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Non-electrostatic binding and self-association of amyloid β-peptide on the surface of tightly packed phosphatidylcholine membranes

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ABSTRACT

Self-association of amyloid β-peptide (Aβ) is considered to be an initial step in the development of Alzheimer's disease and is known to be promoted by negatively charged lipid membranes. We have examined the possibility of non-electrostatic Aβ–membrane interaction by using neutral phosphatidylcholine lipids. Fluorescence and circular dichroism spectra have clearly shown that Aβ binds to the phosphatidylcholine membrane in the lamellar gel phase but not in the ripple gel or liquid crystalline phase, indicating the importance of the tight lipid packing characteristic of the lamellar gel phase. The Aβ–membrane binding occurs at both low and high salt concentrations, ensuring the non-electrostatic nature of the interaction. The membrane-bound Aβ molecule takes a monomeric α-helical or self-associated β-sheet structure depending on the temperature, peptide/lipid ratio, and salt concentration. The flat surface of tightly packed phosphatidylcholine membranes appears to serve as a platform for non-electrostatic binding and self-association of Aβ.

One of the key events in the development of Alzheimer's disease is self-association of amyloid β-peptide (Aβ) into a fibrillar β-sheet form [1,2]. The 39–43-residue peptide Aβ is produced by proteolytic cleavage of a large transmembrane precursor protein, and the self-association of Aβ is accelerated in the presence of cellular membranes [3]. Among the membrane components, Aβ is known to show affinity for negatively charged lipids such as phosphatidylglycerol and ganglioside, and the peptides bound to negatively charged lipid membranes self-associate into β-sheets [4–6]. Electrostatic interactions of positively charged amino acid residues of Aβ with membrane surface negative charges are thought to play a role in the peptide–membrane binding. In this study, we have examined the possibility of non-electrostatic interaction between Aβ and lipid membranes by using the neutral (zwitterionic) lipid phosphatidylcholine (PC), which is a major component of neuronal cell membranes [7]. Fluorescence and circular dichroism (CD) spectra recorded at varied temperatures have clearly shown that Aβ binds and self-associates on PC bilayer membranes in the lamellar gel ($L_{\beta'}$) phase, but not in the ripple gel ($P_{\beta'}$) or lamellar liquid crystalline (L_{α}) phase at higher temperatures. The flat surface of tightly packed PC membranes characteristic of the $L_{\beta'}$ phase appears to serve as a platform for binding and self-association of $A\beta_{1-40}$. This study gives evidence that non-electrostatic interaction, which has hitherto been disregarded, promotes membrane binding and self-association of Aβ.

Materials and methods

The 40-residue peptide $A\beta_{1-40}$ (DAEFRHDSGYEVHHQKLVFFAED VGSNKGAIIGLMVGGVV) was synthesized on an automated peptide synthesizer (Applied Biosystems, model 431A) by using Fast-Moc chemistry. The crude peptide was incubated in hexafluoroisopropanol for 24 h at room temperature to dissolve possible aggregates. After removal of the alcohol by evaporation, the peptide was purified by HPLC on a reversed-phase column (Nacalai Tesque ODP50-10E). The purified peptide was dissolved in ammonium hydroxide (pH 11), dispensed into aliquots, lyophilized, and stored in a freezer. The amount of peptide in each aliquot was determined from the UV absorption intensity of tyrosine ($\varepsilon_{275} = 1410\ M^{-1}\ cm^{-1}$) at pH 7.4.

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) were obtained from commercial sources and used without further purification. Unilamellar liposomes were prepared by sonication as described previously [8].

Samples for spectral measurements were prepared as follows. Lyophilized $A\beta_{1-40}$ was mixed with liposome suspension at a peptide/lipid molar ratio of 1/300 (16.7 μM peptide and 5 mM lipid) or 1/1200 (2.5 μM peptide and 3 mM lipid). The peptide–liposome mixture was incubated for 15 min above the main phase transition temperature of the lipid. Lipid-free aqueous solutions of $A\beta_{1-40}$ were prepared by dissolving the peptide with sodium phosphate buffer (5 mM, pH 7.4) at a concentration of 16.7 μM. In the absence of lipid, $A\beta_{1-40}$ gave a CD spectrum ascribable to an irregular

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structure, which remained unchanged for 4 days, ensuring the absence of self-associated oligomers and polymers leading to fibril formation.

Fluorescence and CD spectra were recorded on a Jasco FR-6500 spectrofluorometer and a Jasco J-820 spectropolarimeter, respectively. The temperature of the sample was controlled with a constant-temperature circulating bath and each spectral measurement was started after 15 min of thermal equilibration. The background signal due to liposome suspension (or buffer only) was recorded separately in the same cell and subtracted from the spectra of the peptide samples. The CD intensity was expressed as molar ellipticity [θ] per residue.

Results

Binding to DPPC membrane

As a representative of PC lipids, we employed DPPC with two saturated acyl chains of 16 carbon atoms (di-C16:0). The DPPC bilayer membrane undergoes a pretransition between the lamellar gel and ripple gel phases ($L_{\beta'} \leftrightarrow P_{\beta'}$) at 34.4 ± 2.5 °C ($T_p$) and a main phase transition between the ripple gel and lamellar liquid crystalline phases ($P_{\beta'} \leftrightarrow L_\alpha$) at 41.3 ± 1.8 °C ($T_m$) [9].

Fig. 1 shows fluorescence spectra of $A\beta_{1-40}$ at 945 °C in the presence of DPPC liposomes (peptide/lipid molar ratio, 1/300). The fluorescence was excited at 280 nm and the band at 303 nm is assigned to Tyr. With decrease of the temperature, the fluorescence intensity at 303 nm shows a steep rise around 35 °C and continues to grow at lower temperatures. This is in sharp contrast with a weak and linear temperature dependence of the fluorescence intensity in the absence of lipid (see the inset of Fig. 1). The marked growth of fluorescence intensity below 35 °C is ascribed to an interaction of $A\beta_{1-40}$ with the membrane. The $A\beta_{1-40}$–membrane interaction is suggested to be specific to the $L_{\beta'}$ phase because it occurs below T_p.

To further examine the membrane binding of $A\beta_{1-40}$, CD spectra were recorded for $A\beta_{1-40}$–DPPC (1/300) mixtures at temperatures from 46 to 11 °C (Fig. 2). At 46 °C (above T_m) and 36 °C (between T_m and T_p), the CD spectrum is dominated by a negative peak at 198 nm characteristic of an irregular structure [10]. The spectrum is identical to that observed in lipid-free aqueous solution (see Supplementary Fig. S1), suggesting that $A\beta_{1-40}$ does not interact with the membrane at temperatures higher than T_p, where the membrane is in the L_α or $P_{\beta'}$ phase. The absence of $A\beta_{1-40}$–membrane binding above T_p is consistent with the fluorescence spectra in Fig. 1 and with the results of previous studies that used PC membranes in the liquid crystalline phase [4–6].

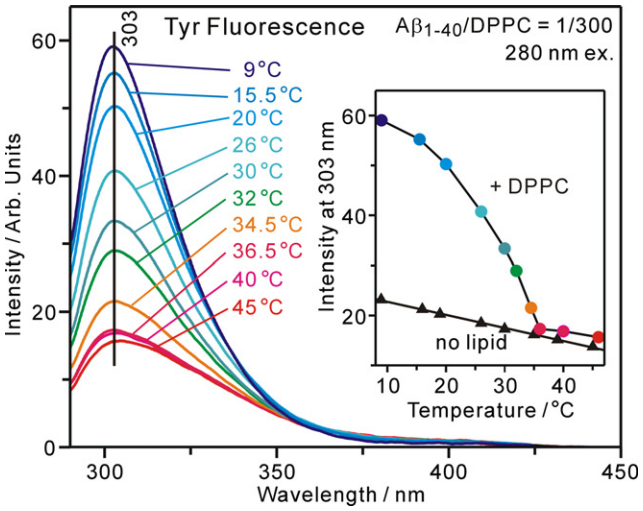

Fig. 1. Fluorescence spectra of $A\beta_{1-40}$ (16.7 μM) at varied temperatures in the presence of DPPC liposomes (5 mM in lipid). The solution was buffered at pH 7.4 with 5 mM sodium phosphate and the fluorescence was excited at 280 nm. The intensity of the Tyr fluorescence band at 303 nm is plotted against temperature in the inset (+DPPC), together with that in the absence of lipid (no lipid).

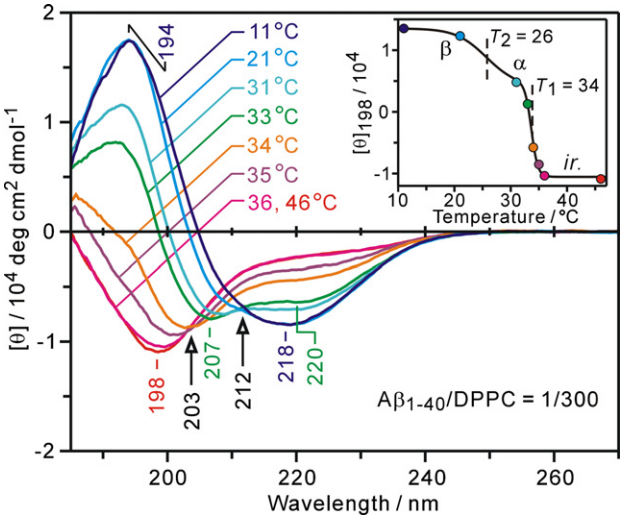

Fig. 2. Temperature dependence of the CD spectrum of $A\beta_{1-40}$ (16.7 μM) in the presence of DPPC liposomes (5 mM in lipid) at pH 7.4 (5 mM sodium phosphate buffer). The arrows at 203 and 212 nm indicate isodichroic points. In the inset, the molar ellipticity at 198 nm $[\theta]_{198}$ is plotted against temperature together with a curve calculated by assuming two sequential transitions at temperatures T_1 and T_2.

Upon crossing T_p (35–33 °C, Fig. 2), the 198-nm negative peak in the CD spectrum loses intensity and instead there grows a double minimum at 207 and 220 nm ascribable to an α-helical conformation [10]. The isodichroic point at 203 nm observed during the spectral change indicates that the irregular-to-α-helical transformation is a two-state transition. A further cooling of the $A\beta_{1-40}$–DPPC mixture down to 11 °C causes another spectral change attributable to a conversion from the α-helical structure to a β-sheet one characterized by a negative peak at 218 nm and a positive peak at 194 nm [10] (Fig. 2). This second transition also involves only two states as judged from the existence of an isodichroic point at 212 nm.

The two sequential transitions of $A\beta_{1-40}$, irregular-to-α-helical and α-helical-to-β-sheet, in the presence of DPPC liposomes were fully reversible with temperature (see Supplementary Fig. S2). To quantitatively analyze the transitions, the molar ellipticity at 198 nm ($[\theta]_{198}$) was plotted against temperature (inset of Fig. 2). The midpoint temperatures of the individual transitions (T_1 and T_2 from high-temperature side) were evaluated from the plot of $[\theta]_{198}$ by assuming that the equilibrium constants can be described by the integrated form of the van't Hoff equation [11]. The T_1 value obtained (34 °C) is in agreement with the pretransition temperature T_p, whereas the T_2 value (26 °C) is not related to any phase transitions of the DPPC membrane [9]. $A\beta_{1-40}$ binds to the DPPC membrane in the $L_{\beta'}$ phase below T_p and the binding is dependent on the membrane structure. By contrast, the conformation of the membrane-bound $A\beta_{1-40}$ (α-helical or β-sheet) seems to be controlled by factors other than the membrane structure.

Effects of the peptide/lipid ratio and salt concentration

The conformation of membrane-bound $A\beta_{1-40}$ is affected by the peptide/lipid ratio and salt concentration. Dilution of the peptide to a 1/1200 peptide/lipid ratio eliminates the second transition

and the CD spectra exhibit only one isodichroic point at 203 nm due to the irregular-to-α-helical transition (Fig. 3A). Addition of 150 mM NaCl produces a single new isodichroic point at 208 nm, indicating a direct conformational transition from irregular to β-sheet (Fig. 3B). These observations are consistently accounted for by assuming a close link between the β-sheet formation and the self-association of the peptide. At low peptide/lipid ratios, the peptide molecule must be isolated in an α-helical conformation stabilized by a single molecular interaction with the membrane. At high salt concentrations, on the other hand, salt ions diminish the electrostatic repulsion among peptide molecules by neutralizing the electric charge, and the peptide molecules self-associates in a β-sheet structure through intermolecular hydrogen bonding. Thus, the β-sheet formation of membrane-bound $A\beta_{1-40}$ reflects the self-association of the peptide, which is greatly enhanced at physiological salt concentration (150 mM NaCl).

In contrast to the conformational change of membrane-bound $A\beta_{1-40}$, neither the peptide/lipid ratio nor the salt concentration affects the initiation temperature of the $A\beta_{1-40}$–membrane binding. This point is clearly seen by comparing the plot of $[\theta]_{198}$ against temperature for three different samples (CD data in Figs. 2 and 3). As Fig. 4 shows, a sharp rise of $[\theta]_{198}$ due to membrane binding occurs at 34 °C irrespective of the peptide/lipid ratio and salt concentration (central three plots). Although the conformation of $A\beta_{1-40}$ at the time of membrane binding differs from α-helical (Figs. 2 and 3A) to β-sheet (Fig. 3B), the peptide binds to the same DPPC membrane structure, i.e. in the $L_{\beta'}$ phase, at the same temperature. Furthermore, the membrane binding is purely non-electrostatic because it is not affected by the salt concentration (Fig. 4).

Binding to other PC membranes

To test whether the coincidence of the membrane binding and the $L_{\beta'}$ phase formation is accidental or not, we have examined by CD spectroscopy the interaction of $A\beta_{1-40}$ with two other PC's having shorter and longer acyl chains: dimyristoyl (di-C14:0, DMPC) and distearoyl (di-C18:0, DSPC). The CD spectra also exhibited temperature-dependent changes as seen in the plots of $[\theta]_{198}$ in Fig. 4 (see Supplementary Figs. S3 and S4 for the original spectra). The T_1 values obtained form the $[\theta]_{198}$ plots are 14 and 46 °C for DMPC and DSPC, respectively, which are in agreement with the T_p values of these lipids within experimental error (13.7 ± 2.2 °C for DMPC and 49.1 ± 2.9 °C for DSPC) [9]. This observation confirms that $A\beta_{1-40}$ specifically binds to the PC membranes in the $L_{\beta'}$ phase.

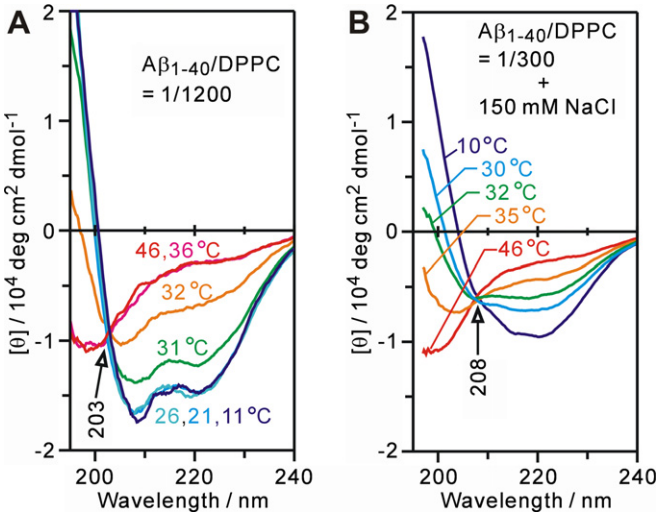

Fig. 3. Effects of the peptide/lipid ratio and salt concentration on the temperature dependence of the CD spectrum of $A\beta_{1-40}$ in the presence of DPPC liposomes. (A) At a peptide/lipid ratio of 1/1200 (2.5 μM $A\beta_{1-40}$ and 3 mM DPPC). (B) In the presence of additional 150 mM NaCl (peptide/lipid ratio, 1/300). The other solution conditions are the same as in Fig. 2.

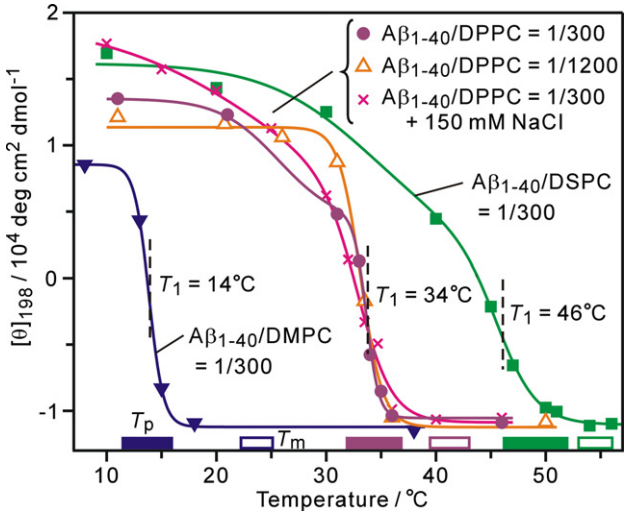

Fig. 4. Molar ellipticity at 198 nm ($[\theta]_{198}$) plotted as a function of temperature for $A\beta_{1-40}$ in the presence of DMPC, DPPC, and DSPC liposomes. The solution conditions are the same as in Figs. 2 or 3. The midpoint temperature of the transition between membrane-bound and unbound states (T_1) is indicated with a vertical line. Filled and empty bars on the temperature axis indicate the regions of reported T_p and T_m values, respectively [9].

Discussion

The specific binding of $A\beta_{1-40}$ to the $L_{\beta'}$ phase described above may be related to structural properties of the membrane. In the high-temperature L_α phase, the membrane structure rapidly fluctuates with rotation and diffusion of conformationally disordered lipid molecules [12]. Although the fluctuation is reduced in the $P_{\beta'}$ phase, the membrane still remains somewhat disordered with bearing periodic ripples on its surface. In the $L_{\beta'}$ phase, on the other hand, the lipid acyl chains are stretched in a predominantly all-*trans* conformation and the lipid molecules are closely packed together to form a tight lamellar structure [12]. The $L_{\beta'}$ phase is characterized by a dense bilayer structure with a flat surface. The binding of $A\beta_{1-40}$ in the $L_{\beta'}$ phase is likely to involve such flat membrane surfaces because the dense acyl chain packing would prevent insertion of the peptide into the bilayer. Actually, $A\beta_{1-40}$ does not affect the *trans-gauche* acyl chain conformational transition as monitored by Raman spectroscopy (see Supplementary Fig. S5).

On the surface of PC membranes in the $L_{\beta'}$ phase, the phosphocholine head groups $-PO_3^-–(CH_2)_2–N^+(CH_3)_3$ are arranged in a two-dimensional lattice with restricted orientational freedom [12,13]. Despite the positive charge, the choline moiety can serve as a non-electrostatic interaction site because the charged ammonium nitrogen is shielded by three hydrophobic methyl groups. According to a model for the gel phase PC membrane [14], the choline moieties are closely arranged and fully exposed on the outermost surface of a tightly packed lipid bilayer. $A\beta_{1-40}$ is likely to bind to such a choline-exposed surface by using hydrophobic amino acid residues in the C-terminal half of the peptide. The binding and self-association of $A\beta_{1-40}$ on the PC membrane surface is consistent with a recent finding by atomic force and fluorescence microscopy that $A\beta_{1-42}$, which has a much stronger propensity

for aggregation than $A\beta_{1-40}$, accumulates on the surface of gel phase PC membranes to form nm-sized aggregates [15].

The non-electrostatic binding and self-association of $A\beta_{1-40}$ on the surface of tightly packed PC membranes revealed here is a new type of interaction that can occur at physiological temperature (~37 °C), pH (~7.4), and salt concentration (~150 mM). This study raises the possibility that such non-electrostatic interaction promotes self-association of Aβ on neuronal cell membranes.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.08.093.

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