Distinguishing the cross- β spine arrangements in amyloid fibrils using FRET analysis

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Abstract

The recently published microcrystal structures of amyloid fibrils from small peptides greatly enhanced our understanding of the atomic-level structure of the amyloid fibril. However, only a few amyloid fibrils can form microcrystals. The dansyl-tryptophan fluorescence resonance energy transfer (FRET) pair was shown to be able to detect the inter-peptide arrangement of the Transthyretin (105–115) amyloid fibril. In this study, we combined the known microcrystal structures with the corresponding FRET efficiencies to build a model for amyloid fibril structure classification. We found that fibrils with an antiparallel structural arrangement gave the largest FRET signal, those with a parallel arrangement gave the lowest FRET signal, and those with a mixed arrangement gave a moderate FRET signal. This confirms that the amyloid fibril structure patterns can be classified based on the FRET efficiency.

Keywords: amyloid fibril; FRET; classification; cross- β ; structure elucidation

Supplemental material: see www.proteinscience.org

Amyloid fibrils are characterized by a common structural component, the cross- β spine. Although this core structure was identified some time ago by X-ray diffraction, the detailed arrangement of the β spine was difficult to determine because of the fibrillar nature of the material. Several methods, such as nuclear magnetic resonance (NMR), hydrogen-deuterium (H/D) exchange, Fourier transform infrared (FTIR), and deep ultraviolet resonance Raman spectroscopy, have contributed to the available structural information (Hiramatsu and Kitagawa 2005;

Toyama et al. 2007; van der Wel et al. 2007; Xu et al. 2007). The recently published crystallographic study on the microcrystal structures of amyloid fibrils formed from small protein segments greatly advanced our understanding of the structure of the fibrils at the atomic level (Sawaya et al. 2007). The authors determined that the arrangement of the cross- β spine was diverse and that the known structures fell into five classes. These classes are distinguished by whether the strands in the sheets are parallel or antiparallel, whether the sheets pack with the same or different surfaces adjacent to one another, and whether the sheets are oriented parallel or antiparallel with respect to one another. However, this method is still limited because only a few amyloid fibrils can form microcrystals.

We previously reported that the dansyl-tryptophan FRET pair could be used to study the inter-peptide arrangement of the amyloid fibril formed from a Transthyretin peptide (105–115) (Deng et al. 2007). In that study, we conjugated the dansyl-tryptophan FRET pair to the terminals of the same peptide, and the change of the inter-peptide distance was detected by FRET signal. By combining existing crystal

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Abbreviations: FRET, fluorescence resonance energy transfer; NMR, nuclear magnetic resonance; FTIR, Fourier transform infrared; H/D, hydrogen-deuterium; AP, antiparallel; HP, half parallel; P, parallel; $I_{504\text{nm}}$, intensity at 504 nm; $I_{360\text{nm}}$, intensity at 360 nm.

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structure information with the FRET signal of the corresponding amyloid fibrils formed from small peptides, we may develop a model for fibril structure prediction based only on the magnitude of the FRET signal. This model will be useful for peptide fibril structure study because many of them cannot form crystals.

Results and Discussion

Structure classification of amyloid fibril

As the FRET study could not distinguish sheets packing with the same or different surfaces adjacent to one another, based on the inter-peptide arrangements, we grouped the amyloid fibril structures into three classes: the antiparallel pattern (AP), in which the peptide is arranged antiparallel within the same β sheet; the parallel pattern (P), in which the peptide is arranged parallel both in the same sheet and between neighboring sheets; and the half-parallel pattern (HP), in which the peptide is arranged parallel in the same sheet and antiparallel between the neighboring sheets (shown in Fig. 1).

Synthesis of peptides with FRET probes and fibril incubation

Fourteen peptides that could form amyloid fibril and are six or seven amino acid residues in length had been selected for the FRET study from the crystallographic study of Sawaya et al. (2007) (listed in the Supplemental material). These short peptides were chosen for FRET study because (1) each of these short peptides formed straight β strands that did not turn in the amyloid fibril, which made the data analysis convenient; (2) the Förster distance of the dansyl-tryptophan FRET pair was 21 Å and the length of six or seven amino acid residues is about

26 Å, which could effectively reduce the intra-peptide FRET; and (3) these segments covered all the classes that have been observed in the crystal structures.

The dansyl-tryptophan probes were conjugated to the terminals of these peptides. After incubation, 9 of the 14 peptides formed amyloid fibril under pH 2 (most amyloid fibrils form under low pH environment and pH 2 is similar to the crystal incubation condition) as confirmed by electron microscopy (EM). The detailed incubation conditions are shown in the Supplemental material. The other five peptides can also form amyloid fibrils under other pH conditions, for example, the modified SSTSA sequence under pH 4; these peptides were not included in our analysis to avoid the uncertainties that may be brought by different incubation conditions.

Fluorescence study for peptides with known fibril microstructure

We measured the fluorescence spectroscopies of the pure amyloid fibrils from all the samples. An excitation wavelength of 295 nm was used to excite tryptophan. Similar to results we have reported before (Deng et al. 2007), FRET signal can be observed at 504 nm corresponding to the emission of the dansyl group (Fig. 2). The fluorescence spectra and the EM images of the representative amyloid fibrils are shown in Figure 2. The three representatives are the same representative peptide segments used in the crystallographic study (Sawaya et al. 2007): SNQNNF, VQIVYK, and VEALYL. In this study, they represent the P, HP, and AP structure patterns, respectively.

We compared the FRET intensity of the pure amyloid fibrils using the ratio of the acceptor peak value $I_{504\text{nm}}$ and the donor peak value $I_{360\text{nm}}$. According to the rule of FRET (Stryer 1978), this ratio is correlated with the distance of the FRET pair; the larger the ratio, the closer the distance between the two probes. We did not use the Förster formula to calculate the accurate distance of the two probes (Stryer 1978; Piston and Kremers 2007). This is partly because the concentrations of the centrifuged amyloid fibril samples were difficult to keep the same (as two sequences need to be compared for the accurate calculation).

According to the cross- β X-ray diffraction pattern, there are two X-ray reflections of amyloid fibrils: one at about 4.7 Å along the fibril, which is the distance between two β strands in the same β sheet, and the other at about 10 Å perpendicular to the fibril direction, which is the distance between two neighboring sheets. Thus, in the AP pattern, the FRET probes are very close within one sheet and the intensity should be strong, whereas in the P pattern it should be weak since all the peptide segments are parallel and the two terminals are far away. For the HP pattern, the FRET signal should mainly come from the

Figure 1. Three inter-peptide arrangement models of the amyloid fibril. (A) The antiparallel model (AP); (B) the half-parallel model (HP); (C) the parallel model (P).

Figure 2. The fluorescence spectroscopy and the EM images of the representative amyloid fibrils of the parallel pattern (dotted line, peptide sequence SNQNNF), half-parallel pattern (dashed line, peptide sequence VQIVYK), and antiparallel pattern (solid line, VEALYL).

intersheet so the FRET intensity should lie between the AP pattern and the P pattern. As the FRET intensity is also affected by the flexibility of the probes and their conformations in the amyloid fibril, which are difficult to consider, we expected that the FRET intensities can only make qualitative distinctions among these different patterns.

Table 1 shows the FRET ratios of peptides with known amyloid fibril structures. The two peptides that were found to form antiparallel structures, VEALYL and MVGGVV, have a large $I_{504\text{nm}}/I_{360\text{nm}}$ ratio (>30), whereas the peptide with a parallel structure, SNQNNF, has a low $I_{504\text{nm}}/I_{360\text{nm}}$ ratio (<5). The half-parallel peptides, VQIVYK and GGVVIA, have $I_{504\text{nm}}/I_{360\text{nm}}$ ratios in between. Although an accurate definition may not be 100% reliable because of the limited data, it is true that the fibril structures can be classified according to the FRET intensities: AP pattern $>$ HP pattern $\gg$ P pattern. Our estimation is the following: When $I_{504\text{nm}}/I_{360\text{nm}}$ ratio > 32 , we can be confident that the structure is AP; when the ratio is between 32 and 24, there are two possible structures of AP and HP patterns; when the ratio is between 24 and 5, the structure should be HP; and when the ratio is <5 , the structure should be P.

Predicting peptide amyloid fibril structure based on FRET signal

Table 2 shows the FRET ratios of four peptides with unknown fibril structures whose microcrystal structures were difficult to solve. In our FRET study, their amyloid fibril structure patterns could be predicted according to

their FRET intensities based on the relationship summarized above. Since these peptides were all six residues in length and they were all incubated under the same pH condition, their FRET magnitude could be compared directly with the known structure segment. The fibril formed by peptide NFGAIL showed an $I_{504\text{nm}}/I_{360\text{nm}}$ ratio as large as 93, so it should form an antiparallel structure. The fibril formed by peptide ILQINS showed an $I_{504\text{nm}}/I_{360\text{nm}}$ ratio as low as 0.3, thus it should have a parallel structure. The FRET ratio of the TFQINS fibril was between that of VQIVYK and GGVVIA, so its structure might be half parallel. The FRET ratio of the NNQNTF fibril was also close to that from peptides with half-parallel structures, so we assigned it a half-parallel structure. Of course, these predicted structures need to be confirmed further by crystallographic studies.

Our study shows that there is a strong relationship between the amyloid fibril structures with the FRET

Table 1. The FRET ratio for the peptides with known amyloid fibril structure

Peptide segment ^a	Crystal structure	$I_{504\text{nm}}/I_{360\text{nm}}$ ^b
Dansyl-VEALYL-W	Antiparallel	42.5 ± 3
Dansyl-MVGGVV-W	Antiparallel	30 ± 2
Dansyl-VQIVYK-W	Half parallel	24 ± 3
Dansyl-GGVVIA-W	Half parallel	12 ± 2
Dansyl-SNQQNY-W	Parallel	3.4 ± 0.7

^aThe original segment is modified by a dansyl group attached to the N-terminal and a tryptophan at the C-terminal.

^bThe values of the ratio were averages of three measurements.

Table 2. The FRET ratio for peptides with unknown amyloid fibril structure

Peptide segment	I_{504nm}/I_{360nm}	Predicted structure ^a
Dansyl-NFGAIL-W	93 ± 7	Antiparallel
Dansyl-TFQINS-W	20 ± 4	Half parallel
Dansyl-NNQNTF-W	11 ± 2	Half parallel
Dansyl-ILQINS-W	0.3 ± 0.1	Parallel

^aThe structures were predicted by the magnitude of FRET ratio.

magnitudes they induced. By attaching a pair of FRET probes to the terminals of a peptide, the structure of the corresponding amyloid fibrils can be predicted based on the FRET efficiency. This method provided a convenient approach to the structure classification of amyloid fibril. More data can be accumulated to build a comprehensive peptide sequence and FRET efficiency database for a full-scale amyloid fibril structure class prediction.

Materials and Methods

All the modified peptides were synthesized by standard Fmoc synthesis (PS3 automated peptide synthesizer, Protein Technologies, Inc.). The dansyl group was conjugated to the N-terminal of the peptide as described previously (Deng et al. 2007). Briefly, the peptide with tryptophan at the C-terminal was synthesized by solid-state peptide synthesis method first and then the deprotected peptide was mixed with the dansyl chloride in dichloromethane for the conjugation of the dansyl group at the N-terminal. The synthesis and purification were confirmed by mass spectroscopy. The lyophilized, modified peptides were solved at 4 mM in water/acetonitrile mixed solvent (details are listed in the Supplemental material), with the pH adjusted to 2 by adding a small amount of concentrated HCl. The peptide solutions were first sonicated with 5-sec pulse for 8 min with a microtip sonicator, then incubated for two days at 37°C, and then 3 wk at 25°C. The mature fibril was characterized by EM.

The FluoroLog 3 Spectrofluorometer (HORIBA, Jobin Yvon, Inc.) was used to measure the fluorescence intensity of FRET. The samples were diluted and put into a 2-mm-path-length quartz cuvette and measured with the exciting wavelength of

295 nm and scan range from 310 nm to 580 nm. Pure fibrils were obtained by centrifuging the mature fibril solutions at 14,000g for 30 min at 4°C. Then, the supernatant was removed and the amyloid fibril was washed three times with the same solvent. During the fluorescence measurement, the concentrations of pure amyloid fibril were controlled to lower than 10 μM by dilution to reduce the fluorescence quenching. Each sample was measured three times in parallel to be averaged.

Electronic supplemental material

The Supplemental material includes additional details on incubation conditions for the 14 peptides (Supplemental Table S1) and the EM photos of the 9 amyloid fibrils (Supplemental figures).

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