Cross-Linking and Binding of Fibronectin with Asymmetric Acetylcholinesterase†

Mark R. Emmerling, Carl D. Johnson, Deane F. Mosher, Bruce H. Lipton, and Jack E. Lilien*

ABSTRACT: The similarities between the tail of asymmetric acetylcholinesterase (AcChE) and collagen prompted us to investigate if asymmetric AcChE, like collagen, can interact with fibronectin. Gradient centrifugation studies revealed that asymmetric, but not globular, AcChE bound to fibronectin and could be cross-linked covalently to fibronectin by plasma transglutaminase. The interaction of asymmetric AcChE with fibronectin paralleled the interaction of fibronectin with collagen. These results raise the possibility that fibronectin may be involved in attaching asymmetric AcChE to cell surfaces.

Recent studies have demonstrated that acetylcholinesterase (AcChE)[1] is present on the cell surface of muscle fibers in vitro (Rubin et al., 1979; Rotundo & Fambrough, 1980) and an integral component of basal laminae within the synaptic clefts of neuromuscular junctions (McMahan et al., 1978). It has been suggested that one way by which AcChE could become attached to these sites is by interactions with other molecules in the extracellular matrix (Lwebuga-Mukasa et al., 1976; Bon et al., 1978). This possibility has led us to investigate whether the molecular forms of AcChE which possess collagen-like tails can interact with the cell-surface molecule fibronectin.

Fibronectin is a glycoprotein of 440 kilodaltons made up of two disulfide-bonded 200–250-kilodalton subunits. It is found on some cell surfaces and in body fluids, connective tissues, and basal laminae [for reviews, see Yamada & Olden (1978) and Vaheri & Mosher (1978)]. Considerable evidence from studies in cell cultures indicates the involvement of fibronectin as an adhesive component in cell–substrate attachment, cell spreading, and cell motility [for reviews, see Grinnell (1978) and Yamada & Olden (1978)]. The basis for fibronectin acting as an adhesive molecule appears to reside in its ability to interact with cell-surface components such as glycolipids (Kleinman et al., 1979), glycosaminoglycans (Yamada et al., 1980), and collagens (Dessau et al., 1978; Engvall et al., 1978).

Considerable effort has been devoted to exploring and understanding the nature of the interactions between collagen and fibronectin. Fibronectin can bind with interstitial collagens types I, II, and III and to a lesser extent with basement membrane collagens (Dessau et al., 1978; Engvall et al., 1978). Transglutaminase-catalyzed covalent cross-linking of fibronectin to types I and III collagen has also been demonstrated (Mosher et al., 1979a). Cross-linking results in the formation of a peptide linkage between γ-carboxyl groups of glutaminyl residues in fibronectin and ϵ-amino groups of lysyl residues in collagen (Mosher et al., 1980).

AcChE in a variety of vertebrate species exists as multiple molecular forms which, based on physical properties, can be separated into two classes, globular and asymmetric. The asymmetric forms differ from globular forms in that they possess a collagen-like tail. The tail is collagenase sensitive (Johnson et al., 1977; Bon et al., 1979), contains the amino acids hydroxyproline and hydroxylysine (Rosenberry & Richardson, 1977; Anglister & Silman, 1978), and is cross-reactive with antibodies raised against collagen (Anglister et al., 1979).

Because asymmetric AcChE possesses a collagen-like tail, the present study examines whether the enzyme can interact with fibronectin in a fashion similar to collagen. The findings of our studies demonstrate that fibronectin can under appropriate conditions interact with asymmetric AcChE both covalently and noncovalently. These interactions require the presence of the collagen-like tail and are similar to fibronectin–collagen interactions. These observations provide additional evidence for the collagen-like nature of the tail and suggest a possible means by which asymmetric AcChE can become associated with cell surfaces.

Materials and Methods

Triton X-100, ethylenediaminetetraacetic acid (EDTA), spermidine, and bovine serum albumin (BSA) fraction V were purchased from Sigma Chemical Co. Crystalline trypsin (3090 units/mg was obtained from Miles Laboratories. [^{3}H]-Acetylcholine was purchased from Amersham. Purified human thrombin (2000 units/mg) and cyanogen bromide fragment 7 of the α1(I) chain of calf skin type I collagen [1-(I)-CB7] were generous gifts from Dr. John Fenton, II, New York State Department of Health, and Dr. Hynda Kleinman, National Institute of Dental Research. Purified human plasma fibronectin, factor XIII (plasma transglutaminase), and an 180-kilodalton fragment of lightly trypsinized fibronectin were prepared as described previously (Mosher et al., 1980).

AcChE. Fertilized quail eggs (*Coturnix coturnix japonica*) were obtained from the University of Wisconsin Poultry Science Laboratory. The eggs were hatched, and the quails were allowed to grow to 4 days of age. Muscle from the quails were excised and homogenized in 10 volumes of cold 20 mM borate buffer (pH 8.8) with a Brinkmann Polytron. The homogenate was centrifuged for 30 min at 50000*g*. The supernatant was discarded and the pellet rehomogenized in cold 20 mM borate buffer (pH 8.8) containing 0.5% Triton X-100 and 1.0 M NaCl. The homogenate was centrifuged as before, and the supernatant was collected for isolation of AcChE forms.

Separation of multiple forms was performed by velocity sedimentation on 5 mL of linear 5–20% sucrose gradients in

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[1] Abbreviations used: AcCh, acetylcholine; AcChE, acetylcholinesterase; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid.

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20 mM borate buffer (pH 8.8) containing 1.0 M NaCl, 0.5% Triton X-100, and 1 mg/mL BSA. Gradients were centrifuged in a Beckman SW 50.1 rotor at 50 000 rpm at 3 °C for 5 h. Gradients were divided into 18-drop fractions and assayed for AcChE activity. Fractions containing asymmetric AcChE were pooled and dialyzed overnight at 4 °C against 0.3 M NaCl in 0.02 M borate buffer (pH 8.8).

Globular forms of AcChE were isolated from quail muscle cell cultures prepared from 9-day quail embryos. Cultures were prepared according to Lipton (1977). AcChE was extracted and isolated from cultures in the same manner described for muscles except the initial homogenization in borate buffer was omitted.

Three predominant forms of AcChE are present in muscle and muscle cell cultures with the approximate sedimentation values of 20.5, 12.4, and 7.5 S (M. R. Emmerling, C. D. Johnson, and B. H. Lipton, unpublished experiments). The 20.5S form is collagenase sensitive and asymmetric, while the smaller forms are not collagenase sensitive and are globular. Although asymmetric AcChE from muscle cell cultures interacts with fibronectin (M. R. Emmerling, unpublished experiments), muscle tissue was used for the isolation of asymmetric AcChE because it provided a richer source of the enzyme.

Assays for AcChE activity were performed according to Johnson & Russell (1975) with the following modifications. 1-Butanol was substituted for isoamyl alcohol in the scintillation fluid. Also, no cold acetylcholine (AcCh) was added to the substrate mixture of [^{3}H]AcCh. AcChE activity is expressed as the percent fractional conversion of total substrate. For the correction of substrate depletion, the formula % $F_c = -\ln [1 - (C_0 - B)/(C_t - B)] \times 100$ (Segel, 1975) was used where F_c is the fractional conversion, C_t is the total number of counts by complete hydrolysis of substrate, C_0 is the number of counts produced by the sample containing AcChE, and B is the background.

Cross-Linking. Cross-linking of AcChE and fibronectin was performed by mixing 100 μL of AcChE with an equal volume of Tris-buffered saline (10 mM Tris and 0.14 M NaCl, pH 7.4) containing fibronectin, 50 μg/mL transglutaminase, and 4 μg/mL thrombin, which is needed to activate the transglutaminase. The reaction was started by adding 20 μL of 100 mM $CaCl_2$.

Analysis of samples containing AcChE and fibronectin was performed by velocity sedimentation. Sample (200 μL) was loaded on 5 mL of linear 5–20% sucrose gradients in 20 mM borate buffer (pH 8.8) containing 1.0 M NaCl, 0.5% Triton X-100, and 1 mg/mL BSA. Gradients were centrifuged in an SW 50.1 rotor at 50 000 rpm and 3 °C for either 2 or 2.5 h. Gradients were divided into 18-drop fractions and assayed for AcChE activity. Any modifications of these procedures are described in the figure legends. Sedimentation of AcChE in all figures is from right to left.

Results

Cross-Linking of Asymmetric AcChE to Fibronectin. Because collagen can be covalently attached to fibronectin by plasma transglutaminase (Mosher et al., 1979a), experiments were undertaken to determine if transglutaminase could also catalyze cross-linking between asymmetric AcChE and fibronectin. Cross-linking was demonstrated by velocity sedimentation following incubation of asymmetric AcChE with fibronectin, transglutaminase, thrombin, and $CaCl_2$. As shown in Figure 1, asymmetric AcChE so treated sedimented as a broad peak of activity ahead of unreacted asymmetric AcChE. Altered sedimentation did not occur if fibronectin, transglutaminase, thrombin, or $CaCl_2$ was omitted from the mixture. Altered sedimentation was affected by fibronectin concentration (Figure 1) and the time of incubation (Figure 2). The reaction was also observed to occur at 4, 23, and 37 °C (data not shown). These results suggest strongly that transglutaminase can cross-link asymmetric AcChE to fibronectin but not to other proteins in the incubation mixture.

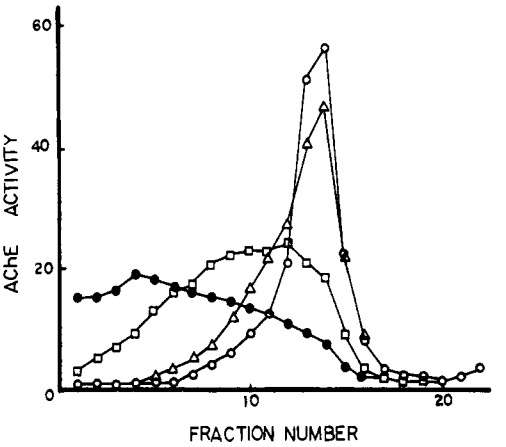

FIGURE 1: Effect of fibronectin concentration on cross-linking with asymmetric AcChE. Asymmetric AcChE was cross-linked with different amounts of fibronectin under the conditions described under Materials and Methods. Gradients were centrifuged for 2.5 h. No fibronectin (○); 125 μg/mL (△); 625 μg/mL (□); 1250 μg/mL (●).

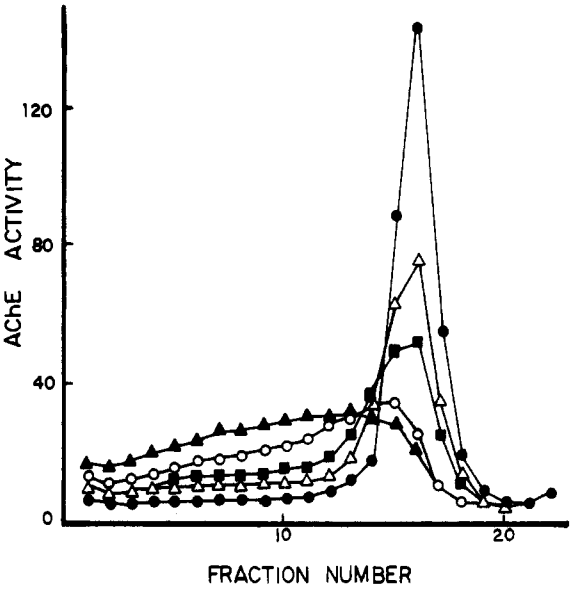

FIGURE 2: Time course of fibronectin–asymmetric AcChE cross-linking. Asymmetric AcChE was mixed with fibronectin (final concentration 750 μg/mL) under cross-linking conditions. At appropriate times, the reaction was stopped by the addition of 20 μL of 0.02 M borate buffer (pH 8.8) containing 20 mM spermidine and 200 mM EDTA. Gradients were centrifuged for 2 h. Control (●); 15 min (△); 30 min (■); 60 min (○); 120 min (▲).

Globular forms of AcChE were studied in a similar manner to determine if forms without collagen tails can interact with fibronectin. The sedimentation profile of globular AcChE incubated with fibronectin under cross-linking conditions sedimented the same as unreacted AcChE (Figure 3). Thus, the collagen tail of AcChE appears to be essential for the cross-linking to fibronectin.

Further experiments were done to establish parallels between fibronectin–collagen and fibronectin–asymmetric AcChE cross-linking. α1(I)-CB7, a cyanogen bromide fragment of type I collagen, contains a site which binds and cross-links to fibronectin (Kleinman et al., 1978). α1(I)-CB7, as well as

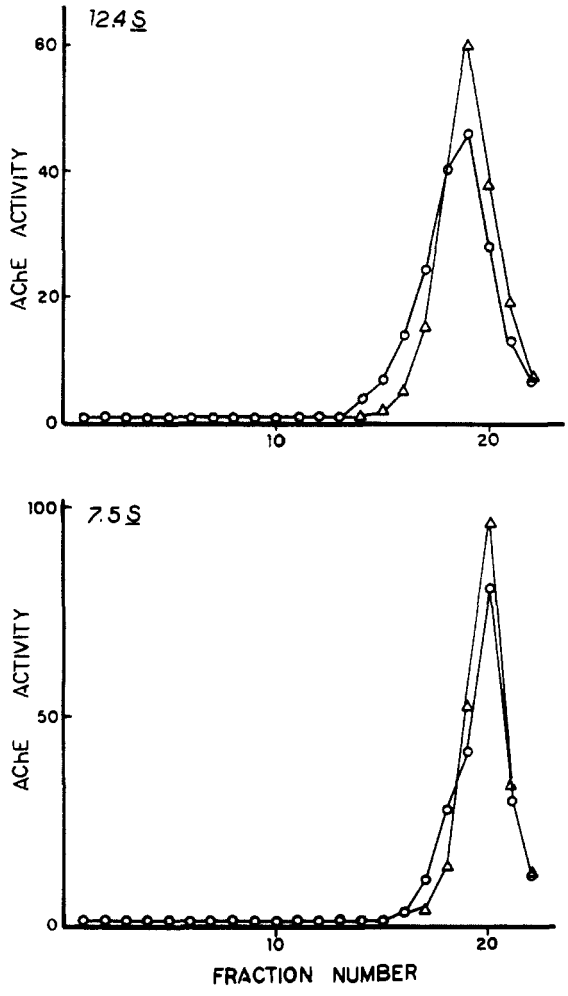

FIGURE 3: Cross-linking of fibronectin with globular forms of AcChE. Globular AcChE forms isolated from muscle cell cultures were mixed with fibronectin (final concentration 850 μg/mL) under cross-linking conditions. Gradients were centrifuged for 2 h. Upper graph, 12.4 S; lower graph, 7.5 S. Globular form (○); EDTA control (△).

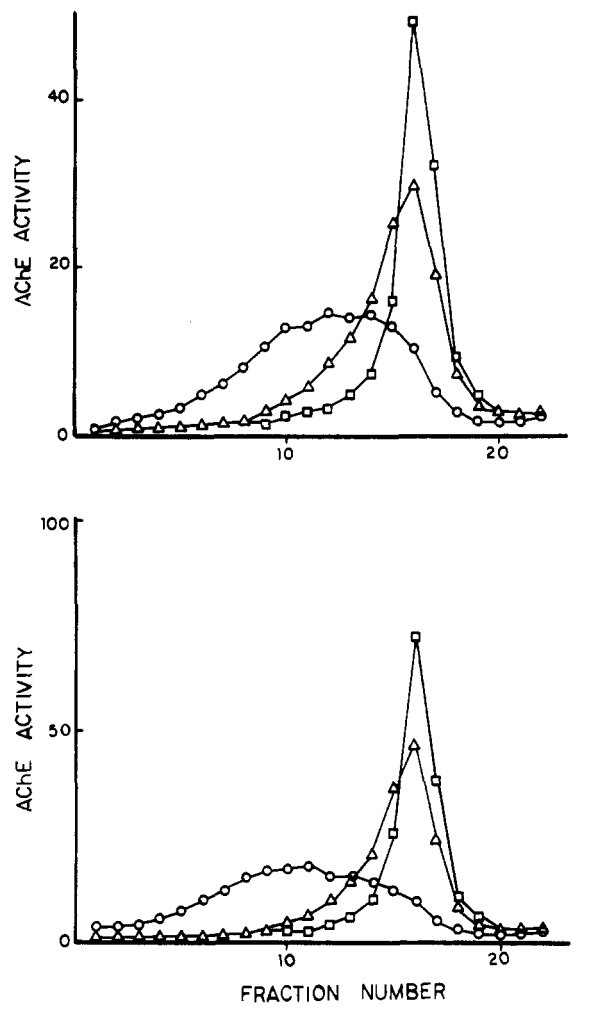

FIGURE 4: Inhibition by α1(I)-CB7 and spermidine of cross-linking between asymmetric AcChE and fibronectin. Asymmetric AcChE was cross-linked with fibronectin (final concentration 625 μg/mL) in the presence of either (upper) 10^{-5} M α1(I)-CB7 (△) or (lower) 10^{-5} M spermidine (△). Cross-linking control (○); EDTA control (□). Gradients were centrifuged 2 h.

the polyamine spermidine, can inhibit cross-linking between collagen and fibronectin (Mosher et al., 1979a,b). This inhibition results from the formation of covalent bonds between α1(I)-CB7 or spermidine and a discrete region of fibronectin which, when occupied, prevents collagen from cross-linking (Mosher et al., 1980). Both α1(I)-CB7 and spermidine inhibited the interaction between asymmetric AcChE and fibronectin (Figure 4), suggesting that, like collagen, asymmetric AcChE becomes covalently attached to fibronectin at the same site as collagen.

Binding of Asymmetric AcChE and Fibronectin. A noncovalent interaction between asymmetric AcChE and fibronectin could be demonstrated by velocity sedimentation under conditions of low ionic strength (Figure 5). Under these conditions, asymmetric AcChE aggregates with itself (Bon & Massoulié, 1978); therefore, it is unclear whether the observed interaction is due to trapping of fibronectin by aggregating AcChE or to actual binding. Spermidine was added to gradients to inhibit fibronectin binding (Vuento & Vaheri, 1978) to resolve this problem. The presence of spermidine in gradients prevented the interaction between asymmetric AcChE and fibronectin without affecting the aggregation of AcChE (Figure 6). Thus, the altered sedimentation of asymmetric AcChE mixed with fibronectin is not due to trapping.

The sedimentation of globular AcChE, which does not aggregate in low salt, was not affected by fibronectin (data not shown). This finding again demonstrates the importance of

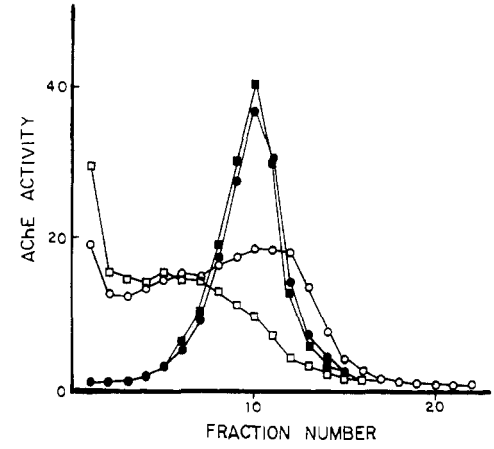

FIGURE 5: Effect of NaCl concentration in gradients on binding between asymmetric AcChE and fibronectin. Asymmetric AcChE was mixed with fibronectin (final concentration 625 μg/mL) or an equal volume of 0.01 M Tris buffer with 0.15 M NaCl (pH 7.4). Gradients were centrifuged for 2.5 h. No NaCl in gradients: AcChE (○); AcChE + fibronectin (□). NaCl (0.2 M) in gradients: AcChE (●); AcChE + fibronectin (■).

the collagen tail of AcChE in interacting with fibronectin.

Analysis of the Asymmetric AcChE–Fibronectin Complex. When the broad peak of asymmetric AcChE cross-linked to fibronectin was divided into five fractions and each fraction

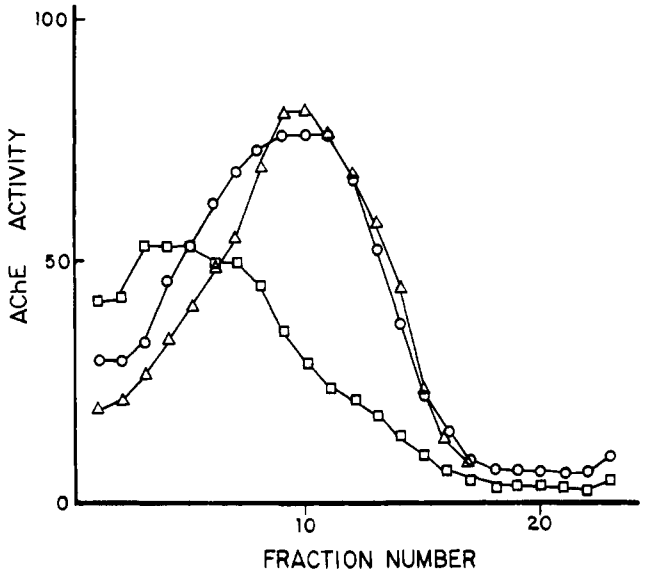

FIGURE 6: Inhibition by spermidine of binding between asymmetric AcChE and fibronectin. Asymmetric AcChE was mixed with fibronectin (final concentration 3.0 mg/mL) or an equal volume of 0.01 M Tris buffer with 0.15 M NaCl (pH 7.4) and loaded on sucrose gradients containing either no or 2.5×10^{-4} M spermidine. Gradients were centrifuged for 2 h. AcChE + fibronectin (□); AcChE ± spermidine (△); AcChE + fibronectin in spermidine (○).

was resedimented, the fractions ran true to their original relative position in the gradients (Figure 7). These results demonstrate that cross-linking gives rise to stable complexes of asymmetric AcChE–fibronectin and that the broad peak of activity in gradient is not due to breakdown of larger complexes into smaller complexes.

The sedimentation behavior of the complexes could be changed to that of slower sedimenting complexes by mild trypsinization with 0.1 μg/mL trypsin (Figure 8). The change was not due to breakdown of quail asymmetric AcChE, because asymmetric AcChE alone underwent only a slight reduction in its sedimentation value. Presumably, the reduced sedimentation rates of trypsin-treated complexes are due to degradation of fibronectin, which is known to be extremely trypsin sensitive (Chen, 1977).

Modification of Fibronectin and Effect on Cross-Linking and Binding. Mild trypsinization of fibronectin produces a 180-kilodalton fragment which contains the site for noncovalent binding to collagen although it does not contain the cross-linking site (Mosher et al., 1980). Asymmetric AcChE could bind with the 180-kilodalton fragment (Figure 9) but did not cross-link to the fragment (data no shown). As with intact fibronectin, the binding was sensitive to inhibition by spermidine.

Discussion

The interactions between fibronectin and asymmetric AcChE are similar to those observed for collagen and fibronectin (Mosher, 1980). Like collagen, asymmetric AcChE can serve as a substrate for transglutaminase to cross-link with fibronectin or in the absence of transglutaminase to bind fibronectin. Furthermore, these interactions are inhibited by α1-(I)-CB7 and spermidine, as are collagen–fibronectin interactions (Mosher et al., 1979a,b). The similarity with which collagen and asymmetric AcChE interact with fibronectin can be attributed to the collagen-like tail of asymmetric AcChE. The importance of the tail is demonstrated by the inability of AcChE forms without tails to cross-link or bind with fibronectin.

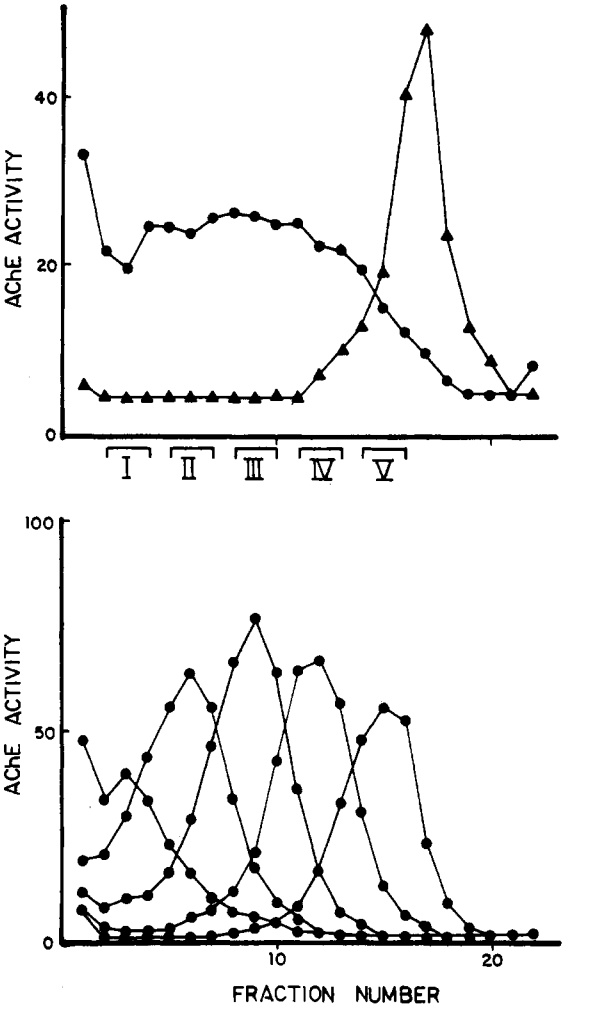

FIGURE 7: Resedimentation of asymmetric AcChE–fibronectin complexes. Upper panel: asymmetric AcChE was cross-linked with fibronectin (final concentration 850 μg/mL) and loaded on gradients, which were then centrifuged for 2 h. Lower panel: fractions from gradients (I–V) were collected, dialyzed overnight at 4 °C against 0.02 M borate buffer (pH 8.8) containing 0.3 M NaCl and rerun on gradients. Gradients were centrifuged for 2 h. Cross-linked AcChE (●); EDTA control (▲).

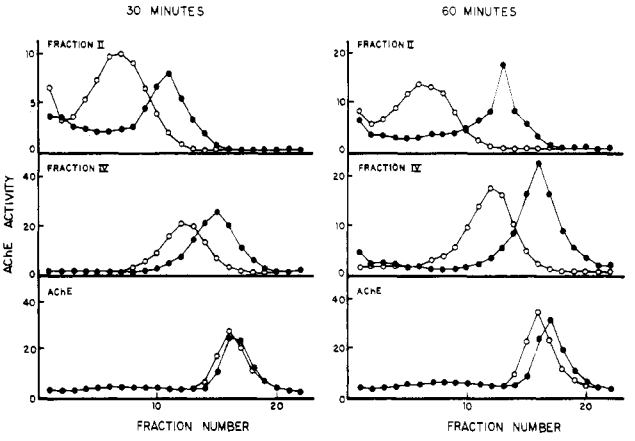

FIGURE 8: Effect of trypsin on asymmetric AcChE–fibronectin complexes. Complexes (fractions II and IV) and asymmetric AcChE were treated with trypsin (final concentration 0.1 μg/mL) at room temperature for 30 and 60 min. Gradients were centrifuged for 2 h. Trypsin treated (●); untreated control (○).

The similarities between asymmetric AcChE and collagen in their interactions with fibronectin suggest that they use the same sites on fibronectin for binding and cross-linking. In studies on collagen, it has been shown that α1(I)-CB7 and

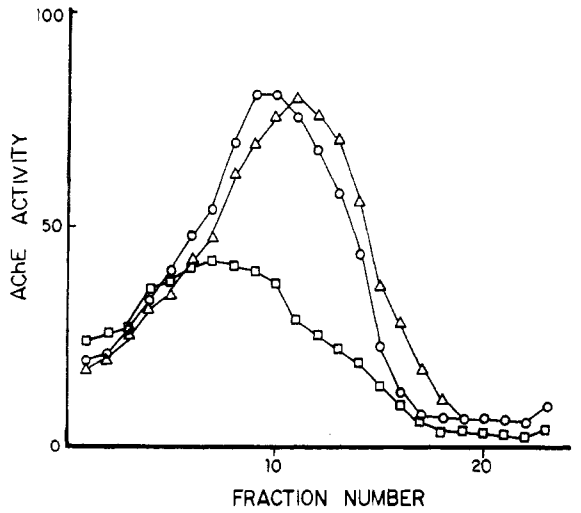

FIGURE 9: Binding of asymmetric AcChE with an 180-kilodalton fragment of fibronectin and its inhibition by spermidine. Asymmetric AcChE was mixed with the 180-kilodalton fragment (final concentration 3.0 mg/mL) or an equal volume of 0.01 M Tris buffer with 0.15 M NaCl (pH 7.4) and loaded on sucrose gradients, as described in Figure 6. Gradients were centrifuged for 2 h. AcChE + 180-kilodalton fragment (□); AcChE in spermidine (○); AcChE + 180-kilodalton fragment in spermidine (△).

spermidine inhibit cross-linking by competing for the same sites on fibronectin as collagen (Mosher et al., 1980). By analogy, inhibition of cross-linking between asymmetric AcChE and fibronectin by α1(I)-CB7 and spermidine demonstrates that asymmetric AcChE also uses the collagen cross-linking site. This conclusion is consistent with studies using the 180-kilodalton fragment of fibronectin. The fragment does not cross-link with α1(I)-CB7, collagen, spermidine (Mosher et al., 1980) or asymmetric AcChE but can still bind α1(I)-CB7, collagen (D. F. Mosher, unpublished experiments), and asymmetric AcChE. Therefore, it appears that the 180-kilodalton fragment has lost the common cross-linking site for collagen and asymmetric AcChE but still retains a common binding site.

The broad peak of AcChE activity present in gradients after cross-linking asymmetric AcChE with fibronectin represents the formation of various sized complexes as demonstrated by resedimentation experiments. The fact that the complexes are extremely trypsin sensitive suggests that fibronectin is an integral part of the complex. The stoichiometry of fibronectin and AcChE in the complexes is unknown. However, it is reasonable to assume that differences in the sedimentation properties of the complexes result from the amount of fibronectin and AcChE present. Fibronectin is a dimer and presumably possesses two identical cross-linking sites for collagen (Mosher, 1980). Under appropriate conditions, fibronectin can also become covalently cross-linked to itself (Mosher, 1975). Therefore, the heterogeneity of complexes formed may result both from attachment of several molecules of asymmetric AcChE to one molecule of fibronectin and from attachment of several fibronectin molecules to one molecule of asymmetric AcChE.

AcChE is an integral part of basal laminae in motor end plates (McMahan et al., 1978). It has been suggested that the attachment of asymmetric AcChE to basal laminae can be accounted for by the interaction of the tail of asymmetric AcChE with molecules in the extracellular matrix (Lwebuga-Mukasa et al., 1976; Bon et al., 1978). Bon and her colleagues (Bon et al., 1978) demonstrated that asymmetric AcChE from electric eel interacted with glycosaminoglycans, which are present in the extracellular matrix of tissues. Thus, it is possible that glycosaminoglycans might bind asymmetric AcChE to basal laminae. However unlike collagenase and papain (Sketelj & Brzin, 1979), hyaluronidase, an enzyme which degrades glycosaminoglycans, does not release AcChE from motor end plates.

The observed interaction between fibronectin and asymmetric AcChE provides another possible molecular basis for the attachment of AcChE to neuromuscular junctions. Fibronectin is found in the basal laminae of a variety of tissues, including muscle fibers (Stenman & Vaheri, 1978). Fibronectin has also been localized to the cell surfaces of myotubes formed by L6 myoblasts in culture (Furcht et al., 1978). The fact that asymmetric AcChE can both bind and cross-link with fibronectin may imply a means by which asymmetric AcChE can become reversibly and irreversibly bound to motor end plates.

Acknowledgments

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Taxol Assembles Tubulin in the Absence of Exogenous Guanosine 5′-Triphosphate or Microtubule-Associated Proteins†

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ABSTRACT: Taxol increases the rate and extent of microtubule assembly in vitro and stabilizes microtubules in vitro and in cells [Schiff, P. B., Fant, J., & Horwitz, S. B. (1979) *Nature (London) 277*, 665–667; Schiff, P. B., & Horwitz, S. B. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1561–1565]. We report herein that taxol has the ability to promote microtubule assembly in the absence of microtubule-associated proteins, rings, and added guanosine 5′-triphosphate (GTP) or organic buffer. The drug enhances additional microtubule assembly when added to microtubules at apparent steady state. This additional assembly can be attributed to both elongation of existing microtubules and spontaneous nucleation of new microtubules. Taxol-treated microtubules have depressed dissociation reactions as determined by dilution experiments. The drug does not inhibit the binding of GTP or the hydrolysis of GTP or guanosine 5′-diphosphate (GDP) in our microtubule protein preparations. Taxol does not competitively inhibit the binding of colchicine to tubulin.

Microtubules are an important component of the cytoskeleton of most eukaryotic cells. They are an integral part of the mitotic spindle, cilia, flagella, and cytoplasm of interphase cells. Drugs which interact with tubulin, the subunit of microtubules, have been useful tools in understanding the regulation of microtubule assembly in cells and the wide variety of cellular functions mediated by microtubules [see Dustin (1978) and Roberts & Hyams (1979)].

Our previous work (Schiff et al., 1979; Schiff & Horwitz, 1980, 1981) has demonstrated that taxol, an experimental antitumor drug (Wani et al., 1971), enhances microtubule assembly and stabilizes microtubules in vitro. Microtubules polymerized in the presence of taxol are resistant to depolymerization by cold (4 °C) or $CaCl_2$ (4 mM). The drug increases the rate, yield, and nucleation phase of the assembly reaction maximally when the tubulin dimer to taxol ratio is 1 and it decreases the critical concentration of microtubule protein (MTP)[1] required to initiate polymerization. The taxol binding site is present on the intact microtubule.

Our observations that the drug blocks cell replication predominantly in the mitotic phase of the cell cycle, inhibits fibroblast cell migration, and stabilizes microtubules in cells clearly relate the cytotoxic activity of the drug to our in vitro studies. Cytoplasmic microtubules in taxol-treated cells resist depolymerization by cold (4 °C) and by antimitotic drugs such as colchicine that normally disrupt microtubules. The drug may also promote microtubule assembly in cells.

We now report further in vitro studies to better characterize the microtubule assembly reaction in the presence of taxol. The effect of taxol on microtubules has been examined at apparent steady-state conditions, and dilution techniques have been used to investigate microtubule depolymerization in the presence of taxol. The relationship of microtubule-associated proteins (MAPs), rings, organic buffer, and colchicine to the effects of taxol on microtubule assembly is reported.

Experimental Procedures

Materials. Taxol and podophyllotoxin were obtained from the National Cancer Institute and dissolved in dimethyl sulfoxide (Me_2SO). [^{3}H]Colchicine (19.6 Ci mmol^{-1}) and Aquasol were from New England Nuclear, and Norit was from Eastman.

Preparation of Microtubule Protein. Calf brain MTP was prepared by two cycles of assembly–disassembly (Shelanski et al., 1973) and stored at −20 °C in Mes buffer [0.1 M 2-(*N*-morpholino)ethanesulfonic acid (Mes), 1 mM EGTA, and 0.5 mM $MgCl_2$, pH 6.6] containing 1 mM guanosine 5′-triphosphate (GTP) and 4 M glycerol. Prior to the onset of each experiment, the MTP was dialyzed for 3 h at 4 °C against 100 volumes of Mes buffer and centrifuged at 120000*g* for 20 min at 4 °C. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

MTP with a sedimentation coefficient of 6 s was prepared by ultracentrifugation (230000*g*) of MTP in Mes buffer for 90 min at 4 °C (Johnson & Borisy, 1977).

Microtubule seeds, which typically had an average length of 0.57 ± 0.34 μm, were prepared by passing a solution of polymerized microtubules (4 mg mL^{-1}) through a 22-gauge

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[1] Abbreviations used: MAP, microtubule-associated protein; MTP, tubulin plus microtubule-associated proteins; Mes, 2-(*N*-morpholino)-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′N′*-tetraacetic acid; Mes buffer, 0.1 M Mes, 1 mM EGTA, and 0.5 mM $MgCl_2$ at pH 6.6; $NaDodSO_4$, sodium dodecyl sulfate; PBS, Dulbecco's phosphate-buffered saline; Me_2SO, dimethyl sulfoxide.

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