Interaction between human amphipathic apolipoproteins and amyloid β-peptide: surface plasmon resonance studies

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Abstract Several apolipoproteins including apoE and apoA-I are known to be associated with amyloid β-peptide, a major component of senile plaques in Alzheimer's disease. In the present study the interaction between three human amphipathic apolipoproteins apoE3, apoA-I and apoA-II and immobilized amyloid β-peptide (1–40) was quantified by plasmon resonance. The interactions were saturable and reversible. The results demonstrated a high affinity of the binding of amphipathic apolipoproteins to amyloid β-peptide. On the other hand, only a small population of synthetic amyloid β-peptide participated in the interaction. The apparent equilibrium dissociation constants K_D were 10 nM for apoE3, 25 nM for apoA-I and 80 nM for apoA-II under physiological conditions. The affinity of the apoE3-amyloid β-peptide binding was not affected by pH in the range 6.0–8.0 but was significantly increased by high salt concentration. ApoA-I mainly followed similar patterns. A major participation of hydrophobic forces in the binding of apoE3 and apoA-I to amyloid β-peptide was suggested.

Key words: Alzheimer's disease; Amyloid beta-protein; Apolipoprotein E; Apolipoprotein A-I; Apolipoprotein A-II; Protein binding

1. Introduction

Apolipoprotein E (apoE) is a key protein in the plasma lipid-transport system and an important cholesterol-transport protein in the brain. Human apoE is a polymorphic 34-kDa protein which has 3 common isoforms: ancestral apoE4, apoE3, the most common isoform, and apoE2 [1–3]. The apoE gene has been linked to Alzheimer's disease (AD) as a genetic risk factor due to the increased ε4 allele frequency found among patients with late-onset familial and sporadic AD [4]. However, the mechanism by which ε4 allele increases the risk of AD is still unknown.

In vitro, apoE binds in isoform-specific manner with 'high avidity' to amyloid β-peptide (Aβ), a major protein component of the senile plaques [5]. Aβ is a small fragment of 39–43 amino acid residues from a transmembrane glycoprotein, the amyloid precursor protein [6]. Most of the biological fluids, including cerebro-spinal fluid and plasma, contain a soluble form of Aβ, mainly Aβ(1–40), with secondary structure distinct from Aβ in amyloid deposits. Aβ of amyloid deposits has β-sheet structure while soluble Aβ demonstrates random coils and α-helical structures [7,8].

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Abbreviations: Apo, apolipoprotein(s); AD, Alzheimer's disease; Aβ, amyloid β-peptide; SPR, surface plasmon resonance; K_D, equilibrium dissociation constant; k_{diss}, k_{ass}, rate constants of dissociation and association, respectively; RU, refractive units.

Several proteins including apoE and apoA-I were co-localized with Aβ in senile plaques. Moreover, it has recently been shown that numerous proteins can bind to synthetic Aβ in vitro, such as apoJ, apoE, apoA-I, apoA-II, amyloid P component, transthyretin and others [9–13]. The binding of Aβ with these proteins is under extensive investigation due to its possible involvement in the pathogenesis of AD. It was suggested that apoJ, transthyretin and apoE may play a role in soluble Aβ transport or in the formation of Aβ fibrils [6,10,14,15], while a possible role of apoA-I and apoA-II in Aβ turnover is not clear yet. Nevertheless, the binding of apoE and other amphipathic apolipoproteins to Aβ has not yet been quantified. In this work we have quantitatively assessed and investigated the binding of human apoE3, apoA-I and apoA-II to Aβ(1–40) by surface plasmon resonance (SPR). SPR, an analytical system based on biosensor technology, allowed characterization of biospecific interactions of label-free compounds [16,17]. The binding kinetics and affinity of Aβ interactions with human apoA-I and apoA-II, important protein components of lipoproteins in plasma and cerebro-spinal fluid, were studied for comparison with apoE since all these apoproteins have similar amphipathic nature [18,19]. Neither apoA-I nor apoA-II has an effect on assembly of Aβ(1–42) into filaments, in contrast to apoE [20]. However, levels of both apolipoproteins were decreased in plasma of late-onset AD patients [9,21]. We showed that these apolipoproteins bound to Aβ with high affinity, with apoE3 having the highest affinity. The effects of pH and salts on the binding of the apolipoproteins to Aβ suggest that hydrophobic forces predominantly participate in the apoE-Aβ and apoA-I-Aβ interactions.

2. Materials and methods

2.1. Materials

Synthetic Aβ(1–40) (lot 506063) was purchased from Bachem (Switzerland). Sensor chips CM5, 100 mM *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide, 400 mM *N*-hydroxysuccinimide and 1.0 M ethanolamine (pH 8.5) were obtained from Pharmacia Biosensor (Sweden). Bovine albumin (essentially fatty acid free) and guanidine were obtained from Sigma (USA).

2.2. Purification of human apolipoproteins

ApoE was purified from human plasma very low density lipoproteins of normal ε3/ε3 individuals. Very low density lipoproteins were obtained by ultracentrifugation at a density 1.006 g/ml and were delipidated in ethanol:diethyl ether mixtures and dissolved in 6 M guanidine-HCl. ApoE was isolated by gel filtration on Sepharose CL-6B in 10 mM Tris-HCl (pH 8.0), 1 mM dithiotreitol with 4 M guanidine-HCl and affinity chromatography on heparin-Sepharose in 0.15 M NaCl, 1 mM EDTA, 0.02% sodium azide, 10 mM Tris-HCl (pH 7.4) [22]. Apolipoproteins A-I and A-II were purified from human plasma high density lipoproteins, isolated by ultracentrifugation over the density range 1.065–1.21 g/ml. High density lipoproteins

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were delipidated in chloroform/methanol 2:1. Apolipoproteins were isolated by gel filtration on Toyopearl HW-55F and then separated by anion-exchange chromatography on DEAE-Toyopearl 650M in 6 M urea [23]. All purified proteins showed a single band on SDS electrophoresis [24].

2.3. Immobilization of Aβ on sensor chip

Aβ(1–40) was dissolved in deionized water to a concentration of 1 mg/ml, and small aliquots were frozen until use. SPR studies were performed on BIAlite apparatus (Pharmacia Biosensor). The immobilization protocol mainly followed the recommendations of the manufacturer. A sensor chip was activated by a misture of 100 mM *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride/400 mM *N*-hydroxysuccinimide mixture 1:1 (v/v), to allow the subsequent covalent cross-linking of the injected peptide through primary amine groups. Then 35 ml of 50 mg/ml Aβ in 10 mM acetate buffer (pH 4.0) was injected at a flow rate of 5 ml/min and unreacted active sites were blocked with 1.0 M ethanolamine, pH 8.5. After immobilization, non-specifically bound Aβ was removed with 4 M guanidine-HCl, 10 mM Tris-HCl (pH 8.0). Since 1000 resonance units (RU) determined by SPR corresponds 1.0 pg/mm^2 of bound ligand, the total immobilized mass of Aβ under these conditions was approx. 7.0 pg/mm^2.

2.4. Surface plasmon resonance experiments

All binding experiments were performed at 25°C and at a flow rate of 5 ml/min. 30 ml of protein in running buffer (10 mM phosphate buffer, 0.15 M NaCl, pH 7.4) were injected for association (contact time 6 min). Dissociation was followed in the same buffer for 6 min. After each run, the sensor chip was regenerated with 10 ml of 4 M guanidine-HCl, 10 mM Tris-HCl, pH 8.0 (contact time 2 min) and washed with running buffer for 5–10 min prior the next injection. In some experiments the pH varied from 6.0 to 8.75 or NaCl concentration varied up to 1.0 M. All buffers were filtered through 0.22 mm filters (Millipore, USA) and degassed before use. The injection system was rinsed to change one buffer for another when necessary.

Rate association and dissociation constants k_{ass} and k_{diss} and other kinetic parameters for apolipoproteins were estimated by the BIAevaluation software (Pharmacia Biosensor) using the model A + B = AB to fit the data. The dissociation rate constant was calculated as the slope of a curve using the log of the drop in response during dissociation phase against time interval $\ln(R_o/R) = k_{diss}(t - t_o)$, where R_o is the response at an arbitrarily chosen starting time t_o, and R is the response at current time t. The association rate constant was calculated from the log transformation of the association phase $\ln(dR/dt)$ against time in accordance to the equation $\ln(dR/dt) = \ln(k_{ass}CR_{max}) - (k_{ass}C + k_{diss})t$, where C is the molar concentration of analyte, R_{max} is the maximal response, and k_{diss} is the known rate dissociation constant. K_D was calculated as the ratio k_{diss}/k_{ass}.

3. Results

The binding of apoA-I, apoA-II, apoE3, and albumin used as a control protein, to Aβ was studied in 10 mM phosphate buffer, 0.15 M NaCl, pH 7.4. All apolipoproteins readily bound to Aβ whereas albumin did not (Fig. 1). Obviously, the interactions between the apolipoproteins and Aβ were saturable and reversible. In the representative experiment, maximal response R_{max} calculated from association phase was 440 RU for apoE3, 400 RU for apoA-I, and 210 RU for apoA-II. It corresponds to the maximal binding, for example, of 0.44 pg/mm^2 of apoE on a sensor chip which contained 7.2 pg/mm^2 of immobilized Aβ. The maximal molar ratios were estimated as apoE:Aβ 1:130, apoA-I:Aβ 1:120, and apoA-II:Aβ 1:90 under these experimental conditions. Thus, only a minor population of the synthetic Aβ(1–40) was able to interact with these apolipoproteins on the time scale of minutes. To assess the kinetic binding constants of apolipoprotein binding with immobilized Aβ under physiological conditions, samples of apolipoproteins were injected on immobilized Aβ in increasing concentrations (0.13–1.0 mM for apoE3, 0.22–1.78 mM for apoA-I, and 0.36–2.87 mM for apoA-II) as shown in Fig. 2. Table 1 shows rate constants and equilibrium dissociation constants of apolipoprotein-Aβ binding. ApoE3 has the highest affinity for Aβ among these three amphipathic apolipoproteins. ApoE3 showed an association rate close to that of apoA-I, but the dissociation rate for apoE3 was significantly lower. In spite of having the lowest affinity of apoA-II for Aβ among the apolipoproteins studied, apoA-II still showed strong binding to Aβ.

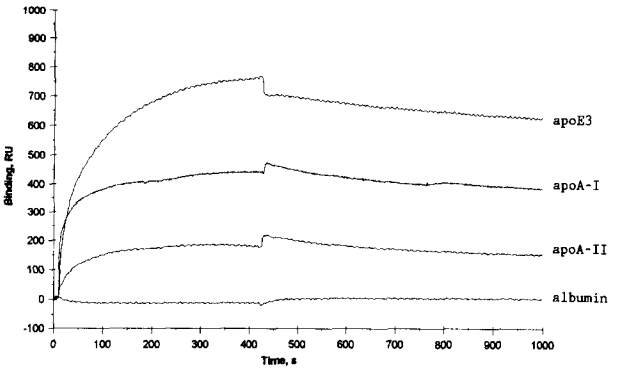

Fig. 1. Kinetic binding curves (sensorgrams) of apolipoprotein and albumin binding to Aβ(1–40) in 10 mM phosphate buffer, 0.15 M NaCl, pH 7.4. ApoE3 was at a concentration of 35 mg/ml (1 mM), apoA-I, apoA-II and albumin being at a concentration of 50 mg/ml (1.8, 2.9 and 0.8 mM, respectively). The binding curves are expressed as resonance units (RU) as a function of time. The spikes indicate the beginning of the dissociation phase. A total of 7200 RU of Aβ was immobilized.

The binding of apolipoproteins to Aβ was carried out at different pH values in 10 mM phosphate buffer, containing 0.15 M NaCl. ApoE3 was used at a concentration of 35 mg/ml (1.0 mM). ApoA-I and apoA-II were at a concentration of 50 mg/ml (1.8 and 2.9 mM, respectively). It was demonstrated that the pH, in the range 6.0–8.0, did not change significantly either the equilibrium dissociation constant K_D (Fig. 3A) or the maximal binding R_{max} (data not shown) of apoE3 and apoA-I interactions with Aβ. A tendency for increasing K_D at pH 8.75 was observed. Rate constants also were not sig-

Table 1
Kinetic binding constants of interactions between apolipoproteins and immobilized Aβ

Apolipoprotein	k_{ass} (s^{-1} M^{-1}) ($\times 10^{-3}$)	k_{diss} (s^{-1}) ($\times 10^4$)	K_D (nM)
ApoE3	25.6 ± 15.1 (n = 7)	2.09 ± 0.55 (n = 8)	10.0 ± 4.6 (n = 7)
ApoA-I	27.7 ± 13.5 (n = 5)	5.69 ± 0.60 (n = 6)	25.0 ± 10.0 (n = 5)
ApoA-II	10.1 ± 3.0 (n = 3)	6.65 ± 1.12 (n = 6)	79.0 ± 22.0 (n = 3)

The buffer contained 10 mM PBS, 0.15 NaCl (pH 7.4). Mean ± SD are shown.

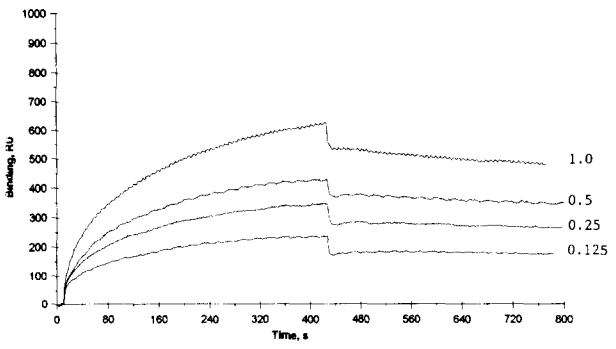

Fig. 2. Binding of human apoE3 to Aβ(1–40). Kinetic binding curves. ApoE3 concentrations are given to the right of each trace in micromolarity.

nificantly changed in this range of pH (data not shown). On the other hand, the affinity of apoA-II-Aβ binding continuously decreased as pH increased from 6.0 to 8.75 (Fig. 3A), due to changes of k_{ass}.

The effects of NaCl were studied in 10 mM phosphate buffer (pH 7.4). The concentrations of the apolipoproteins used were as indicated in pH experiments. It was shown that K_D for apoE3-Aβ interaction was drastically decreased at high NaCl concentration (Fig. 3B). This significant increase of apoE3-Aβ binding affinity was in a major part due to a decrease in k_{diss}. On the other hand, the maximal binding R_{max} decreased as well. NaCl also decreased the K_D of binding of apoA-I and apoA-II to Aβ (Fig. 3B). This was due to a mild increase of k_{ass} for apoA-I and changes of both rate constants for apoA-II.

4. Discussion

The present work demonstrates that human apoE, apoA-I and apoA-II bind to immobilized Aβ with high affinity on the time scale of minutes, whereas albumin as a control protein did not bind. The interaction between these apolipoproteins and Aβ was saturable and reversible. The calculated K_D values were 10 nM for apoE3, 25 nM for apoA-I and 80 nM for apoA-II. ApoJ was shown to have high affinity for Aβ(1–40) (K_D 2 nM by ELISA). All three isoforms of apoE demonstrated significant ability to inhibit apoJ-Aβ(1–40) complex formation and apoE3 was only 6.5 times less efficient than apoJ itself [25]. Thus, the K_D of apoE-Aβ binding obtained by SPR was in good agreement with indirect ELISA data. At the protein concentrations used in the assay (5–50 mg/ml), both apoA-I and apoA-II are predominantly monomeric [26,27], while apoE is mainly tetrameric [28,29]. Nevertheless, analysis of the dissociation phase for apoE-Aβ complex indicated a closely fitting correspondence to a 1:1 complex dissociation model under the experimental conditions, suggesting the absence of allosteric effects.

We estimated that, under our experimental conditions, the binding showed maximum ratios of apo's to Aβ of about 1:100 mol/mol. Although this ratio may apparently depend on the source of Aβ and other conditions [30], it is clear that only a small fraction of the immobilized Aβ molecules are able to bind to apoE on the minute time scale, as followed by SPR. These results confirm the importance of the conformation of Aβ on binding.

The binding of apoE3 to Aβ was not significantly affected in the pH 6.0–8.0 range, while high salt concentrations significantly increased the binding affinity. The latter effect was mainly due to a decreased dissociation rate of the apoE-Aβ complexes in highly polar buffer. The effects of pH and polarity of the buffer were suggested to reflect the major participation of hydrophobic forces in the apoE-Aβ interaction. ApoE has the N-terminal receptor-binding domain and C-terminal domain responsible in major part for lipid binding [28]. Apparently, the Aβ-binding site of apoE is localized in lipid-binding sites. Indeed, delipidation of apoE increases the 'avidity' of apoE-Aβ binding in comparison with lipid-bound apoE [31], indicating the involvement of lipid-binding sites of apoE in the interaction with Aβ. Moreover, it was shown that the C-terminal fragment of apoE 244–272 was critical for formation of apoE-Aβ complexes and the C-terminal domain of apoE was co-purified with Aβ from senile plaques [5,32]. However, the mechanism of the apoE-Aβ binding remains to be elucidated.

The major involvement of hydrophobic forces was also suggested for apoA-I-Aβ but not for the apoA-II-Aβ interaction. The binding of apoA-II to Aβ was affected by pH, indicating significant involvement of other forces in the interaction as well as hydrophobic forces. We suggested that apoA-I may play a role in AD pathogenesis due to its high affinity binding to Aβ.

The SPR technique used in this study is a powerful tool to investigate protein-Aβ interactions. Such a technique may be

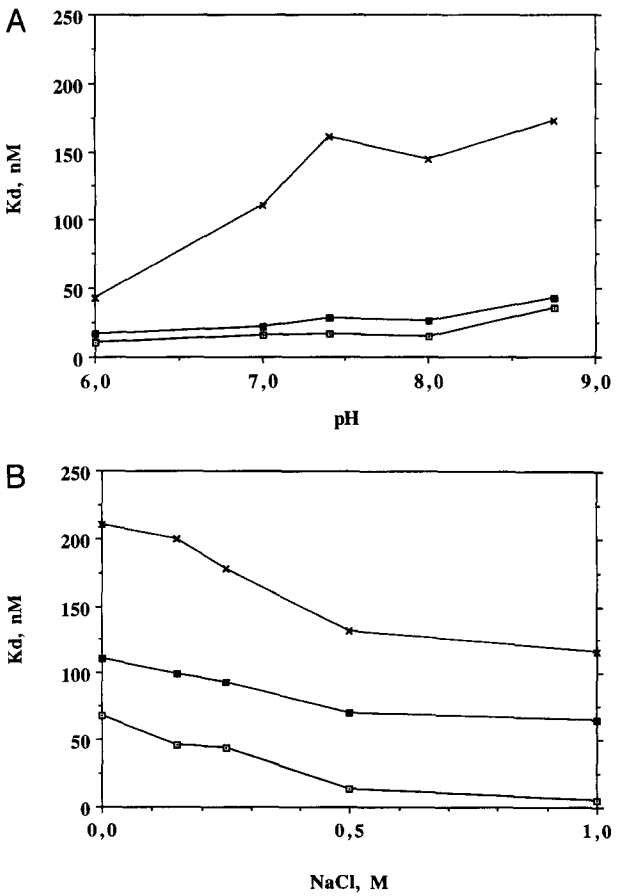

Fig. 3. Effect of pH (A) and NaCl (B) on the equilibrium dissociation constants K_D of the interaction of apoE3 (open boxes), apoA-I (closed boxes) and apoA-II (crosses). The effect of pH was studied in 10 mM phosphate buffer, 0.15 M NaCl and that of NaCl in 10 mM phosphate buffer (pH 7.4).

useful to study the effects of apoE and Aβ polymorphism and to determine potential agents or protein modifications which are able to modulate the interactions.

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