Spin-label EPR on Disordered and Amyloid Proteins

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CHAPTER 3

INTERACTION OF THE AMYLOID β PEPTIDE WITH A MEMBRANE MIMICKING DETERGENT

THE REGIME OF SUB-MICELLAR DETERGENT CONCENTRATION

The amyloid β (Aβ) peptide is important in the context of Alzheimer's disease, where it is one of the major components of the fibrils forming amyloid plaques. Agents that can influence aggregation are important, and of those, membrane mimics are particularly relevant, because the hydrophobic part of Aβ suggests a possible membrane activity of the peptide. We employed spin-label EPR to learn about the aggregation process of Aβ in the presence of the sodium dodecyl sulfate (SDS) detergent as a membrane mimicking agent. In chapter 2 we focus on the overall effect of SDS on the Aβ using a spin label at the N-terminus as a probe. Thereby information on the state of Aβ at high-SDS concentration, i.e., above the critical micelle concentration (CMC) is obtained. In the present chapter we explore the aggregation behavior using two different positions of the spin label. By comparing the two label positions the effect of local mobility of the spin label is eliminated, thereby, we learn about the Aβ aggregation in the SDS concentration regime below the CMC. We demonstrate that at low SDS concentrations the N-terminus of Aβ participates in the solubilization by being located at the particle/ water interface. At higher SDS concentrations an SDS-solubilized state that is a precursor to the one Aβ/micelle state above the CMC of SDS prevails. This study reveals the unique potential of EPR in studying the Aβ aggregation.

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3.1 Introduction

The aggregation of the amyloid β (Aβ) peptide to fibrils and plaques is the chief indicator of Alzheimer's disease [1-7]. The potent pathologic effects of Aβ oligomers provide a compelling reason for elucidating the mechanism(s) leading to the transformation of monomeric Aβ into toxic oligomers and ultimately larger aggregates [8-10]. In this context agents that can influence aggregation are important, and of those, membrane mimics are particularly relevant, because the hydrophobic part of Aβ suggests a possible membrane activity of the peptide. One such agent is the sodium dodecyl sulfate (SDS) detergent [10-14]. Previous studies addressed the aggregation of Aβ under the influence of SDS [15-20]. At high concentrations of SDS, close to the critical micelle concentration (CMC) and above, Aβ is found to have an α-helical conformation [15-18,20]. At submicellar concentrations, SDS seems to accelerate the formation of spherical aggregates [21,22], however, detailed information is missing because samples are heterogeneous, which makes them difficult to study further [9,10,13,23,24]. We use EPR to address this problem [25-28]. Here we employ spin-label EPR to obtain local information about the different sections of the Aβ40 peptide during the process of aggregation. To this aim, two cysteine variants of Aβ40, bearing a nitroxide spin label at the N-terminus or in the middle of the sequence, are examined to study the effect of SDS at various concentrations. The present study suggests that at submicellar concentrations of SDS the Aβ40/SDS aggregates have different shapes. We show that by spin-label mobility EPR local information about Aβ aggregation at a wide range of SDS concentrations can be obtained.

3.2 Materials and methods

The Aβ40 peptide as well as two cysteine-Aβ variants: [cys26]-SL-Aβ (in short: SL26-Aβ) and [cys1]-SL-Aβ (in short: SL1-Aβ), differing in the position of the spin label, were purchased from AnaSpec (purity > 95 %), the solvent DMSO was purchased from Biosolve (purity 99.8 %), the MTS spin label ((1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate) was purchased from Toronto Research Chemicals Inc. (Brisbane Rd., NorthYork, Ontario, Canada, M3J 2J8). Spin labeling was performed and the purified spin-labeled Aβ was analyzed by liquid chromatography as described previously [25]. The peptide was lyophilized and stored in the freezer (-20^0 C) until used.

3.2.1 Sample preparation protocol

Two cysteine variants of the Aβ peptide, SL1-Aβ and SL26-Aβ, differing in the position of the spin label were used. From each Aβ peptide variant six different Aβ sample conditions, differing in SDS concentrations (1.5 mM, 3 mM, 4 mM, 7 mM,

36 mM, and 72 mM) were prepared and compared to a sample into which no SDS was added. The total peptide concentration was kept constant at 0.55 mM. The peptide was a mixture of wild type Aβ and SL-Aβ, which contained 14 % SL-Aβ resulting in diamagnetically diluted samples as reported before [25]. Samples were prepared as described in chapter two of this thesis. In the remainder of the text we use the detergent to peptide (D/P) ratio to refer to each sample condition, i.e., D/P = 0, 2.7, 5.4, 7.3, 12.7, 65.4, and 130.9, which refers to [SDS] = 0 mM, 3 mM, 4 mM, 7 mM, 36 mM, and 72 mM, respectively.

3.2.2 EPR experiments

The X-band cw EPR measurements have been performed at room temperature (20°C) using an ELEXSYS E680 spectrometer (Bruker, Rheinstetten, Germany) equipped with a rectangular cavity. Samples of 10-15 µl peptide solution were drawn into Blaubrand 50 µl capillaries. Measurements were performed using the following parameters: 6.31 mW of microwave power, a modulation amplitude of 1.4 G, and a modulation frequency of 100 kHz. The accumulation time for the spectra was 40 minutes per spectrum. All samples were prepared and measured at least twice.

3.2.3 Simulations of EPR spectra

Matlab (version 7.11.0.584, Natick, Massachusetts, U.S.A) and the EasySpin package [29] were used for the simulation of EPR spectra. For all simulations the following tensor values were used: g = [2.00906, 2.00687, 2.00300] [25,30] and A_{xx} = A_{yy} = 12 and 13 MHz in DMSO and buffer, respectively. For the fast and medium components, different A_{zz} values were used than for the slow component, as discussed before [25]. For each fraction over-modulation effects were taken into account in EasySpin. Usually a superposition of one to three components was required to simulate the spectra. In all cases, isotropic rotation of the spin label was sufficient to reproduce the line-shape observed.

3.3 Results

The spectra of both SL-Aβ variants in DMSO, in which the Aβ peptide is in the monomeric form [31-33], have three narrow lines (figure 3.1). At low field, the first two lines of both SL-Aβ variants in DMSO have similar intensities, whereas the intensity of the third line at high field is larger for the sample of SL1-Aβ compared to that of SL26-Aβ.

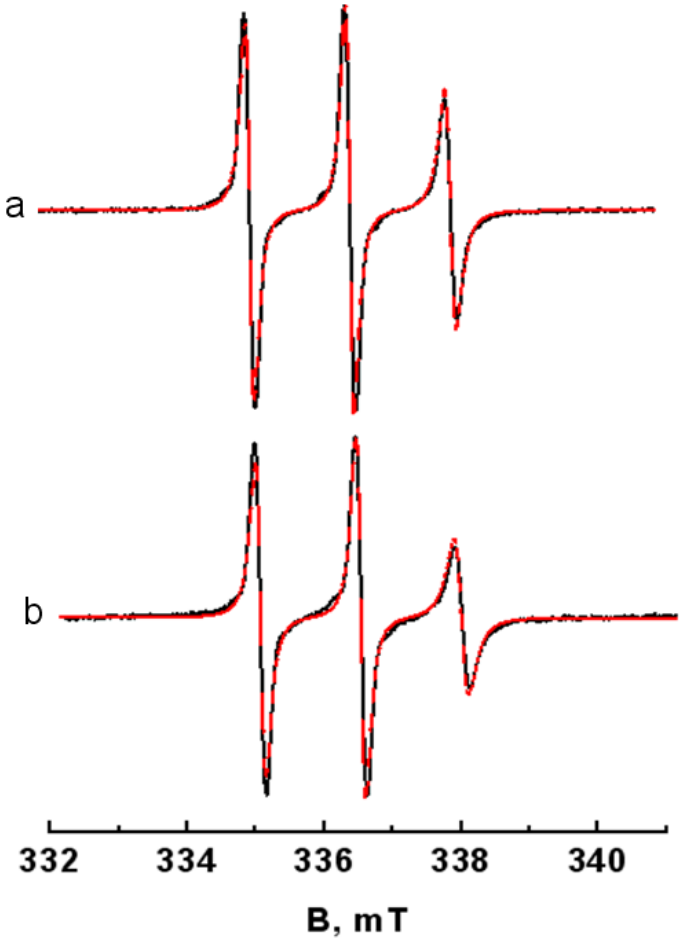

Figure 3.1 Room temperature EPR spectra of monomeric spin-labeled Aβ in DMSO. a: SL1-Aβ. Black line: experiment, red line: simulation. The rotation-correlation time τ_r is 0.19 ns. b: SL26-Aβ. Black line: experiment, red line: simulation. The rotation-correlation time τ_r is 0.27 ns.

Under aggregation conditions [25] (in buffer) and in the absence of SDS (D/P = 0), the lines of both SL-Aβ variants are broadened and additional lines are observed as reported before [25] (figure 3.2.a and 3.2.a'). In the presence of SDS, particularly at low concentrations of SDS (D/P = 2.7, 5.4), the spectra of SL1-Aβ differ from those of SL26-Aβ (figure 3.2.a-c and 3.2.a'-c'), whereas at higher concentrations (above 7 mM, D/P = 12.7), both SL-Aβ variants have identical spectra (figure 3.2.f and 3.2.f'). At D/P ratios of 7.3 and 12.7 the spectrum of SL1-Aβ has narrower lines compared to those of SL26-Aβ.

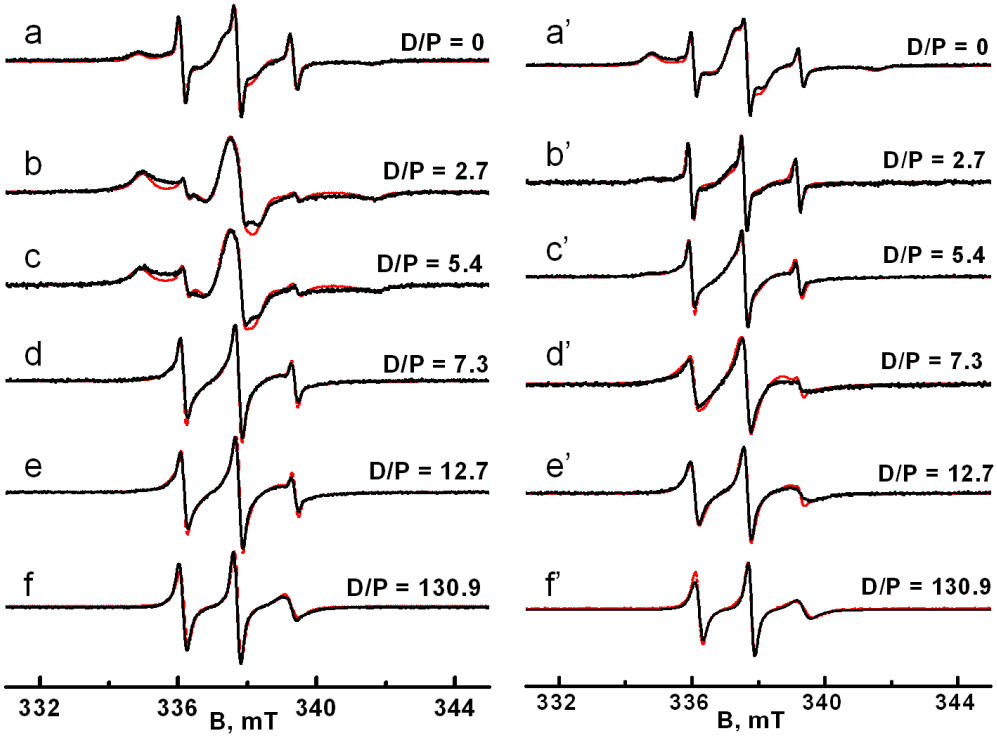

Figure 3.2 Room temperature EPR spectra of SL1-Aβ and SL26-Aβ in buffer for samples with different SDS detergent to peptide (D/P) ratios. a to f : Spectra for SL1-Aβ samples. From a to f the D/P ratio increases. a' to f': Spectra for SL26-Aβ samples organized as in the left part of the figure. Black line: experiment, red line: simulation.

By means of simulation, we quantify the spectral changes. The spectra of both SL-Aβ variants in DMSO are simulated by a single component with a τ_r value of 0.19 ns for SL1-Aβ and 0.27 ns for SL26-Aβ. We attribute the difference in the τ_r values to a slightly lower local mobility of the spin label at position 26 compared to that at position 1. The spectra of both SL-Aβ variants in buffer in the absence of SDS detergent are simulated using three components [25], which, in the remainder of the text, we refer to as fast, medium and slow. Each component is characterized by its τ_r value, and the amount by which this component contributes to the spectrum (table 3.1 and 3.2). We first describe the trends of the amount, and next the corresponding τ_r values.

Table 3.1 EPR parameters obtained form the simulation of cw EPR spectra of the SL1-Aβ samples. Given are: τ_r, rotation-correlation time, A_{zz}, the hyperfine splitting along the z-direction, lw, the component line-width of the simulation, and % stands for the contribution of the component to the total spectrum.

	fast				medium				slow			
D/P	**τ_r (ns)**	**A_{zz} (MHz)**	**lw (mT)**	**%**	**τ_r (ns)**	**A_{zz} (MHz)**	**lw (mT)**	**%**	**τ_r (ns)**	**A_{zz} (MHz)**	**lw (mT)**	**%**
0	0.19 ± 0.02	110	0.14	10 ± 2.00	2.55 ± 0.35	110	0.32	51 ± 2.00	> 50	95	0.50	39 ± 2.00
2.7	0.43 ± 0.02	110	0.14	2.5 ± 0.50	4.80 ± 0.40	110	0.32	64 ± 4.00	> 50	95	0.50	33.5 ± 2.50
5.4	0.43 ± 0.02	110	0.14	2.5 ± 0.50	4.65 ± 0.55	110	0.32	75 ± 3.00	> 50	95	0.50	22.5 ± 2.50
7.3	0.19 ± 0.02	110	0.14	10 ± 2.00	1.76 ± 0.16	110	0.14	90 ± 2.00	-	-	-	-
12.7	0.19 ± 0.02	110	0.14	7 ± 2.00	1.55 ± 0.08	110	0.14	92 ± 3.00	-	-	-	-
65.4	-	-	-	-	0.93 ± 0.03	110	0.06	100	-	-	-	-
130.9	-	-	-	-	0.93 ± 0.03	110	0.06	100	-	-	-	-

Table 3.2 EPR parameters obtained form the simulation of cw EPR spectra of the SL26-Aβ samples. Given are: τ_r, rotation-correlation time, A_{zz}, the hyperfine splitting along the z-direction, lw, the component line-width of the simulation, and % stands for the contribution of the component to the total spectrum.

	fast				medium				slow			
D/P	**τ_r (ns)**	**A_{zz} (MHz)**	**lw (mT)**	**%**	**τ_r (ns)**	**A_{zz} (MHz)**	**lw (mT)**	**%**	**τ_r (ns)**	**A_{zz} (MHz)**	**lw (mT)**	**%**
0	0.27 ± 0.02	110	0.14	6 ± 1.00	3.6 ± 0.10	110	0.32	52 ± 4.00	> 50	95	0.50	42 ± 4.00
2.7	0.26 ± 0.02	110	0.14	24 ± 1.00	2.1 ± 0.10	110	0.32	36 ± 4.00	> 50	95	0.50	40 ± 4.00
5.4	0.26 ± 0.02	110	0.14	13 ± 1.00	2.1 ± 0.10	110	0.32	74 ± 4.00	> 50	95	0.50	13 ± 4.00
7.3	0.26 ± 0.02	110	0.14	4 ± 1.00	2.1 ± 0.10	110	0.14	96 ± 1.00	-	-	-	-
12.7	0.27 ± 0.02	110	0.14	7 ± 1.00	1.4 ± 0.10	110	0.14	93 ± 1.00	-	-	-	-
65.4	-	-	-	-	0.93 ± 0.03	110	0.06	100	-	-	-	-
130.9	-	-	-	-	0.93 ± 0.03	110	0.06	100	-	-	-	-

3.3.1 Effect of SDS on the amount of different components

In the absence of SDS (D/P = 0), the spectra of both SL-Aβ variants are composed of almost equal amounts of the slow and the medium component and a small fraction (about 10 %) of the fast component. The amount of each mobility component at different D/P ratios is represented in figure 3.3. At low concentrations of SDS (between D/P = 0 and 5.4), the trend of the amount of fast and the medium component of SL26-Aβ is different from that of SL1-Aβ. For SL1-Aβ the amount of fast component decreases and the amount of medium component increases. This trend is not evident for SL26-Aβ (figure 3.3). In the same concentration region (between D/P = 0 and 5.4), the amount of slow component decreases in both SL-Aβ variants. Above a D/P ratio of 5.4 the slow component has disappeared leaving only the fast and medium components. At higher concentrations of SDS (above 7 mM SDS, i.e., D/P = 12.7), which is close to the critical micelle concentration of SDS only one component of medium mobility is left, which has the same parameters for both SL-Aβ variants.

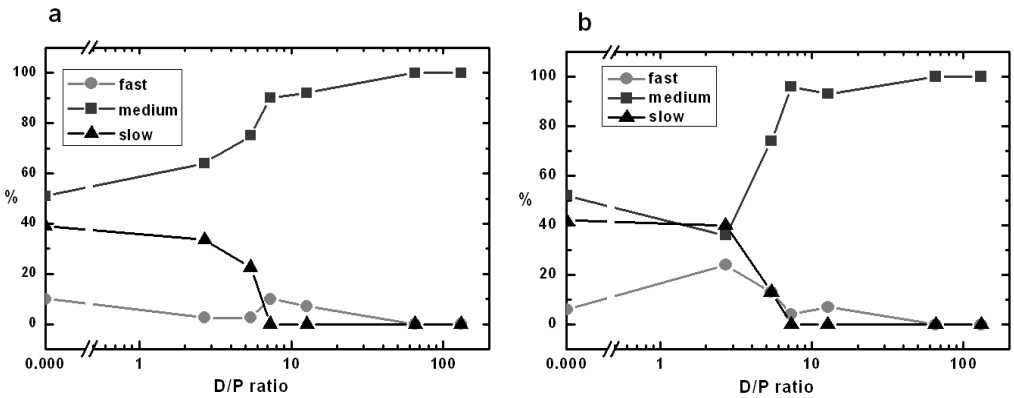

Figure 3.3 Amount of the spectral components as a function of the D/P ratios. a: SL1-Aβ. b: SL26-Aβ.

3.3.2 Effect of SDS on the rotation-correlation time

The τ_r values of the fast component of the EPR spectra of SL1-Aβ and SL26-Aβ in buffer are identical to those of the respective SL-Aβ variants in DMSO. We therefore assign the fast fraction to monomeric Aβ.

In the presence of SDS up to D/P = 5.4 the τ_r values of the fast and the medium component of SL1-Aβ are larger than those of SL26-Aβ. For SL1-Aβ, at D/P < 7.3, the τ_r values of both fast and medium components slightly increase with increasing SDS concentration, whereas those for SL26-Aβ remain constant over that range

(D/P < 7.3). At higher values of D/P (above D/P = 12.7; i.e., 7 mM SDS) no fast component is detected in the spectra of both SL-Aβ variants. The τ_r values of the only observed component in both SL-Aβ variants are identical. This τ_r is longer than the τ_r of both SL-Aβ variants in DMSO, in which the Aβ peptide is in the monomeric form.

3.4 Discussion

We have investigated the aggregation of Aβ at different concentrations of SDS by monitoring two different positions in the Aβ chain; the N-terminal SL1-Aβ and the central SL26-Aβ. From the two label positions we can differentiate spectral changes due to local mobility, analyzing the parameters that differ for SL1-Aβ and SL26-Aβ. Spectral changes because of a change of the aggregation state of Aβ should be reflected in identical parameters for SL1-Aβ and SL26-Aβ.

Figure 3.4 shows the changes in aggregation state: the amount of slow component and the amount of combined fast and medium component. Both amounts agree within the experimental uncertainty for SL1-Aβ and SL26-Aβ. It stands to reason that the Aβ aggregation state changes in a continuous way with the SDS concentration: the amount of slow component decreases, whereas the amount of the more mobile components increases. Considering the mobility to reflect qualitatively the size of the aggregate, the Aβ changes from a more aggregated state with larger particles at low SDS concentrations to a state in which these larger aggregates almost disappear. Concomitantly, the amount of the smaller aggregates increases. Aggregation of Aβ at submicellar SDS concentrations was also concluded from the absence of monomer-NMR signal [13] and from β-sheet signatures found in CD and FTIR spectra, which were attributed to aggregated forms of Aβ [10,13,34]. At higher SDS concentrations (above 7 mM, D/P = 12.7), i.e., close to the CMC, no larger aggregates remain and the sample appears uniform. The higher SDS concentration results agree with previous observations that the predominant species at high-SDS concentrations is a monomeric Aβ, solubilized in an SDS micelle [9,11,13,23,24,35-37].

The difference between the EPR parameters of SL1-Aβ and SL26-Aβ in the submicellar SDS concentration regime far exceeds the sample to sample variation. These differences suggest that the regions of Aβ to which each spin label is attached behave differently in the presence of submicellar SDS concentrations. At the lowest SDS concentrations investigated (D/P = 2.7 and 5.4) the N-terminus goes through a phase of immobilization, as evidenced by the larger τ_r values of the fast and medium component at these SDS concentrations and the smaller amount of the fast component. The spectral parameters of SL26-Aβ reveal an almost inverse behavior, showing that the central part of the peptide becomes slightly more mobile at these

low SDS concentrations. The SL26-Aβ variant reveals an immobilization event of the central region of Aβ at higher SDS concentrations around D/P ratios of 7.3.

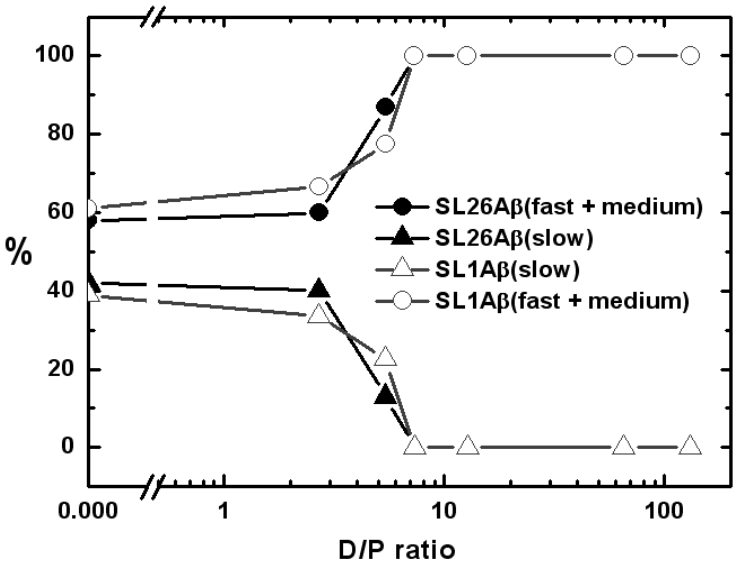

Figure 3.4 Amount of the spectral components as a function of the D/P ratios. The amount of the slow component of the SL1-Aβ and SL26-Aβ variants are shown with triangles, similar to figure 3.3 (filled triangles: SL26-Aβ, non-filled triangles: SL1-Aβ). The amount of the fast and the medium components of the SL1-Aβ and SL26-Aβ variants are shown together with circles (filled circles: SL26-Aβ, non-filled circles: SL1-Aβ). The values for the circles are obtained by adding the amount of the fast to the amount of the medium component at each D/P ratio. In both SL-Aβ variants, the amount of slow component decreases, whereas the amount of the more mobile components (fast plus medium) increases.

At low SDS to peptide ratios, aggregates should be dominated by Aβ-Aβ interactions and these apparently restrict the mobility of the N-terminus. Since this region is not considered to be part of the fibrilization domain of Aβ [38], the aggregates presumably differ from fibrils. Support for compactation involving the N-terminus also comes from Sambasivam et al. [13,39], where FRET distances between residues 1 and 10 suggest an α-helix or a β-turn of the N-terminus of Aβ at a 1.5 mM concentration of SDS, rather than an extended β-sheet. Wahlström et al. [13] interpret their CD data as two β-sheet-type structures, with a transition point around a D/P ratio of 11. This could suggest that at submicellar SDS concentrations (D/P = 2.7 and 5.4) Aβ is an oligomer [13,23] in which the N-terminus is trapped and the middle part is more flexible. Taking the β-sheet character into account [13], that would agree with a non-fibrilar type of β-sheet oligomer. We speculate that at these

low concentrations of SDS the Aβ peptide acts as a kind of detergent (figure 3.5). The hydrophilic N-terminus [38] locates at the water/aggregate interface, which helps to solubilize the aggregate as long as there are not sufficient SDS molecules to perform this task. This position in the interface immobilizes the N-terminus. The SDS could help breaking up the hydrophilic interaction between the aggregation domains of Aβ (residues 25-35) [40], which makes this central part of Aβ more mobile. Above D/P ratios of 5.4, there are sufficient SDS molecules to replace (some of) the Aβ N-termini at the water/aggregate interface. The N-terminus, which is hydrophilic [38], becomes more exposed, as witnessed by the increase in the amount of the fast spectral component and the decrease in τ_r of the medium component. The central part of Aβ becomes buried. Most likely the headgroups of SDS face the aqueous phase and the tails interact with the central hydrophobic part of Aβ. At SDS concentrations above the CMC this form would then transform to the micellar, α-helical form of Aβ. The α-helical form was proposed to be a monomeric Aβ, solubilized in an SDS micelle [9,11,13,23,24,35-37]. This proposal is consistent with the data presented in chapter 2 of this thesis, revealing a single, homogeneous form of Aβ in which Aβ-Aβ interactions are not detectable. Based on the model proposed by Jarvet et al. [23] the N-terminus and the central part of the peptide are not part of the helical domain. Our results show that the N-terminus and central part of Aβ have similar mobilities, which suggests a similar location of these positions with respect to the micelle.

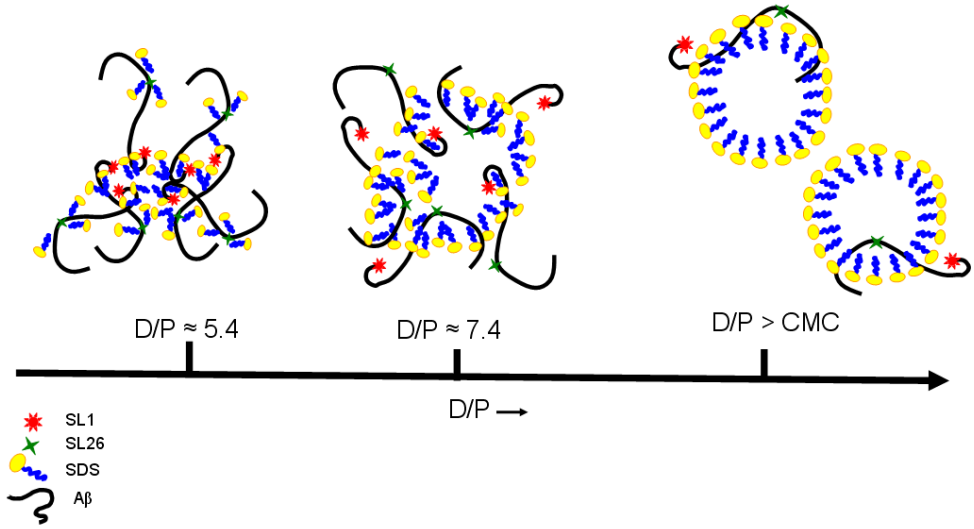

Figure 3.5 Cartoon of the Aβ aggregation at different D/P ratios. On the left side, the Aβ aggregate is shown at a D/P ratios of about 5.4, in which the hydrophilic N-terminus becomes immobilized at the aggregate/buffer interface. This helps to solubilize the aggregate. In the middle, the Aβ aggregate is shown at D/P ratios of about 7.3, where there are sufficient SDS molecules to replace (some of) the Aβ N-termini at the water/aggregate interface. On the right, the Aβ peptide is shown at D/P ratios above the CMC of SDS. Two possible models for Aβ interaction with a micelle are shown, in which both spin labels would have similar rotation correlation times.

In conclusion, we have shown that previously inaccessible detail of the low-SDS form of Aβ can be obtained by spin label EPR. A careful study of two labeling positions in Aβ and the sensitivity of this EPR approach to local mobility reveal a change in the aggregate state. From a particle, in which the N-terminus of Aβ participates in the solubilization by being located at the particle/water interface, the aggregate changes to an SDS-solubilized state that is a precursor to the one Aβ/micelle state above the CMC of SDS. We also demonstrate how from the local mobility parameters global properties of the Aβ-aggregation state are obtained, revealing the unique potential of EPR in studying the Aβ aggregation.

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