ELSEVIER

Chemistry and Physics of Lipids 120 (2002) 57–74

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

www.elsevier.com/locate/chemphyslip

Membrane destabilization induced by β-amyloid peptide 29-42: Importance of the amino-terminus

Marie-Paule Mingeot-Leclercq [a,*], Laurence Lins [b], Mariam Bensliman [a], Françoise Van Bambeke [a], Patrick Van Der Smissen [c], Jacques Peuvot [d], André Schanck [e], Robert Brasseur [f]

[a] *Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, Avenue E. Mounier 73, Bt 7370, B-1200 Brussels, Belgium*

[b] *INSERM U447, IBL, F-59021 Lille Cedex, France*

[c] *Unité de Biologie Cellulaire, Université Catholique de Louvain, B-1200 Brussels, Belgium*

[d] *UCB S.A. Pharma Sector, B-1420 Braine-l'-Alleud, Belgium*

[e] *Unité de Chimie Structurale et des Mécanismes Réactionnels, Université Catholique de Louvain, B-1348 Louvain-La-Neuve, Belgium*

[f] *Centre de Biophysique Moléculaire Numérique, Faculté Universitaire des Sciences Agronomiques, B-5030 Gembloux, Belgium*

Received 28 January 2002; received in revised form 23 May 2002; accepted 22 July 2002

Abstract

Increasing evidence implicates interactions between Aβ-peptides and membrane lipids in Alzheimer's disease. To gain insight into the potential role of the free amino group of the N-terminus of Aβ29-42 fragment in these processes, we have investigated the ability of Aβ29-42 unprotected and Aβ29-42 N-protected to interact with negatively-charged liposomes and have calculated the interaction with membrane lipids by conformational analysis. Using vesicles mimicking the composition of neuronal membranes, we show that both peptides have a similar capacity to induce membrane fusion and permeabilization. The fusogenic effect is related to the appearance of non-bilayer structures where isotropic motions occur as shown by ^{31}P and ^{2}H NMR studies. The molecular modeling calculations confirm the experimental observations and suggest that lipid destabilization could be due to the ability of both peptides to adopt metastable positions in the presence of lipids. In conclusion, the presence of a free or protected (acetylated) amino group in the N-terminus of Aβ29-42 is therefore probably not crucial for destabilizing properties of the C-terminal fragment of Aβ peptides.

Keywords: Amyloid peptides; Fusion; Permeability; NMR; Conformational analysis

1. Introduction

Senile plaques and paired helical filaments are the hallmarks of brain pathology in Alzheimer's disease (Selkoe, 1999). The major component of

* Corresponding author. Tel.: +32-2-764-73-74; fax: +32-2-764-73-73

E-mail address: mingeot@facm.ucl.ac.be (M.-P. Mingeot-Leclercq).

0009-3084/02/$ - see front matter
PII: S0009-3084(02)00108-1

the senile plaque is the β-amyloid peptide (Aβ). This peptide is generated in vivo through proteolytic cleavage of the membrane-anchored β-amyloid precursor protein (APP) (Kang et al., 1987; Selkoe, 1993) by β-secretase (at the luminal side of the membrane) (Sinha and Lieberburg, 1999) and γ-secretase (within its transmembrane domain) (Selkoe, 1999). The latter is heterogeneous but principally leads to 40 (Aβ1-40) and 42 (Aβ1-42) amino acid peptides in roughly 9:1 proportion. β-Amyloid peptides are amphiphilic with an extracellular hydrophilic N-terminus (residues 1-28) and a membrane-spanning C-terminus region (residues 29-40 or 29-42) (Coles et al., 1998). Importantly, Aβ1-42 is more hydrophobic, and considerably more prone to nucleation and fibril formation than Aβ1-40 (Jarrett et al., 1993; Hartmann et al., 1997). Its increase is observed in all genetic mutations leading to autosomal dominant familial Alzheimer's disease (Selkoe, 1997; Hardy, 1997).

Results from several studies suggest that Aβ neurotoxicity might be mediated through direct interaction between Aβ peptides and cellular membranes (Arispe et al., 1993a,b; Terzi et al., 1995, 1997; Cribbs et al., 1997; Hertel et al., 1997). Experimental evidences prove the interaction of amyloid peptides with lipids such as phosphoinositides (Decout et al., 1998; McLaurin et al., 1998a), phosphatidylglycerol (Terzi et al., 1995), cholesterol, phosphatidylcholine, stearic acid (Avdulov et al., 1997), and gangliosides (McLaurin and Chakrabartty, 1996; McLaurin et al., 1998b; Choo-Smith and Surewicz, 1997; Matsuzaki and Horikiri, 1999). By small angle X-ray diffraction approaches, Mason et al. (1996) demonstrated that amyloid peptide (25-35 fragment) intercalates deep into the membrane bilayer hydrocarbon core.

Interactions between Aβ or their fragments with lipids are probably responsible for membrane destabilization in accordance with the effect of Aβ on membrane permeabilization in models of liposomes or in cells (Arispe et al., 1993a,b; Pillot et al., 1996; McLaurin and Chakrabartty, 1996, 1997; McLaurin et al., 1998b; Rhee et al., 1998; Yang et al., 1998), fusion of small unilamellar vesicles (Pillot et al., 1996), imposition of negative curvature strain on ganglioside-containing lipid bilayers (Matsuzaki and Horikiri, 1999), and changes in membrane fluidity (Avdulov et al., 1997; Müller et al., 1995; Mason et al., 1999; Eckert et al., 2000; Kremer et al., 2000, 2001).

Both the extracellular hydrophilic N-terminus and the membrane-spanning C-terminus are involved for the interaction of amyloid peptides with membranes. First, Decout et al. (1998) suggested that electrostatic interactions between the positive charges of the N-terminal domain of the β-amyloid peptides and the negative charges of the cellular membranes may play a role for the amyloid peptide cytotoxicity. The importance of electrostatic interactions has been ascertained by several experiments showing that (i) Aβ1-40 binding to phosphatidylglycerol is completely inhibited by the presence of NaCl (Terzi et al., 1995), (ii) membrane binding of the Aβ1-40 and Aβ1-42 peptides is maximal when these membranes contain acidic phospholipids (McLaurin and Chakrabartty, 1996) and (iii) compounds that decrease the effective negative charge of membranes prevent the association of Aβ1-40 to negatively-charged vesicles and Aβ-induced toxicity (Hertel et al., 1997). Second, it has been demonstrated that the hydrophobic C-terminal domain of Aβ is critical for amyloid aggregation, fibril formation (Lansbury et al., 1995) and apoptosis (Pillot et al., 1999). The C-terminal domains of two opposite dimers could be extended to form antiparallel β-sheet (Chaney et al., 1998). From conformational studies, Pillot et al. (1996) and Brasseur et al. (1997) have suggested that Aβ29-40 and Aβ29-42 interact with lipids as tilted peptides. These peptides penetrate the lipid bilayer at an angle of 30–70° (relative to the bilayer plane) and therefore could induce membrane destabilization (Brasseur, 1991; Martin et al., 1994; Pillot et al., 1996; Brasseur et al., 1997; Peuvot et al., 1999). Recent neutron diffraction experiments have confirmed the existence of oblique-oriented peptides in lipid bilayers (Bradshaw et al., 2000).

Part of these studies have used the 29-42 extremity of the amyloid peptide instead of the entire 1-42 peptide because it is actually the portion of the peptide which interacts with lipids. Our aim was to investigate the importance for membrane destabilization of a free aminated

function generated on the residue 29 of Aβ29-42 fragment. We therefore compare in the present study the membrane destabilizing properties of the 29-42 fragment under an unprotected form and a protected form (obtained by acetylation of the free terminal amine to mimic a peptidic bound).

For this purpose, we examined the capacity of both peptides to induce membrane fusion and alteration of membrane permeability in relation to a potential effect on membrane fluidity or phospholipid organization. For this last point, we investigated by ^{31}P and ^{2}H NMR the influence of the peptide on the spectral shapes. In parallel, we applied a recent computational method called IMPALA (Ducarme et al., 1998) to investigate the behavior of unprotected and N-protected Aβ29-42 peptides to interact with a model membrane.

2. Materials and methods

2.1. Fluorescence, light scattering and electron microscopy studies

These studies were performed on small unilamellar vesicles, which are known to be highly susceptible to the action of membrane destabilizing agents. They were made of phosphatidylcholine (PC):phosphatidylethanolamine (PE):phosphatidylinositol (PI):phosphatidylserine (PS):sphingomyelin (SM):cholesterol (Chol) (30%:30%:2.5%:10%:5%:22.5%) to mimic the composition of the neuronal membrane (Norton et al., 1975; Ansell and Hawthorne, 1964).

2.1.1. Preparation of small unilamellar vesicles (SUV liposomes)

Liposomes were prepared in Tris buffer pH 8 (Tris 10 mM, NaCl 150 mM, EDTA 0.1 mM, NaN_3 1 mM) as described previously (Mingeot-Leclercq et al., 2001). The actual phospholipid concentration of each preparation was determined by phosphorus assay (Bartlett, 1959). Total lipid concentration was calculated assuming a similar recovery of phospholipids and cholesterol. Liposomes were used the day following their preparation.

2.1.2. Fusion of lipidic phase

These studies were made exactly as described earlier (Van Bambeke et al., 1995), using a mixture of labeled and unlabeled vesicles (final concentration in lipids: 5 μM), and recording the increase in fluorescence due to the dequenching of octadecylrhodamine B chloride (R_{18}) upon its dilution (Hoekstra et al., 1984) after liposomes fusion. Labeled and unlabeled liposomes were mixed at a ratio of 1:4. Peptides were added and the fluorescence was immediately recorded at room temperature during 25 min, using an excitation wavelength of 560 nm and an emission wavelength of 590 nm (Perkin-Elmer LS-30, Perkin-Elmer Ltd., Beaconsfield, UK). Results were expressed as fluorescence values calculated as the difference between the fluorescence signal recorded for the mixing of labeled and unlabeled liposomes and that recorded in identical experimental conditions for the same amount of labeled liposomes mixed with buffer. This correction was made to rule out any interference of the peptide with the fluorescence of the probe.

2.1.3. Determination of the size of liposomes

The apparent average diameter of small unilamellar vesicles was determined by quasi-elastic light scattering spectroscopy (Mazer et al., 1979) using a Coulter® Nano Sizer™ N_4MD (Coulter Electronics Ltd., Luton, UK) as described earlier (Mingeot-Leclercq et al., 1990). Data were analyzed using size distribution analysis mode to determine the full size distribution profile of liposomes incubated with the β-amyloid peptides.

2.1.4. Negative staining

Liposomes were prepared as described above, but at a concentration of 0.157 mM in lipids with 2 mM borate buffer pH 8.0. Peptides were added to liposomes at a ratio peptide:lipid of 2 and the mixture was added to a solution of 2% phosphotungstic acid pH 7.0. The solution was thereafter sprayed on a glow-discharged, formvar coated grid and observed in a Philips CM12 electron microscope operating at 80 kV.

2.1.5. Permeability studies

The release of calcein, entrapped at a self-quenching concentration in liposomes, was followed by the increase of fluorescence upon dilution following their leakage from the vesicles (Weinstein et al., 1977; Van Bambeke et al., 2000). The dried lipid films were hydrated with a solution of purified calcein [16.3 mM; 461 mOsm/kg (measured by the freezing point technique (Advanced Instruments, Needham Heights, MA))]. The unencapsulated dye was eliminated by the minicolumn centrifugation technique (Lelkes, 1984) and the liposomes were diluted to a final lipid concentration of 5 μM in an iso-osmotic 231 mM Tris buffer pH 8 (Tris 10 mM, NaCl 220 mM, EDTA 0.1 mM, NaN_3 1 mM) (461 mOsm/kg). After addition of peptides, the percentage of calcein released was immediately determined as described in Van Bambeke et al. (2000).

2.1.6. Fluidity studies

Membrane fluidity was studied by measuring the degree of fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) (Kitagawa et al., 1991; Kaiser and London, 1998) as a function of temperature. Incorporation of the fluorescent markers, at a molar ratio to the lipids of 1:250, was obtained by vigorous mixing followed by a preincubation at 37 °C in the dark during 1 h. Labeled small unilamellar vesicles were mixed with peptides (final concentration of 0.314 mM in total lipid) and preincubated at 37 °C for 30 min. They were thereafter brought to 55 °C over 15 min and maintained at that temperature during 5 min for stabilization before starting measurements. The fluorescence was recorded as described in Mingeot-Leclercq et al. (2001) on a LS-50 Perkin-Elmer fluorimeter (Perkin-Elmer, Beaconsfield, UK), equipped for polarization measurements and operating at an excitation wavelength of 365 nm (for DPH) or 360 nm (for TMA-DPH) and an emission wavelength of 427 nm (for DPH) or 435 nm (for TMA-DPH).

2.2. NMR studies

2.2.1. ^{31}P NMR

Multilamellar vesicles (MLV) of the same composition as that used for fluorescence and light scattering studies were prepared as follows: organic solutions of the lipids were dried under vacuum, hydrated over 1 h at 37 °C in Tris buffer pH 8 (Tris 10 mM, NaCl 150 mM, EDTA 0.1 mM, NaN_3 1 mM) at a concentration of 10 mM in phospholipids, maintained at 37 °C for a further hour and finally submitted to five freeze-thawing cycles.

Peptide-containing samples were prepared by the following procedure: peptides were first dissolved in trifluoroacetic acid (Jao et al., 1997), the solvent removed with N_2 gas and the sample dried under vacuum. The peptide, dissolved in DMSO (6 mg/ml) was added to the organic solution of phospholipids before MLV preparation.

^{31}P NMR spectra were obtained at 101.3 MHz with a Bruker AC 250 spectrometer. Two milliliters of MLV suspension was used in 10 mm NMR tubes. D_2O (15%) was added for locking on the deuterium signal. Fourier transform conditions were: 25 kHz spectral width, 4K data points, flip angle 40° (10 μs), 1.2 s pulse interval. Five thousand scans were accumulated and a 50 Hz line broadening was applied to the free induction decay before Fourier transformation. Powergated ^1H decoupling was applied. Experiments were conducted as a function of temperature: samples were heated from 35 to 65 °C and cooled down to 35 °C with 30 min equilibration times between each accumulation at each new temperature.

2.2.2. ^{2}H NMR

Aqueous solution of binary mixtures of long- and short-chain phosphatidylcholine can form well-oriented, nematic phase of bilayered discoidal mixed micelles called bicelles (Sanders and Landis, 1995). We used dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidycholine (DHPC) in a molar ratio DMPC:DHPC of 3.5:1. The DMPC solution in $CHCl_3$ (containing 25% of DMPC-d_{54}) was evaporated under vacuum, resuspended in ^2H depleted water, vortexed, centrifuged and freeze-thawed to obtain an homogeneous

slurry. Stock solution of DHPC in ^2H depleted water was added to the DMPC suspension and the final phospholipid content of 20% (w/w) adjusted with 50 mM MES (2-morpholinoethane sulfonic acid monohydrate), pH 6.

Peptide-containing samples were prepared using the same procedure as for ^{31}P NMR studies but the Aβ29-42 in DMSO (see supra) was added to a $CHCl_3$ solution of DMPC. The solvents were evaporated under vacuum, the dry peptide-DMPC mixture resuspended in ^2H depleted water and the suspension treated as described above before addition of DHPC solution and buffer.

NMR samples of 200 μl were introduced in short 5 mm tubes and sealed tightly. Before data acquisition, the samples were kept at 37 °C for 1 h to allow equilibrium alignment to be established. Deuterium NMR spectra were recorded at 55.3 MHz on a Chemagnetics CMX 250/360 spectrometer, the standard quadrupole echo sequence (Davis, 1983) with $\tau = 50$ μs, 90° pulse of 2.1 μs and a repetition time of 1.2 s was used. Four thousand scans were accumulated. 4K data points were acquired, fractionally left-shifted to start the record at the top of the echo, baseline-corrected and zero-filled to 16K. A line broadening of 100 Hz was applied to the free induction decay prior to Fourier transformation.

2.3. Molecular modeling

2.3.1. 3D construction of the peptides

Construction of the two helical peptides was carried out as previously described (Brasseur et al., 1992). Conformation of the side chains was optimized by a Simplex energy minimization. The N-terminus of the peptides was either free or blocked by an acetyl group.

2.3.2. The IMPALA procedure

This method allows the study of interactions between a peptide and a modeled membrane using simple restraint functions designed to mimic properties of the membrane as described elsewhere (Ducarme et al., 1998; Lins et al., 2001). Briefly, The interface is described by a $C(z)$ function. $C(z)$ can be considered as the water concentration with a value of 1 outside the membrane and 0 in the membrane core. $C(z)$ varies between 1 and 0 in the region corresponding to the lipid/water interface (18 Å $> z >$ 13.5 Å). Two restraints are used to mimic membrane properties, mainly the hydrophobic effect (named E_{int}) and the lipidic perturbation (named E_{lip}), depending upon the accessible surface of the peptide atoms. For more details, see Ducarme et al. (1998) and Lins et al. (2001).

The starting position of the peptide was obtained using the TAMMO procedure. This algorithm calculates the position of a molecule versus a planar lipid/water interface taking into account the hydrophobic and hydrophilic centers of the peptide (see Brasseur, 1990).

No modification of the internal structure of the peptide was allowed so that the Coulomb, Van der Waals, H bonds and torsion energies are considered as constants.

The interaction of the peptide with the modeled bilayer was analyzed by a Monte-Carlo simulation of 100,000 steps at 298 K. For each peptide, calculations were repeated 10 times. Three freedom degrees were tested (two rotations and one translation along the z-axis). Maximal 5° rotations and 1 Å translations were allowed. The position of the structure with the lowest restraint values was considered as the most stable in the bilayer.

2.3.3. Restraints plots

Diagrams showing the restraint values as a function of the angle between the helix axis and the bilayer normal and as a function of the penetration of the mass center were obtained as follows: for each degree (angle) or for each 1/10 Å (penetration), the lowest restraint value obtained during the Monte-Carlo simulation was taken. All the points were then joined to generate a profile of the simulation.

Calculations were performed on parallel hardware RAMSES Beowulf connected by a 100 MB network and controlled by a HP Vectra VA Pentium Pro cadenced at 200 MHz (RAMSES architecture, Beuve-Mery et al., 2001). The calculation software has been developed at the CBMN (Gembloux, Belgium). Molecular graphs were drawn by using WinMGM 1.0 (Ab Initio Technology, Obernai, France) and Sigmaplot 5.0 (SPSS, Inc.) was used for the data analysis.

2.4. Materials

β-Amyloid peptides 29-42 (Aβ29-42) were synthesized by Polypeptides Laboratories (Wolfenbüttel, Germany). The identity and purity were controlled by mass spectral analysis. Peptides were solubilized in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) except for NMR measurements (cf. supra). The sequence of Aβ29-42 is Gly–Ala–Ile–Ile–Gly–Leu–Met–Val–Gly–Gly–Val–Val–Ile–Ala (referred throughout the paper to Aβ29-42 unprotected) was compared with that of the same peptide with an acetyl protection at the N-terminal (referred to Aβ29-42 N-protected). Egg yolk phosphatidylcholine, wheat germ phosphatidylinositol, and egg yolk phosphatidylethanolamine (grade 1) were purchased from Lipid Products (Nr Redhill, UK). Sphingomyelin and cholesterol were obtained from Sigma Chemical Co. (St Louis, MO). Bovine spinal cord phosphatidylserine was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). Octadecylrhodamine B (R18), 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) were obtained from Molecular Probes (Eugene, OR). Calcein, purchased from Sigma, was purified by chromatography on Sephadex LH-20 following the technique of Lelkes (1984) and the purity of the final product checked by TLC on silica gel G using CH_3OH:NH_4OH 28% (9:1.5, v/v) as mobile phase. 1,2-Dimyristoyl-*n*-glycero-3-phosphocholine (DMPC), deuterated 1,2-di[myristoyl-d_{27}]-*n*-glycero-3-phosphocholine (DMPC-d_{54}) and 1,2-dihexanoyl-*n*-glycero-3-phosphocholine (DHPC) were purchased from Avanti Polar Lipids (Alabaster, AL) and deuterium (^{2}H)-depleted water from Cambridge Isotopes (Cambridge, MA). Other reagents were obtained from E. Merck (Darmstadt, Germany) and were of analytical grade.

3. Results

This work aimed to give more insight about the importance of the free amino group at the N-terminus of Aβ29-42 for the interaction of amyloid peptide with the membrane. The membrane destabilizing properties of the unprotected and N-protected (acetylated fragment) Aβ29-42 peptides were investigated by experimental and molecular modeling methods.

3.1. Experimental studies

3.1.1. Fusion of lipidic phases

The measurement of fluorescence dequenching of octadecylrhodamine B (R_{18}) is an established technique used to study the fast mixing of lipids occurring during fusion of adjacent membranes

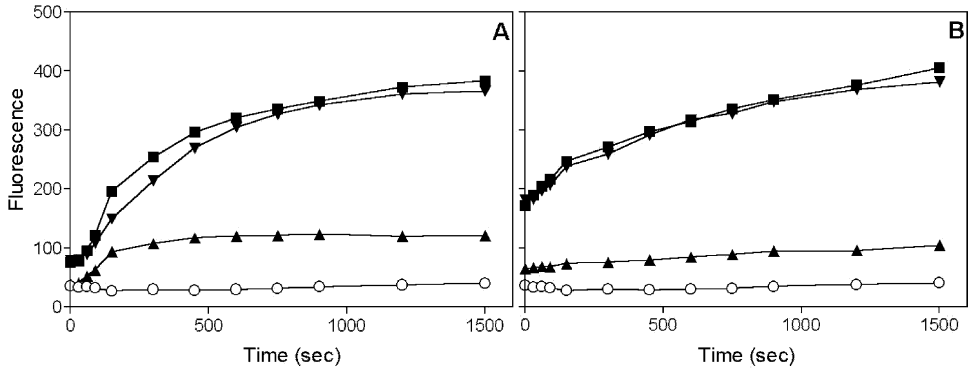

Fig. 1. Effect of Aβ29-42 unprotected (A) and N-protected (B) on fusion of lipidic phases of liposomes (5 μM in lipids) (PC:PE:PI:PS:SM:Chol [30%:30%:2.5%:10%:5%:22.5%]) upon incubation at room temperature in the presence of increasing peptide:lipid ratio. Control [HFP] (○); [peptide:lipid molar ratio] = 0.02 (▲); 0.2 (▼); 0.4 (■). Each point is the mean value of three independent experiments but the S.D. (less than 6% of the fluorescence measured) are not shown for sake of clarity.

(Hoekstra et al., 1984). We have also used it previously to examine the slower exchange of lipids during membrane aggregation induced by drugs such as aminoglycosides (Van Bambeke et al., 1995).

Fig. 1 shows the fluorescence variations observed with R_{18}-labeled liposomes when mixed with unlabeled liposomes and peptides at increasing peptide:lipid ratios (0.02–0.4). In the absence of peptide, no significant increase in fluorescence was seen up to 25 min. In contrast, with both peptides (Aβ29-42 unprotected, and N-protected), a rapid and marked increase in fluorescence over the first few minutes was observed followed by a very slow increment. The effect of both peptides was dose-dependent and was maximal for a peptide:lipid ratio of 0.2 (peptide 1 μM). This increase is faster for liposomes incubated with

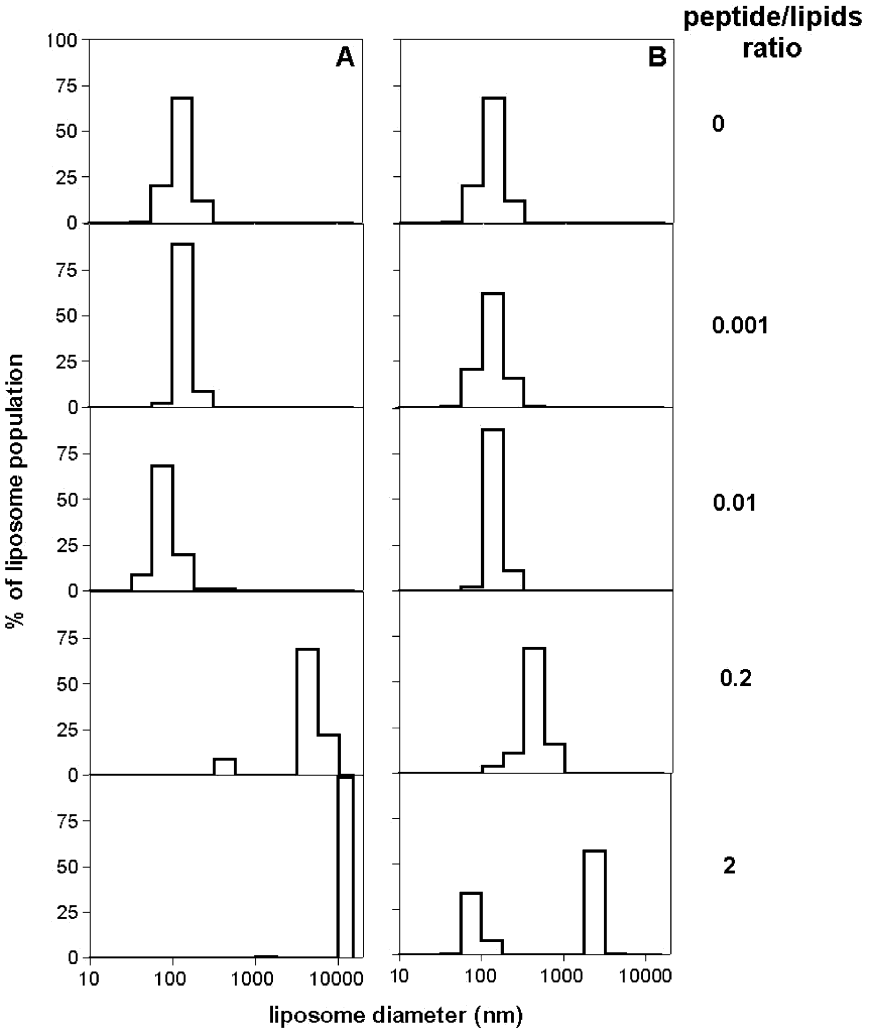

Fig. 2. Effect of Aβ29-42 unprotected (A) and N-protected (B) on the size distribution analysis of the liposomes (65 μM in lipids) (PC:PE:PI:PS:SM:Chol [30%:30%:2.5%:10%:5%:22.5%]) upon incubation at room temperature in the presence of increasing peptide:lipid ratios.

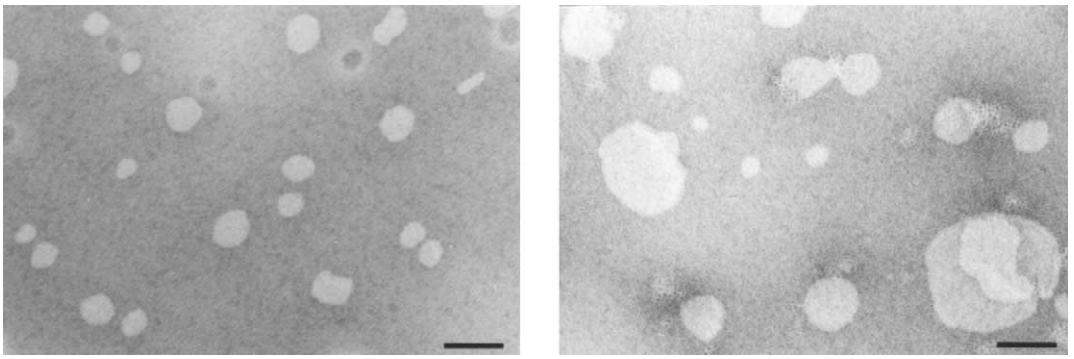

Fig. 3. Electron microscopy pictures of negatively stained liposomes (left panel) upon addition of Aβ29-42 unprotected at a ratio peptide:lipid of 2 (right panel). Bar = 50 nm.

Aβ29-42 N-protected (compare Fig. 1A and B) since a marked fluorescence dequenching is already noted after 15 s.

3.1.2. Size of the liposomes

To further examine the ability of Aβ29-42 peptides to induce fusion of membranes, we investigated their capacity to modify the apparent size and homogeneity of the liposomes preparation by means of a light scattering assay. At a peptide:lipid molar ratio of 0.2 (peptide 13 μM) and 2 (peptide 130 μM), respectively, for Aβ29-42 unprotected (Fig. 2A) and N-protected (Fig. 2B), the peptides caused a striking increase in the apparent diameter of the particles and induced the appearance of two populations. Fusion of liposomes induced by Aβ peptides was further confirmed by negative-staining studies and illustrated in Fig. 3 for Aβ29-42 unprotected. Morphometric analysis, performed on 100 vesicles, gave a mean size of control liposomes of about 17 ± 4 nm versus 28 ± 7 nm for liposomes incubated with the peptide. Discrepancies between the absolute values of the mean liposome diameters recorded by light scattering spectroscopy and by morphometry of the liposomes after negative staining may be accounted for by the fact that the first type of measurement is strongly influenced by the presence of a small number of large particles.

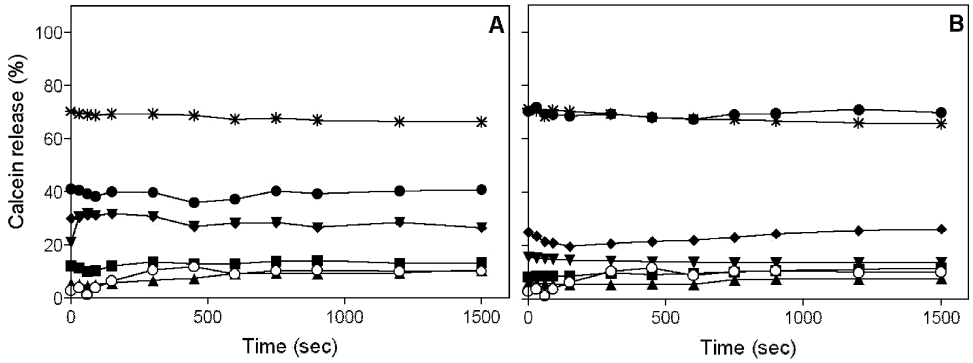

Fig. 4. Effect of Aβ29-42 unprotected (A) and N-protected (B) on calcein release from liposomes (5 μM in lipids) (PC:PE:PI:PS:SM:-Chol [30%:30%:2.5%:10%:5%:22.5%]) upon incubation at room temperature in the presence of increasing peptide:lipid ratio. Control [HFP] (○); [peptide:lipid molar ratio] = 0.3 (▲); 0.6 (■); 1.2 (▼); 3 (◆); 6 (●); 9 (*). Each point is the mean value of three independent experiments but the S.D. (less than 8% of the fluorescence measured) are not shown for sake of clarity.

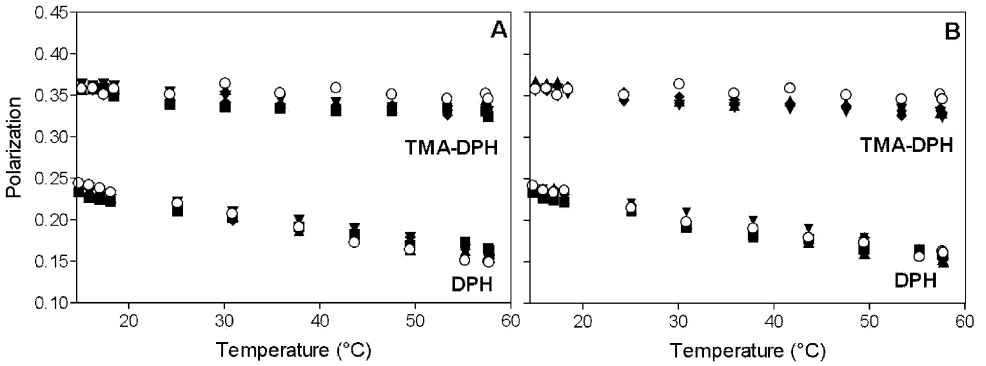

Fig. 5. Effect of Aβ29-42 unprotected (A) and N-protected (B) on the variation of the polarization of the fluorescence of TMA-DPH and DPH incorporated in small unilamellar vesicles (molar ratio to the lipids 1:250) made of PC:PE:PI:PS:SM:Chol (30%:30%:2.5%:10%:5%:22.5%). Control [HFP] (○); [peptide:lipid molar ratio] = 0.001 (▲); 0.01 (■); 0.1 (▼); 1 (◆). Each point is the mean value of three independent experiments but the S.D. (< 0.008) are not shown for sake of clarity.

3.1.3. Calcein permeability

Calcein, a polar molecule with a molecular weight of 622.5, has been widely used to study the permeability of lipid bilayers (Allen and Cleland, 1980). The effect of Aβ29-42 unprotected and N-protected on membrane permeability is shown in Fig. 4 for increasing peptide:lipid ratios ranging from 0.3 to 9. Both peptides induced a fast (< 60 s) and almost complete release of calcein from liposomes as has been observed previously for mellitin (Van Bambeke et al., 1993), a known porogenic agent (Dempsey, 1990). The maximum release was obtained for a peptide:lipid molar ratio of 9 (peptide 45 μM) for Aβ29-42 unprotected (Fig. 4A) and 6 (peptide 30 μM) for Aβ29-42 N-protected (Fig. 4B). The influence of increasing the peptide concentration was greater for the unprotected peptide than for the protected fragment.

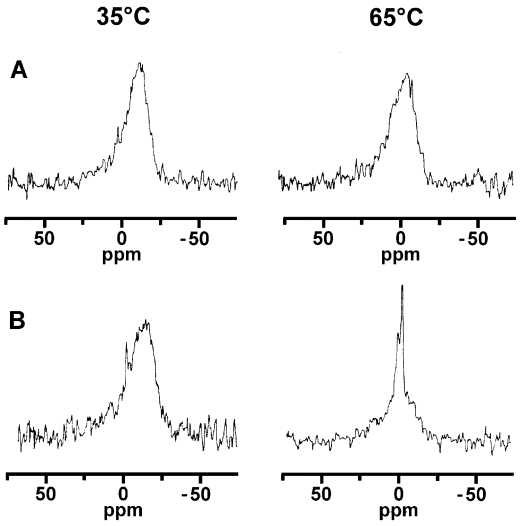

Fig. 6. ^{31}P NMR spectra at 101.3 MHz of multilamellar vesicles made of PC:PE:PI:PS:SM:Chol (30%:30%:2.5%:10%:5%:22.5%). (A) Control vesicles. (B) Vesicles with Aβ29-42 unprotected at a peptide:phospholipid molar ratio of 0.2.

3.1.4. Fluidity

The peptide influence on the fluidity of lipidic domains was examined by the fluorescence polarization of two probes which insert in bilayers at different levels, namely trimethylammoniumdiphenylhexatriene (TMA-DPH) and diphenylhexatriene (DPH). The first molecule inserts between alkyl chains and spans the hydrophilic/hydrophobic interface, whereas the second is totally hydrophobic and is thus embedded in the hydrophobic core of the membrane. The degree of polarization of the two probes linearly decreased in control small unilamellar vesicles when temperature increased but the variation in polarization values with TMA-DPH was less marked than with DPH. This difference indicates a smaller increase in mobility of the alkyl chains close to the interface in comparison to the deeper hydrophobic domain. When liposomes are incubated with Aβ29-42

unprotected (Fig. 5A) and N-protected (Fig. 5B), at increasing peptide:lipid ratios (0.001–1 [peptide concentrations: 0.314–314 μM]), no significant change was seen over the whole range of temperatures investigated. Higher peptide concentrations could not be investigated because they caused marked perturbations of the signal.

3.1.5. Lipid organization

NMR studies were carried out to further characterize the effects of the Aβ29-42 on the lipid organization in relation to alterations of membrane properties described above. Since both the protected and unprotected Aβ29-42 peptides give very similar results in liposome fusion and membrane destabilization experiments, only the unprotected peptide was studied by NMR.

3.1.5.1. ^{3}P NMR. Control MLV (Fig. 6A) at 35 °C gave a broad signal with a maximum at high field and a shoulder at low field. The 0 ppm value corresponds to the isotropic shift that is observed for sonicated vesicles (Seelig, 1978). At 35 °C, the chemical shift value of the maximum was approximately −10 ppm. By increasing the temperature at 65 °C, the maximum peak was displaced to about −7 ppm.

In the presence of the Aβ29-42 fragment (Fig. 6B), a narrow signal appeared superimposed to the broad signal. It became more important at 65 °C than at 35 °C as illustrated for a peptide:phospholipid molar ratio of 0.2. This effect was more marked when the peptide:phospholipid ratio was increased (data not shown).

3.1.5.2. ^{2}H NMR. The deuterium quadrupole echo spectra of chain-perdeuterated DMPC-d_{54} in bicelles were obtained at 37 °C and at pH 6. Spectrum A in Fig. 7 is the peptide-free sample signal typical of well-aligned bicelles made of DMPC and DHPC in a molar ratio of 3.5:1 (Sanders and Schwonek, 1992).

The presence of the Aβ29-42 unprotected induced changes in the spectral shape even at relatively low concentration. Spectrum B of Fig. 7 corresponds to a peptide:DMPC molar ratio of 0.025. The observed quadrupole splittings are not significantly different from those of spectrum A,

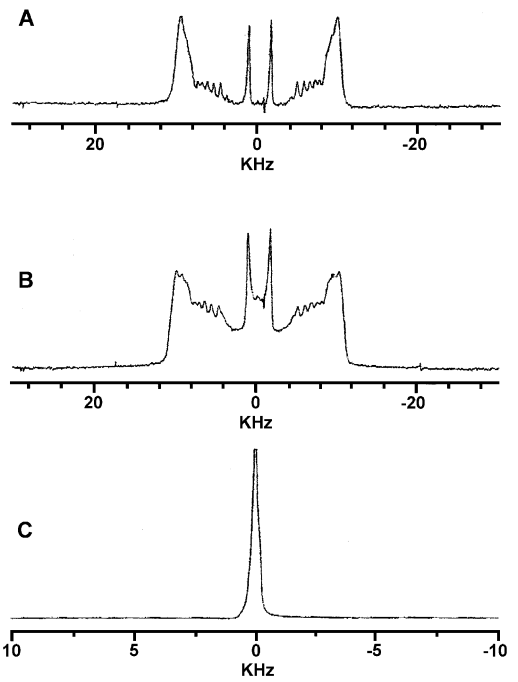

Fig. 7. ^{2}H NMR spectra at 55.3 MHz of DMPC-d54/DHPC (3.5:1) bicelles at 37°C. (A) Peptide free sample. (B) Sample containing Aβ29-42 unprotected incubated for 1 h at a peptide:DMPC molar ratio of 0.025. (C) Sample containing Aβ29-42 unprotected incubated for 1 h at a peptide:DMPC molar ratio of 0.1.

but the broadening of the ^{2}H NMR component is obvious by comparing spectrum B with spectrum A. For higher peptide concentrations (molar ratio peptide:DMPC of 0.1), the effect was dramatic. As illustrated by spectrum C of Fig. 7, the ^{2}H NMR spectrum is reduced to a single narrow signal.

3.2. Molecular modeling

The IMPALA procedure was used to check the influence of the charged group at the N-terminal of the Aβ29-42 peptide on its insertion in a lipid bilayer. Briefly, with this method the interaction between a peptide and a simplified modeled membrane is simulated using a Monte-Carlo minimization procedure on 100,000 steps at 298 K.

Fig. 8A and B shows the most stable position in the modeled bilayer of Aβ29-42 unprotected and the evolution of its mass center during the Monte-Carlo simulation. The peptide adopts an optimal

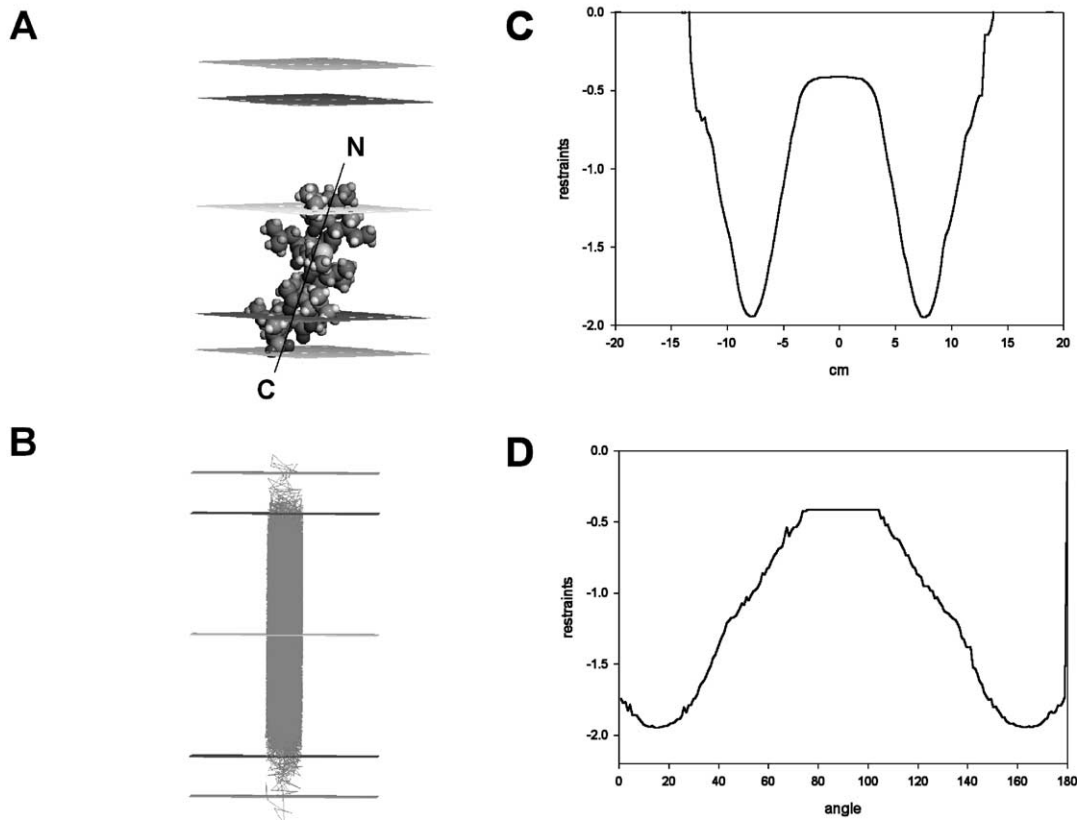

Fig. 8. (A) Best configuration of Aβ29-42 unprotected in ribbon representation after Monte-Carlo simulation (10^5 steps). Middle plane = bilayer center ($z = 0$); first plane = lipid acyl chain/polar headgroup interface at 13.5 Å from the center; second plane = lipid/water interface ($z = 18$ Å). The helix axis and the N to C orientation are shown. (B) Evolution of the mass center during the simulation. Each point corresponds to one mass center position during the MC simulation. (C) Profile of the minimal values of the restraints versus the mass center penetration (cm; in Å) (i.e. the z-coordinate of the molecule). (D) Profile of the minimal values of restraints versus the angle (°). Angles are measured with respect to the bilayer normal.

angle of about 70° between its axis and the bilayer plane, as shown in the plot of the restraints versus the insertion angle (Fig. 8D). Its N-extremity is deeply inserted in the membrane corresponding to an optimal mass center penetration of about 8 Å (Fig. 8C). This configuration is in agreement with the previous calculations on a single interface (Pillot et al., 1996; Brasseur et al., 1997). The Aβ29-42 peptide explores a wide conformational space during the simulation since it is able to translocate in the second lipid layer as shown by the evolution of the mass center during the Monte-Carlo minimization (Fig. 8B). The symmetry observed in the plot of the restraints versus the penetration depth or angle also reflects this phenomenon (Fig. 8C and D). Furthermore, the peptide has access to angle variations of about 40° (from 10° to 50° between the peptide axis and the bilayer normal) with little variation in restraint values, as depicted by the wide minimum on Fig. 8D.

Fig. 9 shows similar results for the Aβ29-42 N-protected: the optimal configuration corresponds to an angle of about 70° (towards the bilayer plane) and a mass center penetration of 8 Å (Fig. 9A and C). Again, the peptide is able to translocate in the second lipid layer (Fig. 9B) and presents a wide minimum when plotting the restraints against the insertion angle (Fig. 9D). A slight difference is noted for the N-protected peptide, i.e.

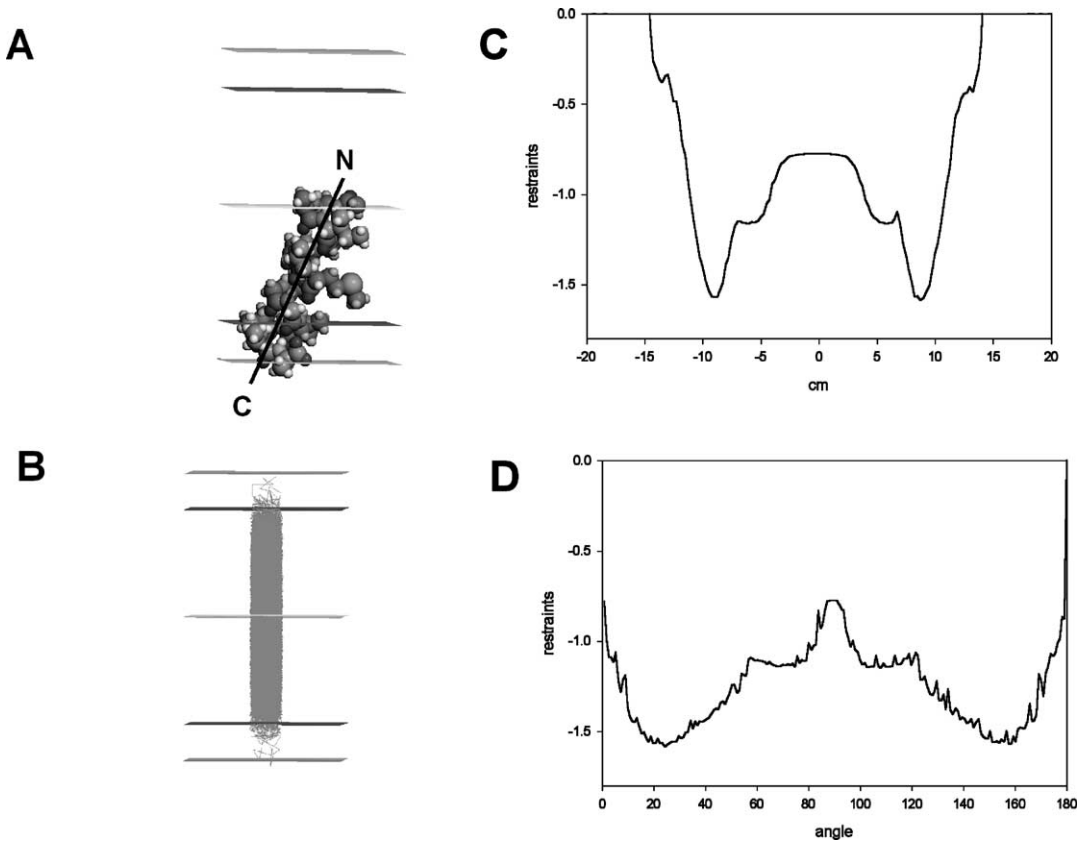

Fig. 9. Best configuration of the Aβ29-42 N-protected peptide (see legend of Fig. 8 for details).

the presence of an additional shoulder in Fig. 9C and D. This should correspond to metastable positions of the peptide more deeply inserted in the lipid core.

4. Discussion

β-Amyloid peptides (Aβ) are formed from APP through β- and γ-secretases (Selkoe, 1994). It was suggested that Aβ could notably exert its toxicity via an interaction with the membrane (Arispe et al., 1993a,b; Terzi et al., 1995, 1997; Cribbs et al., 1997; Hertel et al., 1997). It is commonly stated that residues 1-28 form the extracellular domain whereas the residues 29-40 or 29-42 are located in the cell membrane (Coles et al., 1998). Primary amino acid sequence could determine receptor activating potential (Casal et al., 2002), formation of Aβ aggregates at low pH (Wood et al., 1996) and electrostatic changes in the positively-charged group of histidine, arginine and lysine and the negatively-charged group of glutamic acid and aspartic acid which seem to be crucial for amyloidogenesis (Orpiszewski and Benson, 1999).

By using computer modeling, we have previously shown that the C-terminal domain of Aβ peptide (fragments 29-40 and 29-42) presents physicochemical properties close to those of the putative fusion peptides of viral proteins (Horth et al., 1991; Pillot et al., 1996). These peptides display an asymmetrical distribution of hydrophobic residues along the peptide sequence which is responsible for their oblique insertion in the bilayer (for a review, see Brasseur, 2000). We have previously shown that Aβ29-42 is able to induce liposome fusion (Pillot et al., 1996). In this paper, we have investigated the influence of a potential positive

charge at the N-terminal of Aβ29-42 (free amino group) on destabilizing properties of the membrane since a hydrophilic zone (containing one positive charge) is located upstream of the hydrophobic fragment. The use of an unprotected peptide instead of an elongated fragment avoids interference due to the possible steric hindrance. NMR spectroscopy was used to further characterize the effects of Aβ29-42 on the lipid organization of the membrane.

The in vivo relevance of the experimental model employed in the current work has been ascertained by the literature data. With respect to lipids, McLaurin and Chakrabartty (1997) and Ji et al. (2002) reported that cholesterol and acidic phospholipids are important for membrane insertion of Aβ1-42 peptides. We therefore selected a complex lipid composition including cholesterol and negatively-charged phospholipids and mimicking brain biological membranes to a certain extent (the cholesterol ratio of brain plasma membrane is at least 30% in aged people (Ji et al., 2002), a ratio similar to that used in our study). With respect to peptides, most of our experiments were carried out with Aβ29-42 at peptide concentrations varying from 1 to 50 μM. Under normal conditions, the physiological concentration of Aβ in the cerebrospinal fluid is around 0.5 nM (Seubert et al., 1992; Ji et al., 2002). By aging or under pathological situations, the degradation pathway of Aβ via the low-density lipoprotein receptor-related protein or via a scavenger receptor is reduced to ~45% (Christie et al., 1996) which would increase the extracellular content of Aβ. Moreover, since biological membranes are heterogeneous, locally high peptide concentrations can be obtained transiently and thus, the peptide concentrations used could be similar to those found under in vivo conditions.

We first compared the effects of the two fragments Aβ29-42 unprotected and N-protected on membrane properties of small unilamellar liposomes. Both Aβ29-42 peptides have a similar potency to induce fusion of unilamellar lipid vesicles or to increase their permeability. The critical molar ratio of lipid to Aβ required to observe an effect is similar to that reported by others (Ji et al., 2002).

Changes in the fluidity of the bilayer, an important structural feature of membranes, cannot explain these results since the fluorescence anisotropy experiments failed to show any effect of β-amyloid peptide on membrane fluidity. In this respect, our data are consistent with those obtained by Zubenko et al. (1999) who did not find any significant alterations in the steady-state anisotropy for the membrane hydrocarbon core or the hydrophilic membrane region determined with DPH or TMA-DPH. Other groups, however, have reported an increase (Avdulov et al., 1997; Matsuzaki and Horikiri, 1999; Mason et al., 1999) or a decrease (Müller et al., 1995, 1998; Scheuer et al., 1996; Kremer et al., 2000) in membrane fluidity in response to Aβ peptides. Reasons for these discrepancies are unclear and still under investigation. Calcein release is observed with both unprotected and N-protected Aβ29-42. The effect is fast (within 1 min) similar to that observed with diphtheria toxin fragments (Defrise-Quertain et al., 1989), the *Staphylococcus aureus* toxins, leucocidins and γ-hemolysins (Ferreras et al., 1998), bacterial carotenoids (Hara et al., 1999), gramicidin (Prenner et al., 2001) and butenafine (Mingeot-Leclercq et al., 2001). The calcein release is observed upon addition of peptides at peptide:-lipid ratios higher than those necessary to induce fusion and changes in lipid organization. Because the Aβ29-42 peptides alter membrane permeability and induce vesicle fusion probably as a consequence of the changes they cause in lipid membrane organization, we therefore investigated the potential of these peptides to perturb the membrane bilayer. ^{31}P NMR data of control liposomes are characteristic of a bilayer organization of multilamellar vesicles. Because of the mixed composition of the liposomes used, the low field shoulder is not well defined but the effective chemical shift anisotropy, $\Delta\sigma$ (the difference of chemical shift between the high field maximum and the low field shoulder), can be estimated on the basis of the chemical shift difference between the maximum and the isotropic shift value at 0 ppm, which represents one-third of $\Delta\sigma$ (Seelig, 1978; De Kruijff et al., 1979). The estimated $\Delta\sigma$ values are thus -30 ppm (3×-10 ppm) at 35 °C and -21 ppm (3×-7 ppm) at 65 °C. The

reduction of $\Delta\sigma$ as temperature increases is due to an increase of motional freedom of the phosphate head groups. The peptide influence on the phospholipid organization is revealed by the increase of a narrow signal that becomes more prominent at high temperatures (and with increase in concentration; data not shown).

^{31}P NMR observations are confirmed by the ^{2}H NMR data. The peptide-free sample gives a spectrum where the Pake doublets are well resolved indicating that the bicelles are well oriented with their axis perpendicular to the magnetic field (Sanders and Schwonek, 1992). The peptide effect at low concentration is shown by a broadening of the ^{2}H NMR components resulting most probably from increasing motions which reduce magnetic alignment of the bicelles. At higher concentrations, the peptide induces a drastic reorganization of the DMPC molecules in an isotropic-type phase responsible for the narrow signal at the isotropic shift value.

Our NMR observations are comparable with other NMR data on membranes in the presence of fusion proteins (Yeagle et al., 1991; Epand et al., 1992) and fusion peptides (Schanck et al., 1998a,b) since isotropic-type structures were also observed when bilayer phases were submitted to the action of these fusogenic agents.

The changes in lipid organization induced by Aβ29-42 and the very similar effect of both the unprotected and the N-protected Aβ peptides on membrane properties can be explained by the molecular modeling calculations. The method used implies an empirical restraint term (Ducarme et al., 1998) to calculate the interaction of peptides with a simplified bilayer, a model closer to the natural membranes than the single lipid/water interface used in previous algorithms (Brasseur, 1990, 1991). This approach has been validated by the experimental data obtained with peptides known to interact with membranes (Ducarme et al., 1998; Bradshaw et al., 2000) and has recently allowed us to predict that tilted peptides have access to a wide conformational space in the membrane in contrast to what happens for surface-seeking or transmembrane peptides (Lins et al., 2001). This peculiar behavior could be related to their lipid-destabilizing activity.

Applied to the unprotected and N-protected Aβ29-42, the method shows that the optimal configuration for both peptides is the same with an angle of about 70° towards the bilayer plane, an insertion of the mass center at 8 Å and a preferential orientation of the N-terminus in the lipids; they are both able to translocate through the membrane and have access to a wide conformational space, as previously predicted (Lins et al., 2001). This is in agreement with the NMR results, which show perturbation of the lamellar organization of the phospholipids by Aβ29-42 peptide. The only slight difference between the two peptides is the presence of a supplementary metastable position in the lipid core for the N-protected peptide. This can be explained by a small increase in hydrophobicity due to the presence of an extra methyl group and to the lack of a potential positive charge. However, this difference cannot be observed at the macromolecular level (i.e. in the in vitro assays) probably because it is not sufficient enough to affect fusogenic properties.

In conclusion, the C-terminal domain of the amyloid peptide is able to promote lipidic membrane reorganization and membrane fusion, causing *in fine* modification of bilayer permeability whether its N-terminal residue in position 29 is protected or not. Lipid destabilization induced by Aβ peptides could be due to an ability to adopt metastable positions in the presence of lipids followed by the formation of peptide β-aggregates, as recently suggested (Demeester et al., 2000). These processes might reflect the events occurring during the interaction of amyloid peptides with neuronal cell membranes and thus give insight into the molecular basis of the cytotoxicity of these peptides.

Acknowledgements

R.B., M.-P.M.-L. and F.V.B. are Directeur de Recherche, Maître de Recherche and Chercheur Qualifié of the Belgian Fonds National de la Recherche Scientifique, respectively. This work was supported by the Belgian Fonds de la Recherche Scientifique Médicale (Grants 3.4589.96

and 3.4546.02 to M.-P.M.-L. and Grant 9.4506.99 to A.S.), by the Fonds Speciaux de Recherche (FSR 2002) of the Université Catholique de Louvain to M.-P.M.-L. and F.V.B., and by Région Wallonne (Grant NANOMEMB 011/5020-UCL to M.-P.M.-L. and R.B.). We wish to thank deceased Professor R.R. Vold for her hospitality and fruitful discussions. ^{2}H NMR measurements were performed in her laboratory by one of us (A.S.) during his sabbatical at UCSD (University California of San Diego). The financial support of UCB Pharma is also gratefully acknowledged. We thank F. Andries-Renoird, M.C. Cambier and N. Aguilera for dedicated technical assistance as well as the Unité de Pharmacocinétique, Métabolisme, Nutrition and Toxicologie (UCL) for the free access to the LS-50 Perkin-Elmer fluorimeter and Dr. Roy Massingham for the critical reading of the manuscript.

References

Allen, T.M., Cleland, L.G., 1980. Serum-induced leakage of liposome contents. Biochim. Biophys. Acta 597, 418–426.

Ansell, Hawthorne, 1964. Phospholipids—Chemistry, Metabolism and Function. Phospholipids and the Nervous System. Elsevier, Amsterdam, pp. 278–372.

Arispe, N., Pollard, H.B., Rojas, E., 1993. Giant multilevel cation channels formed by Alzheimer disease amyloid beta-protein (A-betaP(1-40)) in bilayer membranes. Proc. Natl. Acad. Sci. USA 90, 10573–10577.

Arispe, N., Rojas, E., Pollard, H.B., 1993. Alzheimer disease amyloid beta protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminium. Proc. Natl. Acad. Sci. USA 90, 567–571.

Avdulov, N.A., Chochina, S.V., Igbavboa, U., O'Hare, E.O., Schroeder, F., Cleary, J.P., Wood, W.G., 1997. Amyloid beta-peptides increase annular and bulk fluidity and induce lipid peroxidation in brain synaptic plasma membranes. J. Neurochem. 68, 2086–2091.

Bartlett, G.R., 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234, 466–468.

Beuve-Mery, D., Rahman, M., Ducarme, P., Brasseur, R., 2001. Practical aspects of computational chemistry calculations through PC networks: the RAMSES-Beowulf implementation. J. Comput. Chem. 22, 172–177.

Bradshaw, J.P., Darkes, M.J.M., Katsaras, J., Epand, R.M., 2000. Neutron diffraction studies of viral peptides. Physica B 276, 495–498.

Brasseur, R., 1990. TAMMO: theoretical analysis of membrane molecular organization. In: Brasseur, R. (Ed.), Molecular Description of Biological Membrane Components by Computer-aided Conformational Analysis. CRC Press, Boca Raton, FL, pp. 203–219.

Brasseur, R., 1991. Differentiation of lipid-associating helices by use of three-dimensional molecular hydrophobicity potential calculations. J. Biol. Chem. 266, 16120–16127.

Brasseur, R., 2000. Tilted peptides: a motif for membrane destabilization (hypothesis). Mol. Memb. Biol. 17, 31–40.

Brasseur, R., Lins, L., Vanloo, B., Ruysschaert, J.M., Rosseneu, M., 1992. Molecular modelling of the amphipathic helices of the plasma apolipoproteins. Proteins: Struct. Funct. Genet. 13, 246–257.

Brasseur, R., Pillot, T., Lins, L., Vandekerckhove, J., Rosseneu, M., 1997. Peptides in membranes: tipping the balance of membrane stability. Trends Biochem. Sci. 22, 167–171.

Casal, C., Serratosa, J., Tusell, J.M., 2002. Relationship between β-AP peptide aggregation and microglial activation. Brain Res. 928, 76–84.

Chaney, M.O., Webster, S.D., Kuo, Y.M., Roher, A.E., 1998. Molecular modeling of the A beta 1-42 peptide from Alzheimer's disease. Protein Eng. 11, 761–767.

Choo-Smith, L.P., Surewicz, W.K., 1997. The interaction between Alzheimer amyloid beta(1-40) peptide and ganglioside GM1-containing membranes. FEBS Lett. 402, 95–98.

Christie, R.H., Chung, H., Rebeck, G.W., Strickland, D., Hyma, B.T., 1996. Expression of the very low-density lipoprotein receptor (VLDL-r), an apolipoprotein-E receptor, in the central nervous system and in Alzheimer's disease. J. Neuropathol. Exp. Neurol. 55, 491–498.

Coles, M., Bicknell, W., Watson, A.A., Fairlie, D.P., Craik, D.J., 1998. Cholesterol modulates the membrane-disordering effects of beta-amyloid peptides in the hippocampus: specific changes in Alzheimer's disease. Biochemistry (US) 37, 11064–11077.

Cribbs, D.H., Pike, C.J., Weinstein, S.L., Velazquez, P., Cotman, C.W., 1997. All-D-enantionmers of beta-amyloid exhibit similar biological properties to all-L-beta-amyloids. J. Biol. Chem. 272, 7431–7436.

Davis, J.H., 1983. The description of membrane lipid conformation, order and dynamics by 2H NMR. Biochim. Biophys. Acta 737, 117–171.

Decout, A., Labeur, C., Goethals, M., Brasseur, R., Vandekerckhove, J., Rosseneu, M., 1998. Enhanced efficiency of a targeted fusogenic peptide. Biochim. Biophys. Acta 1372, 102–116.

Defrise-Quertain, F., Cabiaux, V., Vandenbranden, M., Wattiez, R., Falmagne, P., Ruysschaert, J.M., 1989. pH-Dependent bilayer destabilization and fusion of phospholipidic large unilamellar vesicles induced by *Diphtheria toxin* and its fragments A and B. Biochemistry (US) 28, 3406–3413.

De Kruijff, B., Verkleij, A.J., van Echteld, C.J.A., Gerritsen, W.J., Mombers, C., Noordam, P.C., de Gier, J., 1979. The occurrence of lipidic particles in lipid bilayers as seen by ^{31}P NMR and freeze-fracture electron microscopy. Biochim. Biophys. Acta 555, 200–209.

Demeester, N., Baier, G., Enzinger, C., Goethals, M., Vandekerckhove, J., Rosseneu, M., Labeur, C., 2000. Apoptosis induced in neuronal cells by C-terminal amyloid beta-fragments is correlated with their aggregation properties in phospholipid membranes. Mol. Membr. Biol. 17, 219–228.

Dempsey, C.E., 1990. The actions of melittin on membranes. Biochim. Biophys. Acta 1031, 143–161.

Ducarme, Ph., Rahman, M., Brasseur, R., 1998. IMPALA: a simple restraint field to simulate the biological membrane in molecular structure studies. Proteins: Struct. Funct. Genet. 30, 357–371.

Eckert, G.P., Cairns, N.J., Maras, A., Gattaz, W.F., Muller, W.E., 2000. Cholesterol modulates the membrane-disordering effects of beta-amyloid peptides in the hippocampus: specific changes in Alzheimer's disease. Dementia Geriatr. Cognitive Disord. 11, 181–186.

Epand, R.F., Cheetham, J., Yeagle, P.L., Richardson, C.D., DeGrado, W.F., 1992. Peptide models for the membrane destabilizing actions of viral fusion protein. Biopolymers 32, 309–314.

Ferreras, M., Hoper, F., Dalla Serra, M., Colin, D.A., Prévost, G., Menestrina, G., 1998. The interaction of *Staphylococcus aureus* bi-component gamma-hemolysins and leucocidins with cells and lipid membranes. Biochem. Biophys. Acta 1414, 108–126.

Hara, M., Yuan, H., Yang, Q., Hoshino, T., Yokoyama, A., Miyake, J., 1999. Stabilization of liposomal membranes by thermozeaxanthins:carotenoid-glucide esters. Biochim. Biophys. Acta 1461, 147–154.

Hardy, J., 1997. The Alzheimer family of diseases: many etiologies, one pathogenesis? Proc. Natl. Acad. Sci. USA 94, 2095–2097.

Hartmann, T., Beiger, S.C., Bruhl, B., Tienari, P.J., Ida, N., Allsop, D., Roberts, G.W., Masters, C.L., Beyreuther, K., 1997. Distinct sites of intracellular production for Alzheimer's disease A beta 40/42 amyloid peptides. Nat. Med. 3, 1016–1020.

Hertel, C., Terzi, E., Hauser, N., Jakob-Roetne, R., Seelig, J., Kemp, J.A., 1997. Inhibition of the electrostatic interaction between beta-amyloid peptide and membranes prevents beta-amyloid-induced toxicity. Proc. Natl. Acad. Sci. USA 94, 9412–9416.

Hoekstra, D., de Boer, T., Klappe, K., Wilschut, J., 1984. Fluorescence method for measuring the kinetics of fusion between biological membranes. Biochemistry (US) 23, 5675–5681.

Horth, M., Lambrecht, B., Chuah Lay Khim, M., Bex, F., Clothilde, C., Ruysschaert, J.-M., Burny, A., Brasseur, R., 1991. Theoretical and functional analysis of the SIV fusion peptide. EMBO J. 10, 2747–2755.

Jao, Sh-Ch., Ma, K., Talafous, J., Orlando, R., Zagorski, M.G., 1997. Trifluoroacetic acid pretreatment reproducibly disaggregates the amyloid beta-peptide. Int. J. Exp. Clin. Invest. 4, 240–252.

Jarrett, J.T., Berger, E.P., Lansbury, P.T., Jr., 1993. The carboxy terminus of the beta-amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. Biochemistry (US) 32, 4693–4697.

Ji, S.-R., Wu, Y., Sui, S.-F., 2002. Cholesterol is an important factor affecting the membrane insertion of β-amyloid peptide (Aβ1-40), which may potentially inhibit the fibril formation. J. Med. Chem. 277, 6273–6279.

Kaiser, R.D., London, E., 1998. Location of diphenylhexatriene (DPH) and its derivatives within membranes: comparison of different fluorescence quenching analyses of membrane depth. Biochemistry (US) 37, 8180–8190.

Kang, J., Lemaire, H.G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.H., Multhaup, G., Beyreuther, K., Muller-Hill, B., 1987. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325, 733–736.

Kitagawa, S., Matsubayashi, M., Kotani, K., Usui, K., Kametani, F., 1991. Asymetry of membrane fluidity in the lipid bilayer of blood platelets: fluorescence study with diphenylhexatriene and analogs. J. Membr. Biol. 119, 221–227.

Kremer, J.J., Pallitto, M.M., Sklansky, D.J., Murphy, R.M., 2000. Correlation of a-amyloid aggregate size and hydrophobicity with decreased bilayer fluidity of model membranes. Biochemistry (US) 39, 10309–10318.

Kremer, J.J., Sklansky, D.J., Murphy, R.M., 2001. Profile of changes in lipid bilayer structure caused by beta-amyloid peptide. Biochemistry (US) 40, 8563–8571.

Lansbury, P.T., Jarrett, J.T., Berger, E.P., 1995. Hot papers—neurochemistry—the carboxy terminus of the beta-amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. Scientist 9, 15.

Lelkes, P.I., 1984. Methodological aspects dealing with stability measurements of liposomes in vitro using the carboxyfluorescein assay. In: Gregoriadis (Ed.), Liposome Technology, Vol. 3. CRC Press, Boca Raton, FL, pp. 225–246.

Lins, L., Charloteaux, B., Thomas, A., Brasseur, R., 2001. Computational study of lipid-destabilizing protein fragments: towards a comprehensive view of tilted peptides. Proteins: Struct. Funct. Genet. 44, 435–447.

Martin, I., Dubois, M.C., Defrise-Quertain, T., Saermark, A., Burny, A., Brasseur, R., Ruysschaert, J.M., 1994. Correlation between fusogenicity of synthetic modified peptides corresponding to the NH2-terminal extremity of simian immunodeficiency virus gp32 and their mode of insertion into the lipid bilayer: an infrared spectroscopy study. Virology 68, 1139–1148.

Mason, R.P., Estermyer, J.D., Kelly, J.F., Mason, P.E., 1996. Alzheimer's disease amyloid beta-peptide 25-35 is localized in the membrane hydrocarbon core: X-ray diffraction analysis. Biochem. Biophys. Res. Commun. 222, 78–82.

Mason, R.P., Jacob, R.F., Walter, M.F., Mason, P.E., Avdulov, N.A., Chochina, S.V., Igbavboa, U., Wood, W.G., 1999. Distribution and fluidizing action of soluble and aggregated amyloid beta-peptide in rat synaptic plasma membranes. J. Biol. Chem. 274, 18801–18807.

Matsuzaki, K., Horikiri, Ch., 1999. Interactions of amyloid beta-peptide (1-40) with ganglioside-containing membranes. Biochemistry (US) 38, 4137–4142.

Mazer, N.A., Carey, M.C., Kwasnick, R.F., Benedek, G.B., 1979. Quasielastic light scattering studies of aqueous biliary lipid systems: size, shape and thermodynamics of bile salt micelles. Biochemistry (US) 18, 3064–3075.

McLaurin, J., Chakrabartty, A., 1996. Membrane disruption by Alzheimer beta-amyloid peptides mediated through specific binding to either phospholipids or gangliosides. J. Biol. Chem. 271, 26482–26489.

McLaurin, J.A., Chakrabartty, A., 1997. Characterization of the interactions of Alzheimer beta-amyloid peptides with phospholipid membranes. Eur. J. Biochem. 245, 355–363.

McLaurin, J., Franklin, T., Chakrabartty, A., Fraser, P.E., 1998. Phosphatidylinositol and inositol involvement in Alzheimer amyloid-beta fibril growth and arrest. J. Mol. Biol. 278, 183–194.

McLaurin, J., Franklin, T., Fraser, P.E., Chakrabartty, A., 1998. Structural transitions associated with the interaction of Alzheimer beta-amyloid peptides with gangliosides. J. Biol. Chem. 273, 4506–4615.

Mingeot-Leclercq, M.P., Piret, J., Brasseur, R., Tulkens, P.M., 1990. Effect of acidic phospholipids on the activity of lysosomal phospholipases and on their inhibition by aminoglycoside antibiotics. I. Biochemical analysis. Biochem. Pharmacol. 40, 489–497.

Mingeot-Leclercq, M.-P., Gallet, X., Flore, C., Van Bambeke, F., Peuvot, J., Brasseur, R., 2001. Experimental and conformational analyses of interactions between butenafine and lipids. Antimicrob. Agents Chemother. 45, 3347–3354.

Müller, W.E., Koch, S., Eckert, A., Hartmann, H., Scheuer, K., 1995. Beta-amyloid peptide decreases membrane fluidity. Brain. Res. 674, 133–136.

Müller, W.E., Eckert, G.P., Scheuer, K., Cairns, N.J., Maras, A., Gattaz, W.F., 1998. Effects of beta-amyloid peptides on the fluidity of membranes from frontal and parietal lobes of human brain: high potencies of A beta 1-42 and A beta 1-43. Amyloid Int. J. Exp. Clin. Invest. 5, 10–15.

Norton, W.T., Toshiaki, A., Poduslo, S.E., DeVries, G.H., 1975. The lipid composition of isolated brain cells and axons. J. Neurosci. Res. 1, 57–75.

Orpiszewski, J., Benson, M.D., 1999. Induction of beta-sheet structure in amyloidogenic peptides by neutralization of aspartate: a model for amyloid nucleation. J. Mol. Biol. 289, 413–428.

Peuvot, J., Schanck, A., Lins, L., Brasseur, R., 1999. Are the fusion processes involved in birth, life and death of the cell depending on tilted insertion of peptides into membranes? J. Theoret. Biol. 198, 173–181.

Pillot, T., Goethals, M., Vanloo, B., Talussot, C., Brasseur, R., Vandekerckhove, J., Rosseneu, M., 1996. Fusogenic properties of the C-terminal domain of the Alzheimer beta-amyloid peptide. J. Biol. Chem. 271, 28757–28765.

Pillot, Th., Drouet, B., Queillé, S., Labeur, Ch., Vandekerckhove, J., Rosseneu, M., PinRon-Raymond, M., Chambaz, J., 1999. The nonfibrillar amyloid beta-peptide induces apoptotic neuronal cell death: involvement of its C-terminal fusogenic domain. J. Neurochem. 73, 1626–1634.

Prenner, E.J., Lewis, R.N.A., Jelokhani-Niaraki, M., Hodges, R.S., McElhaney, R.N., 2001. Cholesterol attenuates the interaction of the antimicrobial peptide gramicidin S with phospholipid bilayer membranes. Biochem. Biophys. Acta 1510, 83–92.

Rhee, S.K., Quist, A.P., Lal, R., 1998. Amyloid beta protein-(1-42) forms calcium-permeable, Zn^{2+}-sensitive channel. J. Biol. Chem. 273, 13379–13382.

Sanders, C.R., Landis, G.C., 1995. Reconstitution of membrane proteins into lipid-rich bilayered mixed micelles for NMR studies. Biochemistry (US) 34, 4030–4040.

Sanders, C.R., Schwonek, J.P., 1992. Characterization of magnetically orientable bilayers in mixtures of dihexanoylphosphatidylcholine and dimyristoylphosphatidylcholine by solid-state NMR. Biochemistry (US) 31, 8898–8905.

Schanck, A., Brasseur, R., Peuvot, J., 1998. Destabilisation of a model membrane by a predicted fusion peptide of fertilin. J. Chim. Phys. 95, 467–473.

Schanck, A., Peuvot, J., Brasseur, R., 1998. Influence of the mode of insertion of SIV peptides into membranes on the structure of model membrane as studied by P-31 NMR. Biochem. Biophys. Res. Commun. 250, 12–14.

Scheuer, K., Maras, A., Gattaz, W.F., Cairns, N., Forstl, H., Muller, W.E., 1996. Cortical NMDA receptor properties and membrane fluidity are altered in Alzheimer's disease. Dementia 7, 210–214.

Seelig, J., 1978. 31P nuclear magnetic resonance and the head group structure of phospholipids in membranes. Biochem. Biophys. Acta 515, 105–140.

Selkoe, D.J., 1993. Physiological production of the beta-amyloid protein and the mechanism of Alzheimer's disease. Trends Neurosci. 16, 403–409.

Selkoe, D.J., 1994. Cell biology of the amyloid a-protein precursor and the mechanism of Alzheimer's disease. Ann. Rev. Cell. Biol. 10, 373–403.

Selkoe, D.J., 1997. Alzheimer's disease: genotypes, phenotypes, and treatments. Science 275, 630–631.

Selkoe, D.J., 1999. Translating cell biology into therapeutic advances in Alzheimer's disease. Nature 399, A23–A31.

Seubert, P., Vigo-Pelfrey, C., Esch, F., Lee, M., Dovey, H., Davis, D., Sinha, S., Schlossmacher, M., Swindlehurst, C., 1992. Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. Nature 359, 325–327.

Sinha, S., Lieberburg, I., 1999. Cellular mechanisms of beta-amyloid production and secretion. Proc. Natl. Acad. Sci. USA 96, 11049–11053.

Terzi, E., Hölzemann, G., Seelig, J., 1995. Self-association of beta-amyloid peptide (1-40) in solution and binding to lipid membranes. J. Mol. Biol. 252, 633–642.

Terzi, E., Hölzemann, G., Seelig, J., 1997. Interaction of Alzheimer beta-amyloid peptide(1-40) with lipid membranes. Biochemistry (US) 36, 14845–14852.

Van Bambeke, F., Mingeot-Leclercq, M.P., Schanck, A., Brasseur, R., Tulkens, P.M., 1993. Alterations in membrane permeability induced by aminoglycoside antibiotics: studies

on liposomes and cultured cells. Eur. J. Pharmacol. 247, 155–168.

Van Bambeke, F., Tulkens, P.M., Brasseur, R., Mingeot-Leclercq, M.P., 1995. Aminoglycoside antibiotics induce aggregation but not fusion of negatively charged liposomes. Eur. J. Pharmacol. 289, 321–333.

Van Bambeke, F., Kerkhofs, A., Schanck, A., Remacle, C., Sonveaux, E., Tulkens, P.M., Mingeot-Leclercq, M.P., 2000. Biophysical studies and intracellular destabilization of pH-sensitive liposomes. Lipids 35, 213–223.

Weinstein, J.N., Yoshikami, S., Henkart, P., Blumenthal, R., Hagins, W.A., 1977. Liposome-cell interaction: transfer and intracellular release of a trapped fluorescent marker. Science 195, 489–491.

Wood, S.J., Maleeff, B., Hart, T., Wetzel, R., 1996. Physical, morphological and functional differences between pH 5.8 and 7.4 aggregates of the Alzheimer's amyloid peptide AP. J. Mol. Biol. 256, 870–877.

Yang, A.J., Chandswangbhuvana, D., Margol, L., Glabe, C.G., 1998. Loss of endosomal/lysosomal membrane impermeability is an early event in amyloid A beta 1-42 pathogenesis. J. Neurosci. Res. 52, 691–698.

Yeagle, P.L., Epand, R.M., Richardson, C.D., Flanagan, T.D., 1991. Effects of the 'fusion peptide' from measles virus on the structure of *N*-methyl dioleoylphosphatidylethanolamine membranes and their fusion with Sendai virus. Biochim. Biophys. Acta 1065, 49–53.

Zubenko, G.S., Kopp, U., Seto, T., Firestone, L.L., 1999. Platelet membrane fluidity individuals at risk for Alzheimer's disease: a comparison of results from fluorescence spectroscopy and electron spin resonance spectroscopy. Psychopharmacology 145, 175–180.