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Methyl dynamics of the amyloid-β peptides Aβ40 and Aβ42

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Abstract

To probe the role of side chain dynamics in Aβ aggregation, we studied the methyl dynamics of native Aβ40 and Aβ42 by measuring cross relaxation rates with interleaved data collection. The methyl groups in the C-terminus are in general more rigid in Aβ42 than in Aβ40, consistent with previous results from backbone ^{15}N dynamics. This lends support to the hypothesis that a rigid C-terminus in Aβ42 may serve as an internal aggregation seed. Interestingly, two methyl groups of V18 located in the central hydrophobic cluster are more mobile in Aβ42 than in Aβ40, most likely due to the paucity of V18 intra-molecular interactions in Aβ42. V18 may then be more available for inter-molecular interactions to form Aβ42 aggregates. Thus, the side chain mobility of the central hydrophobic cluster may play an important role in Aβ aggregation and may contribute to the difference in aggregation propensity between Aβ40 and Aβ42.

Keywords: NMR spectroscopy; Protein dynamics; Methyl dynamics; Aβ; Alzheimer's disease

Alzheimer's disease (AD) is the most common type of senile dementia. AD is characterized by neurofibrillary tangles and senile plaques in the pathology of affected brains. Amyloid-β peptides (Aβ) are the major components of senile plaques and are generated by the sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretase [1]. Aβ40 (40-mer) and Aβ42 (42-mer) are the most common species of Aβ [2]. Aβ40 and Aβ42 share identical amino acid sequence except that Aβ42 has two extra residues (IA) at the C-terminus. However, Aβ42 aggregates much faster [3–7] and is more toxic to neurons in cell culture than Aβ40 [5,8]. Although the mechanism of Aβ toxicity is not well understood, Aβ most likely executes its toxicity through oligomeric and/or fibril forms [9]. Therefore the enhanced toxicity of Aβ42 likely derives from its enhanced aggregation. Characterization of the dynamics of Aβ40 and Aβ42 monomers, which are the starting molecules of aggregation, will provide important insights into the mechanism of Aβ aggregation, Aβ toxicity and the pathogenesis of Alzheimer's disease. Previous studies of the backbone ^{15}N dynamics on ps–ns time scales revealed that Aβ42 has a more rigid C-terminus than Aβ40 [10–12]. It was hypothesized that the rigid C-terminus of Aβ42 may contribute to Aβ aggregation by serving as an internal seed for aggregation and by reducing the entropic cost of aggregation.

Dynamics of protein side chains are of great interest [13,14] due to their essential role in protein interaction and protein aggregation [15,16]. Methyl groups are often involved in hydrophobic interactions. The C-terminal two residues of Aβ42, I41 and A42, which are responsible for its enhanced amyloidogenicity [17], have three methyl groups. Thus methyl groups may play an important role in Aβ aggregation. Although there are several backbone dynamics studies of Aβ [10–12], no side chain dynamics of Aβ has been reported. Here, we present the first study of methyl group dynamics of native Aβ40 and Aβ42 monomers on the ps–ns time scales. We demonstrate that the majority of methyl groups at the C-terminus of Aβ42 are more rigid than those of Aβ40, consistent with previous results from backbone ^{15}N dynamics [10]. In addition, two methyl groups of V18 located in the central hydrophobic cluster are more mobile in Aβ42 than in Aβ40. These

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differences in side chain mobility between Aβ40 and Aβ42 may contribute to the remarkable differences in their aggregation propensity.

Materials and methods

Sample preparation. [U–^{15}N, U–^{13}C]-labeled native Aβ monomers (rPeptide) were prepared using NaOH treatment [18]. Aβ was dissolved in 10 mM NaOH to 1 mg/mL followed by sonication for 30 s. One hundred microliters of this stock solution was diluted with 350 μL of potassium phosphate buffer (20 mM, pH 7.2) to 50 μM. The sample was lyophilized and redissolved in 100% D_2O.

Relaxation experiment. The ^{13}C relaxation rates R_1 and dipole-dipole cross-correlated relaxation rates (Γ) of [U–^{15}N, U–^{13}C]-labeled Aβ monomers were measured on an 800 MHz spectrometer (Bruker) equipped with a cryo-probe. The temperature was calibrated to 273.6 K using 100% methanol. In order to remove the effect of aggregation on relaxation rates, we acquired our data in an interleaved manner as described previously [10]. Relaxation delays (t) were set to 50, 100 200, 300, 400, and 500 ms for R_1 and relaxation rates were obtained by fitting $I(t)/I(0)$ to an exponential decay function. Severely overlapped peaks were excluded from the analysis. The dipole–dipole cross-correlated relaxation rate (Γ) was measured as in the previous study [19] with relaxation delays (Δ) set to 0.6, 1.2, 1.8, 2.4, 3.0, 3.6, 4.2, 4.8, 5.4, 6.4, 7.2, 8.0, 10, 12, 14, 16, 18, and 20 ms. The order parameters of methyl groups (S^2_{axis}) of both Aβ40 and Aβ42 monomers were calculated from the R_1 and Γ for each residue assuming the global correlation time $\tau_m = 4$ ns [10,19].

Results and discussion

To probe the dynamic difference between Aβ40 and Aβ42 in methyl groups, we measured the ^{13}C relaxation rates R_1 and dipole–dipole cross-correlated relaxation rates (Γ) of [U–^{15}N, U–^{13}C]-labeled Aβ monomers on an 800 MHz spectrometer (Bruker) at 273.6 K. Shown in Fig. 1 are the ^{13}C–^{1}H methyl HSQC spectra of [U–^{15}N, U–^{13}C]-labeled Aβ40 and Aβ42 monomers at 273.6 K. Most methyl (CH_3) peaks have similar chemical shifts except for one methyl group in residue V39 and two methyl groups of residue V40.

The average value of R_1 for Aβ40 excluding the C-terminal residue (V40) is 2.48 ± 0.63 at 273.6 K. The average value of R_1 for Aβ42 excluding the C-terminal residues (V40, I41, and A42) is 2.51 ± 0.61 at 273.6 K. As shown in Fig. 2A, most non-terminal residues have similar R_1 values and have R_1(Aβ42)/R_1(Aβ40) ratios very close to

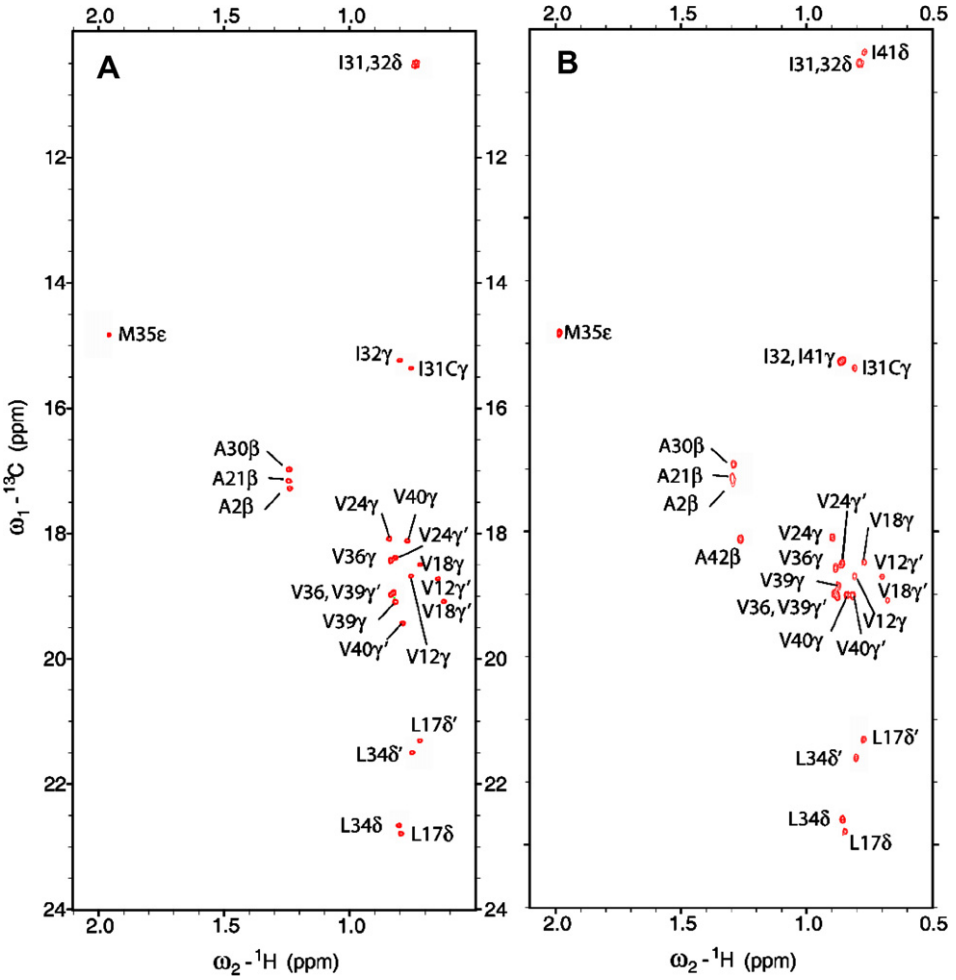

Fig. 1. 2D ^{1}H–^{13}C methyl HSQC spectra of Aβ40 (A) and Aβ42 (B) monomers in potassium phosphate buffer (20 mM, pH 7.2) containing 100% D_2O at 273.6 K. The methyl groups assignment was according to previous report [18] and confirmed with an HCCH-TOCSY. The stereospecific assignments of the methyl groups of Leu and Val were not known and were arbitrarily named as, δ and δ′, and γ and γ′, respectively.

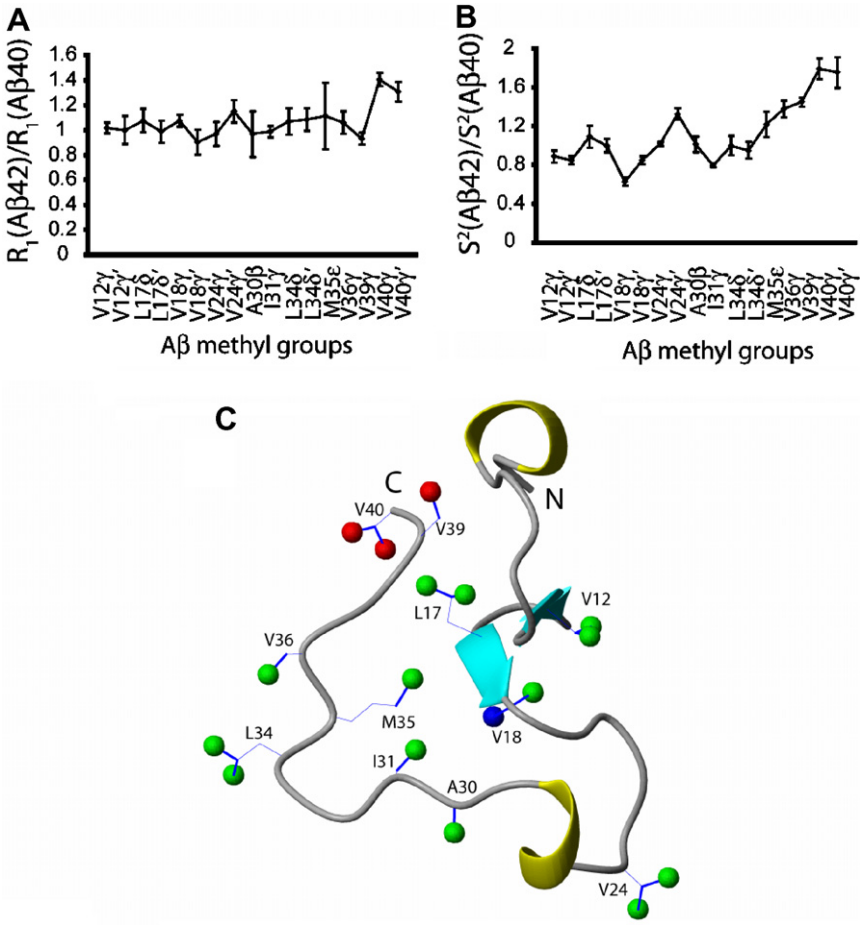

Fig. 2. Comparison of the methyl dynamics between Aβ40 and Aβ42. (A) The ratio of R_1 of Aβ42 over Aβ40. (B) The ratio of order parameter S^2_{axis} of Aβ42 over Aβ40. (C). S^2_{axis}(Aβ42)/S^2_{axis}(Aβ40) values mapped onto the ribbon diagram of a simulated structure of Aβ40 monomer derived from a replica-exchange MD simulation [20]. Methyl carbons are shown in a space-filling representation. Methyl groups are color coded in red if the ratio is bigger than 1.4, green if $0.7 < S^2_{axis}(A\beta42)/S^2_{axis}(A\beta40) < 1.4$, blue if $S^2_{axis}(A\beta42)/S^2_{axis}(A\beta40) < 0.7$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

one. Towards the C-terminus, the R_1(Aβ42)/R_1(Aβ40) increases dramatically, with ratios 1.41 and 1.33 for the two methyl groups of V40, respectively, suggesting reduced mobility of Aβ42 at the C-terminus.

S^2_{axis} values describe the amplitude of the internal motion of the methyl groups on the ps–ns time scale, with higher order parameter corresponding to more rigidity. Here, the order parameters derived were only used for a comparison between Aβ40 and Aβ42. Most methyl groups such as L17δ, L34δ, M35ε, and A30β have quite similar order parameter values. However, the two methyl groups of the C-terminal residue V40 in Aβ42 have order parameter values of 0.36 ± 0.02 and 0.34 ± 0.03, whereas the order parameters of the methyl groups of V40 in Aβ40 are 0.20 ± 0.01 and 0.19 ± 0.01. The order parameter value of V39 is 0.43 ± 0.01 in Aβ42 and is 0.30 ± 0.01 in Aβ40. The order parameter value of V36 is 0.35 ± 0.02 in Aβ42 and is 0.25 ± 0.01 in Aβ40. By taking the ratio of the order parameter values between Aβ40 and Aβ42, the C-terminal residues of Aβ42 display strikingly larger S^2_{axis} values than those of Aβ40 (see Fig. 2B). As shown in Fig. 2C, S^2_{axis}(Aβ42)/S^2_{axis}(Aβ40) values were mapped onto the ribbon diagram structure of Aβ40 monomer from a replica-exchange MD simulation [20]. Towards the C-terminus there is clearly an increase in order parameter values of Aβ42, indicating the reduced mobility of the methyl groups in Aβ42. Due to the different amino acid types, the order parameter values cannot be compared directly with an alignment from the C-terminus. We take the ratio between the order parameter values we measured and the average methyl axis order parameter (S^2_{AV}) derived from a previous study [21] to remove the effect of different residue types and to compare the C-terminal dynamics difference between Aβ40 (V39–V40) and Aβ42 (I41–A42). The average methyl axis order parameter values (S^2_{AV}) equal to 0.75, 0.61, and 0.63 for Ala, Ile, and Val, respectively [21]. The ratios S^2_{axis}/S^2_{AV} of the two methyl groups of V40 in Aβ40 are 0.32 ± 0.01 and 0.31 ± 0.01, similar to that of Alanine 42 (0.32 ± 0.02) in Aβ42. The ratio S^2_{axis}/S^2_{AV} of V39 in Aβ40 equals 0.47 ± 0.01 whereas the ratio S^2_{axis}/S^2_{AV} of I41 in Aβ42 equals 0.62 ± 0.03. Therefore with an alignment from the C-terminus, the methyl groups of the last residue have similar mobility in Aβ40 and Aβ42 while the methyl groups of the second

to last residue have significantly decreased mobility in Aβ42 compare with Aβ40. Overall, the methyl group dynamics data corroborated the findings from backbone ^{15}N dynamics and again demonstrated that the C-terminus of Aβ42 is more rigid than Aβ40. The enhanced rigidity may come from preordering of Aβ42 C-terminus in β-conformation for fibril formation [10].

Interestingly, two methyl groups of V18 in Aβ42 have significantly smaller order parameter values than those in Aβ40 with the ratios S^2(Aβ42)/S^2(Aβ40) of 0.6 and 0.8, respectively (see Fig. 2B and C), suggesting V18 side chain is more mobile in Aβ42 than in Aβ40. V18, located in central hydrophobic cluster (CHC), is an important residue for Aβ aggregation [22,23]. Substitution of V18 with other non-hydrophobic amino acids or β-breakers significantly reduced the rate of aggregation [23]. A recent replica-exchange MD simulation of Aβ showed that V18 is involved in many intra-molecular interactions, in particular with V12, in Aβ40 monomer [20]. We also observed slightly increased order parameters for V12 methyl groups in Aβ40 compared with those of Aβ42 (see supporting table and supporting Fig. 2). Intra-molecular interactions of V18 observed in Aβ40 are largely absent in Aβ42 monomer [20], which may lead to increased mobility of V18 in Aβ42. Therefore V18 in Aβ42 is likely more available for inter-molecular interactions to form oligomers, early aggregation seeds and fibrils. This is also consistent with previous finding that Aβ40 and Aβ42 aggregate through different pathways, in which stable trimer and tetramer were formed by Aβ42 but not by Aβ40 [24]. The reduced mobility of V18 in Aβ40 was not observed in our previous backbone ^{15}N dynamics study [10], probably because V18 intra-molecular interactions are mediated by hydrophobic side chains.

In summary, we studied the methyl dynamics of the native Aβ monomers by measuring cross-relaxation rates. Consistent with our previous backbone dynamics study [10], the side chain methyl groups at C-terminus are in general more rigid in Aβ42 than in Aβ40. In contrast, two methyl groups of the critical V18 residue are more mobile in Aβ42, likely due to reduced intra-molecular interactions in Aβ42. This may increase the probability of inter-molecular interaction involving V18 for the formation Aβ42 oligomer, aggregation seed, and fibrils.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc. 2007.07.198.

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