J|A|C|S COMMUNICATIONS

Published on Web 12/03/2009

Self-Aggregation of a Polyalanine Octamer Promoted by Its C-Terminal Tyrosine and Probed by a Strongly Enhanced Vibrational Circular Dichroism Signal

Thomas J. Measey,[†] Kathryn B. Smith,[§] Sean M. Decatur,[||] Liming Zhao,[‡] Guoliang Yang,[‡] and Reinhard Schweitzer-Stenner*,[†]

Departments of Chemistry and Physics, Drexel University, 3141 Chestnut Street, Philadelphia, Pennsylvania 19104, Department of Chemistry, Mount Holyoke College, South Hadley, Massachusetts 01075, and Department of Chemistry and Biochemistry, Oberlin College, Oberlin, Ohio 44074

Received September 30, 2009; E-mail: rschweitzer-stenner@drexel.edu

Self-assembly of polypeptides and proteins has become the subject of intense research activity for various reasons. First, an understanding of the underlying mechanism is necessary for the development of diagnostic and therapeutic strategies for a variety of diseases, including Alzheimer's, Huntington's, Creutzfield−Jacob, and Parkinson's disease.[1] These diseases are believed to result from misfolding and subsequent aggregation of specific proteins to form rigid fibrils. These fibrils then deposit in tissues to form insoluble plaques.[2] Second, polypeptide/protein self-aggregation results in the formation of novel supramolecular structures (e.g., hydrogels) that are of biomedical and biotechnological relevance.[3]

Elucidating the mechanism of the self-assembly of peptides and proteins is of fundamental importance.[4] However, the rules governing self-aggregation are still debated. Strong experimental evidence suggests that the aggregation process does not primarily reflect the conformational propensities of a peptide's amino acid residues.[5] The pivotal role of aliphatic and aromatic residues in the aggregation of even small peptides suggests that aggregation is strongly driven by hydrophobic forces.[5,6] Gazit[7] demonstrated the importance of aromatic residues for self-aggregation, but it is unclear whether this is due to aromaticity or the propensity of these peptides to adopt β-sheet-favoring conformations. In this regard, conformational disorder has been suggested to be a prerequisite for fibrillation.[4,8,9]

In this communication, we report fibril formation by an eight-residue alanine-based oligopeptide, namely, Ac-A_4KA_2Y-NH_2 (AKY8). Oligoalanines of this size typically exhibit a statistical coil structure in aqueous solution, with a predominant sampling of polyproline II (PPII) conformations.[10] PPII has been hypothesized to be a prerequisite for polypeptide/protein aggregation.[11] However, our results compel us to attribute the self-aggregation of this peptide to favorable stacking interactions between side chains of different strands.

Figure 1 compares the amide I′ regions of the FTIR and vibrational circular dichroism (VCD) spectra of AKY8 and the reference peptide Ac-A_4KA_2-NH_2 (AK7) in D_2O at peptide concentrations of ∼20 and ∼50 mM for AKY8 and AK7, respectively. The negative couplets in the VCD spectra of the two peptides measured immediately after dissolution (Figure 1a) are identical and indicate a statistical ensemble of conformations, with a predominance of PPII-like conformations.[12] The UV-CD spectra of AKY8 and AK7, displayed in Figure 2, corroborate this notion. Overnight incubation of the two peptides, however, resulted in the formation of a gelatinous solution for AKY8 but not for AK7.

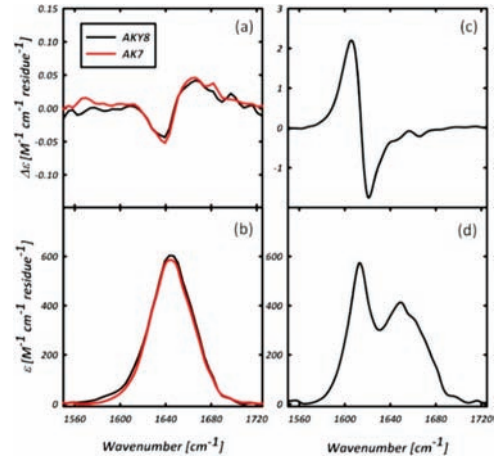

Figure 1. (a) VCD and (b) FTIR spectra of monomeric AKY8 and AK7 in D_2O. (c) FTIR and (d) VCD spectra of an AKY8 fibril solution in D_2O after overnight incubation at acidic pH.

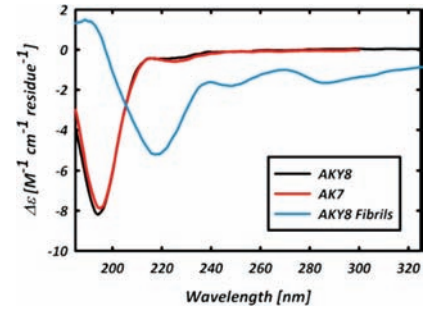

Figure 2. UV-CD spectra of monomeric AKY8 (black) and AK7 (red) and the AKY8 fibril solution (blue).

Representative atomic force microscopy (AFM) images of the resultant AKY8 solution depict rather large fibrils with heights of 22−24 nm (Figure 3). We found that the AKY8 fibrils bind congo red, an indication that they are of the amyloid type.

The IR spectrum of the fibril solution (Figure 1d) shows a band at ∼1616 cm^{-1}, indicative of an antiparallel β-sheet conformation. The corresponding UV-CD spectrum (Figure 2), which shows a minimum at ∼220 nm, also indicates a β-sheet conformation.[13] The broad IR band centered at ∼1650 cm^{-1} is blue-shifted by ∼7 cm^{-1} with respect to the amide I′ band of the monomer and coincides with the amide I′ bands in the isotropic and anisostropic Raman spectra of AKY8 (data not shown). This suggests that both the IR and Raman bands should be assigned to a distorted β-sheet conformation in which the two Raman-active modes become IR-

[†] Department of Chemistry, Drexel University.
[‡] Department of Physics, Drexel University.
[§] Mount Holyoke College.
[||] Oberlin College.

10.1021/ja908324m

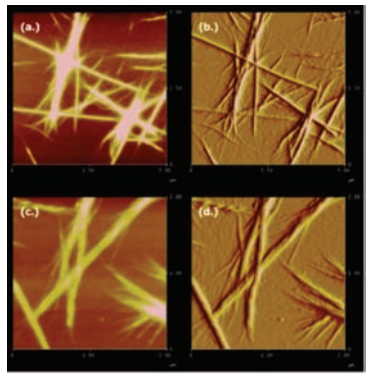

Figure 3. AFM images of AKY8 fibrils: (a) height and (b) amplitude images of a 5 μm $\times$ 5 μm area; (c) height and (d) amplitude images of a 2 μm $\times$ 2 μm area. The AFM images were acquired in air.

active. On the contrary, the IR and VCD spectra of AK7 do not indicate any aggregation, even after overnight incubation.

The most peculiar observation in this study is the VCD spectrum of the AKY8 fibril solution, which exhibits a drastically enhanced positive couplet with an inflection point at 1616 cm^{-1}. Its intensity is nearly 2 orders of magnitude larger than that in the initial VCD spectrum of unaggregated AKY8 in this region (Figure 1). Enhanced VCD signals in the amide I′ region have recently been reported for lysozyme and insulin fibrils.[14]

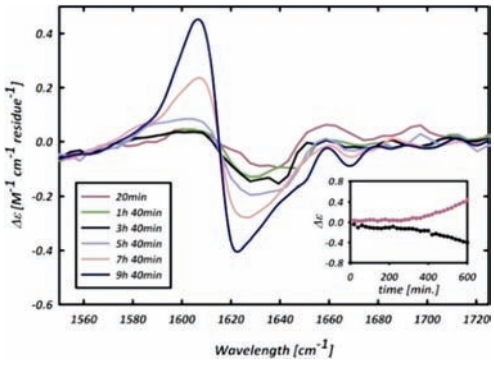

Figure 4. Temporal evolution of the amide I′ VCD signal of the AKY8 fibril solution. Inset: intensity of the VCD signal as a function of time at 1604 cm^{-1} (red) and 1620 cm^{-1} (black).

The giant VCD signal can conveniently be used to probe the kinetics of the aggregation process. Figure 4 shows the formation of the enhanced couplet as a function of time for the first 10 h of sample incubation. The inset shows the VCD intensity of the maximum (red) and minimum (black) of the emerging couplet. A lag time is observed prior to the fibril growth, as is typical for fibrillogenesis,[15] leading us to conclude that the enhanced VCD signal is a direct probe of the fibrillization process rather than of the early β-sheet formation. The UV-CD spectrum of the AKY8 fibril solution (Figure 2) shows clear CD in the region of the tyrosine side chain absorption, which is absent in the spectrum of the monomer. This observation, along with the inability of AK7 to aggregate, clearly proves that tyrosine is pivotal for the observed self-aggregation of AKY8. Even though the role of aromatic residues in aggregation processes is well-documented,[6] our result is very surprising, since it was not expected that a single tyrosine positioned at the C-terminal could aggregate a peptide that does not have other aggregation-promoting sources. The IR spectrum of the AKY8 fibril solution indicates an antiparallel β-sheet conformation, so interactions between the tyrosine residues in adjacent strands of the same sheet can be ruled out. Instead, we propose that sheet formation is caused by cation$-\pi$ interactions between the lysine and tyrosine side chains that yield the following out-of-register arrangement:

AAAAKAAY
YAAKAAAA
AAAAKAAY
YAAKAAAA

It has been shown that cation$-\pi$ interactions, particularly those between a tyrosine and charged lysine, can be important for the stabilization of protein structures.[16] The role of such an interaction in peptide aggregation, however, has not been proposed to date. The fibrils revealed by AFM are consistent with the stacking of β-sheet tapes, which could be stabilized by π stacking between tyrosine residues or by additional cation$-\pi$ interactions. First simulations of the IR and VCD profiles of the amide I band for a very simple two-dimensional model of amide I oscillators in which a one-dimensional chain represents a sheet indicates that efficient packing rather than tilting of the sheet structure might yield the observed enhancement of the VCD signal.

Taken together, the results of the present study show that a single tyrosine residue can promote self-aggregation of a short polyalanine peptide. We propose that cation$-\pi$ interactions and, to a minor extent, $\pi-\pi$ interactions serve as the driving force for the aggregation process. Furthermore, the fibrillization gives rise to a large enhancement of the amide I VCD signal, which may reflect a rather compact facial assembly of β-sheets.

Acknowledgment. We acknowledge financial support from NSF (CHEM 0804492 to R.S.-S. and 0849164 to S.M.D.) and NIH (R01-GM071793 to G.Y.).

Supporting Information Available: Materials and Methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dobson, C. M. *Trends Biochem. Sci.* **1999**, *24*, 329.
(2) Glenner, G. G. *N. Engl. J. Med.* **1980**, *302*, 1283.
(3) Yokoi, H.; Kinoshita, T.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8414.
(4) Dobson, C. M. *Nature* **2003**, *426*, 884.
(5) Kim, W.; Hecht, M. H. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15824.
(6) Gazit, E. *Prion* **2007**, *1*, 32.
(7) Gazit, E. *FASEB J.* **2002**, *16*, 77.
(8) Chiti, F.; Dobson, C. M. *Annu. Rev. Biochem.* **2006**, *75*, 333.
(9) Rochet, J. C.; Landsbury, P. T., Jr. *Curr. Opin. Struct. Biol.* **2000**, *10*, 60.
(10) Graf, J.; Nguyen, P. H.; Stock, G.; Schwalbe, H. *J. Am. Chem. Soc.* **2007**, *129*, 1179.
(11) Blanch, E. W.; Morozova-Roche, L. A.; Cochran, D. A. E.; Doig, A. J.; Hecht, L.; Barron, L. D. *J. Mol. Biol.* **2000**, *301*, 553.
(12) Schweitzer-Stenner, R. *J. Phys. Chem. B* **2009**, *113*, 2922.
(13) MacPhee, C. E.; Dobson, C. M. *J. Am. Chem. Soc.* **2000**, *122*, 12707.
(14) Ma, S.; Cao, X.; Mak, M.; Sadik, A.; Walkner, C.; Freedman, T. B.; Lednev, I. K.; Dukor, R. K.; Nafie, L. A. *J. Am. Chem. Soc.* **2007**, *129*, 12365.
(15) Frieden, C. *Protein Sci.* **2007**, *16*, 2334.
(16) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303.

JA908324M