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ARTICLE

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Cholesterol inhibits the insertion of the Alzheimer's peptide Aβ(25–35) in lipid bilayers

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Abstract The physiological relationship between brain cholesterol content and the action of amyloid β (Aβ) peptide in Alzheimer's disease (AD) is a highly controversially discussed topic. Evidences for modulations of the Aβ/membrane interaction induced by plasma membrane cholesterol have already been observed. We have recently reported that Aβ(25–35) is capable of inserting in lipid membranes and perturbing their structure. Applying neutron diffraction and selective deuteration, we now demonstrate that cholesterol alters, at the molecular level, the capability of Aβ(25–35) to penetrate into the lipid bilayers; in particular, a molar weight content of 20% of cholesterol hinders the intercalation of monomeric Aβ(25–35) completely. At very low cholesterol content (about 1% molar weight) the location of the C-terminal part of Aβ(25–35) has been unequivocally established in the hydrocarbon region of the membrane, in agreement with our previous results on pure phospholipids membrane. These results link a structural property to a physiological and functional behavior and point to a therapeutical approach to prevent the AD by modulation of membrane properties.

Keywords Amyloid peptide · Aβ(25–35) · Alzheimer's disease · Neutron diffraction · Lipid bilayers · Cholesterol

Introduction

The β-amyloid peptides (Aβ) of variable length, containing 39–43 amino acids, are derived by cleavage of the amyloid precursor protein APP in the transmembrane region by a γ-secretase and at its N terminus by a β-secretase (Selkoe 1999). The established view of the pathophysiology of Alzheimer's disease (AD) considers the presence of proteinaceous deposits of Aβ accumulating extracellularly in the brain, the senile plaques, as the cause of the cognitive disorder (Busciglio et al. 1997; Hardy and Selkoe 2002; Hartley et al. 1999). Recently, the direct interaction of soluble oligomeric forms of Aβ with the cell membrane and their insertion in the membrane have also been considered as putative causes of neuronal cell death. It has been shown in vivo that a neurotoxic effect of Aβ(1–42) is independent of plaques formation (Hsia et al. 1999) and that protofibrillar intermediates of Aβ induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons (Hartley et al. 1999). A strong correlation of the neurodegenerative process with the absolute level of soluble Aβ has been found (McLean et al. 1999). Moreover, soluble forms of Aβ may be released from the mature senile plaques, interact with the neurons and cause neuron damage or disruption (Lambert et al. 1998).

In an attempt to explore the molecular basis of interactions between Aβ and biological membranes, we have investigated in previous studies, by neutron diffraction and selective peptide deuteration, the intercalation of a cytotoxic fragment of Aβ(1–42), i.e., Aβ(25–35) (G_{25}SNKGAIIGLM$_{35}$), in phospholipid bilayers of different compositions (Dante et al. 2002, 2003). The technique has been successfully applied to locate the deuterated amino acid $^{2}H_{10}$-Leu34 of Aβ in the hydrophobic core of the lipid bilayer. Perturbing effects of Aβ(25–35) on the membrane structure were also demonstrated (Dante et al. 2003). We have detected the same perturbing effect when investigating the interaction of the long peptide Aβ(1–42) with lipid bilayers (unpublished data). In order to elucidate any influence of the membrane properties on Aβ insertion, we have tested the effect of the membrane constituent cholesterol. Cholesterol is an important component of animal

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cellular membranes and is well known for influencing the membrane order and dynamics, as well as the cellular stability. In nerve cells, where it constitutes up to 25% of the membrane lipids, cholesterol is implicated in neuronal plasticity (Koudinov and Koudinova 2001) and is needed to build the synapses (Eckert et al. 2000). Cholesterol has been shown to regulate the activity of many enzymes and receptors (Albert et al. 1996; Cornelius 1995; Lasalde et al. 1995; Whetton et al. 1983) and the calcium homeostasis (Müller et al. 1997).

A connection between AD and membrane cholesterol has been indicated for the first time by Sparks et al. (1990). A large body of data has been collected since then in an attempt to clarify this link. The principal results are reviewed for instance in Wood et al. (2002), Hartmann (2001), Eckert et al. (2003) and Wolozin (2001) and are highly controversial.

Some studies state that cholesterol promotes the neurodegenerative process. For instance, studies in vitro and in vivo have demonstrated that cholesterol increases the activity of α-secretase, the enzyme that cleaves Aβ from its precursor protein APP (Fassbender et al. 2001; Kojro et al. 2001). Studies on transgenic mice overexpressing APP showed that a high cholesterol diet promotes Aβ plaques formation (Refolo et al. 2000). Epidemiological studies have described that AD is reduced in population taking statins, a class of drugs lowering plasma cholesterol (Jick et al. 2000; Wolozin 2002).

On the other hand, neuronal cells of AD patients have been found to be cholesterol depleted (Mason et al. 1992) and many authors reported a protective effect of cholesterol against neurotoxic properties of Aβ (Yip et al. 2001; Zhou and Richardson 1996). An inhibiting (Lin and Kagan 2002) or stimulating (Micelli et al. 2004) effect of cholesterol on the channel formation of Aβ in lipid bilayers and of Aβ-induced free calcium elevation in cultured cells (Kawahara and Kuroda 2000, 2001) have also been reported. Moreover, cholesterol depletion of neuronal cells causes another of the AD hallmarks, i.e., tau protein deposition inside neurons (Koudinov and Koudinova 2003).

In this scenario, the question whether enrichment or depletion of brain membrane cholesterol is beneficial to AD prevention is raised. Our current investigation, based on a structural study by neutron diffraction, clearly demonstrates that cholesterol inhibits the insertion of monomeric Aβ into the lipid membrane, supporting a beneficial influence or protective effort of cholesterol.

Materials and methods

Sample preparation

1-Palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and the net negatively charged lipid, 1-palmitoyl-2-oleoyl-phosphatidylserine (POPS), were purchased from Avanti Polar Lipid (Alabaster, AL, USA); cholesterol was a product of Sigma (Germany). The lipids were solubilized in chloroform; the solvent was evaporated under a stream of nitrogen and the lipids desiccated under vacuum ($p < 1$ mbar) for 12 h. All samples contained POPC and POPS in a relative ratio of 92:8 and cholesterol in different amounts; in particular, samples containing cholesterol in a ratio of 1, 5, 20 and 40% molar weight with respect to the total lipid amount were prepared. Hydrogenated H-Leu34-Aβ(25–35) and selectively deuterated ^{2}H-Leu34-Aβ(25–35) were products of WITA (Teltow, Germany). The peptide was pretreated with trifluoroacetic acid (TFA) to be kept in the monomer form (Jao et al. 1997). TFA was evaporated under a stream of nitrogen and the peptide was immediately re-dissolved in methanol. The peptide, either hydrogenated or selectively deuterated, was added to the dissolved lipids in a P/L molar ratio of 1:33. The lipid–peptide mixtures (20 mg) were sprayed with an artist airbrush onto quartz slides (65 mm × 15 mm × 1 mm). Samples consisting of POPC/POPS and POPC/POPS/cholesterol were prepared as well, as references. The phospholipid samples in the presence of the deuterated and hydrogenated peptides had been previously investigated and therefore were not measured again. The samples were dried under vacuum for 12 h and re-hydrated in a chamber at a relative humidity of 98% maintained by an aqueous saturated solution of K_2SO_4. Samples prepared in this way consisted of about 2,000 membrane layers and after hydration had a thickness of 1 μm.

Neutron diffraction and data analysis

Neutron diffraction measurements were carried out on the membrane diffractometer V1 at the Berlin Neutron Scattering Center of the Hahn-Meitner-Institute, Berlin (Germany).

The procedure for sample investigation is reported in detail elsewhere (Dante et al. 2002). Briefly, the samples were placed vertically in an aluminum container at controlled temperature ($T = 25.0 \pm 0.1$°C) and relative humidity (r.h. 98%). After reaching the thermodynamic equilibrium condition, diffraction patterns of POPC/POPS/cholesterol bilayers and POPC/POPS/cholesterol bilayers containing either 3% (mol) ^{2}H-Leu34-Aβ(25–35) or 3% (mol) H-Leu34-Aβ(25–35) were measured by rocking the samples around the expected Bragg position θ by $\theta \pm 2°$. Up to five lamellar orders for each sample were measured. After background subtraction, the experimental Bragg reflections were fitted to Gaussians to obtain the integrated intensities. Scaled intensities, corrected with absorption and Lorentz factors, were square-rooted to produce the structure factor amplitudes $F(h)$. The highest absorption concerned the first order reflex and was lower than 2%. The Fourier synthesis of structure factors allowed the reconstruction of the scattering length density profiles $\rho(z)$. The structure factor phase assignment was obtained with the

isomorphous replacement methods, using the D_2O/H_2O exchange at three different ratios (8:92; 20:80; 50:50). However, due to limited measuring time at the neutron source, the structure factors at three different water vapor compositions were not measured in the case of samples containing 5 and 20 mol% cholesterol in the presence of Aβ. These samples were measured at 8:92 D_2O/H_2O and the phases of their structure factors were unambiguously assigned on the basis of their corresponding samples in the absence of peptide. For the localization of the label, only the structure factors obtained at 8% D_2O, where the scattering length density of the water layer is zero, were employed. The position of the deuterated amino acid was determined by the differences in the densities between samples with Aβ containing deuterated and hydrogenated leucines. The difference between these density profiles gives in fact the deuterium distribution in the lipid bilayer. The distribution of the label was fitted in reciprocal space to the position and amplitude of one or two Gaussian functions using the measured difference of structure factors up to the fifth order.

Results

All measurements were repeated twice, with independent sample preparation and data acquisition. The results were reproducible, within the experimental error. The mosaicity of the samples, determined by rocking them around the first Bragg peak, was inferior to 0.5°. Five diffraction orders have been detected for each sample. The spacing of the samples, as calculated from the diffraction patterns, increases linearly from 5.37 to 6.60 nm as a function of the cholesterol molar content. The analytical procedure to derive the scattering length density profiles is described in detail elsewhere (Dante et al. 2002). The calculated structure factors are listed in Table 1 together with the experimental statistical errors ΔF. Figure 1 shows the pure lipid membranes with different cholesterol contents, in the absence of peptide. The patterns represent the elementary cell of the oriented samples, i.e., one lipid bilayer with its hydration shell; the center of the elementary cell is the middle of the bilayer profile ($z = 0$), occupied by the terminal methylene groups of the phospholipids. The water layers are centered at the two edges of the diagram; the two main peaks in the profiles correspond to the polar lipid headgroups, near the glycerol backbone, which contain fewer hydrogen atoms than the hydrocarbon region and have therefore a higher scattering length density. The reported profiles have been calculated at 8% D_2O contrast, where water has a scattering length density equal to zero, hence their offsets have been forced to zero in the water region. The membrane profile in the absence of cholesterol superimposes with the profile of the membrane with the lowest cholesterol content. Cholesterol increases the membrane thickness and the distance between headgroups. The distribution of the headgroups is broader in the presence of cholesterol. Cholesterol is known to have a rigidifying effect on the membrane. This effect is visible in the stretching of the alkyl chains and in the ordering of the methyl groups that results in deeper minimum at the center of the bilayer. Finally, the location of the cholesterol molecule in the alkyl chain region induces a smoothing of the shape of the profiles in this region, as indicated by the disappearance of the secondary maximum at 0.5 nm, which corresponds to the double bond of the unsaturated lipid chains. Figure 2 depicts the effect of cholesterol on the Aβ/membrane interaction. The presence of the peptide did not alter significantly the spacing or the membrane thickness. From the top (Fig. 2a) to the bottom (Fig. 2c) the scattering length density profiles obtained in the presence of increasing amounts of cholesterol are reported. The solid lines refer to the samples containing deuterated peptide, the broken lines refer to the samples with unlabeled Aβ(25–35). The most pronounced difference between the scattering length density profiles in the presence of labeled and unlabeled peptide is visible in Fig. 2a for 1% cholesterol. The two profiles resemble very closely those obtained in the absence of cholesterol, as shown in our previous investigation (Dante et al. 2002). As one can infer directly from Fig. 2a, deuterium distribution, i.e., the difference between the profiles containing ^{2}H-Leu34-Aβ(25–35) or H-Leu34-Aβ(25–35), has two maxima. The difference between the two scattering profiles, calculated in 8% D_2O content, was fitted in the reciprocal space, following the procedure already described (Dante et al. 2002). Due to the similarity of the result with that obtained in the absence of cholesterol we refer to the mentioned paper and to the results discussed therein. We remind here that two Gaussian functions (and their mirror curves in the unit cell) were fitted to the difference profile. To perform the fit we were limited to four free parameters, since we measured only five structure factors. We used the amplitude and position of the Gaussians as free parameters, whilst the experimental resolution was used as the width of the Gaussian. To indicate that the difference between the membrane profiles in the presence of hydrogenated or deuterated Aβ(25–35) is significant, Fig. 2a reports the confidence limits at 95% of the two density profiles, taking into account the experimental errors of the structure factors. Once again the mathematical procedure to calculate the confidence limits is detailed in our previous publication (Dante et al. 2002).

A part of the peptide is intercalated in the lipid membrane and the deuterated label is located in the hydrocarbon region at $\pm$ 0.54 nm from the center of the lipid bilayer. A second label position is localized in the water phase at 2.75 nm from the center of the bilayer. In addition, in the absence of cholesterol, Aβ is divided in two populations, one penetrating into the membrane, the second one confined to the outside of the bilayer. We had interpreted a similar result

Table 1 Structure factors $F(h)$ of the investigated samples measured at 8% D_2O:H_2O contrast

Chol[a] (mol%)	Peptide	$F(1)$	$F(2)$	$F(3)$	$F(4)$	$F(5)$
0[b]		− 0.427 ± 0.005[c]	− 0.663 ± 0.005	+ 0.501 ± 0.005	0	− 0.36 ± 0.01
0[b]	Aβ(25–35) hydrogenated	− 0.475 ± 0.005	− 0.724 ± 0.003	+ 0.447 ± 0.005	0	− 0.22 ± 0.02
0[b]	Aβ(25–35) deuterated	− 0.543 ± 0.004	− 0.638 ± 0.004	+ 0.366 ± 0.007	0	− 0.40 ± 0.02
1		− 0.546 ± 0.003	− 0.639 ± 0.003	+ 0.490 ± 0.003	− 0.113 ± 0.008	− 0.12 ± 0.01
1	Aβ(25–35) hydrogenated	− 0.632 ± 0.003	− 0.671 ± 0.003	+ 0.464 ± 0.003	0	− 0.16 ± 0.01
1	Aβ(25–35) deuterated	− 0.633 ± 0.003	− 0.576 ± 0.004	+ 0.433 ± 0.002	0	− 0.28 ± 0.02
5		− 0.638 ± 0.003	− 0.589 ± 0.004	+ 0.447 ± 0.002	− 0.128 ± 0.008	− 0.17 ± 0.01
5	Aβ(25–35) hydrogenated	− 0.601 ± 0.004	− 0.687 ± 0.004	+ 0.328 ± 0.002	0	− 0.24 ± 0.02
5	Aβ(25–35) deuterated	− 0.657 ± 0.005	− 0.633 ± 0.004	+ 0.359 ± 0.004	0	− 0.19 ± 0.01
20		− 0.663 ± 0.005	− 0.572 ± 0.004	+ 0.404 ± 0.004	− 0.228 ± 0.010	− 0.13 ± 0.01
20	Aβ(25–35) hydrogenated	− 0.646 ± 0.003	− 0.598 ± 0.003	+ 0.404 ± 0.003	− 0.229 ± 0.012	− 0.13 ± 0.01
20	Aβ(25–35) deuterated	− 0.648 ± 0.003	− 0.597 ± 0.003	+ 0.407 ± 0.003	− 0.171 ± 0.008	− 0.17 ± 0.01
40		− 0.625 ± 0.002	− 0.682 ± 0.004	+ 0.313 ± 0.005	− 0.160 ± 0.009	− 0.13 ± 0.01
40	Aβ(25–35) hydrogenated	− 0.653 ± 0.002	− 0.638 ± 0.004	+ 0.337 ± 0.005	− 0.179 ± 0.009	− 0.14 ± 0.01
40	Aβ(25–35) deuterated	− 0.640 ± 0.002	− 0.622 ± 0.004	+ 0.371 ± 0.005	− 0.213 ± 0.011	− 0.14 ± 0.01

[a]The membrane composition consists of POPC/POPS/cholesterol mixtures, with cholesterol in different molar amounts
[b]The structure factors of the samples without cholesterol are retrieved from our previous experiment (Dante et al. 2002) and calculated at 8% D_2O:H_2O contrast by linear regression and scaled to the actual SF
[c]The reported errors are derived from the counting statistic. The listed values are the mean value of two measurements, whereby the single values are inside the error bars and therefore undistinguishable

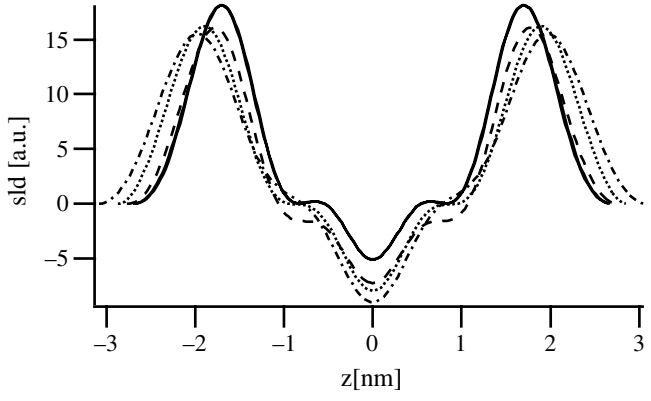

Fig. 1 Scattering length density (*sld*) profiles of POPC/POPS/Chol membranes. The *solid line* refers to the membrane with 0 and 1% cholesterol content. Increasing the molar weight content of cholesterol to 5% (*dashed line*), 20% (*dotted line*) and 40% molar weight (*dash and dotted line*) a progressive change in the shape of the profile is observed. The spacing and membrane thickness increase, and the headgroup region broadens. The stiffening effect of cholesterol results in the ordering of the methyl groups and consequently in a decrease of the scattering length density in the region around $z = 0$. The region most largely affected by presence of cholesterol is the hydrocarbon region

obtained previously as a different behavior of monomeric and aggregated Aβ, the former intercalating in the bilayer, the latter aggregated in the water phase or at the water/membrane interface.

Figure 2b depicts the profiles of a membrane with 5% molar weight cholesterol content, in the presence of hydrogenated (dashed) and deuterated Aβ(25–35) (solid). The difference between the two profiles is much less pronounced than in the samples containing 1% molar cholesterol only. A perturbation of the membrane is present, but the minor difference between the two scattering length density profiles does not allow any localization of the deuterated label.

For cholesterol contents higher than 5% molar weight (i.e., 20 and 40% molar weight), no difference in the membrane profiles in the presence of hydrogenated and deuterated peptides is detected. Even at the highest cholesterol content, no phase separation has been detected. Figure 2c shows the results for a lipid membrane with 20% molar cholesterol, i.e., at the physiological relevant content present in nerve cell membranes. The membrane profiles in the presence and absence of the peptide overlap perfectly. This finding indicates that the peptide does not intercalate into the membrane with 20 and 40 mol% cholesterol. Similar results obtained for the highest cholesterol content are not shown. We have shown (Dante et al. 2002) that the intercalation of the peptide changes the membrane structure substantially. Intercalation of the peptide at different depths would lead to a broad, nearly undetectable deuterium distribution but would nevertheless induce such structural changes, which are not seen. These observations lead to the important conclusion that even a moderate cholesterol content hinders the perturbations of a lipid membrane by Aβ(25–35) and prevents the intercalation of Aβ(25–35) inside the hydrocarbon region. We point out that the presence of cholesterol induces a different behavior of the peptide not only in the membrane region, but also in the water phase. In fact, in the case of 20 and 40% cholesterol molar contents, no deuterated label is detectable in the water phase. This difference in behavior may be explained tentatively by the finding that cholesterol induces Aβ fibril formation (Yip et al. 2001). These fibrils are distributed randomly and no coherent diffraction signal is generated. In the case of low cholesterol content the peptide could be squeezed out of the membrane due to a reduced hydration (98%) but stays monomeric or as small oligomers evenly distributed between the membrane sheets.

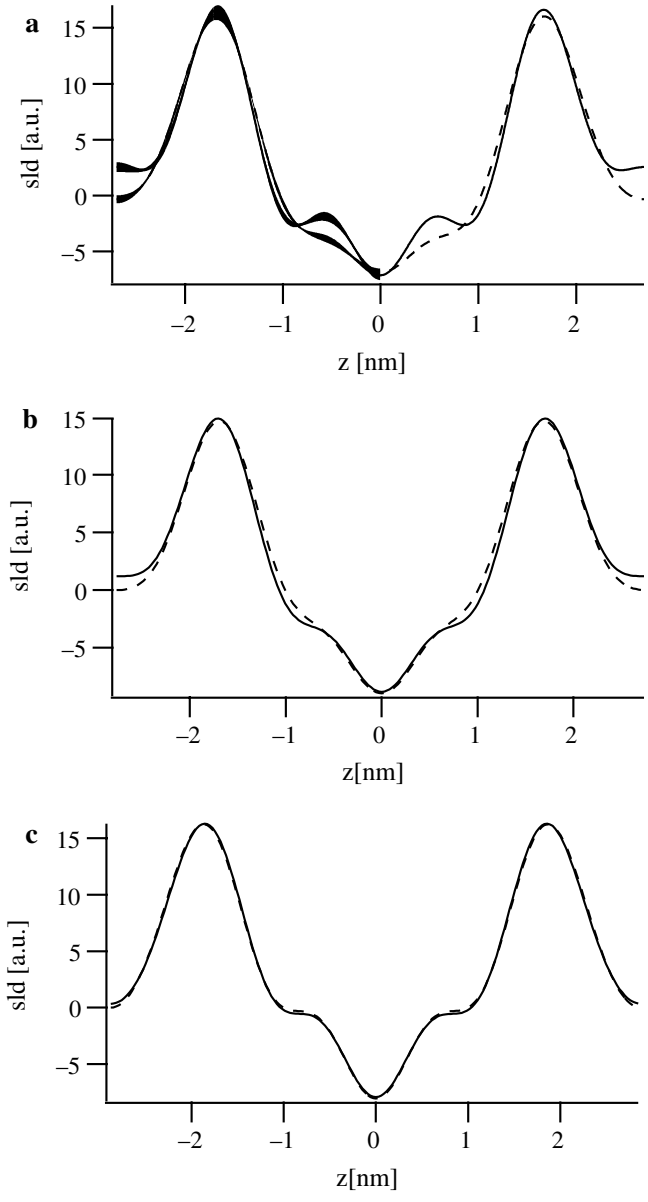

Fig. 2 Scattering length density (*sld*) profiles of lipid membranes in the presence of H-Leu34-Aβ(25–35) (*dashed line*) and ^{2}H-Leu34-Aβ(25–35) (*solid line*). **a** depicts the profile of a membrane containing 1% cholesterol molar weight. The difference between the two profiles in the water region and the hydrocarbon region reveals the location of the deuterated amino acid Leu34. The 95% confidence limit is shown on the *left* as a *gray band*. On increasing the amount of cholesterol to 5% molar weight (**b**) the difference between the profiles in the presence of hydrogenated and deuterated Aβ(25–35) becomes less evident, although a perturbation of the lipid membrane due to the presence of the peptide is still present in the headgroup region and a difference in the water region is also evident. When the amount of cholesterol reaches 20% molar weight, it is not possible to locate the deuterated label, since the two profiles are undistinguishable. In addition (data not shown), they are perfectly equal to the membrane profile of equal composition in the absence of peptide

Model calculation

To get additional information about the distribution of the label in the membrane containing 1% molar weight cholesterol, we have modeled the membrane profiles with Gaussian functions, partitioning the lipid bilayers into appropriate molecular groups. Each part is scaled by equating its area to the sum of the neutron scattering length of the atoms constituting it. This model calculation of the bilayer, fully normalized to the scattering length of the molecular part of the lipids, is also useful to show that the small amount of deuterium contained in the labeled peptide can actually be detected. Therefore, we have modeled separately the profile of a bilayer containing either hydrogenated Aβ(25–35) or deuterated Aβ(25–35). The lipid molecules have been divided into three molecular segments corresponding to their headgroups, their alkyl chains and their terminal methyl groups. The contribution of cholesterol has been summed into the alkyl chains region. Based on our previous knowledge about the peptide location, the overall contribution of hydrogenated Aβ(25–35) has been equally parted between the headgroup and the alkyl chain segments. The contribution of hydrogenated peptide is nevertheless very small. To find the best model fitting our data, the sum of three Gauss functions and their mirrors with fixed area A (the calculated neutron scattering length of the molecular groups) and variable positions and half-width and with an appropriate scaling factor have been fitted to the experimental curve. Modeling the profile with a larger number of Gauss functions, i.e., parsing the lipids in more molecular groups, would result in a more precise simulation of the experimental profiles. Nevertheless, we decided to use only three Gauss functions to keep the number of free parameters limited to a maximum of four. The position of the methyl group ($z = 0$) and the width of the corresponding Gaussian were considered as fixed parameters. The parameters are reported in Tables 2 and 3. The parameters kept fixed in the modeling routine are presented without errors. Modeling the experimental curve obtained in the presence of deuterated Aβ(25–35) yields information about the localization of the deuterated leucine. In this case, the membrane has been modeled with two additional Gauss functions, each with three variable parameters. The three Gauss functions representing the membrane without label have been kept unchanged. The results of the model calculation of the lipid membrane in the presence of Aβ(25–35) and ^{2}H-Leu34-Aβ(2–35) are reported in Fig. 3a and b, respectively. The position of the deuterated leucine at $z = \pm 0.57$ nm estimated from the model is in perfect agreement with the value obtained by the fitting procedure ($z = \pm 0.54$ nm). The area of the Gauss function 4, i.e., the additional scattering length in the alkyl chain region, is 3.15 fm. This is exactly the scattering length of our label, consisting of 10 deuterons per 33 lipid molecules (one H–D exchange corresponds to 10.42 fm). However, to obtain a profile resembling the experimental one, it is necessary to add another Gauss function in the water region corresponding to a scattering length of 2.54 fm. Therefore, 56.4% of the total peptide is found inside the membrane and the rest in the water phase.

Table 2 Parameters of the Gaussian model for the lipid membranes containing 1 mol% cholesterol in the presence of hydrogenated Aβ(25–35)

	Scattering length (area) (fm)	Width (nm)	Position (nm)
Gauss 1: methyl groups $2CH_3$	− 9.142	0.415	0
Gauss 2: alkyl chains + cholesterol + 0.5 peptide 0.99 PO + 0.01 cholesterol + Aβ(30–35)/33	− 17.17	0.57 ± 0.02	0.84 ± 0.02
Gauss 3: headgroups + 0.5 peptide 0.92 PC + 0.08 PS + glycerol + Aβ(25–29)/33	65.64	0.502 ± 0.006	1.69 ± 0.01

The molecular groups have the following composition: PC: $C_6H_{15}NPO_4$; PS: $C_4H_7NPO_6$; glycerol: $C_4H_3O_4$; PO: $C_{30}H_{58}$; cholesterol: $C_{27}H_{46}O$; Aβ(25–29): $C_{17}H_{31}N_7O_8$; Aβ(30–35): $C_{28}H_{50}N_6O_6S$

Table 3 Parameters of the Gaussian model for the lipid membranes containing 1 mol% cholesterol in the presence of ^{2}H-Leu34-Aβ(25–35)

	Scattering length (area) (fm)	Width (nm)	Position (nm)
Gauss 1: methyl groups	− 9.142	0.415	0
Gauss 2: alkyl chains + cholesterol	− 17.17	0.57	0.84
Gauss 3: headgroups	65.64	0.502	1.69
Gauss 4: deuterium label in the membrane core 10 (H–D exchange)/33	3.15	0.23 ± 0.04	0.57 ± 0.01
Gauss 5: deuterium label in the water phase 10 (H–D exchange)/33	2.54 ± 0.07	0.23 ± 0.02	2.75 ± 0.02

The molecular groups of Gaussians 1–3 are defined in Table 2

According to our model the overall contribution of the deuterated label is then equal to 5.69 fm, approximately 1.8 times bigger than the nominal deuterated label. In principle, we cannot rule out that the deuterium presence in the water phase may be due to a slightly erroneous H_2O/D_2O contrast. In fact, a contrast equal to 8.6% instead of 8% H_2O/D_2O would result in a scattering length equal to the area of Gauss 5, assuming 16 water molecules per lipid (Dante et al. 2002). Nevertheless, we believe that the observation of the label in the water region is not due to an artifact, being reproducible and detectable in pure phospholipid membrane as well. Moreover, it is also known that low-resolution diffraction data are well suited for the localization of a label position but not for a precise quantitative determination of the label strength (Papadopoulos and Hauß 2003).

A schematic drawing of the lipid membrane in the presence and absence of Aβ(25–35) is reported in Fig. 4.

Discussion

Controversial indications for modulation by membrane cholesterol in the Aβ interaction with the cell membrane have been recently reported by several studies. Our structural data give an unequivocal confirmation that cholesterol modulates the penetration of Aβ peptide in phospholipid bilayers, supporting and explaining the experimental data described in the quoted literature. The deuterated leucine of the penultimate C-terminal amino acids of the Aβ(25–35) is detected in the hydrocarbon region of the phospholipids at low cholesterol content only (i.e., 1% molar weight). A pictorial view of the phospholipids bilayers containing cholesterol and the deuterated peptide is reported in Fig. 4. The orientation of peptide in the figure is arbitrary, since it is not possible to extract it from our data. A cholesterol content between 5 and 20% molar weight hinders completely the penetration of the peptide in the lipid core, supporting the findings of Curtain et al. (2003) who studied by EPR spectroscopy how metal ions, pH and cholesterol regulate the interactions of Aβ peptides with membrane lipids. They found that the motionally restricted lipid component, characteristic of annular phospholipid surrounding a peptide, is reduced to zero when the cholesterol content in POPC large unilamellar vesicles is about 20% molar weight.

Arispe and Doh (2002), with measurements of cell viability, have observed that cholesterol-deficient membranes are more vulnerable to the action of Aβ(1–40) and Aβ(1–42). Enriching PC12 cells with exogenous cholesterol makes the cells resistant to the cytotoxic action of the peptides. They suggested that cholesterol controls the incorporation of Aβ by modifying the fluidity of the neural membranes. This incorporation would consequently result in the formation of Aβ calcium channels, and thus in cell death. Our data support this view on a direct structural basis.

The channel-forming activity of Aβ(25–35) was investigated by Lin and Kagan (2002) as a function of different physico-chemical parameters, such as ionic strength and membrane composition. It is well known that this peptide forms voltage-dependent, ion-permeable channels in planar lipid bilayers (Arispe et al. 1996; Mirzabekov et al. 1994). These authors highlighted a strong dependence of the channel activity as a function of the cholesterol content of large unilamellar vesicles. In particular, cholesterol dramatically decreased the

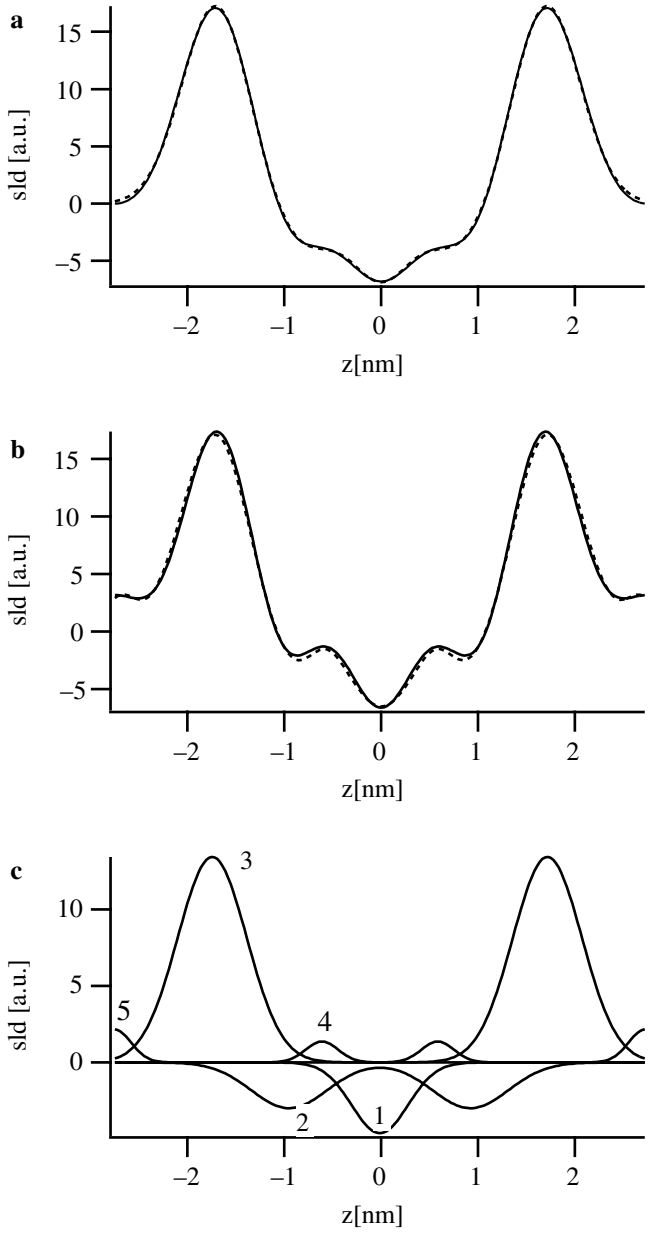

Fig. 3 Scattering length density (*sld*) profiles of a POPC/POPS bilayer with 1% cholesterol molar are shown (*solid lines*) together with their Gaussian models (*dotted lines*) in the case of H-Leu34-Aβ(25–35) (**a**) and ^{2}H-Leu34-Aβ(25–35) (**b**). The single Gauss curves, whose parameters are reported in Tables 1 and 2, are shown in **c**: the Gauss functions *1*, *2* and *3* are the models for the pure lipid membrane containing the hydrogenated peptide, the Gauss functions *4* and *5* represent the contribution of the deuterated Aβ(25–35) to the bilayer profile. Note that the apparent amplitude of Gauss function *5* is the sum of two mirror functions

channel activity of Aβ(25–35), and the measured number of channels dropped for cholesterol concentrations between 0 and 5% molar weight. This is in clear contradiction to the observation by Micelli et al. (2004) that cholesterol facilitates the incorporation of Aβ into membranes and that cholesterol reduces the imbalance of cellular ions, including calcium.

It has to be mentioned that, in conflict to our data, spontaneous insertion of Aβ(1–40) in Langmuir monolayers containing cholesterol has been reported (Ji et al. 2002b) and put in relationship to a conformational change of the peptide between β-sheet and α helix that would enable it to spontaneously insert into the lipid monolayers, which would hinder the fibril formation (Ji et al. 2002a).

Cholesterol is a major component of mammalian cells and is known to influence membrane thickness and fluidity. As already mentioned, a connection between cholesterol and the physiopathology of Aβ has been widely reported, but not explained on a molecular basis. Our data suggest that the rigidifying effect of cholesterol hinders the penetration of the peptide in the membrane and, according to the measurements of other authors, prevents ion-channel formation and cell disruption. Interestingly, in a recent structural investigation by X-ray small angle scattering (Rappolt et al. 2003), cholesterol in molar amount lower than 5% was found to increase the fluidity and compressibility of POPC bilayers. In contrast, at concentration between 5 and 25% molar weight the bilayers became more rigid and an increase in the spacing of about 2 Å was detected. These changes were attributed to the onset of phase separation between liquid-disordered and liquid-ordered phases. Our data are in line with these findings and also suggest that the insertion of the Aβ peptide is linked to the fluidity of the membrane or the lipid pools that may constitute it. It is interesting to note that a hindrance to the penetration of the monomeric Aβ in the membrane could support an accumulation process of Aβ outside the membrane and, perhaps, fibrils and plaques formation. However, besides the presence of cholesterol, many other physico-chemical parameters may influence the Aβ/membrane interaction; membrane composition, transbilayer distribution of cholesterol in neuronal cells, the presence of other lipids, such as glycolipids, and the presence of lipid rafts may play a role in the balance between the incorporation of Aβ and its extracellular segregation. Many of these aspects remain to be investigated and could explain the contradictory results so far collected in the literature.

Our results explain, on the molecular basis, the modulation induced by cholesterol on the Aβ(25–35) insertion into phospholipids bilayers and link this structural property to several observed physiological and functional behaviors. In perspective, this could lead to a better and complete understanding of the mutual effects between cholesterol and Aβ and, in combination with already suggested treatments with cholesterol concentration modifying drugs (Scarpini et al. 2003) or other drugs modulating the membrane properties, to a successful therapeutic approach to cure or prevent AD.

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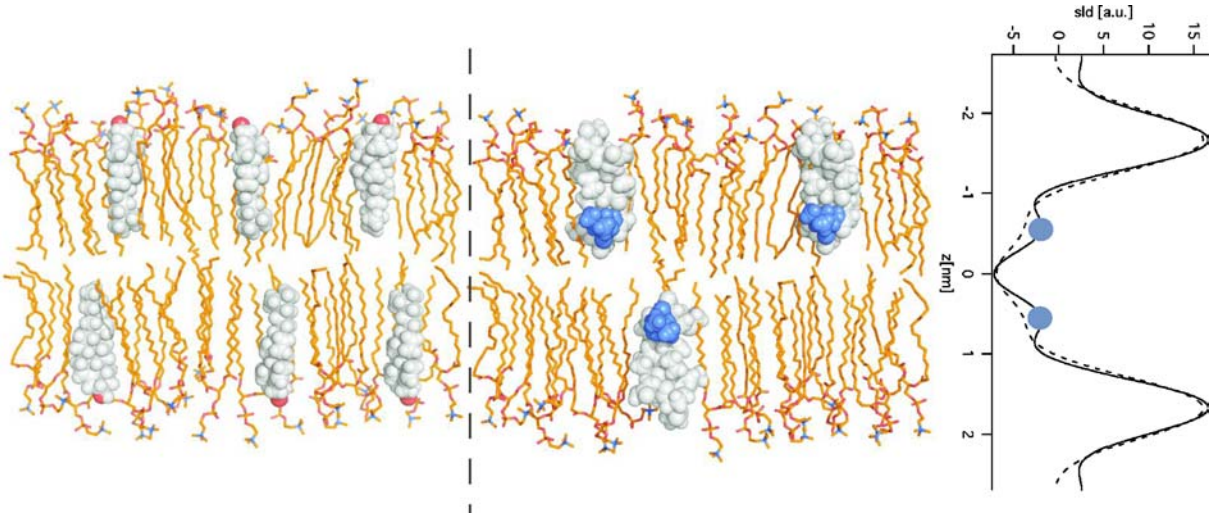

Fig. 4 Schematic drawing of a lipid bilayer containing cholesterol, in which the intercalation of Aβ(25–35) is hindered (*left*). In the absence of cholesterol, Aβ(25–35) can reach the hydrophobic core of the membrane (*right*). The molecular structures of the peptide and the lipids are derived from Kohno et al. (1996) and Heller et al. (1993), respectively. The atoms colored in *blue* correspond to the deuterated labeled Leu34. The scattering length density (*sld*) profiles of the lipid bilayers with protonated and deuterated peptide are drawn at the *right*. The difference between the sld profiles is marked with a *blue dot* in the position of the Leu34

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