Seeding of Aβ Fibril Formation Is Inhibited by All Three Isotypes of Apolipoprotein E

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ABSTRACT: Apolipoprotein E is immunochemically localized to amyloid plaque in Alzheimer's brains, and the allelic distribution of ApoE in individuals is associated with a disposition toward Alzheimer's disease. We show here that all three ApoE isotypes exhibit a strong and specific ability to inhibit both nucleation and seeding of fibril formation by the Aβ peptide *in vitro*. Aβ(1−40) depleted of aggregates requires long incubation times before the onset of fibril formation, but addition of very low levels of Aβ fibrils to such reactions is sufficient to reduce or eliminate this lag time. ApoE added to such seeded reactions extends the lag time in a dose-dependent manner, so that higher levels of seeding require higher levels of ApoE to achieve a given delay time to reaction onset. This effect is observed with all three isotypes produced in *Escherichia coli*, as well as with plasma-derived ApoE and the N-terminal domain of ApoE3 produced in *E. coli*. In contrast, bovine serum albumin and the four-helix bundle protein interleukin-4 are poor inhibitors of seeding. ApoE3 can also inhibit fibril formation by Aβ(1−42). The three full-length isotypes of ApoE produced in *E. coli* are equipotent at inhibition. It is therefore possible that the genetics of ApoE and AD may fundamentally depend on the ability of ApoE to inhibit seeding but that the trends in the genetics must be related to something other than the specific activities of the native ApoE isoforms used in these studies. The data show ApoE to be the first member of a new class of fibril formation inhibitor that acts by blocking the seeding of fibril growth.

Following initial reports that apolipoprotein E (ApoE)[1] is immunochemically located to amyloid plaques in brain tissue of Alzheimer's disease (AD) victims (Namba et al., 1991; Wisniewski & Frangione, 1992), a strong relationship was observed between ApoE isotype profile and susceptibility to early onset AD in humans (Corder et al., 1993). The genetic link between the ApoE4 allele and increased risk of AD, coupled with the association of ApoE with amyloid plaques, supports speculations that fibril formation by the peptide Aβ plays a causative role in the development of AD (Selkoe, 1991; Price et al., 1992). Pathology studies further support a biochemical linkage between ApoE and fibril formation, in showing increased Aβ amyloid loads in ApoE4 individuals with Alzheimer's disease (Ohm et al., 1995; Gomez et al., 1996), cerebral amyloid angiopathy (Greenberg et al., 1995), and inclusion body myositis (Garlepp et al., 1995).

Given the initial suggestions of an ApoE connection to amyloid plaques, a number of laboratories have investigated the interactions between ApoE and Aβ *in vitro* (Strittmatter et al., 1993a,b; Wisniewski et al., 1993, 1994; LaDu et al., 1994, 1995; Ma et al., 1994; Sanan et al., 1994; Castano et al., 1995; Evans et al., 1995). Literature reports on this interaction are greatly inconsistent, however, with some labs reporting stimulation of fibril growth (Ma et al., 1994; Wisniewski et al., 1994) while others report inhibition (Evans et al., 1995). The disparate results may reflect differences in experimental methods for managing and measuring fibril formation, or the source of materials used.

Amyloid formation by Aβ has been shown to take place by a nucleated growth mechanism (Evans et al., 1995). When aggregate is absent, a nucleus[2] must be generated *in situ* in a process that requires relatively high peptide concentrations and significant time delays; when an aggregation seed is present—either due to contamination or due to seeding with exogenous aggregate—the nucleation phase of the reaction is obviated and the fibril extension phase is more quickly engaged (Evans et al., 1995). Using stirred reactions monitored by turbidity, Lansbury's group observed that addition of ApoE extends the time lag to onset of aggregation in an unseeded incubation of Aβ (Evans et al., 1995). They interpret their results to suggest that ApoE acts by interfering with the formation of the nucleus.

In this paper, we extend these findings and report important controls that demonstrate a strong and selective ability of all three ApoE isoforms to inhibit both endogenous nucleation and exogenous seeding of Aβ fibril growth.

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[1] Abbreviations: ApoE, apoliproprotein E; AD, Alzheimer's disease; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; V_L, immunoglobulin light chain variable domain; PBS, phosphate-buffered saline.

[2] In this paper, we discuss aspects of the nucleated growth mechanism using terms according to the following definitions. We refer to peptides that have been freshly exposed to denaturing solvent conditions capable of removing peptide aggregates that can seed fibril formation as being 'disaggregated'. Fibril formation reactions consisting of disaggregated peptide and with no additions of peptide aggregates are 'unseeded'. In such a reaction, the 'nucleus' is the earliest identifiable peptide aggregate which is stable enough to resist dissociation and which therefore serves as the basis for fibril growth. Aggregates transfered into a solution of disaggregated peptide which are capable of initiating fibril growth are called 'seeds' and are characterized as being 'seeding-competent', and experiments involving the use of such seeds are referred to as 'seeded'. Fibril formation reactions conducted in unperturbed solutions are 'unstirred' to distinguish them from fibril formation under 'stirred' conditions.

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MATERIALS AND METHODS

General Materials and Methods. Aβ(1−40) and Aβ(1−42) were obtained from Bachem. Human mixed isotype ApoE was purchased from CalBiochem. Purified human ApoE isotypes 2, 3, and 4 were produced in *E. coli* by secretion into the periplasmic space, and the protein was purified by heparin−Sepharose chromatography as described (Chan et al., 1996a). These proteins were isolated and purified as soluble proteins, without the use of denaturing agents, and appear to be well-behaved in aqueous solution. The production and purification of an N-terminal domain of ApoE3 consisting of amino acids 1−191 have been described (Chan et al., 1996a). Bovine serum albumin was purchased from Sigma. Murine interleukin-4 was obtained from M. Blackburn (SmithKline Beecham). Wild-type REI V_L was prepared by secretion into the periplasm of *E. coli* and purified as described (Chan et al., 1996b). Hexafluoroisopropanol (HFIP) was purchased from Sigma.

Preparation of Seed Stocks. A batch of Aβ fibrils was prepared for the purpose of seeding fibril formation reactions. Three milligrams of Aβ(1−40) (Bachem; lot ZK-600) was solubilized in phosphate-buffered saline (PBS) to a final Aβ concentration of 1 mg/mL. This mixture was incubated at 37 °C and monitored for fibril content over time using Congo red analysis (Wood et al., 1996b). After 2 days, when the Congo red signal reached a maximum, plateau value, fibril formation was considered complete. [The time to maximum Congo red signal can vary, depending on a number of factors: manufacturer of the Aβ, solvent used to solubilize the Aβ, and the amount of preexisting fibrillar aggregates (Howlett et al., 1995) present.] After it was determined that fibrillization was complete, the mixture was vortexed and serially diluted into PBS to prepare stocks of different fibril concentrations, which were then aliquoted into individual Eppendorf tubes and stored at −80 °C. Aliquoting the seed stocks into individual tubes avoids repetitive freeze−thawing of stocks, which might be expected to change the quality of the fibrils.

We have no data on the molar concentration of fibril particles in the seed stocks. We assume, based on previous experience (Wood et al., 1996b), that 100% of the Aβ in this fibrillization reaction was incorporated into fibrils. No attempt was made to fracture the fibrils by sonication or other means to increase seed potency, although this has been reported to be possible (Jarrett & Lansbury, 1992). Since the size and molar concentration of fibrils cannot be determined, we used weight concentrations, or molar equivalents of monomeric Aβ, to characterize the seed stocks. To describe seeding levels of fibril formation reactions, we refer to the weight fraction of Aβ in the added seed compared to Aβ in the reaction.

Fibril Formation Reaction and Detection. For most experiments, Aβ was disaggregated by HFIP treatment as described previously (Wood et al., 1996b). Dried peptide was freshly dissolved in PBS, pH 7.4, and incubated, with or without added seeds, at room temperature.

Fibril formation was monitored by quantifying the red shift observed for the binding of Congo red to fibrils, as described elsewhere (Wood et al., 1996b). It is not possible to use turbidity measurements to follow Aβ fibril formation in unstirred reactions, since such reaction mixtures—which are clearly fibrillar by EM and dye-binding critera—are transparent both to the eye and to the spectrophotometer (Wood et al., 1996b).

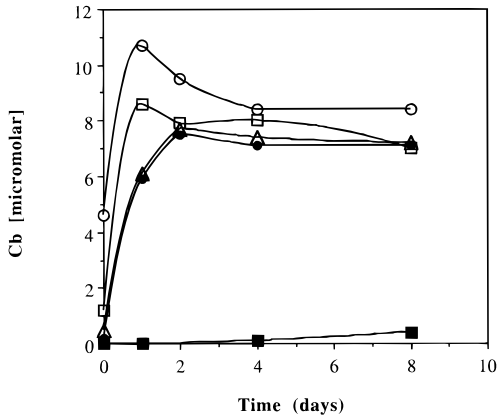

FIGURE 1: Fibril formation initiated with endogenous Aβ aggregate. Fibril formation was quantified by monitoring the red shift in the Congo red spectrum upon binding to fibrils, and is expressed as C_b, the concentration of Congo red bound (see Materials and Methods). Reactions were carried out with 1 mg/mL Aβ(1−40) (Bachem, lot ZK-600) in PBS, pH 7.4 at 37 °C. One reaction was conducted with ZK-600 Aβ directly from the vial, without any disaggregating HFIP pretreatment (○). The other reactions were with ZK600 material that had undergone disaggregation with HFIP (Materials and Methods), and to which was added at time zero either nothing (■) or varying amounts of aggregate-containing ZK600 Aβ direct from the vial [333 μg/mL (□), 100 μg/mL (△), or 33 μg/mL (●)].

RESULTS

Contaminating Aβ aggregate in commercial preparations of the peptide greatly influences the course of fibril formation reactions. Figure 1 shows that material suspended from the vial contains considerable aggregate, as detected by Congo red binding, and that such samples undergo rapid fibril formation reactions under unstirred conditions at room temperature. Exhaustive disaggregation by HFIP treatment eliminates the Congo red signal at time zero and also eliminates fibril formation for up to 8 days. When small amounts of freshly dissolved, aggregate-rich, Aβ(1−40) are added to a reaction set up with disaggregated Aβ(1−40), rapid fibril formation is restored. Thus, the aggregate present in the vial from the manufacturer is capable of seeding fibril formation reactions, consistent with its fibril-like appearance of contaminating aggregates in electron micrographs of fresh commercial peptide (Howlett et al., 1995).

Aggregation seeds not only can be obtained directly from manufacturer's lot of Aβ but also can be generated from *in vitro* fibril formation reactions. Addition of small amounts of such previously generated Aβ(1−40) fibrils to reactions containing disaggregated Aβ also stimulates initiation of fibril formation, with the time to onset of aggregation reduced in a dose-dependent manner (Figure 2A). The seeding efficiency of particular dilutions of such fibril reactions is highly reproducible (Figure 2B).

We have found the fundamental activity of apolipoprotein E in its Aβ interactions *in vitro* is to retard fibril growth by inhibiting seeding. In agreement with previously published results (Evans et al., 1995), small amounts of ApoE3 added to unseeded incubations of disaggregated Aβ(1−40) extend the time lag to onset of aggregation by at least 3 days (Figure 3). In a significant extension of previously published results,

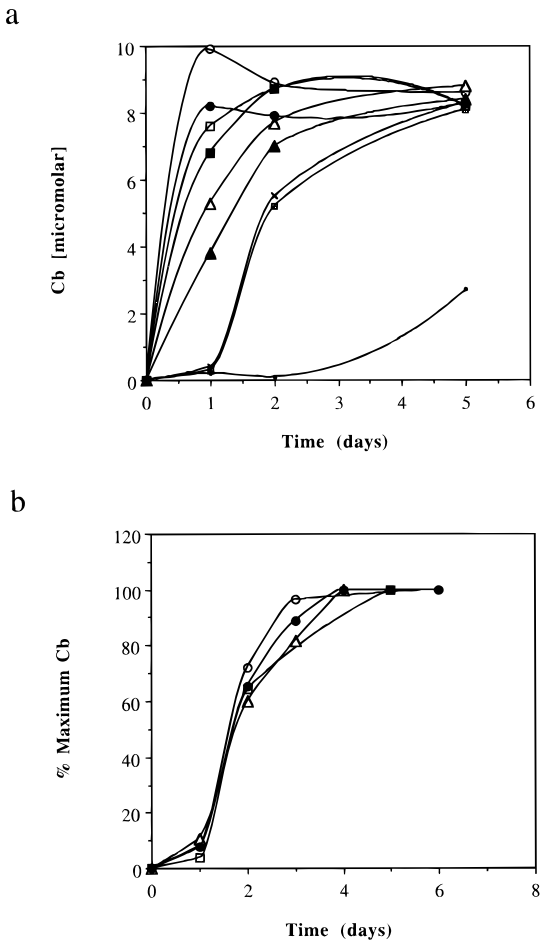

FIGURE 2: Fibril formation with Aβ(1−40) seeded with previously grown fibrils. Fibrils were grown as described under Materials and Methods. Aβ was treated with HFIP and dissolved in PBS to 250 μg/mL, and then incubated at 37 °C. (a) Unseeded (•) or seeded with 25 μg/mL (○), 5 μg/mL (●), 2.5 μg/mL (□), 1.0 μg/mL (■), 0.5 μg/mL (△), 0.25 μg/mL (▲), 0.1 μg/mL (×), or 0.05 μg/mL (open square with plus sign) fibril seed stock. (b) Reproducibility of seeding of 250 μg/mL disaggregated Aβ and 0.05 μg/mL fibril on 3 different days (○, △, and □) and their average (●).

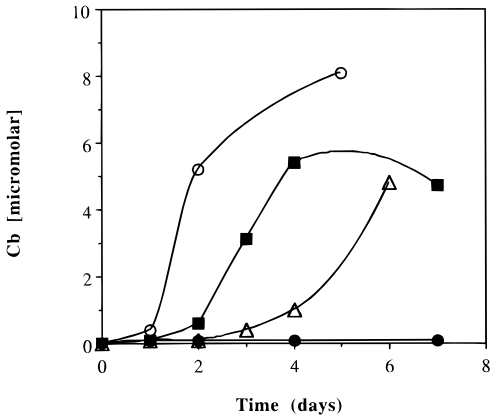

FIGURE 3: Inhibition of seeded and unseeded Aβ fibril formation by ApoE3. For unseeded reactions, Aβ(1−40) was treated with HFIP, then dissolved in PBS at 250 μg/mL, and incubated alone (△) or with 200 nM ApoE3 (●). For seeded reactions, fibrillar Aβ(1−40) (Materials and Methods) was added to 250 μg/mL HFIP-treated Aβ(1−40) to give 0.02 wt % of the total Aβ (i.e., aggregated Aβ was at 0.05 μg/mL), and the mixture was incubated alone (○) or with 30 mM ApoE3 (■).

we also found that small amounts of ApoE3 are capable of extending the time to onset of aggregation in fibril formation reactions seeded by the addition of preformed fibrils (Figure 3).

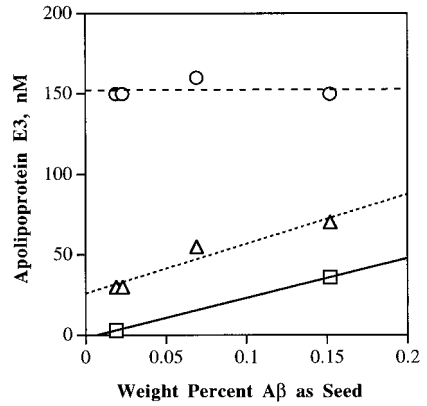

FIGURE 4: Interplay between seeding levels and ApoE3 levels in inhibition of fibril initiation. Reactions contained 58 μM HFIP-treated Aβ(1−40) in PBS at 37 °C. Seeding levels are expressed as the weight percent of total Aβ in the reaction which had been supplied as preformed fibril. ApoE3 concentration is of the tetramer. Maximum ApoE3 concentration which produces no delay of onset of aggregation at various seeding levels (□). Concentration of ApoE3 which produces a 1 day delay of onset of aggregation (△). Minimum concentration of ApoE3 which produces a delay in onset of aggregation of 4 days or more (○).

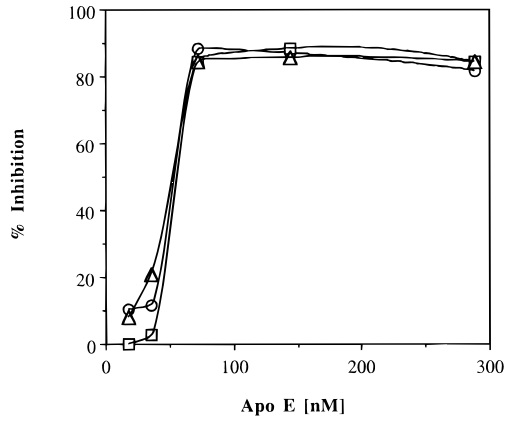

FIGURE 5: Inhibitory activities of ApoE isotypes. Aβ(1−40) was treated with HFIP, and then incubated in PBS at 250 μg/mL at 37 °C with 0.02% fibrillar Aβ(1−40) as seed and varying amounts of apolipoprotein E, subtype E2(○), E3(□), or E4(△). After 1 day, the Congo red signal of ApoE-containing reactions was compared to an ApoE-minus control and the amount of fibril formation suppressed in the ApoE-containing reactions expressed as percent inhibition.

The amount of ApoE required to produce a given effect on the time course of a seeded fibril formation reaction depends on the seeding level. Figure 4 shows that as one increases the weight percent of aggregated Aβ(1−40) added as seed to a fibril formation reaction, the amount of ApoE3 required to produce a 1 day lag in onset of aggregation also increases. In addition, the maximum amount of ApoE3 that can be added without producing an observed effect also increases as seeding dose increases. However, ApoE3 levels of 150 nM seem capable of fully shutting down fibril formation regardless of the seeding dose—at least in the range of seeding levels explored in these experiments.

The three isoforms of human ApoE produced in *E. coli* are equipotent in their abilities to inhibit seeding of Aβ(1−40) fibril formation reactions. Figure 5 shows that, in fibril formation reactions using 250 μM Aβ(1−40), and seeded with 0.02 wt % aggregated Aβ(1−40), equivalent concentra-

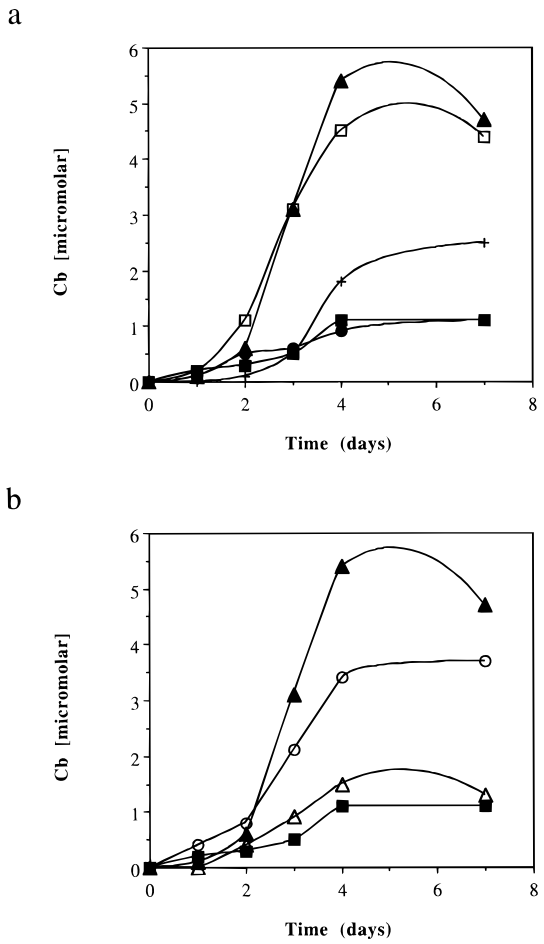

FIGURE 6: Influence of various forms of ApoE on fibril formation monitored by Congo red binding. Reactions (PBS, 37 °C) contained 58 μM Aβ(1−40) and 0.02 wt % Aβ fibril as seed, conditions which consistently produce a 1-day time lag (Figures 2 and 3). (a) Effect of source of ApoE. Tested were full-length *E. coli* ApoE3 (▲, 30 nM; ■, 150 mM), plasma-derived full-length mixed subtype ApoE (□, 70 nM), and delipidated (see Materials and Methods and Results) *E. coli*-produced, full-length ApoE3 (●, 150 nM). Also shown is a nonseeded control (+). (b) Effect of truncation of ApoE. Tested were full-length *E. coli* ApoE3 (▲, 30 nM; ■, 150 nM) and *E. coli*-produced N-terminal domain of ApoE3 (○, 600 nM; △, 3 μM).

tions of the three isoforms are required to produce equivalent levels of inhibition of seeding.

The activity of ApoE isoforms observed here does not seem to be influenced by the fact that it was produced in *E. coli*. Mixed isotype ApoE isolated from human plasma exhibits similar inhibitory activity (Figure 6a). The fact that this *E. coli* material has not been exposed to the delipidation protocol normally carried out in extractions of blood-derived ApoE (Rall et al., 1986) also does not appear to be biasing our results: a mock-delipidation carried out on the *E. coli* ApoE3 leaves its inhibitory activity unchanged (Figure 6a). The monomeric, N-terminal domain of ApoE has been previously reported to be capable of extending the time lag to onset of aggregation in an unseeded fibril formation reaction (Evans et al., 1995), and we show here that the domain produced in *E. coli* can also inhibit *seeded* reactions (Figure 6b).

The seeding phenomenon and the inhibitory activity of ApoE are also observed in reactions of the more hydrophobic, more aggregation-prone 1−42 version of Aβ. Figure 7 shows that the disaggregation protocol generates a peptide

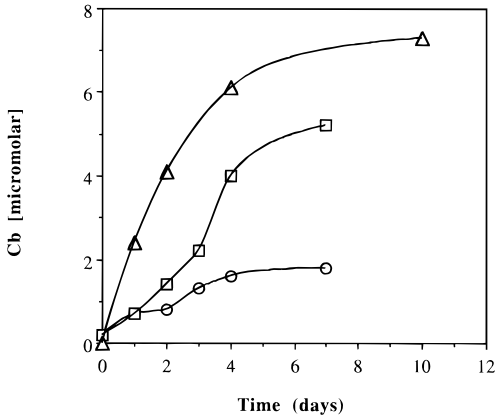

FIGURE 7: Effect of ApoE3 on seeding on Aβ(1−42) fibril formation by Aβ(1−42) fibrils. Seed fibrils and HFIP-disaggregated peptide was obtained as described under Materials and Methods. The reactions contained 55 μM Aβ in PBS at 37 °C. Unseeded disaggregated Aβ(1−42) alone (□) or with seed (△, 1.0 wt %) or seed plus ApoE3 (○, 1.0 wt % Aβ fibril plus 300 nM ApoE3).

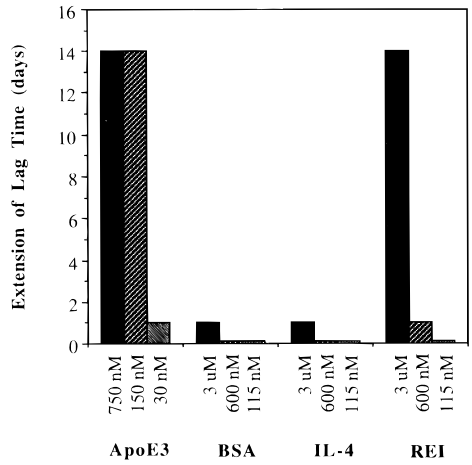

FIGURE 8: Ability of other proteins to delay onset of aggregation seeded by Aβ fibrils. Fibril formation reactions contained 58 μM HFIP-treated Aβ(1−40) and 0.02% of the total weight of Aβ in the reaction volume as preformed fibril. Reactions were monitored for up to 2 weeks by the Congo red assay, and reactions were considered to have initiated when the C_b surpassed 20% of the value reached by a noninhibited control after 2 days. The number of days transpired to reach this stage of the reaction were recorded and ultimately compared to the time to onset of aggregation of a seeded, noninhibited control.

preparation that undergoes a 1−2 day lag before reaching a maximal aggregation rate, and that seeding with previously prepared Aβ(1−42) fibrils abrogates that lag time. Addition of ApoE3 to the seeded reaction delays onset of aggregation by at least 7 days.

The potent activity of ApoE isoforms in inhibiting seeding of fibril formation is a property not found in most other proteins. To produce a 1 day delay in onset of aggregation from a seeded reaction with Aβ(1−40) requires a 100-fold higher molar concentration of bovine serum albumin or interleukin-4 compared to the amount of ApoE required (Figure 8). The only control protein tested capable of generating a delay in onset of aggregation of greater than 1 day, the immunoglobulin V_L domain REI, requires a molar concentration 20-fold higher than ApoE3 to produce an equivalent effect (Figure 8). Other apolipoproteins were not tested, however.

DISCUSSION

Devising reproducible and physiologically relevant conditions for the formation and detection of anyloid fibrils is a challenge in the *in vitro* study of any amyloid system. For example, the contamination of batches of Aβ with seeding-competent (Figure 1) Aβ aggregate (Howlett et al., 1995) clearly presents problems in the study of a nucleated growth system such as fibril formation. As is clear from the results presented here, the inhibitory effect of ApoE can easily be masked if the level of contaminating aggregate is high enough to overwhelm the ApoE (Figure 4). In addition, we have shown elsewhere that turbidity measurements are much more sensitive to nonfibrillar, pH 5.8, Aβ aggregates than to the fibrils produced in unstirred aggregation reactions at neutral pH, while Congo red is more sensitive to *bona fide* fibrils (Wood et al., 1996b). Differences in the aggregation state of Aβ starting material and in methods for monitoring fibril growth may account for some of the differences between our results and previously published studies of the interactions of ApoE and Aβ.

When Aβ(1−40) is exhaustively disaggregated, solutions must be incubated 4 days or more before significant fibril formation begins (Figures 1−3). Amounts of Aβ fibrils equivalent in weight to as little as 0.01% of the total Aβ in the reaction can induce a much more rapid fibril formation (Figure 2). Lansbury's group previously reported the ability of plasma-derived ApoE to extend the time lag to onset of aggregation of an unseeded fibril formation reaction (Evans et al., 1995), and the work reported here for *E. coli*-produced ApoE confirms this result (Figure 3). Significantly, we also show here that ApoE can inhibit the seeding of fibril growth by exogenous Aβ fibrils for both the 1−40 (Figure 3) and 1−42 (Figure 7) versions of Aβ. If this inhibition is based on a direct molecular interaction between ApoE and fibril seeds, it might be expected that the inhibition would be ApoE dose-dependent, and that the required dose for different inhibitory effects would depend on the amount of fibril added as seed. Both effects are observed (Figure 4).

Elsewhere we have reported that all three isotypes of ApoE bind equally well to Aβ in the solution phase, and that this binding proceeds through three distinct phases (Chan et al., 1996a). Within minutes of mixing 20 μM ApoE with 20 μM Aβ, one observes formation of a complex of the ApoE tetramer with about four molecules of Aβ. On further incubation, soluble, high molecular weight complexes are formed which contain about 100 molecules of Aβ per ApoE tetramer. On still further incubation, especially at very high Aβ concentrations, coprecipitates are formed exhibiting about the same Aβ:ApoE ratios as the soluble, high molecular weight aggregates. These high molecular weight aggregates bind thioflavin T as effectively as Aβ fibrils, but are relatively poor seeds of Aβ fibril formation (W. Chan, S. J. Wood, and R. Wetzel, unpublished observations). Thus, at relatively high ApoE levels, aggregates are observed, but these are ApoE-Aβ co-aggregates that are functionally different from mono-component Aβ fibrils. In contrast, at intermediate ApoE concentrations such as those explored in this paper, inhibition of Aβ fibril formation is observed.

The results suggest that, as seeding-competent Aβ aggregates form in reaction mixtures containing ApoE, the aggregates bind to ApoE in such a way that fibril extension is precluded. This phenomenon is identical to the ability of ApoE to extend the time delay to onset of aggregation in an unseeded reaction, as reported here and by the Lansbury group (Evans et al., 1995). Our additional observations, that ApoE inhibits the ability of added fibrils to act as seeds for fibril formation, suggest that ApoE can bind at or near the extension sites of an amyloid fibril, and that these sites may be essentially identical to the binding site on the emerging nucleus in an unseeded reaction.

It might be argued that the disparate results reported previously on the effect of ApoE isotypes on Aβ fibril formation could be attributable to the source of the ApoE, or the chemical form of the Aβ, used in particular experiments. The controls reported here, however, suggest that these factors do not influence the nature of our results.

A large and growing number of studies now have confirmed a strong association between the 4 allele of ApoE and increased risk of AD, with the predominant allele E3 being relatively neutral and E2 perhaps somewhat protective (Katzman, 1994). There are a number of possible explanations for our finding that the specific activities of the ApoE isotypes in inhibiting fibril formation do not correlate with these genetic trends. As suggested previously (Evans et al., 1995), the correlation of risk with Cys content of the three ApoE isotypes suggests the possibility that disulfide-linked aggregates of ApoE (Weisgraber & Shinto, 1991) may be the relevant molecules *in vivo*. Another attractive possibility is that ApoE4 levels might be lower than E3 and E2 levels in the brain—as seems to be the case in the peripheral circulation (Gregg et al., 1986); examination of ApoE levels in cerebrospinal fluid does not support this, however (Lehtimaki et al., 1995).

It is also possible that binding to lipoprotein particles might modulate the ability of ApoE to interact effectively with Aβ aggregates, as has been shown for other measures of the ApoE−Aβ interaction (LaDu et al., 1995). Although there is no evidence that ApoE exists in the plasma as a free protein unassociated with lipid, the state of ApoE in the brain, especially when astrocytes produce large amounts in response to nerve injury (Ignatius et al., 1986), is unclear.

Compared to other control proteins investigated, REI V_L is a relatively potent inhibitor of seeding of Aβ fibril formation. This is intriguing given that immunoglobulin light chain variable domains serve as the basis for light chain amyloidosis (Buxbaum, 1992). Although wild-type REI V_L itself does not seem to be capable of aggregate formation *in vitro*, the antiparallel β-sheet structure of this domain, plus the ability of this class of proteins to sometimes adopt a fibril structure, suggests the possibility that some proteins might be capable of inhibiting fibril extension by incorporating into a growth fibril while at the same time restricting further extension. Despite their high α-helical structure, other apolipoproteins like serum amyloid A and apolipoprotein A1 can also form amyloid fibrils (Pepys, 1988); this suggests that ApoE might be able to inhibit Aβ fibril extension in a manner analogous to the model proposed for REI, that is, to incorporate into the growing fibril but prevent further extension. Other mechanisms are possible, however. For example, an attractive possibility is that ApoE binds to the growing face of the fibril in a manner analogous to the model for the binding of apolipoproteins to lipoprotein particles (Weisgraber, 1994)—by unbundling of the four-helix bundle and subsequent association of the isolated amphipatic helices with the hydrophobic fibril growth face.

If the accumulations of Aβ fibrils and plaques plays a key role in promoting Alzheimer's disease, suppression of amyloid formation may be a viable approach to AD therapeutics (Wetzel, 1996). One type of inhibitor of Aβ fibril formation *in vitro* seems to function by titrating Aβ molecules in solution, blocking their ability to form fibrils (Wood et al., 1996a); effective use of such an inhibitor would therefore require deployment of inhibitor in amounts equal in molar terms to the levels of solution phase Aβ found at deposition sites. In contrast, ApoE inhibition of fibril formation requires only enough ApoE to block the growth sites on the preexisting fibrils or seeds in the system. This constitutes another class of fibril formation inhibitor which might be effective at lower concentrations compared to the "titration" type of inhibitor. Our recognition that ApoE can inhibit fibril extension suggests that inhibitory molecules which function in a similar way might be effective therapeutics, even on patients with established amyloid plaques.

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