Alzheimer’s Peptide Aβ1–42 Binds to Two β-Sheets of α1-Antichymotrypsin and Transforms It from Inhibitor to Substrate*

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The serpin α1-antichymotrypsin is a major component of brain amyloid plaques in Alzheimer’s disease. In vitro α1-antichymotrypsin interacts with the Alzheimer’s amyloid peptide Aβ1–42 and stimulates both formation and disruption of neurotoxic Aβ1–42 fibrils in a concentration-dependent manner. We have constructed a new hybrid model of the complex between Aβ1–42 and α1-antichymotrypsin in which both amino and carboxyl sequences of Aβ1–42 insert into two different β-sheets of α1-antichymotrypsin. We have tested this model and shown experimentally that full-length and amino-terminal segments of Aβ1–42 bind to α1-antichymotrypsin as predicted. We also show that Aβ1–42 forms both intramolecular and intermolecular SDS-stable complexes with α1-antichymotrypsin and that the binding of Aβ1–42 to α1-antichymotrypsin abolishes the inhibitory activity of the latter and its ability to form stable complex with chymotrypsin. The existence of both inter- and as well as intramolecular complexes of Aβ1–42 explains the nonlinear concentration-dependent effects of α1-antichymotrypsin on Aβ1–42 fibril formation, which we have re-investigated here over a broad range of Aβ1–42/α1-antichymotrypsin ratios. These data suggest a molecular basis for the distinction between amorphous and fibrillar Aβ1–42 in vivo. The reciprocal effects of Aβ1–42 and α1-antichymotrypsin could play a role in the etiology of Alzheimer’s disease.

α1-antichymotrypsin (ACT)† is a serpin serine proteinase inhibitor with specificity for cathepsin and chymotrypsin-like enzymes (1). Its physical properties (2), mechanism (3), and structure (4, 5) conform to the general model (6, 7) that has been established for serpins. These inhibitors present a flexible reactive site loop, which is cleaved at a susceptible residue by target proteinase (8, 9) to form a highly stable, covalent complex (10, 11). As in other serpins (12), the reactive site loop in the cleaved form of ACT is inserted into a preexisting, flexible β-sheet (sA) (4), and this cleaved form is stabilized to denaturation relative to the uncleaved native form (2). There is evidence (13, 14) consistent with proposals (10, 11) that insertion of the reactive site loop into sA is a prerequisite for stable proteinase-serpin complex formation. In further support of this, it has been shown that exogenous peptides of restricted sequence can insert into sA of the uncleaved serpin (15–17). These binary peptide-serpin complexes are similarly increased in stability to denaturation but have lost inhibitory activity, becoming substrates instead that do not form a stable complex with target proteinase.

A possible link between ACT and Alzheimer’s disease was established by the observation that ACT occurs in the senile plaques characteristic of this disease (18). Subsequently, it was shown in vitro that ACT can either stimulate formation of the neurotoxic fibrillar form of Alzheimer’s peptide Aβ1–42 (19, 20) or destabilize preformed Aβ1–42 fibrils (21, 22), depending on the stoichiometry of ACT to Aβ. Two models for the binding of Aβ1–42 to ACT (20, 23), based on the known structure and properties of the latter, suggested how amino and carboxyl segments of Aβ1–42 might bind into different β-sheets of ACT. These two models are not mutually exclusive, and we have synthesized them into a new model presented here in which Aβ1–42 binds to ACT through insertion of amino- and carboxy-terminal segments of Aβ1–42 into different β-sheets of ACT. We have experimentally tested this model and its implications to understand the nature of ACTAβ1–42 complexes and their possible relationship to the polymerization of Aβ1–42, which is closely associated with Alzheimer’s disease.

We show here that full-length Aβ1–42 and also its amino-terminal segments (Aβ2–9 and Aβ1–11) bind to ACT. This supports our bimodal model for the binding of Aβ1–42 to ACT in which the amino-terminal segment of Aβ1–42 inserts into sC of ACT and the carboxy-terminal segment into sA. The binding of Aβ1–42 to ACT results in loss of ACT inhibitor activity and its transformation from an inhibitor into a proteinase substrate, an observation consistent with the effects of the insertion of other peptides into sA of serpins. The bimodal model for the intramolecular 1:1 ACTAβ1–42 complex also suggested that intermolecular complexes of higher molecular weight between these molecules may form. We present experimental evidence for the occurrence of complexes of higher multiplicity as well as the 1:1 intramolecular complex. This model for independent, bimodal insertion of the amino- and carboxy-terminal sequences of Aβ1–42 into ACT also offers a structural basis for the distinct effects of low and high concentrations of ACT on Aβ1–42 fibril formation and stability. We have tested this hypothesis with measurements of the polymerization of Aβ1–42 at a

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**RESULTS**

Direct demonstration of complex formation between ACT and Abα1-42 was obtained from SDS gels of 1:100 (ACT:Abα1-42) mixtures incubated for 3 days. A slightly higher molecular weight (lower mobility) band is observed with the expected molecular weight of an SDS-stable 1:1 complex of Abα1-42 with α1-antichymotrypsin (Fig. 1A, lanes a and b).

Our earlier study showed that the carboxy-terminal segment of Abβ2-9 binds into sA of ACT (20) and that full-length Abβ2-9 is necessary for this binding. This implied that the amino-terminal segment of Abβ2-9 contributes to complex stability and model building showed how residues 2–9 of Abβ2-9 could insert into sC of ACT(23). Confirmation of the binding of Abβ2-9 was obtained from competition experiments in which Abβ2-9 was shown to block binding of full-length Abβ2-9 peptide. Preincubation of ACT with Abβ2-9 (Fig. 1A, lane f) followed by incubation with Abβ1-42 showed no formation of the higher molecular weight (lower mobility) ACT:Abα1-42 band. If ACT is incubated first with Abβ2-9 followed by Abβ1-42, the higher molecular weight band is observed (Fig. 1A, lane e), indicating that the ACT:Abβ2-9 complex is stable once formed in prolonged incubation. The low molecular weight (high mobility) band in lane e is unreactive with ACT antibodies in the Western blot and is an Abβ species. Control incubation of ACT with Abβ2-9 did not detectably change the mobility of ACT from that of the native (Fig. 1A, lane d), and the possibility of confounding interactions between Abβ2-9 and Abβ1-42 was eliminated by showing that a preincubated (overnight) equimolar mixture of these two peptides did not alter the effects of Abβ1-42 on ACT (data not shown).

The complex between the amino-terminal segment of Abβ1-42 and ACT inferred from the above competition experiments was directly confirmed by separation of the complex of radiiodinated Abβ1-11 with ACT on agarose gel. Fig. 1B shows the autoradiogram of a 1% agarose gel in which the incubated mixture of ACT with 125I-labeled Abβ1-11 was run. The band for Abβ1-11 detected by autoradiography is coincident with that for ACT. We conclude from these experiments that the amino-terminal segment as well as the carboxy-terminal segment of Abβ1-42 binds to ACT and that both are essential for formation of stable complex.

Variations in time of incubation and stoichiometry of Abβ1-42:ACT result in different forms of the complexes between these two molecules. A stable high molecular weight (low mobility) dimer having the approximate molecular weight of an ACT dimer (Fig. 1C, lane c) is observed on prolonged (3 weeks) incubation with Abβ1-42. This dimer is stable to reduction by β-mercaptoethanol and is blocked by preincubation with Abβ2-9 (Fig. 1C, lane f). The identity of this complex was confirmed in the gels by autoradiography, which detected 125I-labeled Abβ1-42 complexes with ACT (Fig. 1D, band 2 = dimer), and by Western blot using anti-ACT antibody (data not shown) to detect coincident ACT. This autoradiograph also shows that smaller amounts of higher polymers of the ACT:Abβ1-42 complexes are formed (Fig. 1D, band 3). We conclude that Abβ1-42 can link two molecules of ACT into a highly stable closed dimer through intermolecular bimodal binding to the two different β-sheets, sA and sC, in different molecules and that this is a slow process. The blocking of ACT:Abβ1-42 complex formation by Abβ2-9 confirms the requirement for complex formation of binding of the amino terminus of Abβ1-42. This experiment parallels the diminished neurotoxicity and Abβ2-9 fibril formation of ACT:Abβ1-42 observed in the presence of Abβ2-9 (27), suggesting that the complex we have characterized here is relevant to the neurotoxic and fibrillogenic properties observed by others toward cells in culture. Shorter incubation time (24 h) of Abβ1-42
with ACT is insufficient to produce SDS stable complexes (Fig. 1E, lane c versus Fig. 1A, lane b). We infer that formation of the full bimodal complex is necessary to observe SDS-stable complex and that the kinetics for full complex formation are multistep and slow.

The mode of binding of Aβ_{1-42} to ACT proposed here leads to the prediction that ACT in complex with Aβ_{1-42} will lose its proteinase inhibitor activity. Inhibition of serine proteinase activity by serpins occurs with formation of an irreversible proteinase-serpin complex whose structure is stabilized by insertion of the proteinase-linked reactive site loop into sA. If Aβ_{1-42} is inserting into the same site in sA as the reactive site loop, we predict that ACT in complex with Aβ_{1-42} will lose its proteinase inhibitor activity. Fig. 1E shows that overnight pre-incubation of ACT with Aβ_{1-42} abolishes formation of stable complex between ACT and chymotrypsin. Fig. 1E, lane b, shows the higher molecular weight inhibitory complex formed on incubation of ACT with chymotrypsin (band 1), whereas lane d shows that, when ACT is preincubated overnight with a 50-fold molar excess of Aβ_{1-42} prior to incubation with chymotrypsin, no ACT-chymotrypsin complex is formed and only intact (band 2) and cleaved ACT (band 3), along with the cleaved low molecular weight carboxyl-terminal peptide band, are observed. This is consistent with our observations (see below) and earlier ones of other investigators (27) that incubation of ACT with Aβ_{1-42} results in loss of ACT inhibitor activity. It also confirms our earlier conclusion that the stabilization of intact ACT to denaturation after incubation with Aβ_{1-42} is because of
insertion of Aβ1-42 into sA as a substitute for s4A, the reactive site loop strand.

Direct assay of proteinase inhibitor activity of the ACT/Aβ1-42 complex lent further weight to the interpretation of the above experiments. Preincubation of ACT with Aβ1-42 results in a loss of 20% and 50% of ACT inhibitor activity versus chymotrypsin at molar concentration ratios of 1:100 and 1:200, respectively (ACT:Aβ1-42) (Fig. 2, sample 3, light and dark gray bar, respectively). This effect parallels the loss of inhibitor activity of the serpins α1-antitrypsin (15), antithrombin (16), and plasminogen activator inhibitor 1 (17) on incubation with exogenous peptides that can insert into sA. We exploited this assay system to further test whether the amino-terminal Aβ2-9 binds in the complex between Aβ1-42 and ACT. If Aβ2-9 is essential for the binding of full-length Aβ1-42 to ACT, then Aβ2-9 should reverse the loss of inhibitor activity that occurs when Aβ1-42 binds to ACT. Parallel assays of ACT inhibitor activity in the presence of Aβ1-42 with and without overnight preincubation with Aβ2-9 at a 200:1 molar ratio (Aβ2-9:ACT), followed by incubation with Aβ1-42 at 100:1 and 200:1 (Aβ1-42:ACT) ratios, the loss of inhibitor activity is reduced to 5 and 20% from 20 and 50% (sample 4, light and dark gray). These data further support the model for interaction of Aβ1-42 with ACT in which the amino-terminal Aβ2-9 segment as well as the carboxyl-terminal segment of Aβ1-42 bind to ACT.

The above experiments show that there are multiple complexes between ACT and Aβ1-42, whose stability is a function of incubation time. Formation of SDS-stable ACT:Aβ1-42 complex is slow, like that of binary complex formation between serpines and exogenous peptides that bind in sA (15, 16). Slow rates of complex formation may also reflect slow structural isomerizations that are known to occur in Aβ peptides converting from their soluble form to the β-rich fibrillogenic form (28), or the conversion of the ACT structure from the uncleaved, native conformation to the more stable Aβ1-42 strand-inserted conformation, which occurs in cleaved and proteinase-complexed serpines.

The model for bimodal insertion of Aβ1-42 into ACT (Fig. 3), which was the hypothetical basis for the above experiments, is validated by these results. Our earlier observation that Aβ1-42 interaction with ACT stabilizes ACT to denaturation implied

![Fig. 2](image2.png)  
**Fig. 2.** Competition of Aβ2-9 with Aβ1-42 for binding to ACT measured by changes in ACT inhibitor activity. Sample 1, ACT alone; sample 2, ACT + Aβ2-9 (1:200); sample 3, ACT + Aβ1-42 (1:100) (light gray hatched bar) and ACT + Aβ1-42 (1:200) (dark gray bar); and sample 4, ACT + Aβ2-9 (1:200 molar ratio) + Aβ1-42 (1:100) (light gray hatched bar) and 1:200 (dark gray bar). Each bar represents the mean of three independent determinations.

![Fig. 3](image3.png)  
**Fig. 3.** Model for the bimodal binding of Aβ1-42 to one molecule of ACT. Aβ1-42 amino (N) and carboxyl (C) strands (black) inserted into sC and sA (labeled), respectively. Dotted lines for the reactive site loop (RSL) of ACT and residues 10 to 27 (Aβ10-27) of Aβ1-42 indicate that the conformations of these polypeptide segments cannot be modeled reliably.

![Fig. 4](image4.png)  
**Fig. 4.** Aβ1-42 fibril formation as a function of ACT concentration at different times measured using thioflavin T fluorescence. Ordinate scale is expressed as percent multiples of fluorescence of Aβ1-42 alone. ●, 45 min.; ▲, 1 h; ▼, 4 h; ▼, 24 h; ▲, 48 hrs.
that $\alpha\beta_{1-42}$ is inserting into sA of ACT, a property already observed in several serpins. Our results here further confirm this interpretation by showing that ACT loses its inhibitor activity as a result of $\alpha\beta_{1-42}$ binding to ACT, another property previously associated with the insertion of exogenous peptides into sA of inhibitory serpins. Our earlier observation that full-length $\alpha\beta_{1-42}$ is necessary for stabilization of ACT to denaturation is now also explained by the proposed model incorporating the second binding site for the $\alpha\beta_{2-9}$ segment of $\alpha\beta_{1-42}$ to sC of ACT.

Bimodal insertion of $\alpha\beta_{1-42}$ into ACT suggested a possible explanation for the concentration-dependent effects of ACT on $\alpha\beta_{1-42}$ fibril formation and stability. ACT stimulates $\alpha\beta_{1-42}$ fibril formation (19, 20) at relatively low molar ratios of ACT to $\alpha\beta_{1-40}$ or $\alpha\beta_{1-42}$, but at higher concentrations of ACT, preformed $\alpha\beta_{1-40}$ fibrils are destabilized and show signs of disintegration (21, 22). We revisited this phenomenon by determining the dependence of $\alpha\beta_{1-42}$ polymerization on a broader range of ACT concentration than had been explored before. Using the thioflavin T fluorescence assay, we found that higher concentrations of ACT do not stimulate $\alpha\beta_{1-42}$ polymerization and that there appears to be a threshold ratio of ACT to $\alpha\beta_{1-42}$ below which aggregate formation is stimulated (Fig. 4). Our earlier work and that of others (27) showed by electron microscopy that these aggregates are overwhelmingly fibrils. This threshold effect may be a consequence of a switch between intramolecular and dimeric and higher multiplicity intermolecular complexes of ACT with $\alpha\beta_{1-42}$. The intramolecular complex consisting of a single molecule of $\alpha\beta_{1-42}$ bound through its carboxyl and amino terminus in sA and sC, respectively, of a single molecule of ACT (Fig. 3) is more likely to occur at lower ACT concentrations. The closed ACT dimer circularly linked in complex by two molecules of $\alpha\beta_{1-42}$ would not aggregate, whereas open, noncircular, head-to-tail complexes formed through binding of different $\alpha\beta_{1-42}$ molecules to multiple different ACT molecules, which is more likely at high relative ACT concentration, could immobilize $\alpha\beta_{1-42}$ in aggregates.

**DISCUSSION**

We have shown here that $\alpha\beta_{1-42}$ binds into two different $\beta$-sheets of ACT. Binding to one of these $\beta$-sheets, sA, abolishes the inhibitor activity of ACT, transforming it into a substrate. We speculate that the promotion of $\alpha\beta_{1-42}$ fibril formation by ACT in vitro is a result of the imposition of $\beta$-sheet conformation on both amino and carboxyl-terminal sequences of $\alpha\beta_{1-42}$ as a result of its binding to ACT. Fibrillar $\alpha\beta_{1-42}$ is predominantly in $\beta$ conformation, but soluble, nonfibrillogenic $\alpha\beta_{1-42}$ at low concentrations appears to require a conformational isomerization to a more $\beta$-rich conformation to become fibrillogenic (28–30). The conformational transition leading to polymerization may be the result of cooperative interactions among $\alpha\beta_{1-42}$ molecules at high concentration (30) or could be provided by a chaperone, such as ACT, whose complex with $\alpha\beta_{1-42}$ we have modeled here.

Our observation of a nonlinear dependence of $\alpha\beta_{1-42}$ fibril formation on ACT suggests that the relative amounts of $\alpha\beta_{1-42}$ deposited in amorphous plaque or fibrils in Alzheimer’s disease brains may depend on fluctuating ratios of ACT and $\alpha\beta_{1-42}$. Elevated $\alpha\beta_{1-42}$ levels, which occur as a result of specific mutations in the amyloid precursor protein or of raised gene dosage as in Down’s syndrome, correlate with early onset Alzheimer’s disease (31). In the case of late onset Alzheimer’s disease, ACT levels, which rise during inflammation, may also be an important determinant of the neurotoxic fibril load in the brain and therefore be an important parameter in the etiology of Alzheimer’s disease.

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