Electronic Supplementary Material (ESI) for Soft Matter
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Electronic Supplementary Information

Amyloid fibril formation by pepsin in neutral pH at room temperature

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Experimental Details

Material

Pepsin from porcine gastric mucosa were purchased from Sigma-Aldrich (lyophilized powder, catalog no. P7012). Thioflavin T (ThT) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and was used directly from the bottle. The purity of ThT was confirmed by measuring absorption and fluorescence excitation spectra. Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, sodium acetate, and acetic acid were purchased from SRL. Phosphate buffer (20 mM) of pH 3, 6,7,8,9 and acetate buffer (20 mM) of pH 4 and 5 were prepared in milli Q water (~18 MΩ).

Methods and instrumentation

Fluorescence measurements

All the Steady-state fluorescence measurements were carried out with a Perkin-Elmer LS-55 luminescence spectrometer equipped with a filter polarizer and a thermostating cell holder. All measurements were carried out at ~30 ^{o}C unless otherwise mentioned.

The ThT fluorescence was measured to monitor amyloid aggregation of pepsin as a function of time and concentration. Pepsin concentration was kept constant at 0.2% and ThT concentration was ca. 10^{-5} M. The following parameters were adjusted for monitoring the ThT fluorescence intensity during aggregation experiment: λ_{ex} = 410 nm, λ_{em} = 475 nm, excitation band-pass = 2.5 nm and emission band-pass = 5 nm.

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The steady-state fluorescence anisotropy (r) of tryptophan (Trp) residue was calculated employing the equation:

$$r = \frac{I_{VV} - I_{VH}}{I_{VV} + 2GI_{VH}}$$

where I_{VV} and I_{VH} are the fluorescence intensities polarized parallel and perpendicular to the excitation light, and G (= I_{VV}/I_{VH}) is the instrumental grating factor. The software supplied by the manufacturer automatically determined the G factor and fluorescence anisotropy. For each measurement, the anisotropy value was recorded over an integration time of 10 s. Five readings were recorded for each sample and the average value was accepted as the anisotropy value. The sample was excited at 295 nm and the emission intensity was followed at 360 nm using excitation and emission slits with band-pass of 2.5 nm and 4 nm, respectively. A 350 nm cut-off filter was placed in the emission beam to eliminate the effect of scattered radiation.

The conformational change of pepsin was monitored by tryptophan anisotropy measurement at room temperature. The variation of fluorescence anisotropy as a function of pH is presented in Fig. S1. The first part (pH < 5) of the titration curve represents conformational change of pepsin due to deprotonation of amino acid residues, while the sigmoid decay of anisotropy at pH > 6 corresponds to alkaline denaturation.

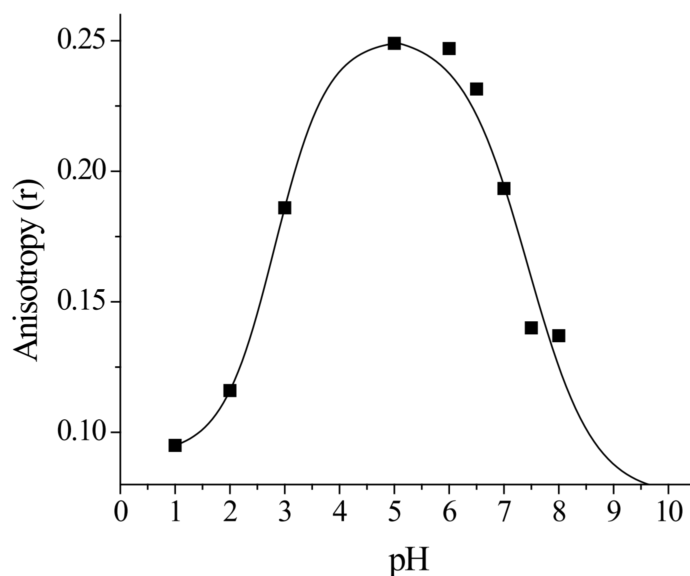

Fig S1. Variation of fluorescence anisotropy (r) of Trp with pH at ~30 °C.

Dynamic light scattering

The dynamic light scattering (DLS) measurements were performed with Zetasizer Nano ZS (Malvem Instrument Lab, Malvern, U.K.) light scattering spectrometer equipped with a He-Ne laser operated at 4 mW (λ_o= 632.8 nm) at 25 °C, a digital correlator, and a computer-controlled and stepping-motor-driven variable angle detection system. All measurements were performed at room temperature. The data acquisition was carried out for at least 100 counts and each experiment was repeated two or three times.

Circular dichroism spectra

A Jasco J-810 spectropolarimeter was used to measure the circular dichroism (CD) spectra using quartz cells of 1 mm path length. For each spectrum, an average of three scans was taken under the conditions of 1 nm bandwidth, 2-second response time. Each spectrum was

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baseline corrected using appropriate reference solution. All measurements were carried out at 25 ^{o}C. The spectrum was recorded in the range of 190 nm to 260 nm. An accumulation of three or more scans with a speed 50 nm/min was performed and data were collected.

Transmission electron microscopy

The morphological structures of the fibril were investigated by transmission electron microscopy (TEM) by without straining method. A drop (5 μL) of solution was poured on a carbon-coated copper grid (400 mesh) and it was kept for drying in desiccators until before use. The specimen was visualized on a JEOL-TEM 2100, Japan, transmission electron microscope operated at 200 kV at 25 ^{o}C.