An ApoE–Aβ inhibition complex in Aβ fibril extension

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Background: Literature reports differ dramatically in showing that apolipoprotein E either facilitates or inhibits Aβ aggregate formation *in vitro*. Resolution of the nature of the ApoE–Aβ interaction is critical for progress towards understanding its possible role in the modulation of Alzheimer's disease.

Results: Here, we show that purified ApoE–Aβ co-aggregate is a poor seed of fibril formation. We also demonstrate ApoE inhibition of Aβ fibril growth in four independent aggregation assays, arguing that the poor fibril formation observed under these conditions is real and not an analytical artifact. We also directly show ApoE binding to immobilized Aβ fibrils by surface plasmon resonance.

Conclusions: The results suggest a unifying model in which ApoE binds to Aβ fibril seeds and nascent nuclei to generate stable complexes that inhibit the rapid extension of mono-component Aβ fibrils but at the same time can foster continued slow growth of mixed ApoE–Aβ aggregates. *In vivo* co-aggregate formation may be important in many examples of pathological protein misassembly.

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Introduction

One of the genetic loci associated with Alzheimer's disease (AD) is the apolipoprotein E (ApoE) gene. Individuals carrying copies of the relatively rare E4 allele (ApoE4) are more at risk for developing AD than carriers of the E2 (ApoE2) and E3 (ApoE3) alleles [1]. Molecular explanations for the genetics have been prompted by the immunochemical detection of ApoE as a component of the Aβ plaques that are a hallmark of Alzheimer's pathology [2–4]. The role of Aβ in the etiology of Alzheimer's disease (AD) is unproven [5,6], however, and it remains possible that ApoE affects the development of AD independently of an Aβ-related mechanism. At the same time, the recent identification of ApoE as a risk factor in Aβ amyloid diseases unrelated to — or independent of — Alzheimer's disease [7–9], as well as a correlation of enhanced plaque load and AD progression with the E4 isoform of ApoE [10], add further support to arguments that ApoE modulation of amyloid formation is important in determining the severity of AD.

The possibility of a relevant molecular association between ApoE and Aβ has inspired a number of investigations on the *in vitro* interactions of these two polypeptides [4,11–22]. No consistent model for the role of ApoE in fibrillogenesis has emerged, however, with some groups reporting stimulation, and others inhibition, of aggregate formation in the presence of ApoE isoforms.

Previously we produced the three major human isoforms of ApoE in *Escherichia coli* and studied their interactions with chemically synthesized Aβ under native solution conditions *in vitro* [21,22]. We conducted two kinds of experiments that gave seemingly contradictory results — results that mirror, in some ways, the conflicting literature reports on the ApoE–Aβ interaction. Working at relatively high ApoE concentrations, we found that all three ApoE isoforms form soluble high molecular weight co-aggregates with Aβ that can be isolated by gel filtration chromatography [21]. At much lower ApoE concentrations, we found that all three ApoE isoforms strongly inhibit the initiation of Aβ fibril formation conducted either with the addition of pre-formed Aβ aggregates ('seeding') or in the absence of exogenous seeds ('nucleation') [22].

Here we report results linking these two previous studies, in which we demonstrate that the high molecular weight complex of ApoE and Aβ isolated by gel filtration has little or no seeding ability for Aβ fibril formation. These new data have led us to propose a model which may help explain some apparently conflicting results reported previously. The model implies that it should be possible to develop therapeutics which, at relatively low concentrations, might be able to slow the growth of pre-existing fibrils, as well as block the nucleation of new ones. Selective aggregation and deposition of normally well-behaved proteins via their misfolding and misassembly is now recognized as an important component of a number of human diseases and other biological functions [23]. The ability of a growing protein aggregate to incorporate other protein molecules into the matrix may account for examples of cross-nucleation, inhibition, and aggregate toxicity in some cases of pathological protein deposition [24].

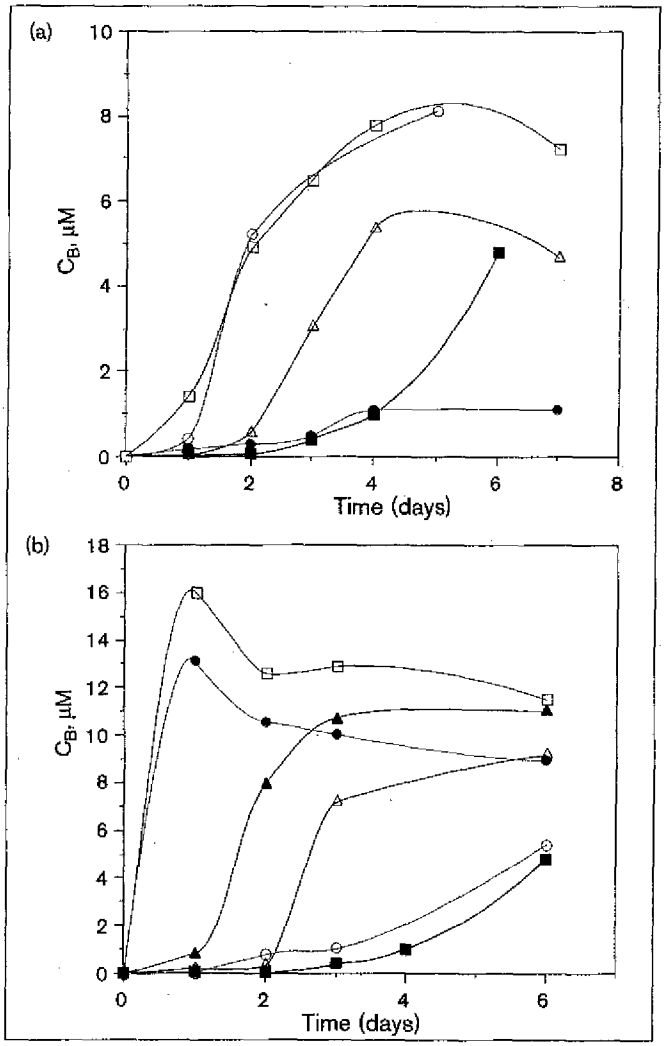
Results

ApoE isoforms are effective inhibitors of the seeding of fibril formation by pre-formed Aβ fibrils [22, 25]. This is true for both 1–40 and 1–42 versions of Aβ, and for plasma-derived and *E. coli*-derived human ApoE [22]. Figure 1 shows that Aβ — if it has been previously treated with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to remove pre-existing aggregates [22,26] — must be incubated for four days before significant fibril formation begins. If the reaction is supplemented with a small amount (0.02 % by weight of the amount of Aβ in the fibril formation reaction) of Aβ fibril, however, fibril formation begins after one day. Addition of nM concentrations of ApoE delays the onset of Aβ fibril formation in such seeded reactions, with 150 nM providing a delay of over seven days — well beyond the lag time normally seen in an unseeded reaction (Fig. 1a). This effect cannot be due to the sequestration of Aβ by ApoE, since the binding capacity of ApoE for non-aggregated Aβ is only about four molecules Aβ per ApoE tetramer [21]; this would, at most, neutralize 1 % of the Aβ in these fibril formation reactions. The effect is also not due to the ability of ApoE to compromise the dye binding assays. When ApoE was added to a Congo red assay of pre-formed fibrils, at three times the concentration at which it is typically present in these assays, no difference in signal was observed (C_B = 6.8 ± 0.2 in the absence of ApoE, C_B = 6.7 ± 0.1 in the presence of ApoE).

Since different aggregated states of Aβ have different responses in the Congo red binding assay [26], we were concerned that the apparent lack of fibril growth during the extended lag phase of ApoE-inhibited reactions might be an artifact of the technique for following fibril formation. To address this, we conducted a fibril-growth experiment in the presence and absence of ApoE, monitoring the reactions using Congo red binding, thioflavin T binding, and HPLC analysis for residual soluble peptide. The results of the three analyses are in excellent agreement (Fig. 2), showing that, in the presence of ApoE, there is no significant growth of Aβ fibrils over the course of the experiment. In principle, it might be possible that significant ApoE–Aβ co-aggregates form over the course of the experiment which are refractory to the detection methods used. The thioflavin T response to isolated ApoE–Aβ co-aggregate is identical to its response to mono-component Aβ fibrils, however (see below). This would appear to rule out the formation of significant levels of both soluble and insoluble high molecular weight ApoE–Aβ co-aggregates in the ApoE-containing reaction (Fig. 2).

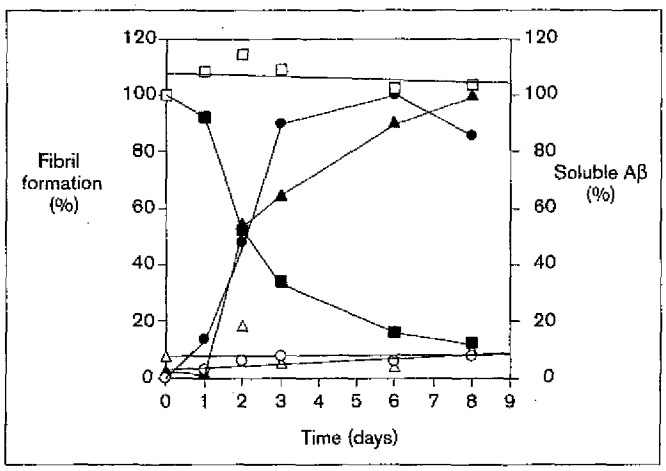
As previously described [26], Aβ fibrils grown under unstirred conditions do not exhibit appreciable turbidity. For this reason, turbidity measurements were not useful in monitoring the reactions shown in Figure 2. But, for reasons that are unclear, fibril formation reactions do generate turbid suspensions when conducted under stirred conditions [18]. To further confirm our findings with the aid of the turbidity assay, and to confirm the observations

Figure 1



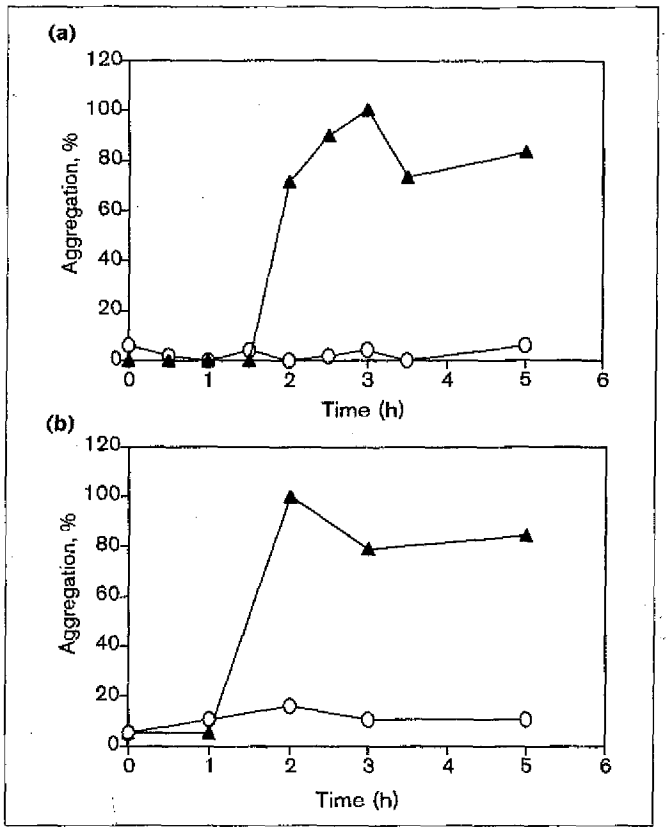
Amyloid fibril formation of 58 μM HFIP-treated Aβ in PBS at 37° C as monitored by Congo red binding. **(a)** ApoE3 inhibition of nucleation of fibril formation by exogenous fibril seed. An unseeded reaction (black ■) requires four days before fibril formation begins. Other time courses shown are from reactions seeded with 0.02 %, by weight, of the amount of HFIP-treated Aβ in the reaction, and in addition containing 0 nM (magenta ○), 0.3 nM (green □), 30 nM (purple △), or 150 nM (cyan ●) tetrameric ApoE3. **(b)** Inability of isolated Aβ-ApoE3 complex to efficiently nucleate fibril formation by Aβ. With no additions (black ■), onset of fibril formation was delayed for four days. Addition of 0.02 % by weight of Aβ fibrils (red ▲) gives onset of fibril formation after a one-day lag, while addition of 10 % by weight of fibrillar Aβ (green □) gives immediate fibril formation. Freshly prepared ApoE3-Aβ complex was assessed for its Aβ content and added to an unseeded fibril formation reaction to deliver 0.02 % by weight (purple △) or 10 % by weight of Aβ (magenta ○). Prior formation is critical for the poor seeding ability of complex: independent addition of 10 % by weight of Aβ fibrils, along with 23 nM ApoE3 tetramer (a proportion identical to that found in isolated complex) exhibits normal, efficient seeding of fibril formation (cyan ●).

Figure 2



Course of fibril formation in unagitated reactions, by multiple methods of detection. Fibril formation reactions were set up as described in the legend to Fig. 1 with 58 μM HFIP-disaggregated Aβ and 0.02 % by weight Aβ fibril as seed. Reactions were conducted in the absence (closed symbols) or presence (open symbols) of 150 nM ApoE3, and were monitored for fibril formation by Congo red binding (○,●) and thioflavin T binding (△,▲), and for soluble Aβ by HPLC (□,■).

Figure 3



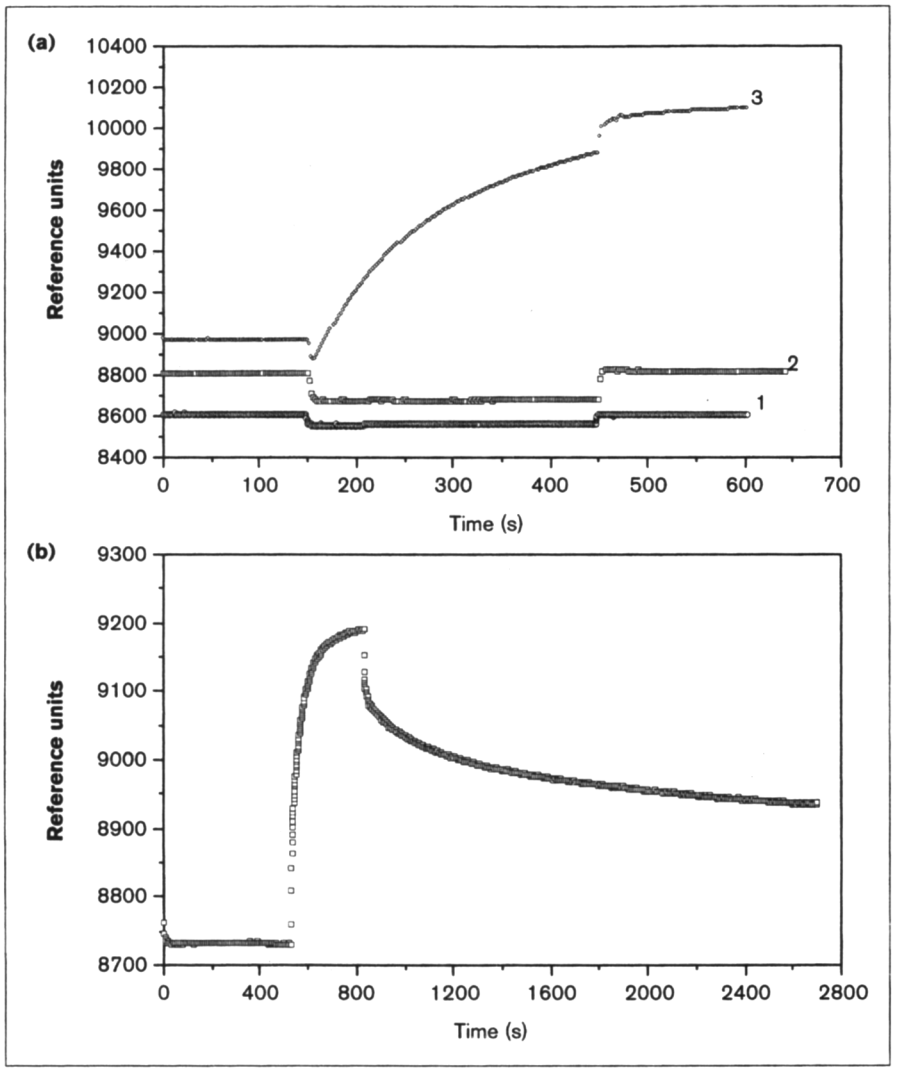
Course of fibril formation in shaken reactions, by two methods of detection. Reactions were conducted with shaking in a microtitre plate in the presence (○) or absence (▲) of ApoE, as described in Materials and methods. **(a)** Reactions monitored by turbidity (apparent absorbance at 405 nm). **(b)** Reactions monitored by Congo red.

of Lansbury and coworkers [18], we conducted fibril formation reactions in the presence and absence of ApoE in a shaken microtiter plate. Aβ aggregation was monitored by turbidity and Congo red binding. In a shaken reaction containing 420 μM HFIP-treated Aβ, rapid aggregation takes place after a time lag of 1.5 h (Fig. 3). Although delays in the generation of detectable turbidity in aggregation reactions can sometimes be attributed to the nonlinearity of light scattering with respect to particle size [27], the time lag observed in the turbidity profile in Figure 3a is probably associated with a nucleation event, since a similar lag is observed when the same reaction is monitored by Congo red binding (Fig. 3b). Aggregation of Aβ, as measured by both turbidity and Congo red binding, is suppressed for at least five hours under these conditions when the reaction is conducted in the presence of 4 μM ApoE3 (Fig. 3).

We have previously demonstrated that ApoE is able to co-aggregate with Aβ in the solution phase [21]. The ability of ApoE to inhibit Aβ fibril extension suggests that ApoE must also bind to extension-competent Aβ fibrils in the solid phase. We used surface plasmon resonance (SPR) to explore this possibility. Figure 4a shows that aggregated Aβ non-covalently adds to a biosensor chip onto which Aβ has been chemically attached. As the aggregated Aβ in this preparation has the ability to seed fibril formation [22] and as it reveals fibrils that can be detected by electron microscopy [28], we interpret these results to indicate that the aggregated material bound to the covalent-Aβ sensor chip is fibrillar. The fact that HFIP-disaggregated Aβ does not detectably bind to the covalent-Aβ chip (trace 2 of Fig. 4a) also suggests that the strong binding found for untreated Aβ solutions is due to the binding of pre-existing Aβ aggregates to the chip. Low molecular weight forms present in HFIP-treated Aβ may also bind to the Aβ attached to the sensor chip, but may give a very low signal due to their low mass.

Figure 4b shows that ApoE binds strongly to the sensor chip containing this aggregated Aβ. The sensogram shows a rapid binding of ApoE to a plateau value that persists until the end of the ligand addition phase. In the buffer wash phase, a portion of the bound ApoE dissociates, but the majority of the ApoE remains more tightly bound, suggesting two classes of binding sites for ApoE on the fibrils. The slowly dissociating ApoE site may reflect additional integration of the ApoE into the Aβ fibril matrix, generating a complex that may resemble the previously reported solution phase ApoE–Aβ [21]. There are a limited number of total ApoE sites on the

Figure 4



Binding of ApoE3 to aggregated Aβ evaluated by surface plasmon resonance (SPR) [41]. Sample flowed over the sensor chip for 300 s (from 150 s to 450 s on the time axis shown) at a rate of 5 μl min^{-1}. The drop in reference units (RU) at 150 s and recovery of RU at 450 s are due to a refractive index difference between equilibration/running buffer and sample buffer. The sensor chip was modified by covalent attachment of Aβ (Materials and methods). **(a)** Non-covalent binding of aggregated Aβ to a sensor chip previously modified by covalent attachment of Aβ. Curve 1 shows that injection of 100 μl min^{-1} Aβ, in Hepes-buffered saline (HBS), pH 7.4 containing 0.1 % Tween-20, over a native, unmodified chip gives no binding. Curve 3 shows that, in contrast, an identical sample of Aβ exhibits strong, irreversible binding to a sensor chip which was previously modified with 500 RU of covalently attached Aβ. If the Aβ is rigorously disaggregated by HFIP pretreatment, however, it displays no detectable binding to the sensor chip containing covalently attached Aβ (curve 2). **(b)** Binding of 25 μl min^{-1} ApoE3 in HBS-Tween to a sensor chip containing immobilized aggregated Aβ (see (a)). The sharp drop in RU at about 900 s is due to a solvent associated refractive index change. The slow decay in RU from 900 s to about 2600 s is attributed to dissociation of a portion of the bound ApoE3 (non-covalently attached Aβ does not dissociate from the chip under these conditions). After 2600 s essentially no further dissociation takes place.

immobilized fibrils: immediate injection of a second aliquot of fresh ApoE gives essentially no additional binding (data not shown). Previous studies have demonstrated binding of ApoE to Aβ immobilized on an SPR chip under native buffer conditions [29]. In these previous experiments, however, neither the aggregation state of the immobilized Aβ, nor the percentage of immobilized Aβ that is covalently attached to the chip, are well defined.

To gain a better understanding of the interaction of ApoE and Aβ, a sample of solution phase ApoE–Aβ co-aggregate was prepared and characterized [21]. The amount of Aβ in this isolated complex was determined by dot blot [21] analysis against an Aβ standard curve. In the thioflavin T assay (Materials and methods), 10 μg of Aβ fibrils gave a relative fluorescence signal of 7.4, while 10 μg Aβ-equivalents (dot blot estimate) of the isolated ApoE–Aβ complex gives a signal of 8.0. As stated above, the equivalent sensitivity of Aβ fibrils and ApoE–Aβ co-aggregate to thioflavin T supports our interpretation that no appreciable aggregate formation occurs in the lag phase of the ApoE-containing reaction shown in Figure 1.

Despite the structural similarity between Aβ fibrils and ApoE–Aβ co-aggregates suggested by the thioflavin T data, there is a significant functional difference between the two aggregates. To examine the ability of ApoE–Aβ complex to seed Aβ fibril formation, a fresh preparation was isolated and quantified. In this experiment, the time lag to onset of aggregation for an unseeded Aβ mixture is four days (Fig. 1b). Consistent with our previous report [22], this time lag is cut to one day (i.e. fibrils form more rapidly) when seeded by the addition of 0.02 % by weight of aggregated monocomponent Aβ fibrils, and is

entirely eliminated when the reaction is seeded with 10 % by weight of aggregated Aβ.

In contrast, when parallel fibril formation reactions are treated with isolated ApoE–Aβ complex, instead of Aβ fibrils, quite different results are obtained (Fig. 1b). When ApoE–Aβ complex is added to a level of 0.02 % by weight of Aβ, the time lag to onset of fibril formation is increased to two days, compared to the one day lag seen for mono-component Aβ fibrils. This is a significant effect; Figure 1a shows that it is necessary to increase the amount of inhibitory ApoE by 100-fold in order to obtain the same increase in time-lag from one day to two days. When the amount of ApoE–Aβ complex is increased to 10 % by weight of Aβ, the time lag to onset of fibril formation is four days, the same as for an unseeded reaction. Thus, in the experiment with 0.02 % by weight aggregated Aβ, added co-aggregate stimulates fibril formation compared to an unseeded control, but does so significantly less well than an equivalent amount of mono-component Aβ fibrils. In the experiment with 10 % by weight aggregated Aβ, added mono-component Aβ fibrils produce strong seeding of fibril formation, while added ApoE–Aβ coaggregate is entirely inert, exhibiting neither seeding (shorter lag time) nor inhibition of nucleation (longer lag time).

Complex formation appears to be relatively slow compared to the fibril extension reaction, but the ApoE–Aβ complex, once formed, seems to be quite stable. When aggregated Aβ (10 % by weight) and free ApoE (23 nM tetramer, equivalent to the amount found in the control co-aggregate) are added to an Aβ incubation without prior co-incubation, fibril formation begins essentially immediately, giving the same kinetics as the reaction with a comparable amount of aggregated Aβ seed alone (Fig. 1b). This indicates that, under these conditions, formation of ApoE–Aβ inhibition complex *in situ* is relatively slow and cannot compete kinetically with Aβ fibril extensions. In contrast, when isolated ApoE–Aβ complex is incubated in PBS at 37° C for 24 h, and then added to a fresh fibril formation reaction, it produces the same time lag to onset of fibril formation as does a comparable amount of freshly isolated complex (data not shown). This indicates that the ApoE–Aβ complex, once formed, is quite stable.

Although protein aggregation reactions generally exhibit significant variability, the experiments described here are highly reproducible, perhaps because of our ability to rigorously eliminate pre-existing aggregates from commercial lots of Aβ [26], and because of the presumed high degree of regular structure in amyloid fibrils [23,30]. The excellent reproducibility of the fundamental seeding phenomenon and its inhibition by ApoE has been demonstrated previously [22]. Fibril seeds stored for over six months produce the same aggregation kinetics as freshly generated fibril seeds (see Materials and methods). Three independently isolated samples of ApoE–Aβ complex produce identically diminished abilities to seed fibril formation reactions (data not shown). The SPR experiments were also repeated at least three times with identical results and ApoE isotypes gave similar binding to aggregates of Aβ(1–42) bound to sensor chips (data not shown).

Discussion

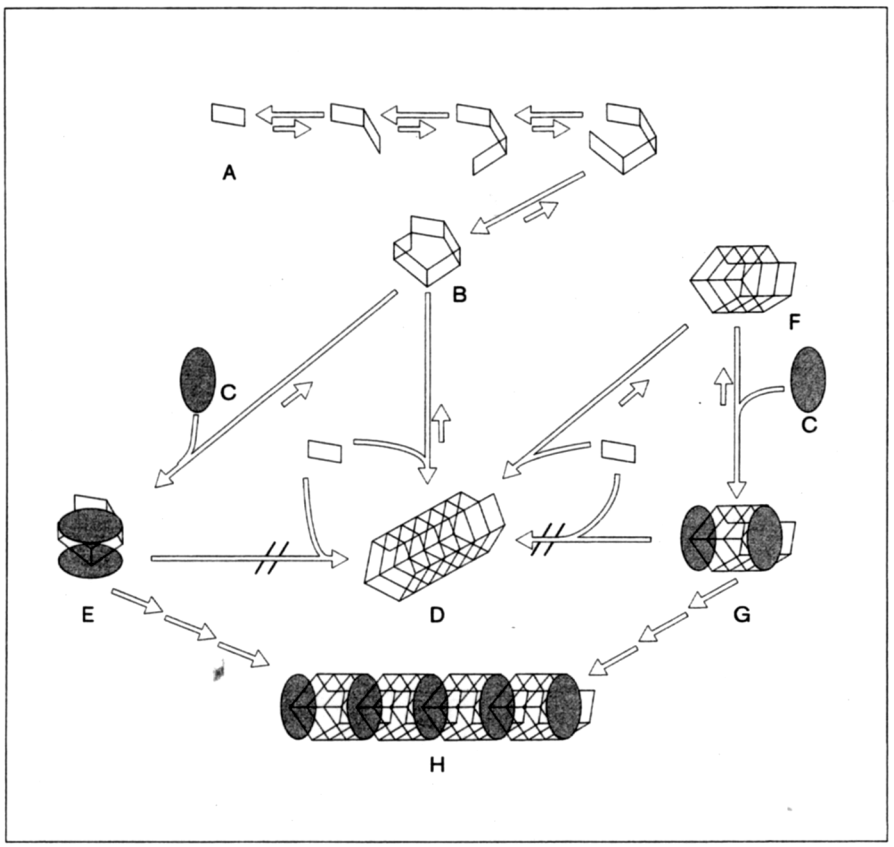
As outlined in the introduction, some previous results from this and other laboratories suggest that ApoE is an inhibitor of Aβ fibril formation, while other reports suggest that it is either a stimulant of Aβ fibril formation, or at least can be incorporated into an ApoE–Aβ co-aggregate. It is important to know whether any of the reported properties of ApoE are responsible for the role of the ApoE gene as a risk factor in AD. It is also important to know whether ApoE is intrinsically helpful or hurtful in the development of Aβ aggregates and AD, since therapeutic application of ApoE, or of an ApoE mimic, is either indicated or contra-indicated depending on the fundamental role of the protein in the AD brain.

The results described here, in addition to previous reports from this laboratory [21,22], can be interpreted according to the model shown in Figure 5. Lansbury and coworkers [31,32] have shown that fibril formation in simple peptide systems displays attributes of a nucleated growth mechanism, in which reaction mixtures display variable, concentration-dependent lag times to the onset of an aggregation reaction which, once engaged, proceeds rapidly. A diagram of how nucleation is presumed to proceed is shown at the top of Figure 5. Each step in the assembly of B from units of A (Fig. 5) is thermodynamically unfavorable, until the final step where sufficient internal contacts exist to provide a degree of stability to B. At this point, B has an extended lifetime in solution and subsequent additions of A are rapid, ending the observed lag phase in the kinetics and generating the growing fibril D.

Evans *et al.* [18] reported that, in such an Aβ fibril formation reaction, added ApoE (C in Fig. 5) can delay the onset of aggregation, presumably by binding to fibrillization nuclei as they develop. This result, which we have confirmed ([22] and results described here), is portrayed in the path from B to E to D in Figure 5. The simplest model for this inhibition is direct binding of ApoE to the growth face(s) of the nascent nucleus to form E.

In agreement with the nucleated growth mechanism, it is possible to reduce or eliminate the lag time before onset of fibril formation by the addition of Aβ fibrils ('seeding'; the F to D path Fig. 5). We have shown that ApoE can also inhibit this seeding reaction by associating with the growing fibril to form a complex and prevent its extension into fibrils (F to G to D in Fig. 5) [22,25].

Figure 5



A model for the observed effects of ApoE on co-aggregate formation with Aβ and on inhibiting fibril formation by Aβ. Aβ components (A) self-associate weakly and reversibly until a critical size is reached which possesses some stability. This is the nucleus for fibril formation (B), which at this point can add further Aβ components in a rapid growth phase to form fibrils (D). Association of ApoE (C) with B to form complex E blocks the growth termini of the nucleus, thus preventing its extension into fibrils. Fibril formation (D) can also be seeded by the addition of pre-formed fibrils (F) to a solution of Aβ. The model shows that ApoE can also associate with the growth surfaces of the added seed to form a complex (G), thus preventing its extension into fibrils. Finally, the model shows that under appropriate conditions super-co-aggregates (H) of ApoE and Aβ can assemble from E or G. Thus, as indicated in Figure 1b, newly generated nuclei B, even in the absence of additional ApoE, can add to co-aggregates E to produce larger aggregates (H) with concommitant suppression of fibril nucleation. This schematic model is not meant to suggest any particular degree of linearity, regularity, or stoichiometry in the structures of ApoE–Aβ co-aggregates. For example, the tetrameric structure of the native ApoE molecule may introduce branch points in an ApoE–Aβ complex for recruitment and binding of additional Aβ complexes or other molecules.

Here we report that the gel-filtration-isolated co-aggregate of Aβ and ApoE is itself a poor seed for fibril formation. This result is consistent with the model in Figure 5, suggesting that the isolated co-aggregate may resemble the presumptive inhibition complexes E and G. These ApoE–Aβ complexes might be able to recruit additional ApoE from the reaction mixture, as well as additional emerging Aβ nuclei, thus supporting conversion of almost all free Aβ into large co-aggregates H, as we have observed [21].

The picture that emerges from our *in vitro* studies can be summarized as follows: ApoE is an effective inhibitor of fibril formation, acting via formation of co-aggregates of ApoE and Aβ that are seeding- and nucleation-inert, and hence represent inhibition complexes of fibril extension. As inhibition proceeds, given sufficient ApoE, levels of ApoE–Aβ inhibition complex can accumulate as the sole reaction product. This co-aggregate displays some of the dye-binding attributes of Aβ fibrils (see above), but it is distinctly different from mono-component Aβ fibrils in our only available measure of fibril function — the ability to seed further fibril formation. Therefore, at high, non-physiological concentrations of ApoE and Aβ, it might be possible to detect aggregation by thioflavin T binding — but it would be the formation of seeding-incompetent ApoE– Aβ co-aggregates, and not mono-component Aβ fibrils, that are actually being measured.

The studies discussed here show that the observation of aggregate formation with mixtures of ApoE and Aβ depends on the concentrations of Aβ and ApoE used and the means by which the aggregate is detected. We have not observed an acceleration of aggregate growth by added ApoE under any conditions. Nonetheless, it remains possible that even higher Aβ concentrations than those used here might produce such an acceleration. Alternatively, reports of stimulation of Aβ aggregation by ApoE may be based on other critical differences in the aggregation or detection protocols. As discussed here, some methods of detection of Aβ amyloid fibrils can be misleading and might be misinterpreted.

Our results argue that the aggregates formed in mixtures of ApoE and Aβ are not fibrils of Aβ alone, but are intimate co-aggregates of Aβ and ApoE, and that these co-aggregates are functionally different from monocomponent Aβ fibrils. Since the ApoE–Aβ co-aggregates display some of the dye-binding properties of Aβ fibrils (see above), our results are not incompatible with the observed

fibrillar structure of ApoE-containing amyloid plaque isolated from brain tissue [4]. The basic fibrillar structure of the ApoE–Aβ co-aggregates makes their poor seeding ability all the more interesting.

Significance

The cytotoxicity in cell culture that has been demonstrated for both aggregated [33] and disaggregated [34] Aβ has been cited as evidence for the importance of Aβ in disease processes. It may also be relevant, however, to examine the cellular effects of co-aggregates such as the ApoE–Aβ complex. Not only are brain plaques probably co-aggregates of Aβ and ApoE [4], but in addition the soluble ApoE–Aβ co-aggregates that can be generated *in vitro* [21] may also exist in the brain, where their (probably transient) solubility might bestow on them unique cellular activities.

Our observation that ApoE can bind to preformed fibrils and prevent their ability to be extended in a fibril-forming growth phase suggests that it is not only an inhibitor of nucleation, but also of seeding (i.e. fibril extension). This has important implications for therapeutic strategies for control of amyloid plaque growth. Thus, ApoE, or another molecule with the same activity as ApoE, might be expected to effectively reduce the progress of plaque formation, even in brains already containing substantial quantities of amyloid.

The ability of a growing fibril to incorporate other proteins into its structure may prove to be an important general mechanism not only of aggregate formation, but also of aggregate seeding, inhibition and toxicity. For example, we have suggested elsewhere [22] that other proteins that also inhibit fibril growth by binding to the growing fibril [22,35] may do so by becoming incorporated into the developing β-sheet network, providing an 'end-cap' that precludes extension. At the same time, fibrils derived from some other polypeptide sequences are apparently so structurally similar to Aβ that they are capable of 'cross-seeding' formation of Aβ fibrils [36]. Finally, the ability of a growing protein aggregate to remove active protein molecules from solution may have a major role in the biological consequences of prion formation [24]. Thus, as we become increasingly aware of the high molecular selectivity of many forms of amyloid formation, it is important to recognize that the process may not be absolutely specific, and that the potential misin-corporation of proteins into the growing fibril may have important biological consequences.

Materials and methods

Materials

The E3 variant of ApoE was produced *in E. coli* [37] from cloned cDNA and purified under native conditions as described [21]. Aβ was purchased from Bachem. The experiments reported here were with the 1–40 version of Aβ.

Complex formation

The general method for isolation of complexes under native conditions has been described [21]. To make the complex used in the experiments described here, Aβ(1–40) (45 μM) was incubated with 5.3 μM (of tetramer) ApoE3 in PBS for 16 h at room temperature, centrifuged for 5 min at 15 000 rpm in an Eppendorf microfuge, filtered through a 0.45 μm filter, and applied to a SMART (*Pharmacia*) microisolation system equipped with a Superose 12 column equilibrated and run in phosphate-buffered saline, 1 mM EDTA, pH 7.4 at 40 μl ml^{-1} at room temperature. Fractions containing the high-molecular weight co-aggregate peak were collected, their content of Aβ quantified by dot blot analysis [21], and their content of aggregated Aβ assessed by thioflavin T (thioflavin T assay) [38] and Congo red binding [39]. These fractions were used to evaluate the seeding capability of the ApoE–Aβ co-aggregate.

Fibril formation

To prepare Aβ depleted of aggregates for fibril formation reactions, peptide as obtained from the manufacturer was dissolved at 5–15 mg ml^{-1} in HFIP (Sigma) and incubated at room temperature for 24 h then stored at −70° C in this solvent, as described previously [26]. A batch of fibril seeds [22] was prepared by incubating a 1 mg ml^{-1} PBS (Sigma) solution of Aβ (prepared by dilution from an acetic acid stock solution of a non-HFIP-treated vial lot) at 37° C for 3 days. Seed suspensions were aliquoted and stored at −70° C. To observe a nucleation dependent time-lag to the onset of fibril formation, it is important to remove the HFIP and resuspend the dry peptide rather than dilute into PBS from the HFIP stock [26]. Unless otherwise stated, fibril formation reactions were conducted unstirred and monitored by Congo red binding [26].

The Aβ fibril seeds used in this experiment were stored for >6 months at −70° C. The functional stability of these seeds under these conditions is quite good, as indicated by the reproducible fibril formation progress curves conducted when the seeds were fresh [22] and after 6 months storage (Fig. 2).

Fibril formation reactions under shaken conditions were conducted as follows. Aβ (400 μg) was treated with HFIP and the solvent was subsequently evaporated under vacuum. The peptide was suspended in 200 μl PBS to generate a 460 μM solution, which was filtered in a 0.2 μm centrifugation filtration unit. The 180 μl of filtrate was split into two wells of a microtitre plate. Into one well 20 μl ApoE3 (0.8 mg ml^{-1} in PBS) was added resulting in a final ApoE3 concentration of 1 μM (tetramer). Into the control well was added 20 μl of PBS. The final concentration of Aβ was 380 μM in both wells. The plate was incubated, with shaking, at room temperature for 5 h. During this time, the microtitre plate absorbance at 405 nm was monitored. Aliquots (10 μg) were removed for Congo red binding analysis.

Surface plasmon resonance

Aβ, either with or without disaggregation by HFIP (see above), was covalently bound to the sensor chip via the peptide's amino groups using the standard surface-activation chemistry (*N*-ethyl-*N'*-[3-(dimethylamino)-propyl]carbodiimide hydrochloride plus *N*-hydroxysuccinimide) for modification of the carboxymethyldextran layer of the biosensor chip [40].

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