The Self-aggregation of A Polyalanine Octamer Promoted by Its C-Terminal Tyrosine And Probed By A Strongly Enhanced VCD Signal

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Supporting Information

Materials and Methods

Materials and Sample Preparation:

Congo red was obtained from Fluka (> 97% purity). AKY8 was synthesized via Fmoc solid-phase peptide synthesis using a CEM Liberty microwave accelerated peptide synthesizer. An acetyl group was added to the N-terminus after the final deprotection. Cleavage of the peptide product from the resin was carried out in a cocktail consisting of 95% trifluoroacetic acid (TFA), 2.5% H2O, and 2.5% triisopropanol. Crude peptide was precipitated from the cleavage cocktail using cold t-butyl methyl ether, collected by centrifugation, and lyophilized. The crude peptide was purified using reverse phase high-performance liquid chromatography (RP-HPLC) on an AKTA system (Amersham Pharmacia Biotech) equipped with a C18 prep column (Vydac). The peptide was eluted using a linear gradient of acetonitrile, with 0.85% TFA. Electrospray mass spectrometry (performed at the University of Massachusetts at Amherst Mass Spectrometry Facility) was used to confirm the peptide identity and purity. Prior to use, all samples were dialyzed in a Spectra-Por Float-A-Lyzer Dialysis Bag, with a molecular weight cut-off of 500 Da (Spectrum Labs), to remove residual trifluoroacetic acid (TFA), which overlaps with the amide I' band in the FTIR spectrum. The peptides were then lyophilized overnight, and redissolved in D_2O. For AKY8, the exact peptide concentration was determined from the tyrosine absorption at 275 nm, which has an extinction coefficient of 1450 M^{-1} cm^{-1}, for experiments involving the monomeric, non-aggregated state. To form the fibril solution of AKY8, the peptide was incubated overnight at room temperatures in the presence of deuterium chloride (DCl). This procedure resulted in a gelatinous solution, containing AKY8 fibrils. To determine if the fibrils were of the amyloid type, a previously prepared 100 μM congo red in D_2O solution was added to the resulting gelatinous AKY8 solution, and the subsequent UV/visible absorption spectrum was measured. Upon binding of congo red to amyloid fibrils, a red-shift of the visible absorption band ca. 498 nm is observed.

Vibrational Spectroscopy

Vibrational Circular Dichroism (VCD) and FTIR spectra were measured with a ChiralIR™ spectrometer with a single PEM from BioTools (Jupiter, FL). The peptide solutions were placed in a 20 um CaF_2 BioCell™ obtained from BioTools. The VCD and IR spectra of the unaggregated peptide were collected for 324 and 36 minutes, respectively, to improve signal-to-noise ratio, while the VCD and IR spectra of

the supernatant fibril solution were obtained for 18 and 2 minutes, respectively. Both VCD and IR spectra were collected using 8 cm-1 resolution.

UV-CD Spectroscopy
All UV-CD spectra were obtained on a Jasco J-810 spectropolarimenter, using a data pitch of 0.05 nm, a response time of 1 second, a bandwidth of 5 nm, a scan speed of 500 nm/min, and a wavelength window of either 180 – 300 or 180 – 500 nm. The instrument was purged with N_2 during the course of the measurements. For all UV-CD measurements, peptide stock solutions were diluted 10-fold with D_2O, and were placed in a 50 um Q Silica UV-Grade demountable cell (International Crystal Laboratories).

Atomic Force Microscopy (AFM)
All AFM experiments were performed at room temperature, using a multimode atomic force microscope (Nanoscope IIIa; Digital Instruments, Santa Barbara, CA), equipped with an E-type piezoscanner. Peptide samples were applied to a freshly-cleaved mica surface for 15 min, and subsequently dried with a stream of N_2 gas. Contact-mode imaging was carried out with a silicon nitride cantilever (180 * 18 micrometer; Park Scientific Instruments, Sunnyvale, CA). Height and deflection images of the peptide fibrils were obtained with a scan rate of 4 Hz and an integral gain of 2-4.