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High Density Lipoprotein Inhibits Assembly of Amyloid β-Peptides into Fibrils

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The extracellular deposition of amyloid beta (Aβ) in senile plaques constitutes one of the defining hallmarks of Alzheimer's disease. Aβ peptides can aggregate spontaneously to highly insoluble amyloid fibrils, but several components are likely to influence the kinetics of fibrillogenesis *in vivo*. We report here that high density lipoprotein (HDL), the predominant lipoprotein in the human brain, reduces amyloid formation *in vitro* as determined by thioflavin T fluorescence and high speed sedimentation assays. The inhibition occurred in a dose dependent manner, and with concentrations of HDL above 1% resulting in more than 70% inhibition. We also examined the combined effect of apolipoprotein E (apoE) and HDL on Aβ fibrillogenesis. We found that HDL particles enriched with any of the three apoE isoforms inhibited Aβ fibrillogenesis as their native counterparts. Taken together, these findings suggest that HDL-like particles in the brain may prevent the formation of Aβ fibrils.

Key Words: **Alzheimer's disease; high density lipoprotein; apolipoprotein E; amyloid-β peptide.**

Alzheimer's disease is characterised by a progressive cerebral accumulation of senile plaques consisting of the 4.3 kDa amyloid β-peptide (Aβ) as the principal component (1). Synthetic analogues of Aβ can adopt a β-sheet structure and aggregate spontaneously *in vitro* to form amyloid fibrils similar to those of senile plaques (2). Several studies have demonstrated that various proteins and non-protein components can affect the kinetics of Aβ amyloid formation. These components include α-synuclein (3), acetylcholinesterase (4), heparan sulfate proteoglycan (5), and trivalent zincions (6) that all enhance amyloidogenesis, whereas apolipoprotein J (7), tetradecyltrimethylammonium bromide (TDMA) (8), and congo red (9) inhibits or strongly reduces the formation of amyloid fibrils.

Apolipoprotein E (apoE), a 299 amino acid protein with a prominent role in the transport and metabolism of plasma cholesterol and triglycerides, displays a unique feature with respect to its interaction with Aβ. ApoE exists in three major isoforms, apoE2, apoE3 and apoE4, which are products of the ϵ2, ϵ3 and ϵ4 alleles respectively. Several studies have demonstrated that the ϵ4 allele of apoE correlates with an increased risk of both sporadic and late onset familial Alzheimer's disease (10). The biochemical mechanism underlying the genetic association between apoE and Alzheimer's disease remains unclear, but an isoform-specific interaction between apoE and Aβ has been suggested to play a role. Delipidated apoE4 has thus been shown to associate more avidly than E3 with Aβ to form complexes that resist separation on SDS-PAGE (14). Other studies have further demonstrated that delipidated apoE4 rather than apoE3 enhances the amyloidogenesis of Aβ (15). In their lipidated versions, however, apoE4 binds less avidly to Aβ than apoE3 (16). This indicates that association with lipid particles induces structural changes in apoE that may affect its biochemical properties and isoform specific characteristics towards Aβ.

In the brain, apoE is produced and secreted by astrocytes and microglial cells (11). Neurons do not secrete apoE, but express the low-density lipoprotein receptor-related protein by which it can be internalised (12). Similar to its role in the plasma, apoE may thus play an important role in the transport of lipoproteins in the CNS. Physiologically, apoE is associated to lipoprotein particles in both plasma and cerebrospinal fluid (CSF). The only lipoproteins present in the brain resemble the high-density lipoproteins (HDL) of plasma, and examinations have confirmed an association between apoE and HDL-like particles in the CSF (13).

HDL can also associate directly with Aβ and HDL-Aβ complexes have been isolated from CSF (17). Aβ has recently been shown to mediate the cellular

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uptake of lipoproteins (18) and other studies have demonstrated that HDL increases the cellular degradation of Aβ in cultured rat microglia (19). In accordance with this, HDL has been shown to decrease the Aβ induced neurotoxicity in cortical cell cultures (20). Taken together, these findings suggest that HDL particles may influence the biochemical properties of Aβ and amyloid formation directly.

To explore this further, we have studied the effect of HDL particles on amyloid fibril formation *in vitro*. We find that HDL can inhibit the spontaneous aggregation of Aβ into fibrils. Purified apoE has previously been shown to enhance the formation of Aβ fibrils (21). We confirm this finding but demonstrate that the accelerating effect of apoE on Aβ fibril formation can be completely abolished by the presence of HDL particles.

MATERIALS AND METHODS

Materials. Peptide Aβ(1-42) was synthesized by Bachem (CH) and obtained as lyophilised powder. ^{125}I-Aβ(1-40) was purchased from Amersham (UK). Thioflavin T, congo red, and tetradecyltrimethylammonium bromide (TDMA) was purchased from Sigma. Isolated low and high density lipoproteins (LDL and HDL) were obtained from Sigma. Recombinant apoE proteins from both PanVera (WI) and Calbiochem (CA) were used with similar results.

Aggregation of Aβ peptides. A 5 mg/ml stock solution of Aβ(1-42) was freshly prepared before each experiment by dissolving the lyophilised peptide in 0.01 M HCl, followed by subsequent dilution 1:1 with 0.01 M NaOH to yield a neutral pH. Aliquots of Aβ(1-42) were dissolved in PBS (pH 7.4) to 100 μM and incubated at a total volume of 30 μl for 24 h at room temperature. For co-incubation experiments, aliquots of HDL (0.4 mg cholesterol/mg) and/or apoE were added to final concentrations as indicated in the figure legends. The Aβ(1-42) rather than the shorter Aβ(1-40) was used throughout this study, since the Aβ(1-42) and the Aβ(1-43) forms of Aβ are specifically found in all kinds of AD plaques, indicating that those forms are critically important in AD pathology.

Thioflavin T based fluorometric assay. The amyloid formation was quantitated by the thioflavin T fluorimetric assay. Thioflavin T binds specifically to amyloid and this introduces a shift in its emission spectrum and a fluorescent signal proportional to the amount of amyloid formed (22). After incubation, Aβ peptides were added to PBS (pH 6.0) and 3 μM thioflavin T in a final volume of 1 ml. Fluorescence was monitored at excitation 454 nm and emission 482 nm using a Fluoroscan II fluorometer (Molecular devices, UK). A time scan of fluorescence was performed and three values after the decay reached a plateau (around 5 min) were averaged after subtracting the background fluorescence of 3 μM thioflavin T. For co-incubation experiments, fluorescence of test compound alone was determined. Samples were run in triplicate and each experiment was repeated three times. The mean ± SD for the typical experiment is shown in figures.

Quantification of Aβ aggregation by sedimentation. Aliquots of Aβ were incubated as described above, either alone or in the presence of different concentrations of HDL particles. To allow subsequent analysis by autoradiography, each incubation was supplemented with traces of radiolabelled ^{125}I-Aβ(1-40) to a final specificity of 5,000 cpm. The soluble and aggregated peptide in each sample was separated by centrifugation at 16,000 g for 20 min. The pellets were resuspended and boiled for 3 min in sample buffer, electrophoresed on 10–20% Tris-tricine gels, and subjected to autoradiography.

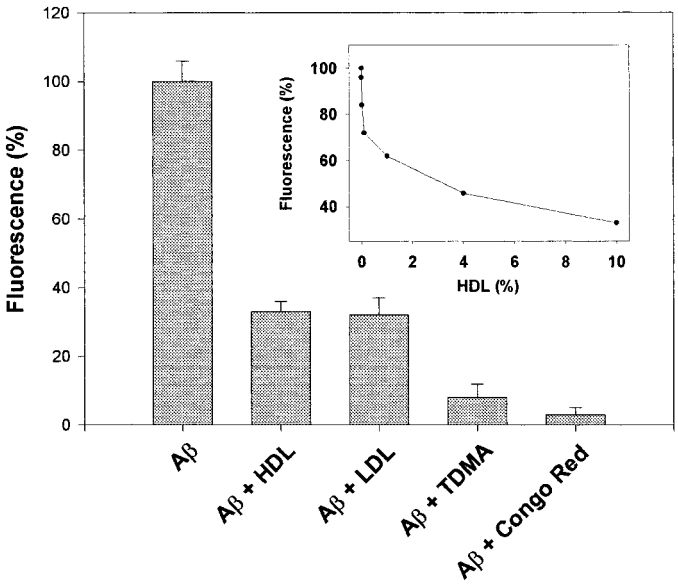

FIG. 1. Inhibition of amyloid formation by HDL as detected by the thioflavin T assay. Aβ(1-42) was incubated for 24 h, either alone (Aβ), in the presence of 10% (v/v) LDL or HDL or in the presence of equimolar concentrations of TDMA or congo red, which are both well-established inhibitors of amyloid formation. Data are expressed in relation to the spontaneous amyloid formation by Aβ alone. The bars represent the mean ±SD of a typical experiment performed in triplicate. Insert shows the dose-dependent effect of HDL on amyloid formation.

RESULTS

Aβ Aggregates Spontaneously

The thioflavin T fluorescence assay was used to investigate the effect of HDL on Aβ amyloid formation. Freshly suspended Aβ(1-42) had little specific thioflavin T fluorescence immediately after dilution in the fluorescence buffer. Following a 24 h incubation period, the fluorescence in samples containing Aβ(1-42) alone had increased significantly, indicating the formation of amyloid structures (Fig. 1). The formation of amyloid fibrils from Aβ can be inhibited by TDMA (8) and the dye congo red (9). We therefore used these compounds as controls in the thioflavin T assay. In the presence of equimolar concentrations of Aβ(1-42) and either congo red or TDMA, the amyloid formation was reduced to less than 5% of the spontaneous amyloid formation observed with Aβ(1-42) alone. Co-incubation of Aβ(1-42) with either HDL or LDL particles resulted in significantly less amyloid formation than in the presence of Aβ(1-42) alone. The amyloid inhibiting effect of the HDL increased in a dose-dependent manner. Concentrations of HDL particles of 0.01% and lower had virtually no effect, whereas concentrations higher than 1% reduced the amyloid formation by up to 70% relative to the incubations with Aβ(1-42) alone.

The results above were confirmed by examining the presence of aggregated peptide in the incubations of Aβ

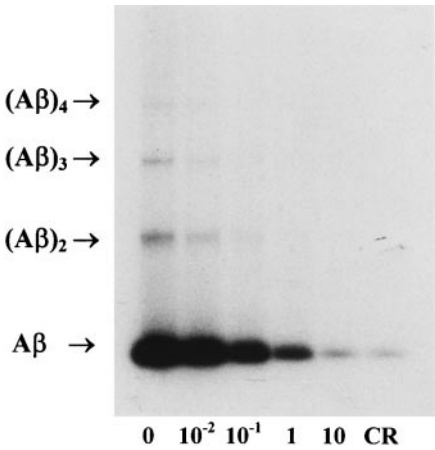

FIG. 2. Time course experiment of Aβ aggregation as analyzed by autoradiography following high-speed sedimentation and electrophoretic separation. Aβ(1-42) containing trace amounts of 125I-Aβ(1-40) was incubated at room temperature for 24 h, either alone (0), with equimolar concentrations of congo red (CR) or in the presence of HDL, ranging from concentrations of 0.01% to 10% (v/v).

and HDL. During a 24 h incubation period, the Aβ peptides formed aggregates that could be isolated by high speed centrifugation. Following separation by SDS-PAGE, the aggregates were visualised by autoradiography and analysed. The vast majority of the sedimented material appeared with an electrophoretic mobility of approximately 4 kDa, corresponding to monomeric Aβ peptide. However, in accordance with previous reports (23), the Aβ peptides also formed SDS-stable oligomers with electrophoretic mobilities of 8, 12 and 16 kDa (Fig. 2). As expected from the findings above, significantly less Aβ could be sedimented from incubations containing HDL. Furthermore, fewer oligomers were formed. Even after 24 h incubation, virtually no oligomers were detectable in co-incubations of Aβ and high concentrations of HDL.

It has previously been documented that delipidated apoE can affect amyloid formation in an isoform specific manner (15). We confirmed these findings in our own assays and extended our experiments to study the combined effect of apoE and HDL (Fig. 3). We found that apoE4 increased amyloid formation by more than 50% relative to the spontaneous aggregation obtained by incubating Aβ alone. ApoE3 increased amyloid formation to a lesser extent, whereas apoE2 had no detectable effect on the amyloid formation in our experiments. Co-incubation of Aβ with HDL effectively prevented the enhancing effect of all apoE isoforms on amyloid formation. The amyloid formation in samples containing both HDL and apoE was even reduced to the same level as incubations with HDL alone. The sedimentation assay confirmed that addition of apoE to co-incubations of Aβ and HDL had no detectable effect on the amount of sedimentable material (not shown).

DISCUSSION

In the present paper we have shown that HDL particles interact with Aβ to prevent its aggregation into amyloid. We found that both native as well as apoE-enriched HDL were equally capable of inhibiting the amyloid formation. Previous studies have demonstrated that Aβ can associate directly with HDL (17), while others have reported that HDL particles decrease the neurotoxicity of Aβ peptides (20). Taken together, these results indicate that HDL may strongly influence the structural and biochemical properties of Aβ. Through a direct interaction with HDL, the tendency of Aβ to self-aggregate may thus be deactivated and the toxic effect of Aβ on neurons prevented.

Protein binding studies have previously shown that Aβ interacts with apoE in an isoform specific manner (14, 16), and fibril formation of Aβ(1-40) as well as Aβ(1-42) is enhanced by the presence of delipidated apoE (21). This has implicated that apoE may act as a pathological chaperone that induces conformational changes in Aβ and facilitates its aggregation into amyloid. In its physiological state, apoE is normally present in the brain in association to HDL-like particles. It was therefore relevant to examine the effect of co-incubating apoE with Aβ in the presence of HDL particles.

We confirmed that amyloid formation of Aβ is clearly enhanced by recombinant, purified human apoE in an isoform specific manner. ApoE4 and to a lesser extent apoE3 increased the amount of amyloid

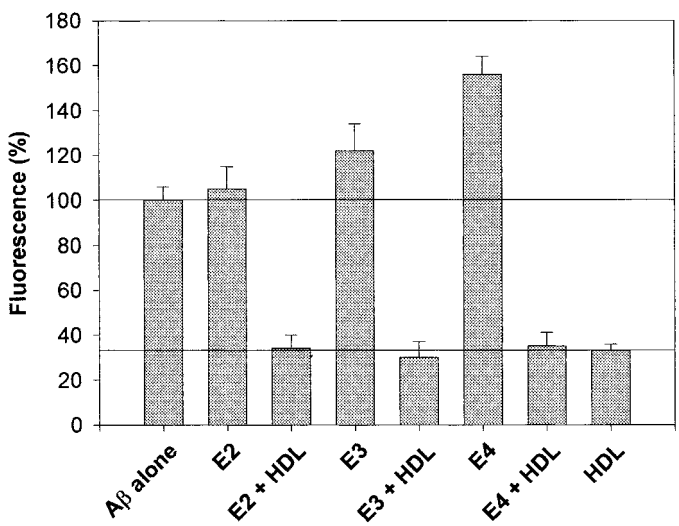

FIG. 3. Combined effect of apoE and HDL on Aβ amyloid formation. Aβ peptide was incubated alone (Aβ alone), in the presence of individual, delipidated apoE isoforms (E2, E3, and E4), in the presence of both apoE and 10% HDL (v/v), or in the presence of 10% HDL only (HDL). Delipidated apoE enhanced amyloid formation in an isoform specific manner. However, both native and apoE-enriched HDL particles inhibited the spontaneous formation of Aβ amyloid formation to a similar extent.

structures formed by Aβ. In the presence of HDL particles, we found no direct effect of apoE on amyloid formation. The HDL particles completely neutralised the amyloid enhancing effect of either apoE isoform. This suggests that although delipidated apoE may increase the spontaneous amyloid formation of Aβ, HDL-bound apoE may actually decrease amyloid formation due to the strong amyloid inhibiting effect of HDL.

The apoE genotype may, however, influence the formation of amyloid in another manner *in vivo*. Rather than direct interaction with Aβ, the apoE isoform may influence the amyloid formation by affecting the levels of HDL-like particles in the brain. In human plasma, the Apoϵ4 allele is associated with lower levels of HDL than the Apoϵ3 allele (24), and an analogous relationship between apoE genotype and HDL levels is likely to occur in the brain. The lower level of HDL particles associated with the Apoϵ4 genotype may thus be less capable of preventing the formation of Aβ amyloid fibrils. Furthermore, HDL particles display a preference for binding to apoE3 in comparison with apoE4 (25). The low level of HDL particles associated with the Apoϵ4 genotype, combined with the modest affinity of apoE4 for HDL, may result in a fraction of apoE4 existing in a delipidated form in the brain. The strong amyloid enhancing effect of delipidated apoE4 makes this potentially harmful, and could contribute to an increased deposition of aggregated Aβ.

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