Perspectives on Solid State NMR in Biology (Eds. S.R. Kühne & H.J.M. de Groot), Kluwer Academic Publishers, Dordrecht, pp. 203-214.

Structural insight into the interaction of amyloid-• peptide with biological membranes by solid state NMR.

Gerhard Gröbner[a], Clemens Glaubitz[b], Philip T. F. Williamson[c], Timothy Hadingham[d], Anthony Watts[d]

[a] *Biophysical Chemistry Department, Umea University, SE-90187 Umea, Sweden; gerhard.grobner@chem.umu.se*

[b] *Physical Chemistry Department, University of Stockholm, Sweden.*

[c] *Physical Chemistry Department, ETH Zurich, Switzerland.*

[d] *Biomembrane Structure Unit, University of Oxford, UK.*

Introduction

Alzheimer's disease (AD) is a chronic dementia, affecting an increasingly large number of old people worldwide [1-3]. AD together with mature onset diabetes and prion-transmissible spongiform encephalopathies, belongs to a category of amyloid diseases, which are all categorized by an abnormal folding of a normally soluble protein into neurotoxic aggregated structures [3-5]. The key event in AD is the metabolism of amyloid precursor protein to amyloid-• -peptide (A•) and the following deposit of A• as plaques in the brain of patients. This 39-42 amino acid long peptide has been linked to the apoptosis of neuronal cells, and its neurotoxicity seems to be associated with its ability to convert from a non-toxic monomeric form into toxic aggregates [5-7]. However the cellular mechanism involved in mediating the toxic effect of Aβ peptide remains unclear [6-11]; and also the process of transformation into insoluble, neurotoxic peptide aggregates. Due to the complexity and dependency of this process from physiological parameters, various models for fibril formation are studied at present including aggregation in solution [7,8], lipid-mediated aggregation of Aβ in contact with cell membrane surfaces [10-13], and formation of transmembrane ion channel-like structures in neuronal membranes [9,14,15]. Structural and physiological studies of the self-assembly of Aβ-peptide into fibrillar structures found this process strongly depending on the physical conditions present [5,6-8,16]. While earlier studies proposed antiparallel-β-sheet structures for the amyloid fibrils [6], more recent work indicates a in register, parallel organization of β-sheets propagating and twisting along the fibrillar axis [5,17]. However, there is growing evidence that not mature fibrils are the toxic agent itself but their precursors socalled diffusible "protofibrils" [3,4,8].

Various experimental evidence indicates, that non-specific interactions of Aβ with cell membranes may play an important role [9-15]. Since Aβ peptide comprises an extracellular and transmembrane (28-42 position) domain, its association with membranes has been shown to induce and accelerate formation of prefibrillar and fibrillar structures. It also can insert into lipid bilayers and form cation-selective channels [9,14,15]. The mechanism of self-assembly of A• on membrane surfaces or as ion-channels into membranes is not understood yet, primarily due to the lack of structural information at an atomic level. However, to extract this information is very challenging since any structural biology method including NMR has to deal with a very complex, non-cyrstalline, and disordered system.

Here, we report a strategy for structure determination where first, lipid-modulated structural and aggregational features of Aβ and its interactions with membranes are characterized by circular dichroism (CD) and ^{31}P MAS NMR spectroscopy; and secondly, rotational resonance (RR) ^{13}C CP MAS NMR recoupling techniques [19-22] give a first first insight into the membrane-bound secondary structure of the peptide, before major aggregation occurs. In this context also limits and future prospects of solid state NMR methods for structure determination on these systems are discussed.

Methods

Materials: L-α-Dimyristoylphosphatidylcholine (DMPC) and Dimyristoylphosphatidylglycerol (DMPG) were obtained from Sigma (UK), DMPC-d_{67} from Avanti Polar Lipids (US). $A\beta_{1\text{-}40}$ was synthesized by standard solid-phase FMOC chemistry (NSR Centre, Nijmegen, Netherlands), subsequently purified by HPLC and quality checked by MALDI MS. $A\beta_{1\text{-}40}$ containing 1-^{13}C-Ile_{31} and 2-^{13}C-Gly_{33} (Promochem, UK) was prepared in the same way. To obtain a monomeric, soluble form of the peptide, 10 mg peptide were dissolved in 500 µl TFA (trifluoroacetic acid). After removal of TFA by nitrogen stream, TFE (trifluoroethanol) was added to resuspend the protein film and then evaporated under fine vacuum to remove any traces of acidic TFA. For binding studies of Aβ to membrane surfaces, peptide was added to vesicles of various DMPC/DMPG compositions to give a final 30:1 P/L molar ratio. The mixture was incubated for 30 min at 310K, three times freeze-cycled and pelleted. For reconstitution trials, incorporation of Aβ into various DMPC/DMPG bilayers at 30:1 L/P molar ratio, was carried out as described before [20]. For incorporation of Aβ peptide in a nonaggregated state into membranes at a 20:1 L/P molar ratio for NMR experiments, 15 mg peptide film was dissolved in TFE (2 ml) subsequently mixed with DMPC-d_{67}, dried as a lipid/peptide film and resuspended in buffer (10 mM NaH_2PO_4, 0.2 mM EDTA,140 mM NaCl, pH 7.8). After sucrose density purification, vesicles were pelleted into MAS NMR rotors and kept frozen prior to measurements.

CD-measurements: Samples were sonicated under cooling using a probe-type sonicator and metal debris removed by centrifugation. CD-spectra (Jasco, USA) were obtained using a 1mm path length quartz cell (Hellma, Germany). CD-spectra were corrected for the lipid vesicle background and analyzed using the k2d software [23].

NMR experiments: ^{31}P MAS NMR experiments were carried out under efficient proton decoupling (30 kHz), at 81 MHz phosphorous frequency on a 200 MHz Infinity (Chemagnetics, USA) using double resonance 7 mm MAS NMR probe (Bruker, D). ^{13}C MAS NMR experiments were performed at 25.18 MHz, 100.6 MHz and 125.7 MHz ^{13}C frequencies on Chemagnetics and Bruker spectrometers using double resonance 7 mm and 4 mm MAS Probes. Cross polarization (CP) contact time was 0.6 ms for solids and 1.0 ms for membrane samples. Decoupling power varied between 60-80 kHz. The C_α-glycine ^{13}C resonance was selectively inverted by applying a DANTE pulse sequence [20], followed by a variable mixing time (0.5 ms – 30 ms).

Results and Discussions

The secondary structural features and aggregation properties of Aβ-peptide are very sensitive to the physical conditions, especially to the kind of interaction between the peptide and its membrane-environment. CD- and ^{31}P MAS NMR experiments were carried out using lipid vesicles of various composition to study the structural changes in the peptide as a function of its interactions with membranes either by contact to the surface or by incorporation. In this way suitable starting conditions were found to perform first ^{13}C RR CP MAS NMR experiments to explore structural features in the transmembrane part of the $A\beta_{1\text{-}40}$ peptide before major aggregation occurs.

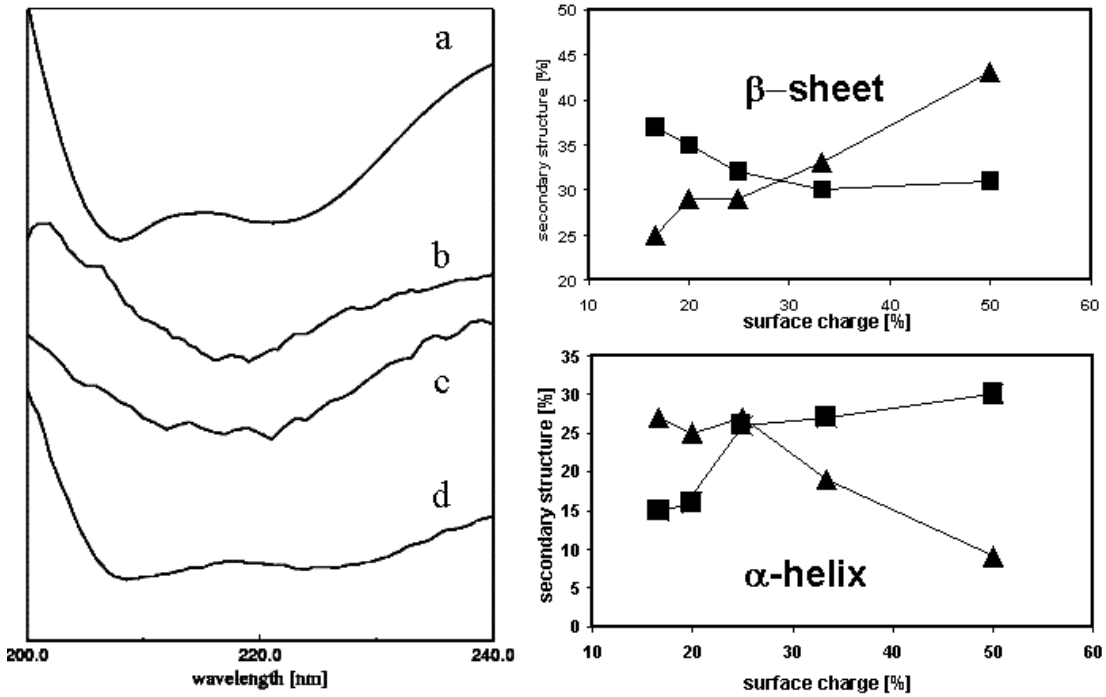

Figure 1. Left: CD-spectra for $A\beta_{1\text{-}40}$ peptide at RT: a) Aβ in TFE; b) Aβ bound to mixed DMPC/DMPG (2:1 PC/PG ratio) membrane surfaces at 30:1 L/P molar ratio; c) Aβ incorporated into mixed DMPC/DMPG (2:1 PC/PG ratio) at 30:1 L/P molar ratio by dialysis reconstitution; d) Aβ incorporated into DMPC membranes by cosolubilization at 20:1 lipid/peptide molar ratio. Right: Lipid-induced fraction of β-sheet and α-helix structures for Aβ added to (•) or incorporated into (•) mixed bilayers at 30:1 L/P molar ratio. Membrane surface charge varied between 50% and 17%.

CD-Measurements:

How the different lipid-peptide interactions affect the structure of Aβ-peptide can be seen in Figure 2 where results are shown for CD-measurements carried out on $A\beta_{1\text{-}40}$ either bound to various membrane surfaces or incorporated into them under different conditions. In Figure 2 (left), CD spectra are displayed for the spectral region between 200 – 240 nm at RT. Trace a) reveals a typical α-helical structure of Aβ, prepared as a monomer in TFE after HPLC purification. Trace b) reveals a significant amount of β-structures for Aβ added to charged membrane surfaces (33mol% negatively charged DMPG) at a 30:1 P/L molar ratio. A similar situation is seen in Trace c) when Aβ was

incorporated at the same ratio into the same membrane matrix by reconstitution via dialysis. To the contrary, reconstitution of the peptide into DMPC bilayers by cosolubilization using the membrane mimicking solvent TFE showed dominantly helical features (Trace d). These results are not surprising since TFE is stabilizing helical structures while in aqueous conditions a conversion from an initially random coil form into β-sheet state can easily take place [10]. Occuring interactions of Aβ in aqueous environment with charged membrane surfaces accelerate then this conversion as described in detail by Terzi et. al. [10] and others. The spectrum obtained for Aβ upon incorporation via dialysis (Trace c) is therefore not unexpected. Reconstitution trials using neutral DMPC alone failed to incorporate Aβ-peptide, probably due to the missing stabilization of the positively charged peptide in the micellar detergent system by negatively charged lipid headgroups.

To study in more detail the relationship between the structural properties of Aβ and the relevant lipid-peptide interactions, comparative binding and incorporation studies were performed under a systematic variation of the lipid environment. The amount of negatively charged DMPG lipid in the DMPC bilayer was varied between 50 mol% and 17 mol%. The analyis of the CD-studies of Aβ either added or incorporated into these membranes are displayed in Figure 2 (right). Adding $A\beta_{1-40}$ to mixed liposomes, reveals a strong relationship between the amount of β-sheet aggregates and bilayer surface charge density. In contrast, $A\beta_{1-40}$ incorporated into liposomes of the same composition shows an opposite behaviour.

^{31}P MAS NMR:

Since CD-spectroscopy does not provide a detailed view at a molecular level for the interactions of the peptide with membrane surfaces, ^{31}P MAS NMR was used complimentary to study the nature and specificity of interactions of Aβ with the various lipid components when bound to charged membrane surfaces. Samples were prepared as for CD-spectroscopy except for sonication, and the corresponding NMR lineshapes are shown in Figure 3 together with the spectra obtained for pure vesicles of different PC/PG content. Two resonances corresponding to DMPG and DMPC lipids can clearly be resolved with the intensity ratio changing from 1:1 to 5:1 PC/PG composition as expected. Upon addition of Aβ peptide, no specific interaction between the peptide and a lipid component were observed, only effects seen in both resonances in the same way. Line narrowing occurs for both resonances, most likely reflecting an increased fluidity of the system. A close inspection of the isotropic chemical shift values shows two detectable effects as summarized in Figure 3 (right) where the chemical shifts are plotted against the lipid composition before and upon binding of peptide. First, the chemical shift values change for both lipids in relation to the contents of charged lipids. Secondly, upon binding, Aβ induces a change in the chemical shift values for both resonances in the same direction, identical to the one observed when lowering the amount of charged vesicles. This effect reflects a partial compensation of membrane surface charge and suggests a mainly electrostatic binding of Aβ to the membrane surface.

The CD and ^{31}P MAS NMR studies clearly show that a precise control over the occurring lipid-peptide interactions and related parameters are essential to extract by

any biophysical method high resolution structural information for this complex, disordered system, where the conversion from a monomeric form into toxic aggregates has serious neurotoxicological implications.

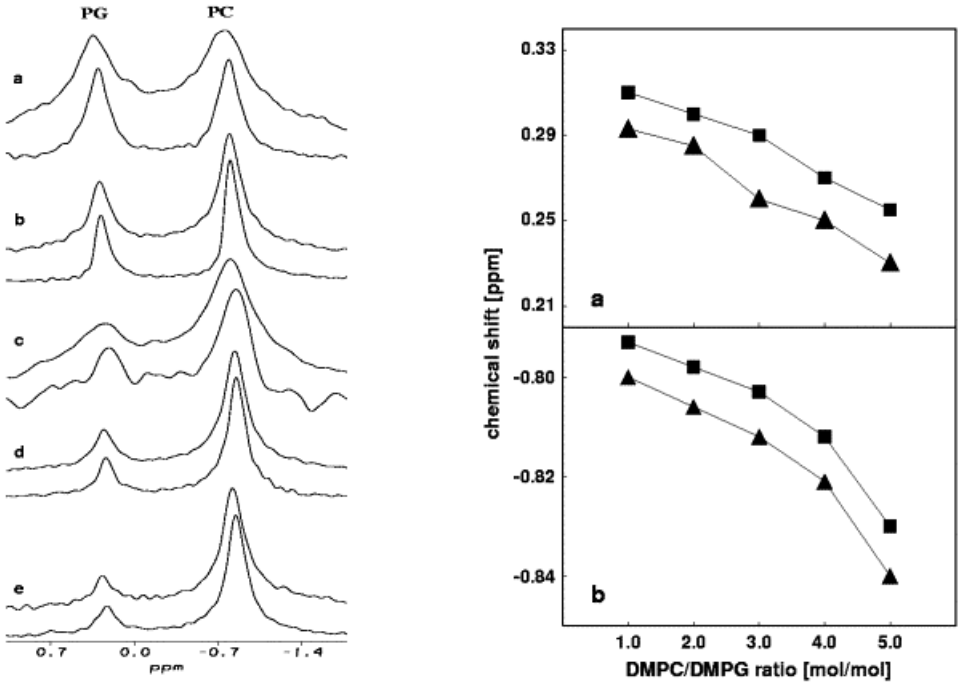

Figure 2. Left: ^{31}P MAS NMR spectra (2kHz spinning speed, RT) of DMPC/DMPG vesicles before (top trace of each panel) and upon addition of $A\beta_{1-40}$ peptide at 30:1 L/P ratio (bottom trace). DMPC/DMPG molar ratios: 1:1 a); 2:1 b); 3:1 c); 4:1 d); 5:1 e). Right: Isotropic chemical shift values at RT for DMPG (a) and DMPC (b) as a function of DMPC/DMPG molar ratios of the membrane before (•) and upon addition of peptide (•).

^{13}C CP RR MAS NMR:

To get first high resolution structural data for membrane-bound Aβ, it was incorporated in a predominantly α-helical form into membranes with the purpose to study the secondary structure of the transmembrane part of the peptide before major aggregation occurs. ^{13}C CP RR MAS NMR experiments were carried out on $A\beta_{1-40}$ specifically labellel as indicated in Figure 3, and reconstituted into DMPC-d_{67} bilayers at a 20:1 lipid/peptide molar ratio by cosolubilization.

In Figure 4 ^{13}C CP MAS NMR spectra are shown for labelled $A\beta_{1-40}$ peptide before (100.6 MHz) and after incorporation into membranes (125.7 MHz), respectively. In the spectrum for the solid peptide at 293 K (Trace a) the resonance at 175 ppm can be assigned to the labelled 1-^{13}C-Ile_{31} position and the resonance at 44 ppm to the 2-^{13}C-Gly_{33} position, both situated in the transmembrane part of the peptide. The spectrum of labelled Aβ upon incorporation into DMPC bilayers obtained at RT and 5 kHz spinning speed is shown in Trace b) of Figure 4. It is immediately obvious that the use of perdeuterated DMPC reduces the natural abundance signal arising from lipid carbon atoms drastically due to the missing CP conditions and reveals otherwise hidden resonances from the peptide and a few signals from the lipid glycerol backbone carrying protons [19,20]. In the membrane the resonance of the carbonyl

group of Ile_{31} in the transmembrane region is shifted upfield to 172 ppm and the $13C_\alpha$ resonance of Gly_{33} up to 42 ppm at 293K. At 213K (see Trace c), the resonance positions from both labelled residues remain unchanged but overlap partially with lipid resonances which are now broadened due to restricted motional mobility [20].

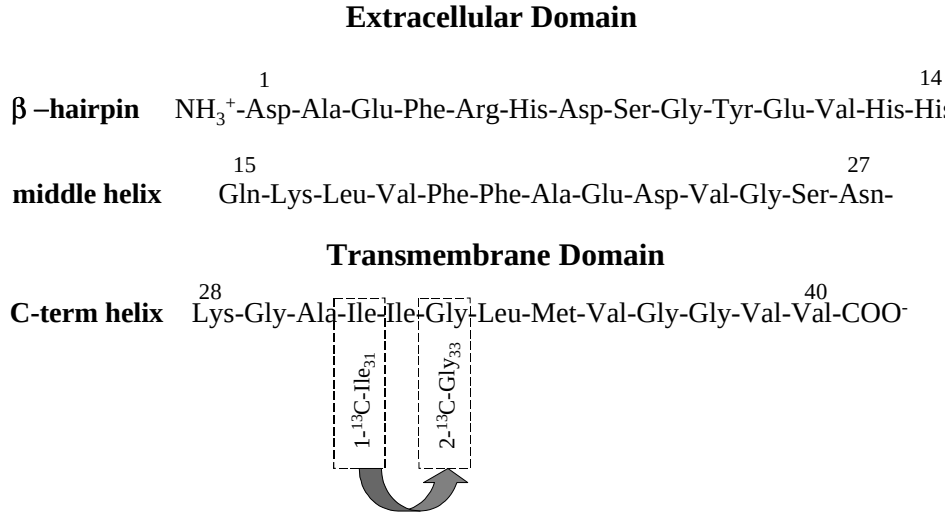

Figure 3. Amino acid sequence of amyloid-β peptide. The three domains of the monomeric membrane-bound secondary structure of $A\beta_{1-40}$ are indicated as predicted from modelling approaches [24]. Synthetically introduced residues carrying ^{13}C labels as used for rotational resonance distance measurements are marked.

Comparison of the spectrum at 293 K with the one obtained at 213 K shows the same inhomgeneous linebroadening for both resonances arising from the peptide when incorporated into membranes. Therefore for this peptide segment, a distribution of conformational states must exist accompanied by a highly restricted dynamics; a phenomen already observed before in other transmembrane peptide systems like melittin or the M13 coat protein [20,25,26].

Despite the already restricted dynamics of the peptide at RT, rotational resonance experiments on the multilamellar DMPC lipid vesicles containing Aβ peptide were carried out at 213 K to exclude dynamical effects completely, who could interfer with the correct measurement of dipolar couplings and hence distances. At 213 K the peptide is also prevented from undergoing secondary structural changes into β-sheet structures. Magnetization exchange experiments under rotational resonance n=2 conditions were carried out for the membraneous system at 125.7 MHz ^{13}C frequency and 8172 Hz spinning speed between the 1-^{13}C-Ile_{31} and 2-^{13}C-Gly_{33} resonances. For off-resonance experiments a spinning speed of 7171 Hz was used. For comparison, experiments were also performed on pure solid labelled $A\beta_{1-40}$ at n=1 rotational resonance condition (25.18 MHz). To analyse the magnetization exchange correctly, the amount of natural abundance signal arising from non labelled residues of the peptide was determined. Experiments identical to the one shown in Figure 4a were carried out on unlabelled solid $A\beta_{1-40}$ (spectra not shown) and compared to the one obtained for labelled peptide. For the carbonyl resonance, the percentage of natural abundance was found to be 33% and for the C_α region a fraction of around 30% was estimated.

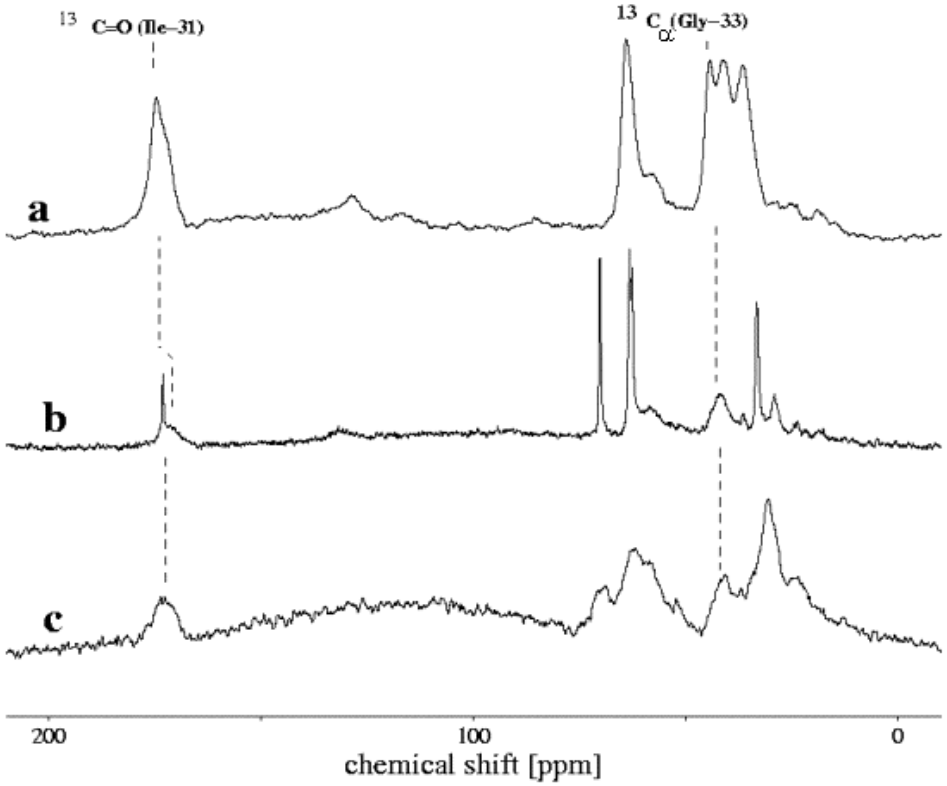

Figure 5. ^{13}C-CP-MAS NMR spectra of $A\beta_{1-40}$, isotopically ^{13}C labelled at positions as indicated: a) Spectrum (100.6 MHz) obtained at 9 kHz spinning speed and 293 K for solid $A\beta_{1-40}$; b) spectrum obtained at 125.7 MHz frequency for $A\beta_{1-40}$ incorporated into DMPC-d_{67} lipid bilayers at 293 K and 5 kHz spinning speed; c) spectrum as in b) but at 213 K and 8 kHz spinning rate.

The magnetization exchange curves obtained for the labelled Aβ in DMPC membranes at 213 K and n=2 conditions are shown in Figure 5a. For comparison, Figure 5b displays the data obtained for the pure peptide before incorporation. A significant signal decay can be clearly seen in both cases indicating a short distance between both labels in the transmembrane part of the peptide. Due to the much higher signal to noise ratio and the n=1 condition, data for the pure solid peptide could be obtained for more mixing times and with smaller error limits (< 20%) than for the peptide in the membrane (error < 30%). However, the observed large linewidth seen for the solid peptide (see Figure 4a) indicates a not surprising range of conformational states and related distribution of distance constraints. Therefore RR conditions are not fulfilled for all conformational states at the same spinning speed due to the large inhomogeneous linebroadening, a problem already discussed before [20]. Together with the distribution of distance constraints and in the case of membrane-bound peptide also pure signal/noise ratio makes a proper measurement of magnetization exchange and precise determination of an internuclear distance very difficult, as seen in the error margin and fluctuating magnetization exchange values. Despite the variation in the individual values, a clear trend of signal decay can be seen in the membrane-bound Aβ. Simulation of the magnetization decay curve still provides a lower and upper limit for the distance constraints [19-22]. However, due to the limited number of time points and the larger errors in the individual intensities, only a rough

estimation of the internuclear distance can be done. Nevertheless the signal decay as seen in Figure 5a indicates an α-helical structural feature for the transmembrane segment of Aβ in agreement not only with the CD-measurements (s. Figure 1d) but also with similar measurements obtained for the transmembrane helical part of M13 coat protein in membranes [20]. Any major β-sheet structure can be excluded to the measured distances and the relevant CD spectrum. This result is not surprising since the method of incorporation relies on a water-free technique using the membrane mimicking solvent TFE. Due to the cosolubilization with lipids, the peptide is already in a stable lipid environment before resuspension in an aqueous medium and, through the lipid matrix, protected against any conformational changes into β-sheet like structures when kept at low temperatures.

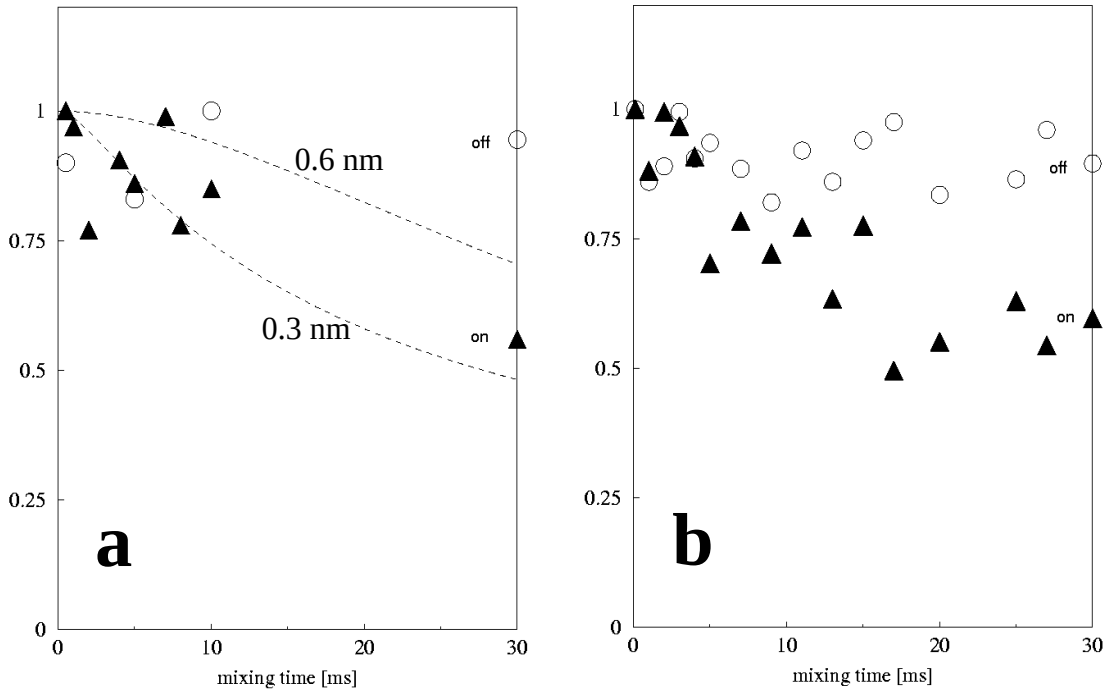

Figure 5: Magnetization exchange curves between 1-^{13}C-Ile$_{31}$ and 2-^{13}C-Gly$_{33}$ in Aβ$_{1-40}$ peptide incorporated into DMPC membranes (a) and as solid (b). The difference magnetization of both spin pairs $<I_z$-$S_z>$ is plotted over the mixing time. Experimental error indicated in text: Measurements were carried out for a) at 125.7 MHz at 213K and n=2 RR conditions, for b) at 25.18 MHz at 293 K and n=1 RR conditions.

In this report first data are presented on the structure of the membrane anchored part of the Aβ$_{1-40}$ peptide when embedded in lipid bilayers. A combination of CD-spectroscopy and rotational resonance NMR methods using specifically ^{13}C labelled Aβ-peptide revealed, that its transmembrane part exhibits a mainly α-helical secondary structure. But the experiments clearly show the problems of acquiring distance constraints for non-crystalline, disordered and heterogeneous systems by means of solid state NMR.

Limits and Future Prospectives

For a precise determination of the secondary structure for Aβ-peptide in membranes and changes occuring upon onset of aggregation, the limits of RR and standard solid state NMR approaches are severe, and new NMR strategies have to be used, to obtain the required information for non-crystalline, disordered biological polymers. These strategies should also include the application of multiple or uniformly labelled systems to extract a huge wealth of structural information and avoid the expensive and sometimes not possible synthesis of specifically labelled molecules, to obtain a single distance or torsion angle.

To obtain information about the modulation of the structural and aggregational properties of Aβ-peptide, various problems have to be addressed; problem often avoided in current solid state NMR studies by using peptides in a well defined crystalline environment to obtain good resolution. However, this approach doe not only avoid the problemes related to the presence of many conformational states, but often also any relevance to the biological situation. Since the aggregation process in nature is not directed towards a highly ordered system but like in many other similar diseases into disordered and aggregated systems, NMR methodology has to adapt to this situation.

As seen in the ^{13}C MAS NMR spectra presented here, two sets of problems have to be addressed. First, the high natural abundance occurring from the lipid matrix (perdeuterated lipids not always available) and other peptide residues makes accurate identification and intensity measurements of labeled residues difficult. Secondly, non-crystalline solids or peptides associated with biological membranes have inhomogeneous broadened NMR resonance that results from structural heterogeneity present already in the non-aggregated state [20,27]. This puts serious limitations on the application of exact rotational resonance conditions and on the proper assignment for uniformly labeled systems.

One way to remove background signals due to natural abundance is the application of double quantum NMR filters, as demonstrated successfully up to medium distances (< 4Å) e.g. by Levitt [28], or Gregory et. al. using a DQ-DRAWS sequence [29]. But the efficiency is extremely low (10% for 3.8 Å [29]) and would be even less for the Aβ-peptide in this study (distance for α-helix: 4,4 Å). And the labeled peptide is still diluted in an excess of lipids and water. Therefore the use of multiple or uniformly labeled peptide by means of molecular biology is more promising for the future. In this way shorter distances e. g. between ^{13}C dipoles are present providing much higher efficiencies for these filters [21]. However due to the multiple strong couplings increased linewidths occur and the observation of weak couplings is more difficult.

The second big advantage of multiple labeled systems is to extract many structural constraints simultaneously, important to describe the structure of larger sytems. However, this requires a full assignment of the occurring resonances despite the increased linewidths. Various groups have already successfully developed appropriate NMR sequences for full assignment for multiple labelled peptides and proteins e.g. on crystalline SH3 using [28], the lyophilized ubiquitin [29], and an chemotactic tripeptide using 2D and 3D ^{15}N-^{13}C-^{13}C chemical shift correlation NMR [30]. Recently, the group of de Huub demonstrated on large chlorosmal antennae

complexes a successful assignment for a system with considerable structural heterogeneity using high-field 2D and 3D dipolar correlation methods [31]. These ongoing developments should enable researchers in the near future by using high-fields and 3D experiments to assign e. g. multiple labelled Aβ in its neurotoxic aggregated state despite the presence of large inhomogenous linebroading.

Another unsolved problem which is the question how to extract many distance and torsion angle constraints from multiple or uniformly labelled molecules with their inherent multiple strong couplings. Various broadband recoupling techniques for homonuclear and heteronuclear systems for distance measurments have been developed over the last years e.g. RFDR (rf-driven recoupling), DRAMA (dipolar recovery at the magic angle), SEDRA (simple excitation for the dephasing of rotational-echo amplitudes), C7, Post-C7 [21]. However, the conversion of cross peak intensities into distance information (similar to NOE constraints in solution NMR) is difficult and therefore the extraction of multiple distance information from these spectra is currently a huge challenge. Similar problems arise for the determination of torsion angles. Recently, Hong and coworkers developed a promising approach which could be suitable for samples with a broad distribution of various secondary structural features like Aβ [34]. Their NMR pulse sequences discriminate between α-helix and β-sheet residues and filter them selectively. In this way it should be possible to determine the amount of α-helix and β-sheet residues in a molecule at various states. In addition, development is also ongoing how to determine the number of molecules arranged into aggregates using static multiple quantum NMR techniques [17].

To obtain a complete structural description of Aβ-peptide associated with membranes at its various aggregational states MAS techniques have to be applied to multiple/uniformly labeled peptide in the future. This requires development in both spectral resolution and sensitivity, and includes high field NMR machines, development of labeling schemes and sample preparation for improved resolution and finally the development of pulse sequences and appropriate algorithms to extract multiple distance and torsion angle constraints from these systems.

Acknowledgements

Financial support from BBSRC (43/B11683), EU (FMRX-CT96-0004) and local funding (Umeå University) is acknowledged. CG is recipient of a DFG Emmy-Noether Fellowship and A. W. of a BBSRC Senior Fellowship (43/SF09211).

References

1. Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L., and Beyreuther, K., Proc. Nat. Sci. USA 82 (1985) 4245.
2. Haass, C., and Selkoe, D. J., Cell 75 (1993) 1039.
3. Rochet, J.-C., and Lansbury Jr., P. T., Curr. Opin. Struct. Biol. 10 (2000) 60.
4. Lansbury Jr., P. T., Proc. Natl. Acad. Sci. USA 96 (1999) 3342.
5. Burkoth, T. S., Benzinger, T. L. S., Urban, V., Morgan, D. M., Gregory, D. M., Thiyagarayan, P., Botto, R. E., Meredith, S. C. and Lynn, D. G., J. Am. Chem. Soc. 122 (2000) 7883.

6. Iversen, L. L., Mortishire-Smith, R. J., Pollack, S. J. and Shearman, M. S., Biochem. J. 311 (1995) 1.
7. Lorenzo, A., and Yankner, B. A., Proc. Natl. Acad. Sci. USA 91 (1994) 12243.
8. Walsh, D. M., Hartley, D. M., Kusumoto, Y., Fezoui, Y., Condron, M. M., Lomakin, A. Benedek, G. B., Selkoe, D. J., and Teplow, D. B., J. Biol. Chem. 274 (1999) 25945.
9. Vargas, J., Alarcón, J. M. and Rojas, E., Biophys. J. 79 (2000) 934.
10. Terzi, E., Hölzemann, G., and Seelig, J., Biochemistry 36 (1997) 14845.
11. Kremer, J. J., Pallitto, M. M., Sklansky, D. J., and Murphy, R. M., Biochemistry 39 (2000) 10309.
12. McLaurin, J., Franklin, T., Chakrabartty, A., and Fraser, P. E., J. Mol. Biol. 278 (1998) 183.
13. Choo-Smith, L.-P., Garzon-Rodriguez, W., Glabe, C. G., and Surewicz, W. K., J. Biol. Chem. 272 (1997) 22987.
14. Kawahara, M., Arispe, N., Kuroda, Y., and Rojas, E., Biophys. J. 73 (1997) 9412.
15. Rhee, S. K., Quist, A. P., and Lal, R., J. Biol. Chem. 29 (1998), 13379.
16. Jarvet, J., Damberg, P., Bodell, K., Eriksson, L. E. G. , and Gräslund, A., J. Am. Chem. Soc. 122 (2000) 4261.
17. Antzutkin, O. N., Balbach, J. J., Leapman, R. D., Rizzo, N. W., Reed J., and Tycko, R., Proc. Natl. Acad. Sci. USA 84 (2000) 13045.
18. Mason, R. P., Jacob, R. F., Walter, M. F., Mason, P. E., Avdulov, N. A., Chochina, S. V., Igbavboa, U., and Wood, W. G., J. Biol. Chem. 274 (1999) 18801.
19. Smith, S. O., Aschheim, K. and Groesbeek, M., Quart. Rev. Biophysics 29 (1996) 395.
20. Glaubitz, C., Gröbner, G., and Watts, A., Biochim. Biophys. Acta 1463 (2000) 151.
21. Griffin, R. G., Nature Struct. Biol. 5 (1998) 508.
22. Raleigh, D. P., Levitt, M. H., and Griffin, R. G., Chem. Phys. Lett. 146 (1988) 71.
23. Andrade, M. A., Chacon, P., Merelo, J. J., and Moran, F., Protein Engineering 6 (1993) 383.
24. Durell, S.R., Guy, H. R., Arispe, N., Rojas, E., and Pollard, H. B., Biophys. J. 67 (1994) 2137.
25. McDonnell, P. A., Shon, L., Kim, Y., and Opella, S. J., J. Mol. Biol. 233 (1993) 447.
26. Naito, A., Nagao, T., Norisada, K., Mizuno, T., Tuzi, S., and Saitô, H., Biophys. J. 78 (2000) 2405.
27. Tycko, R., J. Biomol. NMR 8 (1996) 239.
28. Karlsson, T., Edén, M., Luthman, H., and Levitt, M. H., J. Magn. Reson. 145 (2000) 95.
29. Gregory, D. M., Wolfe, G. M., Jarvie, T. P., Sheils J. C., and Drobny, G. P., Mol. Phys. 89 (1996) 1835.
30. Pauli, J., van Rossum, B., Förster, H., de Groot, H. J. M., and Oschkinat, H., J. Magn. Reson. 143 (2000) 411.
31. Hong, M., J. Biomol. NMR 15 (1999) 1.
32. Rienstra, C. M., Hohwy, M., Hong, M., and Griffin, R. G., J. Am. Chem. Soc. 122 (2000) 10979.
33. van Rossum, B.-J., Steensgaard, D. B., Mulder, F. M., Boender, G. J., Schaffner, K., Holzwarth, A. R., and de Groot, H. J. M., Biochemistry, in press.
34. Huster, D. Yamaguchi, S., and Hong, M., J.Am. Chem. Soc. 122 (2000) 11320.