Concentration and pH Dependence of A$\beta_{11\text{-}25}$ Conformations

Jiho Kim, Jeong Eun Kim, and Minyung Lee*

Department of Chemistry, Division of Molecular Life Sciences, Ewha Womans University, Seoul 120-750, Korea
Received January 9, 2004

Key Words : β-amyloid peptide, Tryptophan, Conformation

β-amyloid(Aβ) peptide, consisting of 39-43 amino acid residue fragments, are produced from amyloid precursor protein (APP) in the course of metabolic process in the cell.[1,2] Some unknown risk factors induce the highly toxic Aβ *in vivo* self-assembling in the brain, which has been known to be the major pathogenesis of Alzheimer's disease.[3] Although self-assembling processes of Aβ and its fragments have been subjected to intensive studies under various conditions,[4,5] conformations of their aggregates are not fully understood at the molecular level. In this communication, we have chosen A$\beta_{11\text{-}25}$, an Aβ fragment containing the aggregation motif[6-9] of the full length amyloid, and investigated the conformation change by the time resolved fluorescence. It was observed that the fluorescence decay characteristics of tryptophan attached to the N-terminus of A$\beta_{11\text{-}25}$ were varied markedly at different aggregate concentrations and pHs, due to conformation switching.

The peptide used in this work has the amino acid sequence of WEVHHQKLVFFAEDVG (Trp-A$\beta_{11\text{-}25}$). It contains the key aggregation motif, KLVFF(16-20), in the central region and the tryptophan residue was introduced at N-terminus as the fluorescence probe. The fluorescence decays of Trp-A$\beta_{11\text{-}25}$ in pH 7.4 and in pH 5.0 buffer solutions were measured by employing the picosecond time-correlated single photon counting (TCSPC) system. The sample was excited at 284 nm and fluorescence photons were collected at 340 nm. We carried out the experiments at physiologically equivalent pH 7.4 and at pH 5.0: The acidic pH was chosen because recent investigations on Aβ showed that intracellular production and accumulation of Aβ occur in lysosomes, whose pH is known to be around 5.[10,11] As shown in Figure 1, tryptophan lifetime was increased at higher concentration in both pHs. The decays were fitted to the double exponential form and the deconvoluted data were listed in Table 1. The average fluorescence lifetime of Trp-A$\beta_{11\text{-}25}$ was increased from 3.05 ns to 4.74 ns and the amplitude of long lifetime component was also increased at pH 7.4 with increasing the peptide concentration. Therefore, the long lifetime component is originated from the tryptophan protected from water molecules. However, at pH 5.0, the increase of the average lifetime was not pronounced and the amplitude was maintained as almost the constant value. These experimental results are expected to be correlated with the conformational changes of A$\beta_{11\text{-}25}$. It has been reported that the full length

*Corresponding Author. Tel: +82-2-3277-2383; Fax: −82-2-3277-2384; e-mail: mylee@ewha.ac.kr

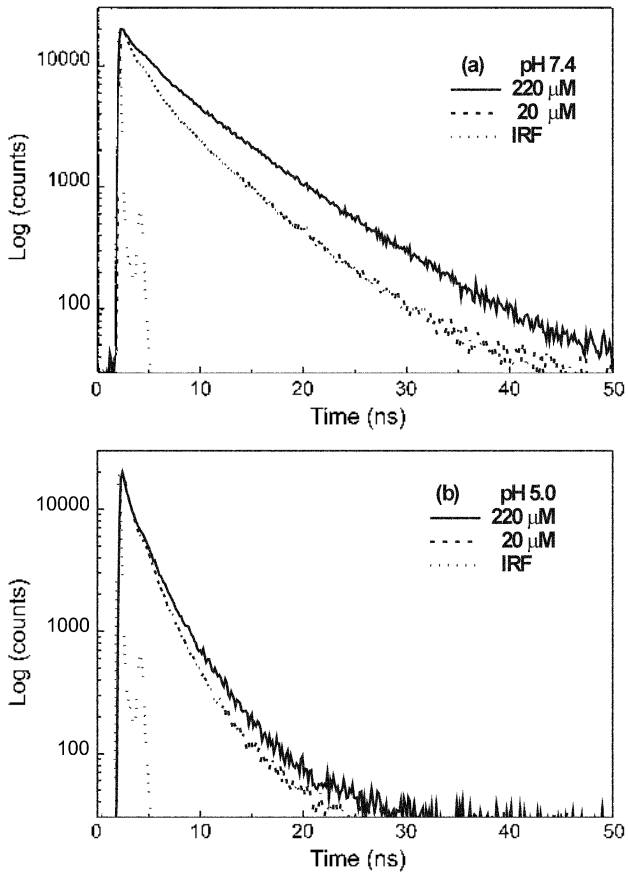

Figure 1. Fluorescence decay curves of Trp-A$\beta_{11\text{-}25}$ in pH 7.4 (a) and pH 5.0 (b) buffer solution at two different concentrations.

Aβ monomer and multimer have the random coil or collapsed coil structure with no sign of α-helix.[12,13] It is expected that A$\beta_{11\text{-}25}$ exists as a multimeric state with the collapsed coil structure at 20 μM at pH 7.4. Therefore, the major fraction (A_1) of N-terminus tryptophan was exposed to the water molecule that quenches the tryptophan fluorescence. At 220 μM, A$\beta_{11\text{-}25}$ is in the fibril state with extended β-sheet structures in PBS buffer. The buried tryptophan fraction (A_2) is much higher than that exposed to water molecules, which leads to the increase of the average fluorescence lifetime of Trp-A$\beta_{11\text{-}25}$ at pH 7.4. It is expected that the A$\beta_{11\text{-}25}$ exists already as a fibril state with the extended β-sheet structure at 20 μM concentration at pH 5.0. Therefore, the amplitude of long lifetime component and average lifetime were not changed pronounced with increasing the peptide concentration at pH 5.0. At both pHs,

Table 1. Fluorescence lifetime data of Trp-A$\beta_{11\text{-}25}$ in pH 7.4 and pH 5.0 buffer solution

	concentration	A_1	τ_1 (ns)	A_2	τ_2 (ns)	$*<\tau>$ (ns)	χ^2
pH 7.4	20 μM	0.67	1.56	0.33	6.07	3.05	1.74
	220 μM	0.45	1.85	0.55	7.10	4.74	1.78
pH 5.0	20 μM	0.80	1.06	0.20	3.22	1.49	1.59
	220 μM	0.74	1.11	0.26	3.51	1.73	1.71

$*<\tau> = A_1\tau_1 + A_2\tau_2$

A$\beta_{11\text{-}25}$ undergoes conformational switching from the collapsed coil to the β-sheet structure but the conformational switching at pH 5.0 couldn't be monitored in our experimental condition.[14]

The fluorescence lifetime shortening of tryptophan at pH 5.0 compared with that at pH 7.4 is due to fluorescence quenching by the protonated histidine in the acidic condition. As listed in Table 1, the long lifetime component was dramatically shortened in the presence of histidine residues at pH 5.0. It has been known that, when the imidazole ring of histidine was protonated, it acts as an excellent electron acceptor.[15-18] The side chains of tryptophan and histidine spatially interact in the α-helical structure (an i, i+4 interaction).[16] Since our model peptide system, Trp-A$\beta_{11\text{-}25}$, has the tryptophan residue at N-terminus and histidine residue at 14 position, the i, i−4 interaction is possible. The tryptophan side chain interacts with the protonated histidine when these residues are helical, and thus electron transfer to the histidine quenches the tryptophan fluorescence in Trp-A$\beta_{11\text{-}25}$. The quenching phenomenon of tryptophan by histidine residues is a potential probe of helix formation. Therefore, we could suggest that A$\beta_{11\text{-}25}$ has some α-helix propensities in the fibril state.

Figure 2 shows the possible molecular model of the parallel β-sheet and antiparallel β-sheet structure of A$\beta_{11\text{-}25}$. If A$\beta_{11\text{-}25}$ fibril has a parallel alignment (Figure 2(a)), the degree of quenching of tryptophan should be increased due to the interstrand electron transfer as increasing the peptide concentration at pH 5.0. However, the decrease in fluorescence lifetime of tryptophan was observed at much higher concentration, which means that the quenching effect originated from the histidine residue is maintained in the fibril structure. Our fluorescence decay time data represents the possibility that A$\beta_{11\text{-}25}$ fibril has the antiparallel arrangement[19,20] as shown in Figure 2(b).

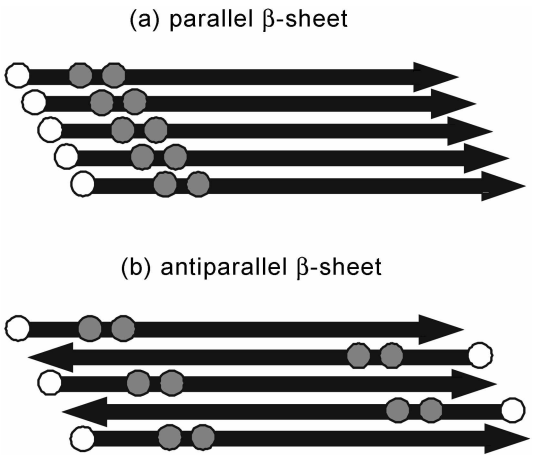

Figure 2. The molecular model of two possible arrangements (parallel and antiparallel) of the Trp-A$\beta_{11\text{-}25}$ fibrils. The labeled tryptophan is shown in white and His 13, 14 are shown in gray.

In conclusion, we observed the conformational changes of A$\beta_{11\text{-}25}$ with increasing the peptide concentration. At pH 7.4, the conformational changes were inferred from the lifetime increase of tryptophan. The amyloid peptide undergoes a conformational change from the collapsed coil (multimers) to the antiparallel β-sheet structure. It is very likely that the A$\beta_{11\text{-}25}$ fibrils should be formed at least one order lower concentrations at pH 5, when compared to the physiological condition.

Acknowledgment. This work has been supported by a grant of the Korea Health R&D project, Ministry of Health and Welfare (02-PJ1-PG3-12109-0001).

References

1. Lee, E. B.; Skovronsky, D. M.; Abtahian, F.; Doms, R. W.; Lee, V. M.-Y. *J. Biol. Chem.* **2003**, *278*, 4458.
2. Walsh, D. M.; Tseng, B. P.; Rydel, R. E.; Podlisny, M. B.; Selkoe, D. J. *Biochemistry* **2000**, *39*, 10831.
3. *Amyloid, Prions, and Other Protein Aggregates* in *Methods in Enzymology*; Wetzel, R., Ed.; Academic Press: San diego, 1999; Vol. 309.
4. Pallitto, M.; Murphy, R. M. *Biophys. J.* **2001**, *81*, 1805.
5. Yong, W.; Lomakin, A.; Kirkitadze, M. D.; Teplow, D. B.; Chen, S.-H.; Benedek, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 150.
6. (a) Huang, T. H. J.; Yang, D.-S.; Plaskos, N. P.; Go, S.; Yip, C. M.; Fraser, P. E.; Chakrabartty, A. *J. Mol. Biol.* **2000**, *297*, 73. (b) Gorman, P. M.; Yip, C. M.; Fraser, P. E.; Chakrabartty, A. *J. Mol. Biol.* **2003**, *325*, 743.
7. Jarvet, J.; Damberg, P.; Bodell, K.; Eriksson, L. E. G.; Gräslund, A. *J. Am. Chem. Soc.* **2000**, *122*, 4261.
8. Kim, J. E.; Lee, M. *Biochem. Biophys. Res. Comm.* **2003**, *303*, 576.
9. Kang, J.; Jo, J. H. *Bull. Korean Chem. Soc.* **2003**, *24*, 1403.
10. Su, Y.; Chang, P.-E. *Brain Res.* **2001**, *893*, 287.
11. Pasternak, S. H.; Bagshaw, R. D.; Gurial, M.; Zhang, S.; Ackerley, C. A.; Pak, B. J.; Callahan, J. W.; Mahuran, D. J. *J. Biol. Chem.* **2003**, *278*, 26687.
12. Findeis, M. A. *Biochim. Biophys. Acta* **2000**, *1502*, 76.
13. Massi, F.; Peng, J. W.; Lee, J. P.; Straub, J. E. *Biophys. J.* **2001**, *80*, 31.
14. Kim, J.; Lee, M. *Biochem. Biophys. Res. Comm.* **2004**, *316*, 393.
15. Chen, Y.; Barkley, M. D. *Biochemistry* **1998**, *37*, 9976.
16. LeVine III, H. *Arc. Biochem. Biophys.* **2003**, *417*, 112.
17. Thompson, P. A.; Muñoz, V.; Jas, G. S.; Henry, E. R.; Eaton, W. A.; Hofrichter, J. *J. Phys. Chem. B* **2000**, *104*, 378.
18. Chin, J. J.; Jhun, B. H.; Jung, C. Y. *Biochemistry* **1992**, *31*, 1945.
19. Li, L.; Darden, T. A.; Bartolotti, L.; Kominos, D.; Pedersen, L. G. *Biophys. J.* **1999**, *76*, 2871.
20. Ma, B.; Nussinow, R. *Proc. Natl. Acad. USA* **2002**, *99*, 14126.