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Alzheimer's Disease Amyloid β Peptide 25-35 Is Localized in the Membrane Hydrocarbon Core: X-Ray Diffraction Analysis

R. Preston Mason,[1] Janet D. Estermyer, Jeremiah F. Kelly,* and Pamela E. Mason

*Neurosciences Research Center, Allegheny Campus, Medical College of Pennsylvania and Hahnemann University, Pittsburgh, Pennsylvania 15212-4772; and *Molecular Physiology and Genetics Section, Gerontology Research Center, National Institute on Aging, Baltimore, Maryland 21224*

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Alzheimer's disease (AD) neuropathology is characterized by neuritic plaques composed primarily of amyloid β peptide (Aβ). An elevation in Aβ in the cerebral cortex has been implicated in the pathophysiology of AD but its mechanism of action is unknown. The addition of Aβ protein to neuronal cell cultures produces changes in the activity of various membrane proteins, including ion channels and receptors, potentially as a result of intercalating into the membrane bilayer. In this study, the interactions of the Aβ fragment 25-35 [Aβ(25-35)] with liposomes were directly examined by small angle x-ray diffraction approaches. One-dimensional electron density profiles generated from the diffraction data demonstrated that the addition of Aβ(25-35) produced a discrete increase in electron density 0–12 Å from the center of the lipid bilayer. Under these conditions, the membrane concentration of Aβ(25-35) was 860-fold higher than in the aqueous buffer. These findings indicate that this peptide is highly lipophilic and inserts into the membrane hydrocarbon core. Following the intercalation of Aβ(25-35) to this location in the membrane, the protein fragment may interact with regulatory membrane proteins.

A characteristic neuropathological lesion in the cerebral cortex of patients with AD is the neuritic plaque, composed of a 39–43 residue protein known as amyloid β peptide (Aβ). The Aβ peptide has been implicated in the pathophysiology of AD, as it has been demonstrated to have potent cytotoxic effects in cultured cortical neurons, potentially as a result of changes in calcium homeostasis (1–3) and membrane integrity (4). The hypothesis that Aβ interacts with the membrane as part of its mechanism of neurotoxicity is further supported by the fact that this protein is able to alter membrane lipid dynamics (5) and the activity of various membrane-bound proteins, including enzymes (3), ion channels (6,7), receptors (1,8,9) and receptor G-proteins (10). In addition, there is evidence that Aβ spontaneously incorporates into the cell membrane to form cation channels (11). These studies indicate that the β/A4 peptide interacts with the membrane as part of its mechanism of action.

In this study, the molecular structure of lipid bilayers in the presence of the neurotoxic fragment Aβ(25-35) was analyzed with the use of small angle x-ray diffraction. Fourier analysis of the x-ray diffraction data indicated that the addition of Aβ(25-35) produced a discrete increase in electron density though a broad region of the membrane hydrocarbon core. The results of this study demonstrated that Aβ(25-35) is lipophilic and spontaneously intercalates into the membrane bilayer.

MATERIALS AND METHODS

All chemicals used were reagent-grade or better and made up in ultra-pure deionized water. 1-Palmitoyl-2-oleoyl phosphatidylcholine (POPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and stored at −80°C. The Aβ(25-35) peptide was purchased from Bachem California (Torrance, CA) and immediately prepared for experiments by dissolving in water at a 1.0 mg/ml concentration.

Preparation of membrane multilayers for x-ray diffraction analysis. Oriented membrane multilayer samples of POPC in the absence and presence of Aβ(25-35) were prepared as follows. Phospholipids dissolved in chloroform were dried down

[1] Corresponding author. Fax: (412)359-6390.

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with a stream of N_2 gas to a thin film on the sides and bottom of a glass 13 × 100 mm test tube while vortexing. Residual solvent was removed under vacuum overnight. A specified volume of buffer (0.5 mM HEPES, 2.0 mM NaCl, pH 7.3) in the absence and presence of freshly prepared Aβ(25-35) was added to the dried lipids while vortexing, yielding a final phospholipid concentration of 5.0 mg/ml. The phospholipid phosphorus content of the samples was determined by previously described methods (12,13). Multilamellar vesicles (MLV) were formed by vortexing the buffer and lipids for three minutes at ambient temperature (14).

Multilayer samples for small angle x-ray scattering were prepared by centrifugation of 250 μg of MLV in a Sorvall AH-629 swinging bucket ultracentrifuge rotor (Dupont Corp., Wilmington, DE) at 35,000 × g for 90 minutes at 5°C in Lucite sedimentation cells, each containing an aluminum foil substrate (15). Greater than 98% of the sample phospholipid was measured in the pellet following centrifugation. The amount of Aβ(25-35) associated with the lipid vesicles was calculated for several protein-to-lipid mass ratios (1:10, 1:5 and 1:2.5) with trials consisting of 6 samples each, except at the 1:5 ratio, in which two trials ($n = 12$) were conducted. Following centrifugation, the supernatant was immediately removed and the concentration of Aβ(25-35) was measured using the Bio-Rad (Hercules, CA) dye-binding procedure. The amount of Aβ(25-35) bound to liposomes is thus the difference between total Aβ(25-35) concentration and the amount of Aβ(25-35) measured in the supernatant. For each trial, a mean of the percent of protein bound to the liposomes and the partition coefficient were calculated, as previously described (16). The final percent of bound protein and partition coefficient value were calculated from the average mean ±S.D. of all four trials.

Prior to x-ray diffraction analysis, the samples were equilibrated overnight in a glass vial containing a saturated salt solution ($K_2C_4H_4O_6$) which served to define a specific relative humidity of 74% at 20°C. The membrane samples were then placed in sealed brass canisters containing aluminum foil windows in which temperature and relative humidity were controlled (15).

X-ray diffraction data collection and analysis. Small angle x-ray scattering was carried out by aligning the membrane multilayer samples at near-grazing incidence with respect to the x-ray beam. The radiation source was a collimated, monochromatic x-ray beam (CuK_α, $\lambda = 1.54$ Å) from a Rigaku RU200 (Danvers, MA) rotating anode microfocus generator. The fixed geometry beamline utilized a single Franks mirror, providing nickel-filtered radiation ($K_{\alpha 1}$ and $K_{\alpha 2}$ unresolved) at the detection plane. The beam height at the sample was ~3 mm.

Bragg's diffraction data from the oriented membrane multilayer samples were recorded on a one-dimensional position-sensitive electronic detector (Innovative Technologies, Inc., Newburyport, MA). In addition to direct calibration of the detector system, cholesterol was used to verify the calibration. The sample-to-detector distance used in these experiments was 150 mm. Each individual lamellar diffraction peak was background-corrected using a linear subtraction routine which averaged the noise. The intensity functions were corrected by a factor of $s = 2\sin\Theta/\lambda$, the Lorentz correction, in which λ is the wavelength of radiation (1.54 Å) and Θ is the Bragg angle equal to one half of the angle between the incident beam and the scattered beam. A swelling analysis was used to assign unambiguous phases to the experimental structure factors (17).

RESULTS

Effect of Aβ(25-35) on Membrane Structure

X-ray scattering from membrane multilayers composed of POPC and Aβ(25-35) produced a highly reproducible meridional diffraction pattern at 5°C and 20°C. In the presence of 2.0 mM NaCl, the control POPC lipid bilayer *d*-space value (the distance between the center of one membrane bilayer to the next, including surface hydration) was 60.8 ± 0.3 Å, and intrabilayer headgroup separation was 48 Å. Following addition of Aβ(25-35) at a 1:5 protein to lipid mass ratio, the *d*-space value was not significantly changed, 61.1 ± 0.3 Å. At 20°C, the POPC membrane *d*-space values were 53.6 Å and 53.2 Å in the absence and presence of Aβ(25-35), respectively, and the intrabilayer headgroup separation was 40 Å. The reduction in membrane width at 20°C is attributed to increased phospholipid acyl chain trans-gauche isomerization as a result of increased thermal energy in the sample.

In Figure 1, centrosymmetric electron density profiles were superimposed for membrane samples at 5°C and 20°C prepared either in the absence or presence of Aβ(25-35). By way of interpretation of the figures, the two peaks of electron density on either side of the figure correspond to phospholipid headgroups, while the minimum of electron density at the center of the membrane is associated with terminal methylene segments. Differences in electron density are indicated by the shaded area in the center of the figure. The addition of Aβ(25-35) peptide produced a discrete increase in electron density 0–12 Å from the center of the membrane lipid bilayer. The greatest area of contrast or increased electron density was observed 7 Å from the center of the membrane bilayer.

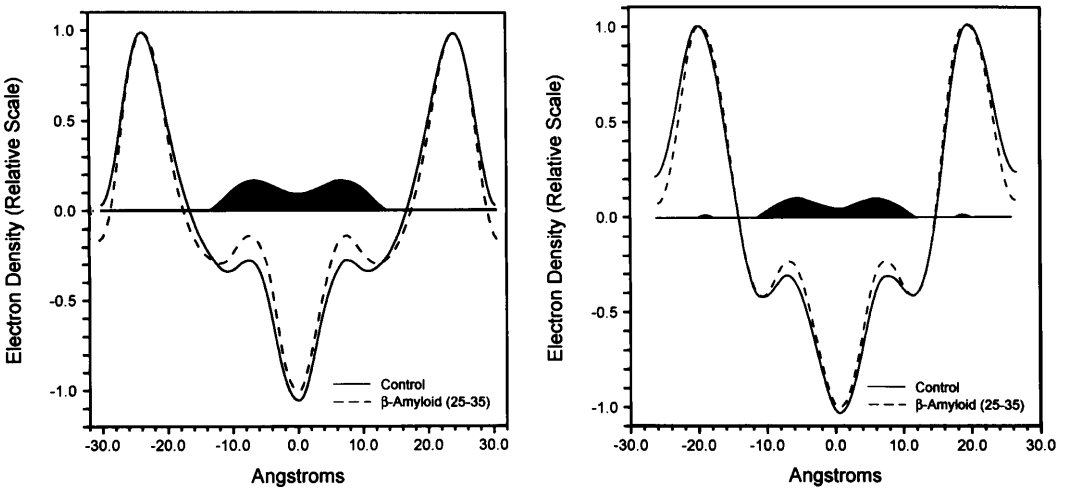

FIG. 1. Superimposed one-dimensional membrane electron density profiles (Å versus electrons/Å^3) of centrosymmetric phospholipid bilayers in the absence and presence of Aβ(25-35) at a 1:5 protein:lipid mass ratio. These experiments were carried out in buffer containing 2.0 mM NaCl and at 5°C (left figure) and 20°C (right figure). The positive difference in electron density corresponding to the time-averaged membrane distribution of the peptide is represented by the shaded region in the centers of the figures.

These data indicate that the equilibrium location of Aβ(25-35) is in the membrane hydrocarbon core and extends from the center of the membrane to near the glycerol backbone of the phospholipid molecules.

The lipid bilayer electron density profiles indicated that the increases in electron density following the addition of β/A4 peptide fragment were restricted to the membrane hydrocarbon core; there was no increase in electron density associated with the hydrated surface of the membrane. Moreover, the center-of-mass positions of the phospholipid headgroups were not altered by the addition of protein. As a control, these experiments were carried out in the absence of salt in the buffer. Under the latter conditions, any increase in electron density following the addition of Aβ(25-35) was observed only in the interbilayer water space and not in the hydrocarbon core (data not shown).

Membrane Binding of Aβ(25-35)

The membrane-based equilibrium partition coefficient of Aβ(25-35) was determined over a range of Aβ(25-35) to phospholipid mass ratios from 1:10 to 1:2.5. The average percent of protein bound to the liposomes was 50.95 ± 4.60% (n = 4, mean ± S.D.) at 5°C. The calculated equilibrium partition coefficient of Aβ(25-35) was 860 ± 170 (n = 4, mean ± S.D.).

DISCUSSION

The Aβ peptide has been implicated in the pathophysiology of AD, as it has been demonstrated to have potent cytotoxic effects in cultured cortical neurons, potentially as a result of changes in membrane function and structure (1–5,20). In this study, the molecular membrane interactions of the neurotoxic fragment of Aβ, Aβ(25-35), was directly examined by small angle x-ray diffraction. Figure 1 is a plot of molecular electron density (electrons/Å^3) as a function of distance across a single membrane bilayer unit cell. A direct subtraction of the control membrane bilayer profile from the protein-containing membrane sample demonstrated a positive difference in electron density 0–12 Å which is attributed to the equilibrium location of Aβ(25-35). The addition of Aβ(25-35) to the membrane did not perturb the overall width of the membrane lipid bilayer, including intrabilayer phosphate headgroup separation. Under these experimental conditions, the

25 26 27 28 29 30 31 32 33 34 35

H_2N--Gly--Ser--Asn--Lys--Gly--Ala--Ile--Ile--Gly--Leu--Met--OH

FIG. 2. The primary amino acid sequence of Aβ(25-35).

concentration of Aβ(25-35) in the liposomes was 860-fold higher than in the aqueous buffer; this partition coefficient was observed over a range of protein concentrations.

The results of these x-ray diffraction experiments and the chemical properties of the Aβ(25-35) fragment, the following molecular model for protein/membrane interaction is proposed. This model is based on the assumption that the Aβ(25-35) fragment assumes is in a monomer conformation in the presence of the phospholipid bilayer. The charged lysine residue may be placed near the hydrocarbon core/water interface or ester linkage of phospholipid molecules, approximately 13–15 Å from the center of the POPC membrane bilayer. The seven non-polar amino acids of the carboxyl terminus (Gly-Ala-Ile-Ile-Gly-Leu-Met-OH) could extend into the hydrocarbon core (including lysine, these eight residues in an α-helical conformation would be approximately 12 Å in length). These amino acids would contribute most to the large contrast or positive difference in electron density observed in the profiles (Fig. 1). The remaining three amino terminus amino acids (Gly-Ser-Asn) would overlap the electron-dense phosphate headgroup region of the membrane bilayer. This model for Aβ(25-35) is consistent with theoretical models for the structure of membrane-bound amyloid β/A4 (Fig. 2) (18).

In the absence of salt, the addition of Aβ(25-35) produced an increase in electron density associated only with the hydrated membrane surface or interbilayer water space. These findings are consistent with titration calorimetry experiments (19) which suggest that Aβ(25-35) has strong electrostatic interactions with membrane phospholipid headgroups only in the absence of NaCl. In these experiments, the addition of 0.1 mM NaCl abolished electrostatic interactions between Aβ(25-35) and the membrane, as evidenced by a change in membrane enthalpy (19). Based on these results, we would suggest that the interactions of Aβ(25-35) fragment with the membrane hydrocarbon core in the presence of salt is governed primarily by entropic as opposed to enthalpic forces.

The results of the x-ray diffraction analysis and equilibrium membrane binding measurements indicate that Aβ(25-35) has strong hydrophobic interactions with the membrane hydrocarbon core. The presence of Aβ(25-35) at elevated concentrations in the membrane may underlie alterations in membrane thermodynamic properties (19) and steric interactions with membrane proteins, leading to altered membrane fluidity, signal transduction and calcium metabolism in AD (Muller et al., 1995; Pettegrew, 1989; Mattson et al., 1992; Roth et al., 1995).

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