating with phosphohexose were observed. Digestion of the DEAE bound hexose(s) with alkaline phosphatase altered the electrophoretic profile such that most of the previously acidic hexose now migrated as a neutral glycitol (Fig. 6).

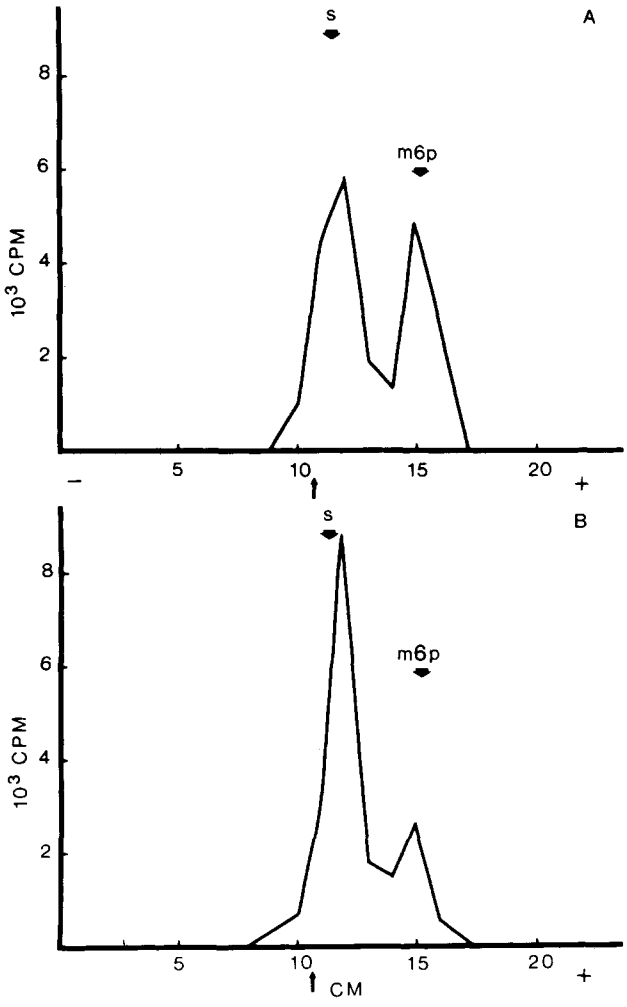

Fig. 6. Paper electrophoretogram in pyridine acetate of tritiated borohydride reduced acidic monosaccharides isolated from ligatin cosolubilized hydrolases. Migration of untreated (A) and alkaline phosphatase treated material (B) is shown relative to sorbitol (s) and mannitol 6-phosphate (m6p).

DISCUSSION

In both the central nervous system and at the neuromuscular junction, acetylcholinesterase removes acetylcholine from the extracellular space by hydrolysis. A significant fraction of this enzyme at the neuromuscular junction is anchored to the basal lamina by a noncatalytic subunit, a collagen-like "tail." In the central nervous system, there is little if any collagen-tailed acetylcholinesterase [14]. Essentially all of the acetylcholinesterase accessible to the extracellular space is attached to the external surface of the neuronal plasma membrane. Brain acetylcholinesterase can be solubilized from isolated membranes by use of chelating agents which leave the membranes relatively intact. We have shown that brain acetylcholinesterase becomes soluble with the release of the filamentous cell surface lectin, ligatin. Brain ligatin is similar in size, in amino acid composition, in polymerization properties, and in filament morphology to ileal ligatin which has been shown to mediate attachment of a cell surface hexosaminidase. In brain, ligatin binds both hexosaminidase and acetylcholinesterase, as evidenced by cosolubilization of these hydrolases with the lectin and by reconstitution of the ligatin-hydrolase complex in vitro by affinity chromatography.

The brain acetylcholinesterase that cosolubilizes with ligatin binds to ligatin affinity columns prepared from either ileal or brain ligatin. No differences in the elution patterns of AChE from these two ligatin matrices is observed. Binding of AChE activity to a ligatin affinity column is diminished by incubation of the enzyme with endoglycosidase H, demonstrating that an asparagine linked oligosaccharide moiety is required for recognition of the AChE by the lectin. The elution of AChE from ligatin affinity columns by phosphorylated hexoses, preferentially GlclP and Man6P, suggests that the endoglycosidase H sensitive oligosaccharide(s) mediating AChE-ligatin binding incorporate one or more such phosphohexose residues. This suggestion is further supported by the loss of internally incorporated ^{32}P label and net negative charge from AChE upon removal of the endoglycosidase H sensitive oligosaccharide chains. Preliminary characterization of the acidic monosaccharides isolated from the ligatin associated hydrolases from brain confirms the presence of phosphohexose.

Contrary to our inital expectations, phosphatase treatment of ligatin associated hydrolases does not affect the net negative charge of 60% of the AChE activity, and only partially diminishes its binding to a ligatin affinity column. The acidic phospho-oligosaccharide chains released from ligatin associated brain hydrolases by endoglycosidase H digestion are similarly insensitive to phosphatase [15]. The presence of a phosphatase insensitive phosphate on the oligosaccharide chain(s) recognized by ligatin may be indicative of a steric conformation which renders an otherwise sensitive phosphomonoester inaccessible to phosphatase. However, phosphatase insensitivity may also result from the presence of a nonterminal phosphate, one in ester linkage to two of the hexose residues in the oligosaccharide. Tabas and Kornfeld [12] have reported that newly synthesized β-glucuronidase isolated from murine lymphoma cells contains phosphorylated oligosaccharides in which the phosphate is present in 6 linkage to the penultimate residue, a mannose, and in α-1 linkage to the terminal residue, an N-acetyl glucosamine. An analogous structure present on the acidic oligosaccharide chains of brain AChE may account for the effectiveness of two relatively dissimilar phosphohexose structures, GlclP and Man6P, in eluting

AChE from ligatin affinity columns. Such a phospho-oligosaccharide structure may represent an important posttranslational modification enabling the cell to direct and localize hydrolases to its surface via the lectin ligatin.

ACKNOWLEDGMENTS

We thank Melanie Skaggs and Amy Canine for technical assistance. We are grateful to Ms. Susan Gurganus for excellent secretarial assistance.

This work was supported by NIH grants EY-02845 and GM-23911 and by NIH predoctoral traineeship GM-07184 awarded to S.M. Gaston.

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