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Preferential Interactions between ApoE-containing Lipoproteins and A β Revealed by a Detection Method that Combines Size Exclusion Chromatography with Non-Reducing Gel-shift

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Abstract

The association between apolipoprotein E (apoE) and amyloid- β peptide (A β) may significantly impact the function of both proteins, thus affecting the etiology of Alzheimer's disease (AD). However, apoE/A β interactions remain fundamentally defined by the stringency of the detection method. Here we use size exclusion chromatography (SEC) as a non-stringent approach to the detection of apoE/A β interactions in solution, specifically apoE and both endogenous and exogenous A β from plasma, CSF and astrocyte conditioned media. By SEC analysis, A β association with plasma and CNS lipoproteins is apoE-dependent. While endogenous A β elutes to specific human plasma lipoproteins distinct from those containing apoE, it is the apoE-containing lipoproteins that absorb excess amounts of exogenous A β 40. In human CSF, apoE, endogenous A β and phospholipid elute in an almost identical profile, as do apoE, exogenous A β and phospholipid from astrocyte conditioned media. Combining SEC fractionation with subsequent analysis for SDS-stable apoE/A β complex reveals that apoE-containing astrocyte lipoproteins exhibit the most robust interactions with A β . Thus, standardization of the methods for detecting apoE/A β complex is necessary to determine its functional significance in the neuropathology characteristic of AD. Importantly, a systematic understanding of the role of apoE-containing plasma and CNS lipoproteins in A β homeostasis could potentially contribute to identifying a plasma biomarker currently over-looked because it has multiple components.

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1. Introduction

Two key proteins involved in Alzheimer's disease (AD) are found circulating in both peripheral and CNS fluids associated with lipoprotein particles: apolipoprotein E (apoE) and amyloid- β peptide (A β). In humans, apoE is expressed as three naturally occurring common isoforms (apoE2, apoE3 and apoE4). ApoE modulates risk for AD, with ϵ 2/2 decreasing risk 4-fold and ϵ 4/4 increasing risk 12 fold [1-3]. ApoE expression is highest in the liver, followed by apoE expression in the brain. ApoE-containing plasma lipoproteins, synthesized primarily by the liver, do not cross the blood-brain barrier (BBB). ApoE is the major lipoprotein forming apolipoprotein produced in the brain (For review, [4]); secreted primarily by astrocytes as nascent apoE-containing discoidal particles [5, 6]. ApoE associates with lipoproteins to provide structural stability and serve as a ligand for receptor-mediated uptake of lipoproteins, facilitating cellular metabolism of cholesterol and lipids (for review, [7]).

Unlike apoE, the function of the association of A β with lipoproteins is less clear, although presumably the peptide associates with particles for its own stability and transport in plasma [8-15] or CSF [14, 16, 17]. The association of amphiphilic A β with lipoproteins would allow the peptide to remain soluble, either via an interaction with the lipids or apolipoprotein components of lipoproteins. Plasma lipoproteins in particular have been implicated in the transport of A β , including specific clearance from the brain [18]. Although the physiological consequence of the association of A β with lipoproteins remains unclear, it is interesting to note that in AD patients there is a decline in plasma lipoprotein-associated A β and an increase in free A β [19], consistent with disturbances in lipoprotein homeostasis that affect plasma A β levels in normolipidaemic AD patients [20].

ApoE/A β /lipoprotein interactions may be important for both clearance of the peptide and as a potential biomarker. Considerable work has focused on the role of apoE in the brain, including its association with A β . ApoE could serve as a chaperone, both in facilitating extracellular amyloid deposition and transporting soluble A β across the BBB to plasma [6, 21, 22]. As a concerted effort has been devoted to develop plasma biomarkers for AD, it is critical to understand the role of apoE-containing plasma and CNS lipoproteins in A β homeostasis during the development and progression of AD pathology. This knowledge could facilitate identification of a plasma biomarker currently over-looked because it has multiple components, apoE/A β /lipoproteins, with possibly distinct patterns of change that effect the overall complex.

A major unresolved issue in this field is the nature of the association between A β and apoE. This interaction is influenced by a number of parameters, with two particularly relevant to the data presented herein. First, apoE interactions with A β depend on the lipidation state of apoE, whether the apoE is purified [23-26], or associated with lipid-poor- [23, 26, 27], reconstituted "HDL"- [26], astrocyte- [28], CSF- [29] or plasma-lipoproteins [10, 24, 25]. Second, the nature and amount apoE/A β complex depends on the method of detecting the association between apoE and A β , whether in the presence of detergent or more "physiologic" buffers. Previous methods include, in an approximate order of descending stringency, gel-shift assay of SDS-PAGE [23-25, 28, 30], density gradient ultracentrifugation [10], non-denaturing gradient gel electrophoresis [28], co-immunoprecipitation (IP) [31] and solid-phase binding assays/ELISA [26, 29, 32-35]. Surprisingly, size exclusion chromatography (SEC)/gel-filtration, perhaps the most gentle method that can be used with physiological buffers, has not been used to directly study apoE interactions with A β . As this method has traditionally been used to separate the different classes of plasma lipoproteins by size, SEC has been used in the detailed characterization of the CNS-relevant lipoproteins found in CSF and secreted by astrocytes [17, 30, 36-38].

The present study examines the association of endogenous and exogenous A β with lipoproteins in solution, utilizing apoE-containing lipoproteins from both sides of the BBB, peripheral (plasma lipoproteins) and CNS-relevant (CSF and astrocyte-secreted lipoproteins). Using SEC by fast protein liquid chromatography (FPLC), apoE and A β elute with lipoproteins from plasma, CSF and astrocyte-conditioned media. The interaction of apoE and A β in specific fractions is further characterized with a non-reducing gel-shift assay to detect the presence of SDS-stable apoE/A β complexes. By this measure, the most robust interaction is between A β and apoE-containing isolated rat astrocyte lipoproteins.

2. Materials and Methods

2.1. Sources of apoE

Informed consent was obtained for experimentation with human subjects. All procedures were performed in compliance with the relevant Laws and Institutional guidelines and were approved by the institutional committee(s), from where the samples were obtained. 1) Human plasma from apoE genotyped (ϵ 2/2, ϵ 3/3, ϵ 4/4) donors was kindly provided by Brad Hyman (Massachusetts General Hospital, Boston Mass). Donors were neurologically intact control subjects who were fasted. 2) Human CSF (ϵ 3/3) was kindly provided by David Holtzman (Washington University, St Louis, Mo.). As previously described, the CSF was collected and concentrated 50-fold (Centriprep-10, Millipore, Bedford, MA) [30]. 3) Primary rat astrocyte conditioned media was generously provided by Linda Van Eldik (Northwestern University). Rat astrocyte conditioned media was prepared from neonatal (1-2 day old) Sprague-Dawley rats, concentrated 50-fold (Centriprep-10, Millipore, Bedford, MA) and prepared as previously described [30]. 4) Isolated rat astrocyte lipoproteins were prepared by pooling and concentrating FPLC fractions 30-45 of rat astrocyte conditioned media. 5) Rat apoE was purified as previously described [25]. Briefly, the lipoproteins were dialyzed against 0.01% EDTA, lyophilized, and delipidated in CHCl₃:MeOH (2:1). Delipidated proteins were pelleted in MeOH and solubilized in 6M guanidine, 0.1M Tris, 0.01% EDTA (pH 7.4), and 1% 2-mercaptoethanol (bME). Proteins were fractionated on a Sephacryl S-300 (Pharmacia, Gaithersburg, MD) column equilibrated in 4M guanidine, 0.1M Tris, 0.01% EDTA (pH 7.4), and 0.1% bME. Fractions containing apoE were dialyzed into 5mM NH₄HCO₃, lyophilized, and resuspended in 0.1M NH₄HCO₃.

2.2. Amyloid- β peptide (A β)

Lyophilized powder of the 40 amino acid isoform of A β (A β 40) peptide was initially solubilized at 5mM in 100% DMSO (Me₂SO) and diluted prior to mixing with apoE, as described below.

2.3. Methods for separating lipoproteins

Some of the apparent disparity in the literature as it relates to interactions among apoE, A β and lipoproteins may be due to the differences in the methods for analyzing lipoproteins. For example, a common method is to isolate classes of lipoproteins. Lipoproteins have been isolated from normal and AD plasma by sequential ultracentrifugation, resulting in VLDL ($d < 1.006$) IDL/LDL ($1.006 < d < 1.063$) and HDL ($1.063 < d < 1.21$) particles [20, 25] and CSF particles further fractionated to densities of 1.063, 1.125, 1.21, and 1.25 [16]. While density centrifugation provides a gradient, rather than a single sample encompassing a wide range of densities, it is known to strip apolipoproteins from lipoprotein particles [30, 39]. Thus, characterization by size gradient (SEC) is perhaps the least disruptive method for both isolating lipoproteins and identifying apoE/A β interactions by co-elution.

2.4. Fast protein liquid chromatography (FPLC)

As previously described, size exclusion chromatography (SEC) by FPLC with tandem Superose-6 columns (Amersham Pharmacia Biotech) was used and samples eluted [0.02 M sodium phosphate, 0.05M NaCl, pH 7.4, 0.03% EDTA, and 0.02% sodium azide] at a flow rate of 0.4 mL/min. For plasma, 80 fractions (70 fractions for CSF and astrocyte media) of 400 μ l each were collected for subsequent analysis of protein and lipid content [30]. 1ml of human plasma was incubated with 0.5% DMSO (Me₂SO) $\pm$ 25 μ M A β 40 for 1 hour at room temperature and fractionated by FPLC. 1ml concentrated CSF was fractionated by FPLC. 1ml of concentrated astrocyte conditioned media was incubated with 0.5% DMSO $\pm$ 25 μ M A β 40 for 1 hour at room temperature and fractionated by FPLC.

In human plasma, apoE concentrations are $\sim$ 1.4mM[40-47] and plasma A β levels are between 0.1 and 5nM [48, 49] although highly variable across studies. Thus, the endogenous plasma apoE:A β molar ratio is likely between 1.7×10^{-5} – 1.3×10^{-3} . Exogenous A β 40 was added to the human plasma at 25mM, which equates to an apoE:A β ratio of between 1:5-1:16. In human CSF, apoE concentrations are $\sim$ 0.2mM [50-52], while A β levels vary between 0.5 and 2nM [48], thus giving an apoE:A β ratio of $1:2.5 \times 10^{-3}$ – $1:1 \times 10^{-2}$. For experiments using rat ACM the apoE:A β ratio was 1:36.

2.5. Immuno-blots and Western Blot

Antisera to human and rat apoE was prepared by immunizing rabbits with purified apoE isolated from human and rat plasma, respectively. A β was detected using monoclonal antibody 4G8 that recognizes amino acids 17-24 of A β (Senetec). As previously described, FPLC fractions were immuno-blotted directly onto Immobilon-P membrane using a vacuum manifold system, probed for human and rat apoE and A β , visualized by enhanced chemiluminescence (ECL) and quantified by densitometry using ImageQuant (Molecular Dynamics, Sunnyvale, CA). As previously described, SDS-stable apoE/A β complex formation was analyzed by non-reducing gel-shift assay (originally described in [23, 24]. Briefly, for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), selected FLPC fractions containing 2x Laemmli buffer (4% SDS) were boiled 5 minutes and electrophoresed on non-reducing 10-20% SDS-tricine gels. Gels were transferred to Immobilon-P (Millipore Corp., Bedford, MA) and probed with human or rat apoE anti-sera, or 4G8 antibody [25, 30, 36, 53].

2.6. Incubation of astrocyte lipoproteins with A β

As previously described, standard binding reaction conditions were used to identify SDS-stable apoE/A β complex using purified rat apoE or isolated apoE-containing rat astrocyte lipoproteins. Specifically, 25 μ g/ml ($\sim$ 700nM) apoE was incubated for 2 hours at room temperature with 5% DMSO $\pm$ 250 μ M A β 40 [23-25, 54].

2.7. Lipid analysis

Aliquots of the FPLC fractions were analyzed for phospholipid (PL) content using an enzymatic assay (Wako, Richmond, VA) [30].

3. Results

3.1. Endogenous and exogenous A β elute with specific human plasma lipoproteins

Human plasma from apoE genotyped donors (ϵ 2/2, ϵ 3/3, ϵ 4/4) was fractionated by SEC using FPLC to determine whether the isoforms of human apoE affect endogenous A β association with plasma lipoproteins. Endogenous A β clearly has an elution profile that indicates a specific preference for certain classes of lipoproteins in the plasma, although this

profile does not match the apoE profile. Indeed, 95% of the endogenous A β elutes in lipoprotein-containing fractions (Figure 1D). The elution profile for endogenous A β from ϵ 2/2, ϵ 3/3, ϵ 4/4 human plasma presents the general profile of a peak at IDL and a broad HDL peak centering on HDL-2, with minor variations among the apoE isoforms (Figure 1-A, B and C). For example, with apoE4, there is also a small A β peak at VLDL. The general elution profile for apoE includes a peak at VLDL that decreases across the IDL/LDL range and then peaks at HDL-1 (large HDL) (Figure 1-A, B and C), although there are isoform-specific variations. ApoE2 has a broad profile from VLDL to HDL-1 with no prominent peaks. ApoE3 has a large, distinct peak at VLDL, a shoulder at IDL and a peak at HDL-1. ApoE4 has a peak at VLDL with a broad shoulder to IDL and a peak at HDL-1. There was also a peak of endogenous A β that eluted in fractions consistent with small apoA1-containing HDL lipoproteins. Furthermore, we have previously demonstrated that apoA1 forms a complex with A β using isolated plasma HDL from human, rat and rabbit [25].

With the addition of 25 μ M A β 40 to the plasma, both the A β and apoE elution profiles are similar for the 3 apoE isoforms, although the elution profile for exogenous A β was not as well-defined as the elution profile of endogenous A β (Figure 1-E, F and G). These results indicate that apoE-containing lipoproteins absorb the majority of A β when the plasma is challenged by incubation with a bolus of A β 40 (~70%; Figure 1-H), consistent with previous reports that both endogenous and exogenous A β can associate with specific plasma lipoproteins [9, 10, 12]. In contrast, Biere and co-workers used density gradient ultracentrifugation to demonstrate that 89% of exogenous I¹²⁵-A β eluted in the free protein fractions, primarily bound to albumin [10]. In the current study, the use of density gradient ultracentrifugation also resulted in >90% of the endogenous A β eluting as free protein (data not shown), a pattern consistent with previous studies showing the loss of actual apolipoproteins from CSF and astrocyte conditioned media [30]. Specifically, the majority of the apolipoproteins from CSF and astrocyte conditioned media eluted as free protein with density gradient ultracentrifugation, while these apolipoproteins were lipoprotein-associated when fractionated by SEC [30]. An additional consideration is the use of I¹²⁵-A β vs. unlabeled A β as we have observed that radioiodinated-A β interferes with interactions between apoE and A β (M.J. LaDu and G. Bu, unpublished results). Thus, there are several explanations for the partitioning of A β with free protein and possible association with albumin.

3.2. Endogenous A β exists mainly in apoE containing HDL-like particles in CSF

The primary lipoprotein present in human CSF is the size of HDL-2 with apoE as the primary apolipoprotein (for review, [55, 56]). The lipid composition of human CSF lipoproteins is not significantly different among the 3 apoE genotypes [17]. Further, A β was primarily associated with CSF lipoproteins [17]. Here, human CSF from an ϵ 3/ ϵ 3 carrier was used to further characterize the nature of the interaction between apoE and endogenous A β . ApoE, A β and phospholipid eluted in a single peak primarily between fractions 35-43 (Figure 2-A), with 100% of the endogenous A β associated with this apoE-containing lipoprotein (Figure 2-B). Western blot analysis of SDS-PAGE of selected FLPC fractions confirmed the co-localization of apoE and A β to the same fractions (Figure 2-C and D). Importantly, a portion of the A β was associated with apoE via an SDS-stable complex, as demonstrated by an A β band at ~45 and ~97 kDa, consistent with apoE monomer/A β and apoE dimer/A β complexes (Figure 2-D)(for example, [25]). In addition, a significant portion of the A β is monomer and dimer by Western blot, suggesting that A β forms a non-SDS-stable complex either with apoE or directly with a lipid component of CSF-lipoproteins.

3.3. A β associates with apoE-containing rat astrocyte lipoproteins

To extend the characterization of A β interactions with apoE-containing CNS lipoproteins, rat astrocyte conditioned media with or without the addition of exogenous A β was fractionated by SEC. Initially, SDS-stable apoE/A β complex formation was analyzed in the SEC fractions that contained both apoE and exogenous A β . In addition, in the absence of exogenous A β SEC fractions containing isolated apoE-containing astrocyte lipoproteins were pooled and incubated with A β for comparison to purified rat apoE.

SEC fractionation of conditioned media from primary rat astrocyte cultures and analysis for apoE and phospholipid revealed a broad peak between fractions 35-45 (Figure 3-A) [30, 36], suggesting particles similar in size to CSF but more heterogeneous (Figure 2-A) [17, 30]. Incubation of this conditioned media with 25 μ M A β 40 prior to SEC resulted in A β eluting primarily in fractions 35-45, with the excess found in the free protein fractions (Figure 3-B), as observed with human plasma incubated with exogenous A β 40 (Figure 1-E, F, G and H). In addition, A β also eluted in fractions 3-8, consistent with large particles or, more likely, large A β aggregates as these fractions contain minimal amounts of apoE. SEC fractionation of serum free media + 25 μ M A β 40 resulted in A β eluting entirely in the free protein fractions, suggesting that a component in the conditioned media facilitated the aggregation of the peptide (data not shown). Thus, the distribution of exogenous A β in SEC of astrocyte conditioned media results in A β eluting in very early fractions (8%), with the apoE/phospholipid peak (53%) and as free protein (39%) (Figure 3-C).

Western blot analysis of SDS-PAGE of selected fractions again confirmed the co-localization of apoE and A β to the same fractions (Figure 3-D and E), where >50% of the A β appears to be in an SDS-stable complex with apoE, although A β monomer and dimer bands are clearly visible between 3-14kDa (Figure 3-E). These results are in apparent conflict with Morikawa and coworkers who incubated conditioned media from immortalized astrocytes from GFAP-apoE transgenic mice [57] with A β and observed very little complex under denaturing conditions but were able to detect apoE/A β complex by nondenaturing gradient gel electrophoresis [28]. While the precise reason for these differences is not entirely clear, the immortalized cell lines described in this initial paper did not become a widely used reagent as the apoE production varied with both isoform and passage number (M.J. LaDu, unpublished observations). Finally, in the absence of exogenous A β , SEC fractions 35-45 from rat astrocyte conditioned media were pooled and concentrated. Standard binding reaction conditions were used to analyze SDS-stable apoE/A β complex formation between A β 40 (250 μ M) and isolated apoE-containing rat astrocyte lipoproteins or purified rat apoE (700nM) (Figure 3-F) [23-25, 54]. While a complex at 45kDa (A β) is visible with the pure rat apoE, the vast majority of the A β runs as a smear between 3-14kDa. However, the majority of the A β incubated with the astrocyte lipoproteins appears as 45 and ~97kDa bands, consistent with apoE monomer/A β and apoE dimer/A β complex [25]. Although it is possible that the higher molecular weight A β species correspond to A β aggregates, the demonstration of these bands with both α -apoE and α -A β antibodies indicates that most if not all of this immunodetection corresponds to an ApoE/A β complex. The majority of the A β incubated with rat astrocyte lipoproteins forms an SDS-stable complex with apoE.

4. Discussion

Aging is a common risk factor for both AD and cardiovascular diseases (CVD). Plasma lipoprotein dysfunction/dysregulation is largely responsible for CVD as people age. The greatest genetic factor modulating AD risk is apoE, a component of plasma lipoproteins. As a concerted effort has been devoted to develop plasma biomarkers for AD, it is critical to understand the association of apoE and A β with plasma lipoproteins. This is possible only

via a systematic understanding of the role of peripheral and apoE-containing CNS lipoproteins in A β homeostasis during the development and progression of AD pathology. This knowledge could potentially contribute to identifying a plasma biomarker currently over-looked because it has multiple components, including a specific lipid profile, apoE conformation and assembly of A β [58, 59].

One of the primary challenges remains identifying apoE/A β interactions, as the method of detection continues to define what constitutes an interaction. This study focused on 2 components of this interaction: 1) the source of the apoE-containing lipoproteins, specifically human plasma, human CSF and rat astrocyte conditioned media; and 2) the method of detection, specifically a combination of co-elution from SEC fractionation (mild) and subsequent analysis of apoE/A β containing fractions for SDS-stable complex via gel-shift assay (stringent). The elution of apoE and A β from human plasma revealed that while the profile of endogenous A β was specific and consistent among plasma from the 3 apoE genotypes, the profile did not align with the apoE profile. However, when an excess of 25 μ M A β 40 was incubated with plasma, the elution profiles for apoE and A β were nearly identical, demonstrating that apoE-containing lipoproteins have the capacity to absorb a large excess of A β as the normal concentration of A β in plasma is below 10nM [48, 49, 60]. In human CSF, the elution profiles for apoE and endogenous A β were virtually identical, while exogenous A β incubated with rat astrocyte conditioned media eluted primarily with apoE, with the excess A β in free protein fractions similar to the pattern of exogenous A β in plasma. Thus, under mild conditions using SEC, the profiles of A β and apoE-containing CNS lipoproteins are virtually identical, specifically for lipoproteins from CSF and astrocyte conditioned media.

A β is both an amphipathic and lipophilic peptide that interacts with several types of lipids, including phospholipids and gangliosides [61-63], and vesicles formed from human brain lipids [64]. Given past reports of other hydrophobic molecules such as serum amyloid A “remodeling” plasma and CSF lipoproteins [65], the addition of exogenous A β could modify lipoprotein size. Surprisingly, interaction with exogenous A β did not induce drastic changes to the overall size of the A β /apoE-containing lipoprotein particles, as indicated by the basically unmodified apoE elution profiles in the presence and absence of excess exogenous A β in plasma and astrocyte conditioned media. However, although the specific biological functions of SDS stable apoE/A β complex remain unclear, the apparent gradient of complex formation using purified apoE (low), apoE-containing CSF lipoproteins (medium), and apoE-containing astrocyte lipoproteins (high) suggests that: 1) the conformation of lipoprotein-associated apoE can facilitate its interaction with A β and 2) lipids are critical to this process although their precise identity and role are not known at present.

While the exact physiological consequence of the association of A β with lipoproteins, specifically apoE-containing lipoproteins, is not entirely clear, evidence suggests that it maintains the solubility of the A β peptide and may facilitate A β clearance and transport across the BBB [21, 22, 66]. Currently A β and apoE are measured in total plasma protein preparations, which may not be sensitive to detect subtle changes in protein levels. In this current study, the interactions between A β and apoE can be detected with specific lipoprotein assemblies, which may reveal differences in early stage AD. Further work is needed to assess whether interactions between lipoproteins and A β could serve as useful biomarkers for pre-clinical diagnosis of AD.

5. Conclusion

In the AD field, a major focus is on identification of a plasma biomarker. An understanding of apoE/A β /lipoprotein interactions may facilitate the identification of a constellation of

markers that meet this need. By focusing on plasma, CSF and astrocyte lipoproteins, we have demonstrated that not only do apoE and A β co-elute with CSF and astrocyte lipoproteins, apoE-containing human plasma lipoproteins can absorb a bolus of excess A β . Combining SEC fractionation with subsequent analysis for SDS-stable complex reveals that apoE-containing astrocyte lipoproteins exhibit the most robust interactions with A β . Further work is needed to assess whether changing patterns in these interactions can serve as useful biomarkers for the pre-clinical diagnosis of AD.

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Highlights

1. SEC is a non-stringent approach to the detection of apoE/A β interactions in solution.
2. By SEC analysis, A β association with CNS and plasma lipoproteins is apoE-dependent.
3. ApoE-containing human plasma lipoproteins absorb excess amounts of exogenous A β 40.
4. A β and apoE-containing astrocyte lipoproteins exhibit the most robust interaction.
5. A β /lipoprotein interactions are important for clearance and as a potential biomarker.

Figure 1. Endogenous and exogenous Aβ elute with specific human plasma lipoproteins
 Elution profiles of apoE and Aβ in human plasma from ε2/2, ε3/3, ε4/4 without (A, B, C) or with (E, F, G) the addition of exogenous Aβ40. Graphs are an average of n = 3 separate experiments. Graph of the average proportion of endogenous Aβ (D) or exogenous Aβ (H) that elute with plasma lipoproteins or as free protein. (Note: the characteristic distribution of human plasma lipoproteins are given on graphs below the units of the X-axis: VLDL, IDL/LDL, HDL, and free protein).

Figure 2. Endogenous Aβ co-elutes with CSF lipoproteins

Elution profiles of apoE, Aβ and phospholipid (PL) in human CSF (ε3/3) (**A**). Graph of the average proportion of endogenous Aβ that elutes with apoE-containing CSF lipoproteins or as free protein (**B**). Representative Western blots of SDS-PAGE of selected FPLC fractions probed for Aβ (**C**) or apoE (**D**).

Figure 3. Exogenous Aβ co-elutes with astrocyte lipoproteins and forms SDS-stable complex with apoE in isolated rat astrocyte lipoproteins

Elution profiles for rat apoE and PL in conditioned media from primary rat astrocyte cultures without (**A**) or with the addition of exogenous Aβ40 (**B**). Graphs are an average of $n = 2$ separate experiments. Graph of the average proportion of exogenous Aβ that elutes with apoE-containing astrocyte lipoproteins or as free protein (**C**). Representative Western blots of SDS-PAGE of selected FPLC fractions probed for Aβ (**D**) or apoE (**E**). Western blots of purified rat apoE and isolated rat astrocyte lipoproteins incubated with Aβ40 and probed for apoE (**F**, right) or Aβ (**F**, left).