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Residue-Specific Dynamics and Local Environmental Changes in A β 40 Oligomer and Fibril Formation

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Abstract

Elucidating local dynamics of protein aggregation is crucial for understanding the mechanistic details of protein amyloidogenesis. Herein, we studied the residue-specific dynamics and local environmental changes of A β 40 along the course of aggregation by using para-cyanophenylalanine (Phe_{CN}) as a fluorescent and vibrational probe. Our results show that the Phe_{CN} residues introduced at various positions all exhibited an immediate decay of fluorescence intensity, indicating a relatively synergistic process in early oligomer formation. The fast decreases in the fluorescence intensities of residues 19 and 20 in the central hydrophobic core region and residue

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Conflict of interest

The authors declare no conflict of interest.

10 in the N-terminal region suggest that they play crucial roles in the formation of the oligomeric core. The Phe_{CN}4 residue exhibits a remarkably slower decrease in fluorescence intensity, implicating its dynamic conformational characteristics in oligomer and fibril formation. Our results also suggest that the N-terminal residues in fibrils are surrounded by a relatively hydrophobic local environment, as opposed to being solvated.

Graphical abstract

Elucidating the site-specific dynamics of protein aggregation remains a formidable challenge while being critical for understanding the mechanistic details of protein self-assembly. By using the unnatural amino acid *para*-cyanophenylalanine as both a fluorescent and Raman-active probe, local environmental changes and dynamics of specific residues along the aggregation pathway of Aβ₄₀ were characterized.

Keywords

aggregation; amyloid-β; local dynamics; oligomers; unnatural amino acids

Alzheimer's disease (AD) is linked to a poorly understood accumulation of amyloid-β (Aβ) peptide in brain tissue. The characteristic cross-β-sheet fibrillar structures of Aβ have been resolved with atomic resolution,^[1] greatly advancing our understanding of the structural basis of this essential hallmark of AD. Moreover, an increasing number of studies suggest that oligomeric assemblies of Aβ are more toxic than mature fibrils.^[2] However, conducting structural and mechanistic studies of Aβ oligomers is technically challenging owing to the dynamic and metastable nature of oligomers. It is therefore critical to elucidate the high-resolution and site-specific dynamics of Aβ self-assembly for a more comprehensive understanding of the mechanisms of Aβ oligomer and fibril formation.

The incorporation of molecular probes, for example, intrinsic fluorophores such as tryptophan (Trp), unnatural extrinsic probes, and isotopically labeled probes, into proteins has been used to explore the dynamics of protein aggregation in a site-specific manner.^[3] Meanwhile, many of these current approaches are still limited by poor sensitivity, temporal restriction, or structural perturbation caused by the incorporation of large extrinsic probes. The characterization of local kinetics of Aβ aggregation towards a mechanistic understanding of Aβ self-assembly remains scarce.

Herein, we used an unnatural amino acid, *para*-cyanophenylalanine (Phe_{CN}), as a fluorescent and vibrational probe to study the local dynamics of Aβ₄₀ oligomer and fibril formation at residue-specific resolution. The quantum yield of Phe_{CN} fluorescence is sensitive to hydrogen-bonding interactions involving the nitrile group, increasing when the nitrile group is hydrogen-bonded to protic solvent and decreasing in an aprotic, hydrophobic

environment.^[4] Additionally, the stretching frequency of the nitrile group is sensitive to its hydration status and local electric field,^[5] making the nitrile moiety also useful as a vibrational probe of the local environment. Phe_{CN} has been employed to study the conformational changes, folding, and hydration states of proteins.^[4c,e, 6] Recently, this probe has also been used to monitor the formation of amyloid fibrils.^[7] Here, we replaced the Tyr10, Phe19, Phe20, and Phe4 residues in A β 40 with Phe_{CN}, respectively (Figure 1A), to explore local dynamics and environmental changes of specific residues along the pathway of A β oligomerization and fibrillization.

We first characterized the fibrillogenic properties of the four A β 40 mutants. Substitution of either Phe or Tyr with Phe_{CN} likely introduces minimal perturbation in terms of the physical properties of the protein because of the structural similarity of Phe_{CN} to Phe and Tyr residues.^[4c,6a] Our results show that the four A β 40 mutants all aggregate into amyloid fibrils. The changes in the secondary structures from random coil to β -sheet upon fibril formation were confirmed by circular dichroism (CD) spectroscopy (Figure 1B, C). The formation of amyloid fibrils of the mutants was confirmed by atomic force microscopy (AFM) imaging (see the Supporting Information, Figure S1). The aggregation kinetics of the peptides were also monitored in terms of the thioflavin T (ThT) fluorescence using a fluorescence plate reader. The half-time (t_{50}) of the growth phase of A β 40 (5 μ m) fibril formation under the conditions of this study was approximately 13.2 h, with A β 40-M1 and A β 40-M4 aggregating at a similar rate (Figure 1D, E). It is well recognized that the central hydrophobic core region “LVFFA” (residues 17–21) in A β is crucial for the formation and stabilization of fibrillar structures.^[8] Our results show that substitution of the Phe19 or Phe20 residue with Phe_{CN} leads to slightly faster aggregation (A β 40-M2 and A β 40-M3 in Figure 1D, E), in accordance with the importance of the central hydrophobic core in A β fibril formation. The similarity in the steepness of the growth phases of the mutants compared with that of A β 40 indicates that these peptides undergo a comparable process in the growth and elongation of the nucleus to fibrils. Together, these results suggest that mutation at these amino acid positions does not change the aggregation pathway or amyloid structures dramatically. Incorporation of Phe_{CN} into A β 40 at some positions, however, may affect the energy barrier for the nucleated conformational conversion and therefore the rate of amyloid formation. The extent of this effect is largely dependent on the local sequence of the position being mutated.

The local conformational dynamics and environmental changes of the mutant peptides along the aggregation pathway were recorded by time-dependent Phe_{CN} fluorescence analysis (Figure 2), with the ThT-binding fluorescence measured in parallel to monitor fibril formation. The fluorescence intensity of Phe_{CN} strongly depends on environmental factors, such as hydrogen bonding and polarity.^[4] As depicted in Figure 2 and Figure 3, the four mutants all show a significant decrease in Phe_{CN} fluorescence intensity over the period of aggregation. It is worth noting that the fluorescence intensity of the free Phe_{CN} amino acid, or the Phe_{CN} residue in an A β fragment peptide mutant that does not aggregate to form amyloids,^[7c] remains stable over time (Figure S2). This suggests that the observed Phe_{CN} fluorescence intensity changes of the A β 40 mutants are not caused by the spectroscopic instability of the Phe_{CN} fluorophore, but rather by aggregation-induced local environmental changes. The decrease in fluorescence intensity begins immediately, prior to the onset of

fibril formation as depicted by ThT fluorescence (curves of ThT vs. Phe_{CN} in Figure 3). The rapid decrease observed here is consistent with previous studies reporting fast A β oligomer formation without a lag time.^[9] The AFM results also show that spherical oligomers of a mutant peptide A β 40-M1 were observed after 1 h incubation, much earlier than the formation of fibrillar structures (Figure S3). Our kinetic results are consistent with a rapid collapse proposed by computational and experimental studies of protein self-assembly.^[10] In the initial collapse process, the condensation of the polypeptide chain into oligomeric assemblies likely results in transit of the examined residues from a solvent-exposed to a more dehydrated and hydrophobic local environment, causing the decrease in the Phe_{CN} fluorescence intensity. Although the molecular structures of oligomers have remained difficult to explore, our results suggest that the early self-assembly of A β is a relatively synergistic process, involving the participation of residues of both the N-terminal and central regions.

It should be noted that the Phe_{CN} fluorescence is also quenched by amino acids, such as deprotonated His, as well as through FRET to Trp and Tyr.^[4d, 6c, 11] An A β 40 derivative with double mutation of Y10F and F4F_{CN} still exhibits a remarkable Phe_{CN} fluorescence decrease after aggregation (Figure S4), implying that the Tyr10 residue does not contribute significantly to the fluorescence loss of Phe_{CN} in this case. The significant Phe_{CN} fluorescence decrease in the aggregation of A β 40-M1, in which Tyr10 was mutated to Phe_{CN}, also consolidates this conclusion. Additionally, the Phe_{CN} fluorescence intensity of A β 40-M1 still shows a pronounced drop after aggregation at an acidic pH of 4.9 (Figure S5). The quenching activity of protonated His at this pH becomes much weaker.^[6c] These results suggest that His quenching is not a major factor either in the Phe_{CN} fluorescence intensity drop in this study.

It has been demonstrated that amyloidogenic proteins can rapidly and efficiently form spherical oligomers that are kinetically competent and slowly converted into amyloid fibrils by a nucleated conformational conversion mechanism.^[9, 12] To further assess the rate of the local changes of specific residues in A β 40 aggregation, the traces of the decreases in the Phe_{CN} fluorescence intensities of the peptides were fitted using an empirical double exponential function for a best fit. The decay time of a slow-decrease phase (τ_{slow}) of the Phe_{CN} fluorescence intensity of the peptides is comparable to their respective t_{50} values for ThT kinetics (Table S1), suggesting that this slow phase is likely related to the later local conformational changes towards the formation of fibrils. The fast-decrease phase obtained here may be putatively attributed to early oligomer formation. As shown in Figure 4, the Phe_{CN}19 and Phe_{CN}20 residues exhibit a short decay time (τ_{fast}) of approximately 1.7 and 1.0 h in the fast-decrease phase of Phe_{CN} fluorescence, respectively. Although the heterogeneity and the transient nature of the oligomers makes it difficult to experimentally elucidate the local structural characteristics in oligomers, computational studies have suggested that A β 40 oligomerization is primarily driven by intermolecular interactions among the central hydrophobic core region.^[13] An in silico study reported an A β pentameric structure with the core made up of amino acids including the LVFFA region.^[13a] In a ring-shaped A β oligomeric structure, Phe19 and Phe20 are both involved in critical hydrophobic interactions.^[14] Most recently, Zheng and co-workers reported a cylindrin-shaped A β oligomeric structure composed of hairpin-shaped monomers, allowing for the central

hydrophobic core to be packed inside the complex.^[15] The high rates for the decay of the fluorescence intensity of Phe_{CN}19 and Phe_{CN}20 observed in our study are consistent with the crucial roles of these residues in early A β self-assembly.

Various NMR studies have shown that in contrast to the well-ordered hydrophobic core and the C-terminal region, the N-terminal residues of A β 40 are structurally disordered and do not display unambiguous distance restraints to the remainder of the core structure in amyloid fibrils.^[11] However, recent mutagenesis studies indicated that the N-terminal residues can dramatically influence the oligomerization and/ or fibrillization properties of A β ,^[16] but the detailed role of this region in A β aggregation still remains poorly understood. The N-terminal Phe_{CN}10 residue in A β 40-M1 interestingly also shows a short decay time ($\tau_{\text{fast}} \approx 2.6$ h) in the fast phase of Phe_{CN} fluorescence intensity decrease (Figure 4 A). Considering the slight differences in the fibril formation rates of the mutants (t_{50} in Table S1), the decay rate of the fast-decrease phase of Phe_{CN} fluorescence can be compared more reasonably by scaling the time axes with the respective t_{50} values of the peptides.^[3b] As shown in Figure 4B, the relative decay ratio ($\tau_{\text{fast}}/t_{50}$) of A β 40-M1 is comparable to that of A β 40-M2 and A β 40-M3. This suggests that the Tyr10 residue, albeit not distinguished well with a constrained conformation in fibrils, is synergistically involved in early self-assembly to form metastable local structures in oligomers, as illustrated in the schematic aggregation model shown in Figure 4C. It is plausible that the N-terminal hydrophobic residues, such as the “SGY” (residues 8–10) cluster, also play an active role in the formation of metastable oligomers.

The Phe4 residue in A β 40 is close to the N-terminus of the sequence. The τ_{fast} value of Phe_{CN}4 (ca. 6.8 h) is significantly larger than that of the other three residues studied (Figure 4). This indicates that the Phe_{CN}4 residue undergoes a more dynamic conformational change in the formation of oligomeric intermediates. The early self-association of the peptide can lead to transient interactions of Phe_{CN}4 with adjacent inter- and intramolecular residues, favoring formation of flexible and loose residual structures. For example, Urbanc and co-workers reported that a β -strand structure involving Ala2–Phe4 may form a pentameric structure of A β 40, and these N-terminal β -strands may shield the hydrophobic core of the oligomer.^[13a] These highly dynamic local structures may dissipate quickly to form new local interactions and residual structures. Such dynamic processes may presumably account for the lower rate of the decrease in the fluorescence intensity of Phe_{CN}4. Perhaps the highly dynamic nature of the N-terminal region may be directly related to the heterogenic properties of A β oligomeric intermediates.

Upon formation of amyloid fibrils, the fluorescence intensities of Phe_{CN}19 and Phe_{CN}20 underwent significant decreases of approximately 90% and 86%, respectively (Figure 5A). This is likely due to the tight packing of the central residual region in fibrils (as indicated in Figure S6), minimizing their solvent accessibility and hydrogen-bond formation. There are also slightly less pronounced drops in the fluorescence intensities of the Phe_{CN}10 (ca. 77%) and Phe_{CN}4 (ca. 76%) residues after fibril formation. This suggests that these N-terminal residues are also surrounded by a relatively hydrophobic environment in fibrillar structures, instead of being exposed to solvent, although the degree of their dehydration is likely lower than that of the residues at the central hydrophobic core region. Furthermore, the CN

stretching vibration band in the Raman spectra of all mutants is centered at approximately 2228 cm^{-1} (Figure 5B and Table S2), suggesting a dehydrated and hydrophobic local environment at the mutating positions in amyloids.^[17] This result is consistent with our fluorescence results. In addition, the band width of the CN stretching vibration is sensitive to the dynamics of the local environment.^[5a,c] The broader Raman band widths of CN in A β 40-M1 and A β 40-M4 (Table S2) indicate a more dynamic and inhomogeneous local environment for the N-terminal residues in fibrils, consistent with previous NMR studies (Figure S6). Interestingly, the values of the fluorescence anisotropy of the Phe_{CN}4 and Phe_{CN}10 residues are about 0.24 after amyloid formation, which is higher than that of the Phe_{CN}19 (ca. 0.12) and Phe_{CN}20 (ca. 0.14) residues (Figure S7). This may indicate that the N-terminal residues are not in proximity to the amyloid core in the fibrillar structures.

In conclusion, we have characterized and distinguished the residue-specific dynamics of A β 40 oligomer and fibril formation using the Phe_{CN} probe. The fast decay of the fluorescence intensity of residues 19 and 20 in the central hydrophobic core region and residue 10 in the N-terminal region suggests that these residues quickly participate in the initial self-association of monomers to form metastable local structures in oligomers, and that they are relatively well-packed and sequestered from solvent in oligomeric structures. The remarkably slower decrease in the fluorescence intensity of the Phe_{CN}4 residue suggests a more dynamic and fluctuating conformational transition at this position in the formation of oligomers. Our results also demonstrate that all of the residues examined, including those at the N-terminal region, are surrounded by a hydrophobic local environment in fibrillar structures, instead of being exposed to solvent, while the conformation of the N-terminal residues appears to be more heterogeneous and dynamic. These results provide new insight into the dynamic details of A β 40 aggregation with residue-specific resolution, and may illuminate future simulation approaches to explore A β aggregation mechanism(s) at an atomic level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. a) Paravastu AK, Leapman RD, Yau WM, Tycko R. *Proc. Natl. Acad. Sci. USA.* 2008; 105:18349–18354. [PubMed: 19015532] b) Lu JX, Qiang W, Yau WM, Schwieters CD, Meredith SC, Tycko R. *Cell.* 2013; 154:1257–1268. [PubMed: 24034249] c) Wälti MA, Ravotti F, Arai H, Glabe CG, Wall JS, Böckmann A, Güntert P, Meier BH, Riek R. *Proc. Natl. Acad. Sci. USA.* 2016; 113:E4976–E4984. [PubMed: 27469165]
2. Selkoe DJ, Hardy J. *EMBO Mol. Med.* 2016; 8:595–608. [PubMed: 27025652]
3. a) Lee JC, Langen R, Hummel PA, Gray HB, Winkler JR. *Proc. Natl. Acad. Sci. USA.* 2004; 101:16466–16471. [PubMed: 15536128] b) Shim SH, Gupta R, Ling YL, Strasfeld DB, Raleigh DP, Zanni MT. *Proc. Natl. Acad. Sci. USA.* 2009; 106:6614–6619. [PubMed: 19346479] c) Campioni S,

- Mannini B, Zampagni M, Pensalfini A, Parrini C, Evangelisti E, Relini A, Stefani M, Dobson CM, Cecchi C, Chiti F. *Nat. Chem. Biol.* 2010; 6:140–147. [PubMed: 20081829] d) Yushchenko DA, Fauerbach JA, Thirunavukkuarasu S, Jares-Erijman EA, Jovin TM. *J. Am. Chem. Soc.* 2010; 132:7860–7861. [PubMed: 20491471]
4. a) Tucker MJ, Oyola R, Gai F. *Biopolymers*. 2006; 83:571–576. [PubMed: 16917881] b) Tucker MJ, Tang J, Gai F. *J. Phys. Chem. B*. 2006; 110:8105–8109. [PubMed: 16610913] c) Aprilakis KN, Taskent H, Raleigh DP. *Biochemistry*. 2007; 46:12308–12313. [PubMed: 17924662] d) Taskent-Sezgin H, Chung J, Patsalo V, Miyake-Stoner SJ, Miller AM, Brewer SH, Mehl RA, Green DF, Raleigh DP, Carrico I. *Biochemistry*. 2009; 48:9040–9046. [PubMed: 19658436] e) Miyake-Stoner SJ, Miller AM, Hammill JT, Peeler JC, Hess KR, Mehl RA, Brewer SH. *Biochemistry*. 2009; 48:5953–5962. [PubMed: 19492814] f) Serrano AL, Troxler T, Tucker MJ, Gai F. *Chem. Phys. Lett.* 2010; 487:303–306. [PubMed: 20419080]
5. a) Getahun Z, Huang CY, Wang T, De Leon B, DeGrado WF, Gai F. *J. Am. Chem. Soc.* 2003; 125:405–411. [PubMed: 12517152] b) Suydam IT, Snow CD, Pande VS, Boxer SG. *Science*. 2006; 313:200–204. [PubMed: 16840693] c) Lindquist BA, Corcelli SA. *J. Phys. Chem. B*. 2008; 112:6301–6303. [PubMed: 18438998] d) Slocum JD, Webb LJ. *J. Am. Chem. Soc.* 2016; 138:6561–6570. [PubMed: 27128688]
6. a) Glasscock JM, Zhu Y, Chowdhury P, Tang J, Gai F. *Biochemistry*. 2008; 47:11070–11076. [PubMed: 18816063] b) Tang J, Yin H, Qiu J, Tucker MJ, DeGrado WF, Gai F. *J. Am. Chem. Soc.* 2009; 131:3816–3817. [PubMed: 19256494] c) Taskent-Sezgin H, Marek P, Thomas R, Goldberg D, Chung J, Carrico I, Raleigh DP. *Biochemistry*. 2010; 49:6290–6295. [PubMed: 20565125] d) Zimmermann J, Thielges MC, Seo YJ, Dawson PE, Romesberg FE. *Angew. Chem. Int. Ed.* 2011; 50:8333–8337. *Angew. Chem.* 2011; 123, 8483–8487. e) Chung JK, Thielges MC, Fayer MD. *J. Am. Chem. Soc.* 2012; 134:12118–12124. [PubMed: 22764745]
7. a) Marek P, Mukherjee S, Zanni MT, Raleigh DP. *J. Mol. Biol.* 2010; 400:878–888. [PubMed: 20630475] b) Inouye H, Gleason KA, Zhang D, Decatur SM, Kirschner DA. *Proteins Struct. Funct. Bioinf.* 2010; 78:2306–2321. c) Liu H, Lantz R, Cosme P, Rivera N, Andino C, Gonzalez WG, Terentis AC, Wojcikiewicz EP, Oyola R, Miksovska J, Du D. *Chem. Commun.* 2015; 51:7000–7003.
8. a) Hilbich C, Kisters-Woike B, Reed J, Masters CL, Beyreuther K. *J. Mol. Biol.* 1992; 228:460–473. [PubMed: 1453457] b) Shivaprasad S, Wetzel R. *J. Biol. Chem.* 2006; 281:993–1000. [PubMed: 16263715]
9. a) Lee J, Culyba EK, Powers ET, Kelly JW. *Nat. Chem. Biol.* 2011; 7:602–609. [PubMed: 21804535] b) Garai K, Frieden C. *Proc. Natl. Acad. Sci. USA*. 2013; 110:3321–3326. [PubMed: 23401512]
10. a) Nguyen HD, Hall CK. *Proc. Natl. Acad. Sci. USA*. 2004; 101:16180–16185. [PubMed: 15534217] b) Auer S, Meersman F, Dobson CM, Vendruscolo M. *PLoS Comput. Biol.* 2008; 4:e1000222. [PubMed: 19008938] c) Jain N, Bhattacharya M, Mukhopadhyay S. *Biophys. J.* 2011; 101:1720–1729. [PubMed: 21961598]
11. Tucker MJ, Oyola R, Gai F. *Phys J. Chem. B*. 2005; 109:4788–4795.
12. Serio TR, Cashikar AG, Kowal AS, Sawicki GJ, Moslehi JJ, Serpell L, Arnsdorf MF, Lindquist SL. *Science*. 2000; 289:1317–1321. [PubMed: 10958771]
13. a) Urbanc B, Cruz L, Yun S, Buldyrev SV, Bitan G, Teplow DB, Stanley HE. *Proc. Natl. Acad. Sci. USA*. 2004; 101:17345–17350. [PubMed: 15583128] b) Urbanc B, Betnel M, Cruz L, Bitan G, Teplow D. *J. Am. Chem. Soc.* 2010; 132:4266–4280. [PubMed: 20218566]
14. Tran L, Basdevant N, Prévost C, Ha-Duong T. *Sci. Rep.* 2016; 6:21429–21441. [PubMed: 26868929]
15. Zheng W, Tsai M-Y, Chen M, Wolynes PG. *Proc. Natl. Acad. Sci. USA*. 2016; 113:11835–11840. [PubMed: 27698130]
16. a) Maji SK, Ogorzalek Loo RR, Inayathullah M, Spring SM, Vollers SS, Condrón MM, Bitan G, Loo JA, Teplow DB. *J. Biol. Chem.* 2009; 284:23580–23591. [PubMed: 19567875] b) Brännström K, Ohman A, Nilsson L, Pihl M, Sandblad L, Olofsson A. *J. Am. Chem. Soc.* 2014; 136:10956–10964. [PubMed: 25014209]
17. Weeks CL, Jo H, Kier B, DeGrado WF, Spiro TG. *J. Raman Spectrosc.* 2012; 43:1244–1249.

Figure 1.

Aggregation of Aβ40 and the mutants. A) The primary sequence of Aβ40 peptide and the mutants. F_{CN} denotes a Phe_{CN} residue. B) CD spectra of monomeric Aβ40 and the mutant peptides (40 μM, pH 7.4). C) CD spectra of Aβ40 and the mutant peptides (40 μM, pH 7.4) after seven days of incubation. D) Aggregation kinetics of Aβ40 and the mutants (5 μM, pH 7.4) followed by ThT fluorescence at 37°C. Four kinetic curves are presented for each peptide. E) Aggregation half-time (*t*₅₀) of the peptides. The data are presented as mean ± standard deviation (SD) of quadruplicate results.

Figure 2.
Phe_{CN} fluorescence spectra of A) Aβ40-M1, B) Aβ40-M2, C) Aβ40-M3, and D) Aβ40-M4 (5 μM, pH 7.4) measured at different periods of incubation time.

Figure 3.

Aggregation kinetics of Aβ40-M1 A) Aβ40-M1, B) Aβ40-M2, C) Aβ40-M3, and D) Aβ40-M4 (5 μM, pH 7.4). The kinetics were followed in terms of the Phe_{CN} fluorescence (squares, intensity at 298 nm) and the ThT fluorescence (circles, intensity at 485 nm). Data are presented as the mean ± SD of triplicate results. The blue curves are fits of the ThT fluorescence kinetics obtained using a sigmoid equation, and the red curves are fits of the Phe_{CN} fluorescence kinetics obtained using a double exponential equation.

Figure 4.

A) The decay time of the fast-decrease phase in the Phe_{CN} fluorescence intensity (τ_{fast}) obtained by fitting the curves using a double exponential equation. B) Ratio of τ_{fast} and t_{50} of the A β 40 mutants. C) Schematic representation of oligomer (middle) and fibril (right) formation of A β 40. The positions of the residues studied are labeled in the sequence.

Figure 5.

A) Relative Phe_{CN} fluorescence intensities of the four mutants after 24 h incubation. The fluorescence intensities of the initial monomer peptides were set to 1. B) Raman spectra of Aβ40 mutants after incubation for 24 h. The samples (1 mM) were incubated in 50 mM phosphate buffer of pH 7.4 at 25°C. The spectra were smoothed, baseline-corrected, and normalized for clarity.