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CHARACTERIZATION OF AN ACTIVE HYDROPHILIC ERYTHROCYTE MEMBRANE ACETYLCHOLINESTERASE OBTAINED BY LIMITED PROTEOLYSIS OF THE PURIFIED ENZYME

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Purified human erythrocyte membrane acetylcholinesterase was subjected to limited proteolysis with papain. This treatment generated a hydrophilic form of the enzyme as determined by charge-shift crossed immunoelectrophoresis and by binding to phenyl-Sepharose. The hydrophilic enzyme was stable and its activity was independent of the presence of amphiphiles. Electroimmunochemical analysis showed no antigenic difference between the two enzyme forms. Although the proteolytic treatment only brought about a small change in molecular weight, marked differences in the hydrodynamic properties were encountered. The Stokes radius decreased from 8.2 to 5.9 nm and the sedimentation coefficient increased from 6.3 to 7.0 S. The results are consistent with the view that a short hydrophobic peptide responsible for the amphipatic character of acetylcholinesterase is removed by the treatment with papain.

Introduction

Detergent soluble acetylcholinesterase (acetylcholinesterase(DS)) from human erythrocyte membranes is an amphiphilic protein that has been purified by affinity chromatography [1,2]. In the presence of micellar amounts of a detergent, the enzyme exists as a dimer of identical subunits that are interlinked by at least one disulfide bridge [3,4]. In the absence of a detergent, the enzyme forms water-soluble protein micelles composed of multiples of the dimer. Readdition of a detergent disaggregates the protein micelle to active dimers [5]. Acetylcholinesterase(DS) was reconstituted in phospholid vesicles [6] and was shown by charge-shift crossed immunoelectrophoresis to bind detergents [7]. The amphiphilic nature of acetylcholinesterase(DS) indicates that the enzyme is associated with the cell membrane by hydrophobic interactions. As other reports claimed solubilization without the use of a detergent [8], the question remained whether the enzyme has a hydrophobic peptide sequence large enough to anchor the enzyme into the lipid bilayer. In the present communication, we report on the conversion by proteolysis of acetylcholinesterase(DS) to an enzymatically active hydrophilic form and on its characterization. A preliminary report on this work has been presented previously [9].

Abbreviations: acetylcholinesterase(DS), detergent-soluble acetylcholinesterase; acetylcholinesterase(p), hydrophilic acetylcholinesterase derived from acetylcholinesterase(DS) by proteolysis; anti-acetylcholinesterase, rabbit anti-human erythrocyte membrane acetylcholinesterase(DS) antibody

Materials

Chemicals

Agarose Type HSA (electroendosmosis factor,

$m_r = -0.13$) was purchased from Litex, Glostrup, Denmark. Phenyl-Sepharose CL-4B, CNBr-activated Sepharose 4B, Sepharose CL-6B and molecular weight marker proteins were from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Bio-Gel HTP hydroxyapatite was from Bio-Rad. Ferritin (from horse spleen), catalase (from bovine liver) and papain (from *Papaya carica*) were from Boehringer Mannheim GmbH, F.R.G. Human fibrinogen (fraction I, citrated) was from Sigma Chemical Company, St. Louis, MO, U.S.A. Acrylamide (2 × crystallized) and *N,N'*-methylenebisacrylamide (2 × crystallized) were from Serva, Heidelberg, F.R.G. All other reagents were obtained either from Fluka, Buchs, Switzerland, or Merck, Darmstadt, F.R.G.

Biological material

Human blood from healthy donors of various blood groups was supplied by the Central Blood Bank of the Swiss Red Cross. Acetylcholinesterase(DS) from human erythrocyte membranes was purified according to Ott et al. [1]. Antibodies against acetylcholinesterase(DS) were raised in rabbits according to Harboe and Ingild [10] and the immunoglobulin fraction was isolated. Rabbit anti-human erythrocyte membrane antibody and rabbit anti-human serum antibody were from Dako-Immunoglobulins, Copenhagen, Denmark. Human erythrocyte membranes were prepared according to Dodge et al. [11]. The membranes were solubilized in a buffer containing 38 mmol/l Tris, 100 mmol/l glycine, pH 8.7, with 1.0% (v/v) Triton X-100 at a protein concentration of 3 mg/ml [12]. Human serum applied in the specificity test for anti-acetylcholinesterase(DS) contained 1.0% (v/v) Triton X-100.

Methods

Papain immobilization

Papain (35 mg) was dissolved in 20 ml of 0.5 mol/l NaCl, 0.1 mol/l $NaHCO_3$, pH 8.3 (coupling buffer) and incubated with 3.5 ml swollen CNBr-activated Sepharose. After the coupling, the gel was treated according to Axen et al. [13]. Activation of immobilized papain took place during 25 min at 25 °C in 1.32 mM cysteine hydrochloride, 0.26 mmol/l EDTA, pH 6.2, saturated with N_2. The activity of the papain suspension was found to be 4.9 IU/ml using 40 mmol/l *N*-benzoyl-L-arginine ethyl ester hydrochloride as substrate in the above solution; and the released acid was titrated with a pH-stat (Radiometer A/S, Copenhagen, Denmark).

Limited proteolysis

An aliquot of acetylcholinesterase(DS) (920 IU/ml) was added to an equal volume of a suspension of papain-Sepharose in 2.64 mmol/l cysteine hydrochloride, 0.52 mmol/l EDTA, pH 6.2, containing 2.0% (w/v) methyl-α-D-mannoside to avoid adsorption of acetylcholinesterase(DS) to the Sepharose matrix [1]. The mixture was incubated with intermittant mixing at 37 °C and the progress of proteolytic digestion was routinely monitored by measuring acetylcholinesterase activity and by crossed hydrophobic interaction immunoelectrophoresis on phenyl-Sepharose/agarose gels [14]. When the total enzyme activity had decreased to 30–40% the reaction was stopped by sedimenting the immobilized papain at 12000 × *g* for 7 min at room temperature using the Eppendorf centrifuge 5414. Separation of the proteolytic fragments in the supernatant was performed by electrophoresis on agarose gels containing phenyl-Sepharose. To elute the fragments from the gel, pieces from selected areas were removed, squeezed and extracted three times for 10 min each at room temperature with buffer containing 10 mmol/l Tris-HCl, pH 7.4, 144 mmol/l NaCl, 1.0% (w/v) mannoside. For the extraction of acetylcholinesterase(DS) and its amphiphilic fragments the buffer contained, in addition, 1.0% (w/v) Triton X-100. To assess the distribution of enzyme among amphiphilic and hydrophilic forms after separation by hydrophobic interaction electrophoresis, selected areas on the phenyl-Sepharose/agarose gel were sequently extracted with (a) 100 mmol/l sodium phosphate buffer, pH 7.4, (b) with the same buffer containing in addition 1.0% (w/v) methyl-α-D-mannoside, and (c) with solution (b) containing in addition 1.0% (w/v) Triton X-100.

Electrophoresis

Crossed immunoelectrophoresis with an intermediate gel was carried out according to Bjerrum and Boeg-Hansen [15]. Human erythrocyte mem-

branes, solubilized in Triton X-100, or human serum (30 μg protein each) were applied to the well. The intermediate gel contained 2.9 μl/cm^2 of rabbit anti-human erythrocyte membrane antibody and 7.8 μl/cm^2 of rabbit anti-human serum antibody. In these conditions, an antigen precipitated by anti-acetylcholinesterase(DS) appears retarded in the second-dimension gel as compared to the control in which the intermediate gel is devoid of anti-acetylcholinesterase(DS). First-dimension electrophoresis was carried out at 10 V/cm until bovine serum albumin, stained with Bromophenol blue, had migrated 4.5 cm. For the second dimension 2 V/cm were applied for 17 h with the anode on top. Staining for esterase activity was performed according to Brogren and Boeg-Hansen [16]. The immunochemical relationship among the different forms of acetylcholinesterase was assessed by crossed-line immunoelectrophoresis as described in Ref. 15. Hydrophobic interaction crossed immunoelectrophoresis of acetylcholinesterase preparations (0.9–2.0 IU) was performed with 0.6–1.2 μl/cm^2 of anti-acetylcholinesterase(DS) in the second-dimension gel. Charge-shift crossed immunoelectrophoresis was carried out as described in Ref. 7.

SDS-polyacrylamide gel electrophoresis was carried out as described by Weber and Osborn [17]. Stainings were performed with Coomassie brilliant blue R-250 or with silver according to Bürk [18].

Gel filtration

Gel filtration on Sepharose CL-6B was carried out in a 0.5 × 40 cm column that was percolated with buffers containing 10 mmol/l Tris-HCl, pH 7.4, 144 mmol/l NaCl, 1.0% (w/v) methyl-α-D-mannoside without or with 0.1% (w/v) Triton X-100. The void volume (V_0) and the total volume (V_t) were determined with Dextran blue and $K_3(Fe(CN)_6)$, respectively. The Stokes radii were determined as described by Siegel and Monty [19].

Density gradient centrifugation

Density gradient centrifugation was performed according to Martin and Ames [20]. Linear 5–30% (w/v) sucrose gradients in 10 mmol/l Tris-HCl, pH 7.4, 0.1 mmol/l NaCl without or with 0.5% (w/v) Triton X-100 were centrifuged at 4°C for 16 h at 39000 rpm, in a 6 × 14 ml titanium swing-out rotor on an MSE superspeed 65 ultracentrifuge. The tubes were emptied from the bottom and fractions of 0.25 ml were collected.

Assays

Acetylcholinesterase activity was determined using the method of Ellman et al. [21] and catalase according to Aebi [22]. Fibrinogen was determined as protein by the assay of Wang and Smith [23]. The other markers in gel filtration were detected spectrophotometrically: ferritin at 400 nm, Dextran blue at 620 nm and $K_3(Fe(CN)_6)$ at 420 nm.

Results

Acetylcholinesterase(DS) was incubated with papain and the course of proteolytic conversion to acetylcholinesterase(p) was monitored by immunoelectrophoresis. This was possible because the rabbit antibody raised against purified detergent-depleted acetylcholinesterase(DS) was specific for the enzyme as tested with solubilized human erythrocyte membranes or human serum. Compared to the control, the only antigen being retarded by anti acetylcholinesterase(DS) in the intermediate gel was acetylcholinesterase (Fig. 1A and B). Fig. 1C and D shows the precipitation of human serum proteins by antibodies against serum proteins. As compared to the control, none of the precipitation lines appear retarded by anti-acetylcholinesterase(DS) antibodies contained in the intermediate gel. Hydrophobic interaction immunoelectrophoresis on phenyl-Sepharose of the papain-digested acetylcholinesterase(DS) showed two immunoprecipitates, both revealed by their esterase activity (Fig. 2). During electrophoresis in the first dimension, undigested acetylcholinesterase(DS) was bound to phenyl-Sepharose and remained at the origin, whereas acetylcholinesterase(p) did not bind to the hydrophobic matrix and thus gave rise to an anodic precipitate in the second-dimension gel. The migration of acetylcholinesterase(DS) into the second-dimension gel was effected by the Triton X-100 present in the second-dimension gel, thus giving rise to the rocket-shaped precipitate above the origin.

As shown previously by charge-shift crossed immunoelectrophoresis, acetylcholinesterase(DS)

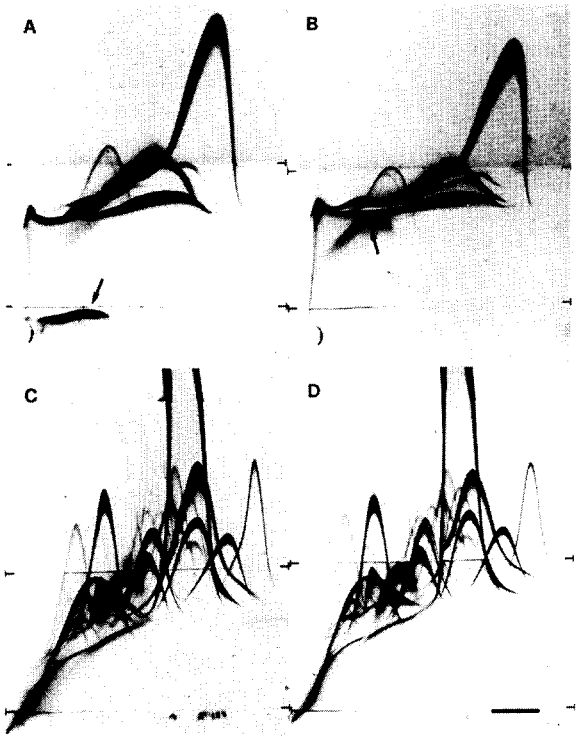

Fig. 1. Test of specificity of the rabbit anti-human erythrocyte membrane acetylcholinesterase(DS) antibody. Crossed immunoelectrophoresis with an intermediate gel of proteins solubilized in Triton X-100 was performed. Panels A + B contained 30 μg of erythrocyte membrane proteins, C + D 30 μg of human serum proteins. The intermediate gels of A and C contained 2.9 μl/cm^2 of rabbit anti-acetylcholinesterase(DS) antibody, the intermediate gels of B and D contained buffer only and served as controls. The reference gels contained in A and B anti-human erythrocyte membrane antibodies (6.5 μl/cm^2), in C and D anti-human serum antibodies (7.8 μg/cm^2). The arrow indicates the position of acetylcholinesterase(DS). The gels were stained for protein and esterase activity as outlined in the Methods section. The bar = 1 cm.

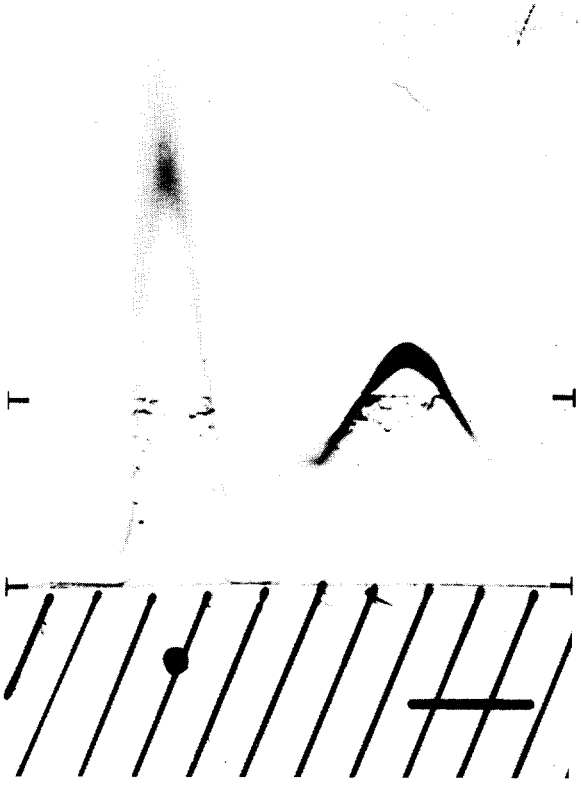

Fig. 2. Crossed hydrophobic interaction immunoelectrophoresis on phenyl-Sepharose of acetylcholinesterase(DS) digested by limited proteolysis with papain. The detergent-free sample, containing 1 IU of enzyme activity, was applied to the well (filled circle) and electrophoresed in the first dimension on a phenyl-Sepharose/agarose gel (hatched area). An antibody-free intermediate gel containing 1% (v/v) Triton X-100 was moulded above the first-dimension gel. The reference gel contained anti-acetylcholinesterase(DS) antibodies (0.6 μl/cm^2) and the plate was stained for esterase activity as outlined in the Methods section.

binds detergent molecules [7]. By the same method it was determined whether acetylcholinesterase(DS) would loose its detergent-binding properties by proteolysis. Fig. 3A, B and C shows the undigested enzyme. Treatment of acetylcholinesterase(DS) with papain gave rise to two precipitates (Fig. 3D, E, F), one shifting 6 mm in the presence of cetyltrimethylammonium bromide and 15 mm in sodium deoxycholate. These values corresponded to those obtained with the untreated control. The other more anodic precipitate displayed no charge-shift at all, indicating that acetylcholinesterase(p) did not bind detergent. Furthermore, charge-shift crossed immunoelectrophoresis showed that acetylcholinesterase(DS) and acetylcholinesterase(p) showed a reaction of immunochemical identity as their lines of precipitation fused. This was corroborated by crossed-line immunoelectrophoresis with acetylcholinesterase(p) in the first dimension and acetylcholinesterase(DS) in the intermediate gel (Fig. 4).

Measurements of enzyme activity during proteolytic digestion showed that parallel to the conversion of acetylcholinesterase(DS) to acetyl-

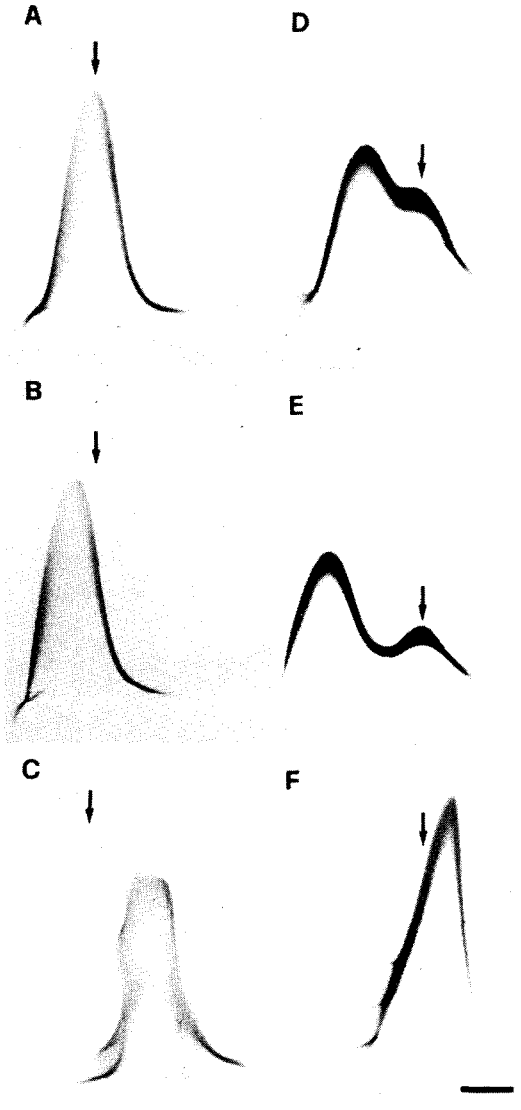

Fig. 3. Charge-shift crossed immunoelectrophoresis of purified acetylcholinesterase(DS) before and after digestion with papain. Panels A, B and C: acetylcholinesterase(DS) (4 IU) before treatment with papain. Panels D, E and F: enzyme (1.25 IU) after 7 min incubation with papain (for details see Methods section). First-dimension electrophoresis was performed in the presence of 0.5% (w/v) Triton X-100 only (A and D); in the presence of 0.5% (w/v) Triton X-100 plus 0.0125% (w/v) cetyltrimethylammonium bromide (B and E) and in the presence of 0.5% (w/v) Triton X-100 plus 0.2% (w/v) sodium deoxycholate. The second-dimension gels contained in A, B and C 2.8 μl/cm^2 and in D, E and F 0.8 μl/cm^2 of anti-acetylcholinesterase(DS) antibodies. The arrows in A, B and C give the position of acetylcholinesterase(DS) in Triton X-100 only; the arrows in D, E and F give the position of acetylcholinesterase(p). Electrophoresis in the first dimension was carried out at 10 V/cm until hemoglobin had migrated 2 cm. Otherwise the conditions were as in Fig. 1. The gels were stained for esterase activity. The bar = 1 cm.

Fig. 4. Crossed-line immunoelectrophoresis of acetylcholinesterase(p) and acetylcholinesterase(DS). The pattern represents the fused precipitates of 2 IU of acetylcholinesterase(p) electrophoresed in the first dimension and subsequently electrophoresed through an intermediate gel containing 2.7 IU of undigested acetylcholinesterase(DS). The reference gel contained anti-acetylcholinesterase(DS) antibodies (0.6 μl/cm^2). Staining and electrophoresis conditions were as in Fig. 2. The bar = 1 cm.

cholinesterase(p) a decrease in enzyme activity occurred. In routine experiments, proteolytic digestion was stopped after the total activity had decreased to 40%. The distribution of activity among the two forms was determined after hydrophobic interaction electrophoresis and elution of the two forms from the phenyl-Sepharose/agarose gel (recovery 84%). It could be shown that the treatment with papain resulted in equal amounts of acetylcholinesterase(DS) and acetylcholinesterase(p) activity.

To elucidate the requirements of acetylcholinesterase(p) for amphiphiles, its activity in the presence and absence of Triton X-100 and upon dilution was examined. The same enzyme activities were obtained when assayed in the absence or presence of 0.1% (w/v) Triton X-100 in the assay medium. Furthermore, dilution of acetylcholinesterase(p) to concentrations below 0.3 ng protein

per ml had no effect on enzyme activity. In contrast, the acetylcholinesterase(DS) was active only in the presence of 0.1% (w/v) Triton X-100, but when the concentration of the detergent was reduced by dilution to 0.00033%, enzyme activity immediately decreased to 36% of the control.

Apparent subunit molecular weights of acetylcholinesterase(DS) and acetylcholinesterase(p) were determined by SDS-polyacrylamide gel electrophoresis. Under recuding conditions the bands corresponding to acetylcholinesterase(DS) and acetylcholinesterase(p) migrated to nearly identical positions and the difference in molecular weights was 2000 or less (results not shown).

To gain information about changes in molecular dimension and shape upon proteolytic conversion of acetylcholinesterase(DS) to acetylcholinesterase(p), the Stokes radii and the sedimentation coefficients of the two enzyme forms were determined. Gel filtration in the presence of 0.1% Triton X-100, on Sepharose Cl-6B (Fig. 5) gave a Stokes radius of 8.24 nm for acetylcholinesterase(DS). The acetylcholinesterase(p) eluted at a position which corresponded to a Stokes radius of 5.87 nm regardless of the absence or present of detergent.

Sedimentation analysis of the undigested enzyme revealed in the presence of Triton X-100 a sedimentation coefficient of 6.3 ± 0.15 S for the undigested enzyme; in the absence of Triton X-100 the enzyme aggregated and sedimented with a broad peak around 11 S (Fig. 6A). After papain digestion the pattern shown in Fig. 6B was obtained. In the presence of Triton X-100 a peak at 6.6 ± 0.4 S was observed, in the absence thereof one peak appeared at 6.9 ± 0.36 S and the other in the range 10–13 S. Acetylcholinesterase(p) was isolated from the phenyl-Sepharose/Agarose electrophoresis gel and subjected to density gradient centrifugation. Identical sedimentation patterns were observed in the absence or presence of 0.5% (w/v) Triton X-100 in the gradients (Fig. 6C). In this experiment a main peak at 7.0 ± 0.16 S and a shoulder at around 5 S was observed.

Discussion

Our results show that purified amphiphilic acetylcholinesterase(DS) could be converted by proteolysis into a hydrophilic form, acetylcholinesterase(p). This parallels observations made on a number of membrane-bound enzymes which could be solubilized by treatment with proteinases without a loss of catalytic activity [24–29]. In our case limited proteolysis with papain not only resulted in the conversion of acetylcholinesterase(DS) to acetylcholinesterase(p), but also in a progressive decrease of acetylcholinesterase activity which led to several proteolytic fragments not investigated further. Other proteolytic enzymes

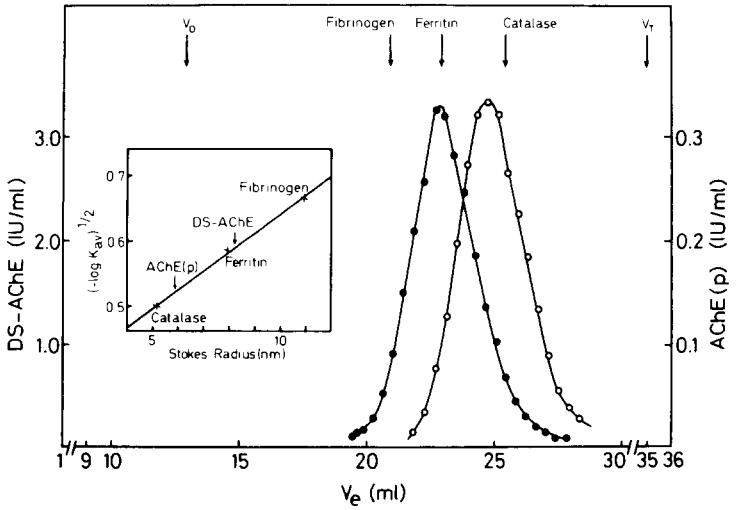

Fig. 5. Gel filtration on Sepharose CL-6B of acetylcholinesterase(DS) (AChE(p)) and acetylcholinesterase(p) (DS-AChE). The diagram represents the elution patterns of the amphiphilic (22 IU in 200 μl) (●) and the hydrophilic enzyme (8.6 IU in 200 μl) (○). Blue Dextran 2000 was used to determine the void volume (V_0) and catalase, ferritin and fibrinogen served as markers to calibrate the column. Inset: relationship of the Stokes radii of acetylcholinesterase(DS) and acetylcholinesterase(p) in 0.1% (w/v) Triton X-100 to the Stokes radii of the marker proteins.

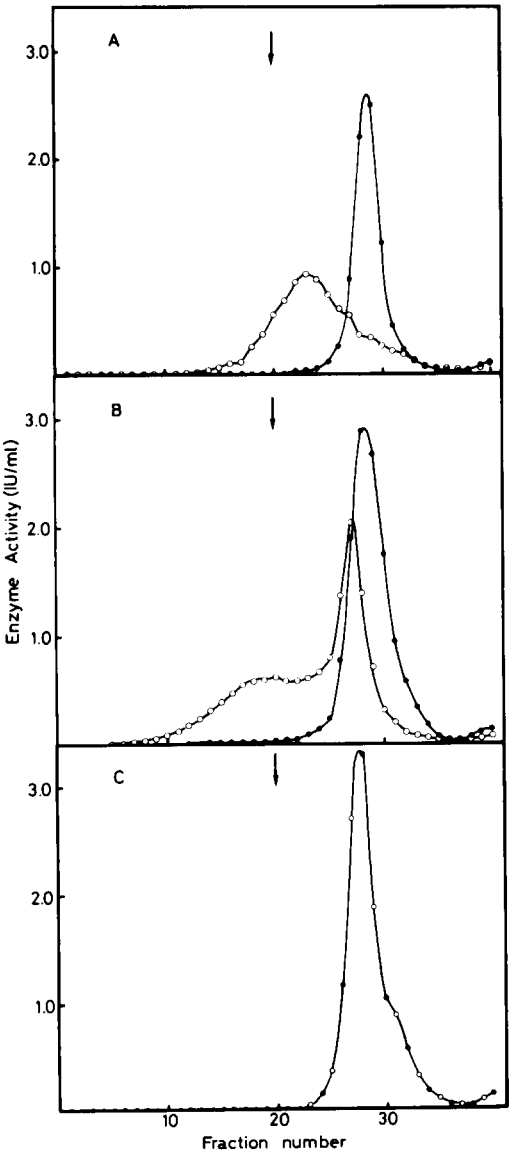

Fig. 6. Sedimentation patterns of acetylcholinesterase(DS) before and after treatment with papain. A: pure acetylcholinesterase(DS) before digestion with papain. B: mixture of acetylcholinesterase(DS) and acetylcholinesterase(p) obtained after 7 min digestion with papain. C: pure acetylcholinesterase(p) after separation from acetylcholinesterase(DS) by hydrophobic interaction electrophoresis on a phenyl-Sepharose/Agarose gel. Sucrose gradients made in the absence (○) and presence (●) of 0.5% (w/v) Triton X-100. Arrow gives the position of catalase ($s_4 = 11.4$ S) in the gradient.

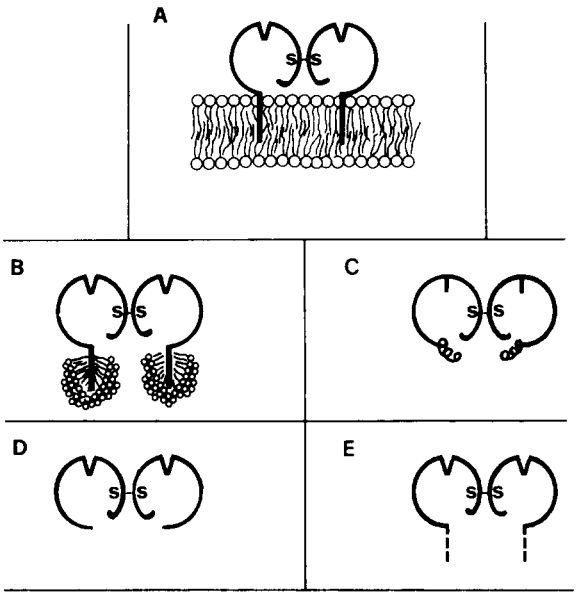

Fig. 7. Diagrammatic representation of acetylcholinesterase(DS) from human erythrocyte membranes. A: native, amphiphilic acetylcholinesterase(DS) anchored in the lipid bilayer of natural or artificial membranes. B: pure acetylcholinesterase(DS) in the presence of Triton X-100. The formation of a protein-detergent mixed micelle fully preserves enzyme activity. C: pure acetylcholinesterase(DS) in the absence of stabilizing amphiphilic interactions (enzyme activity irreversibly lost). E: enzymatically active acetylcholinesterase(p). D: active acetylcholinesterase(DS) in the presence of chaotropic agents.

such as proteinase K, pronase, thermolysin and chymotrypsin were less efficient in the conversion of acetylcholinesterase(DS) to acetylcholinesterase(p) and mainly lead to unspecific degradation of acetylcholinesterase(DS). Collagenase, which converts the asymmetric forms of acetylcholinesterase to globular ones, neither converted acetylcholinesterase(DS) to acetylcholinesterase(p) nor caused a decrease in enzyme activity (M. Weitz, unpublished observations). Our results on the proteolytic conversion of acetylcholinesterase(DS) from human red cell membranes are corroborated by the recent findings of Dutta-Choudhury et al. [30], who also noted that papain was the proteinase most efficient in converting acetylcholinesterase(DS) to acetylcholinesterase(p). They are further sustained by the results obtained with acetylcholinesterase(DS) from *Torpedo marmorata*. This enzyme could either be solubilized as a hydrophilic entity by treating tissue extracts with pronase [31] or by proteolytic conversion of the pure acetyl-

cholinesterase(DS) to acetylcholinesterase(p) by proteinase K [32].

Our characterization of acetylcholinesterase(DS) and acetylcholinesterase(p) is based on immunochemical methods, among others. The results shown in Fig. 1 give evidence that our polyclonal rabbit anti-acetylcholinesterase(DS) antibody was specific for its antigen, as only the precipitin line corresponding to acetylcholinesterase(DS) was retarded in the intermediate gel of the crossed immunoelectrophoresis. No other immunochemical reaction of our antibody was seen either with total erythrocyte membrane antigens or human serum proteins.

Crossed hydrophobic interaction electrophoresis on phenyl-Sepharose proved to be a convenient tool for the separation of acetylcholinesterase(DS) and acetylcholinesterase(p) generated by the treatment with papain. The fact that acetylcholinesterase(DS) was retained at the origin of the hydrophobic matrix gives evidence for the existence of a hydrophobic anchor peptide. This is cleaved off by papain and the resulting product, acetylcholinesterase(p), could migrate towards the anode (Fig. 2). The amphiphilic nature of acetylcholinesterase(DS) and the hydrophilic character of acetylcholinesterase(p) were further corroborated by the results obtained from charge-shift crossed immunoelectrophoresis. Acetylcholinesterase(DS), which binds detergent, showed a bidirectional shift in the presence of positively or negatively charged detergents compared to its migration observed in a neutral detergent. By the same method two fusing precipitin lines were seen after digestion of acetylcholinesterase(DS) with papain. One corresponded to undigested enzyme and, as seen from its charge-shift, still bound detergent (Fig. 3D, E and F). The other, corresponding to acetylcholinesterase(p), showed a higher anodic mobility. It was unaffected by the presence of charged detergents, signifying that acetylcholinesterase(p) had lost its detergent-binding properties. The pattern obtained with sodium deoxycholate shows essentially one precipitin arc (Fig. 3F). Owing to the anodic shift of acetylcholinesterase(DS) in the presence of that detergent, both precipitin lines became superimposed. The result obtained from crossed-line immunoelectrophoresis showed a fusing precipitin line of acetylcholinesterase(DS) and acetylcholinesterase(p), indicating a reaction of immunochemical identiy of the two enzyme forms. Thus proteolytic cleavage of the hydrophobic anchor from acetylcholinesterase(DS) did not affect the immunochemical properties of the enzyme. This result further showed that the hydrophobic segment of acetylcholinesterase(DS) is not antigenic. Similar observations have been made with dopamine-β-hydroxylase [25] and intestinal aminopeptidase [33,34].

Taken together, the results obtained from the different immunochemical experiments give evidence for the existence of a hydrophobic peptide sequence on acetylcholinesterase(DS) that presumably anchors the enzyme into the lipid bilayer of the red cell membrane. The results obtained from SDS-polyacrylamide gel electrophoresis showed a difference in molecular weights of acetylcholinesterase(DS) and acetylcholinesterase(p) in the range of about 2000. This difference corresponds to that reported by Dutta-Choudhury et al. [30] and parallels our observations made on acetylcholinesterase(DS) from *Torpedo marmorata* [32]. Our results further showed that papain did not affect the structural integrity of the hydrophilic part of acetylcholinesterase and that the intersubunit disulfide link was left intact. This again parallels our observations made on the enzyme from *Torpedo* but differs from the degradation pattern observed with acetylcholinesterase(DS) from human [35] and rat brain [36], where the peptide sequence containing the intersubunit disulfide bridge appeared to be sensitive to proteolytic attack.

The results obtained from gel filtration and sucrose density gradient centrifugation show that the conversion of acetylcholinesterase(DS) to acetylcholinesterase(p) brought about changes in the hydrodynamic properties of the enzyme. In the presence of Triton X-100, acetylcholinesterase(DS) had a Stokes radius of 8.2 nm, and this value reflects the size of the enzyme-detergent mixed micelle. Upon proteolytic conversion the detergent-binding peptide was cleaved off and as a consequence the Stokes radius was reduced to 5.9 nm in acetylcholinesterase(p).

On the other hand, the proteolytic conversion of acetylcholinesterase(DS) to acetylcholin-

esterase(p) led to an increase in the sedimentation coefficient from 6.3 ± 0.15 S to 7.0 ± 0.16 S. This increase is consistent with the view that the enzyme-detergent mixed micelle has a lower bouyant density and appears as a non-globular molecule that upon proteolysis becomes globular and increases its density. As seen in Fig. 6C, acetylcholinesterase(p) no longer aggregated in the absence of Triton X-100 in the density gradient medium and its sedimentation coefficient was independent of the presence or absence of micellar amounts of the detergent. Such a result gives additional evidence for the existence of the hydrophobic anchor that is removed by the treatment of acetylcholinesterase(DS) with papain. The shoulder around 5 S in the sedimentation pattern of acetylcholinesterase(p) was consistently observed (Fig. 6C). It indicates that papain also brought about the monomerization of dimeric acetylcholinesterase(DS). In our conditions the amount of monomeric acetylcholinesterase(p) formed was small and was below the limit of detection of SDS-polyacrylamide gel electrophoresis.

Conclusions

From our results we conclude that each subunit of acetylcholinesterase(DS) has a major hydrophilic and a small hydrophobic part, that anchors the enzyme into the lipid bilayer of the native or artificial membranes (Fig. 7A). Acetylcholinesterase(DS) from human red cells belongs to the class of stalked membrane proteins [26,27,37–39]. It has a high lateral mobility within the membrane and shows little or no interaction with the cytoskeletal network [40]. In the presence of Triton X-100 the enzyme exists as a protein-detergent mixed micelle (Fig. 7B), with about one-half of a Triton X-100 micelle attached to each hydrophobic anchor [30]. Removal of the stabilizing detergent molecules in conditions where the enzyme can no longer undergo hydrophobic interactions leads to an irreversible loss in enzyme activity (Fig. 7C and Ref. 41) but enzyme activity is retained upon cleavage of the hydrophobic anchor (Fig. 7D) or in the presence of suitable chaotropic agents (Fig. 7E and Ref. 5).

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