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COMMUNICATION

Transformation of β -sheet structures of amyloid peptide induced by molecular modulators

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2014,
Accepted 00th January 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/

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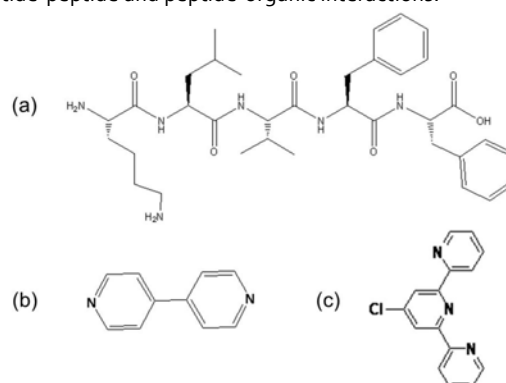
In this work we report the effect of terminus molecular modulators on the secondary structures of the amyloid peptide aggregates. The controlled modulation of the assembly structure and the transformation of β -sheet secondary structures could be beneficial for gaining insight into the aggregation mechanism of peptides. Particularly, the multiple assembling characteristics have been identified as a reflection of peptide-organic interactions.

Amyloid deposit is observed in more than 20 human diseases, including Alzheimer's disease (AD), characteristic of protein misfolding and misassembly into amyloid fibrils with well-defined secondary structures.¹ β -Amyloid (A β) fibril propagation,² closely correlated to the AD pathogenesis,³ are elucidated with a basis parallel β -sheet structure by using X-ray diffraction (XRD) and solid-state NMR.⁴ There is a direct correlation between the percentage of parallel β -sheet structure and the ability of A β peptides to form amyloid, based on different van der Waals, ionic, steric, hydrogen bond, electrostatic intramolecular and intermolecular interactions.⁴ Parallel to these findings, the investigations on recognition of amyloid peptides and interference of peptide-peptide interactions aiming at modulation of the peptide secondary structures offer possibilities for the inhibition of amyloid fibril formation and the treatment of human diseases.

While peptide-peptide interaction is driving force for peptide assembly and aggregation as described in principle of amyloid self-assembly (PASA), the peptide-organic interaction could be critical to develop complementary venues for constructing peptide-organic architectures based on the similar molecular interactions.⁵ In a closely related research topic, with the introduction of organic species as modulators to the peptide assemblies, one could investigate both peptide-peptide and peptide-organic interactions. It is of genuine interest to explore the effect of molecular species on the structural characteristics of peptide assemblies. With a rich variety of molecular designs, the organic species could greatly expand the capability to

modulate peptide assembly structures and secondary structures. As an example, the prevalent conformational polymorphism of organic species could provide possible approach to enriching the secondary structural characteristics of peptide assemblies.^{5a,6}

With the progress in studying peptide assemblies with molecular resolution, it becomes feasible to examine the structural details of peptide assemblies subject to various environments such as ultrahigh vacuum, different solvent polarity, surface chemistry, etc.⁷ In recent scanning tunnelling microscopy (STM) studies, amyloid peptide-organic assemblies have been explored which are dominated by hydrogen bond interactions.⁸ Various heterogeneous assembling characteristics have been identified that could be associated with the peptide-peptide and peptide-organic interactions.^{7,8}



Scheme 1 Schematic illustration of molecular structures of (a) KLVFF, (b) 4, 4'-Dipyridyl (4Bpy), and (c) 4'-chloro-2,2':6',2''-terpyridine (Cl-Ter).

In the present study, we are focusing on the formation of peptide-organic assemblies using analogue of β -amyloid (A β peptide, A β (16–20) (KLVFF) (Scheme 1a), as a representative peptide segment. The pyridine chaperone species interact with the C-terminal of peptides, resulting in uniform peptide-organic assemblies. We report here the assembling behavior of KLVFF and the modulation effect of terpyridine molecules on KLVFF assembly using STM and Fourier transform infrared (FTIR). Two-dimensional (2D) assembling behavior

of the oligopeptides and the fine molecular structures of KLVFF/terpyridine has been demonstrated on highly oriented pyrolytic graphite (HOPG) by using STM. The FTIR results provide indication of parallel β -sheet structure of KLVFF self-assembly, and the transformation from parallel to anti-parallel β -sheet structure by introducing modulators into the peptide assemblies.

Synthetic KLVFF for all the investigations was obtained from Shanghai Science Peptide Biological Technology Co., Ltd. 4,4'-bipyridyl (4Bpy) and 4'-chloro-2,2':6',2''-terpyridine (Cl-Ter) were purchased from Acros (98%, anhydrous). The molecular structures of KLVFF, 4Bpy and Cl-Ter are illustrated in Scheme 1. KLVFF was dissolved in MilliQ water at concentration of $10\mu\text{M}$ as stock solution. 4Bpy and Cl-Ter were dissolved in tetrahydrofuran (AR grade) at concentration of $10\mu\text{M}$: $10\mu\text{M}$ as stock solution. The concentrations of the molecules were adjusted by mixing 4Bpy or Cl-Ter solutions into KLVFF solution at a molar ratio close to 1:1.

The peptide and peptide-organic assemblies were prepared by placing approximately $5\mu\text{l}$ solution on a freshly cleaved atomically flat HOPG surface (quality ZYB). After the solvent was evaporated, the experiments were performed with a Nanoscope IIIA system (Bruker Nano Inc., USA) operating under ambient conditions. The STM tips were mechanically formed Pt/Ir wire (80/20). All STM images were recorded using the constant current mode. The specific tunneling conditions are given in the figure captions. AFM experiments were performed in tapping mode under ambient conditions (Nanoscope IIIA SPM system, Bruker Nano Inc., USA).

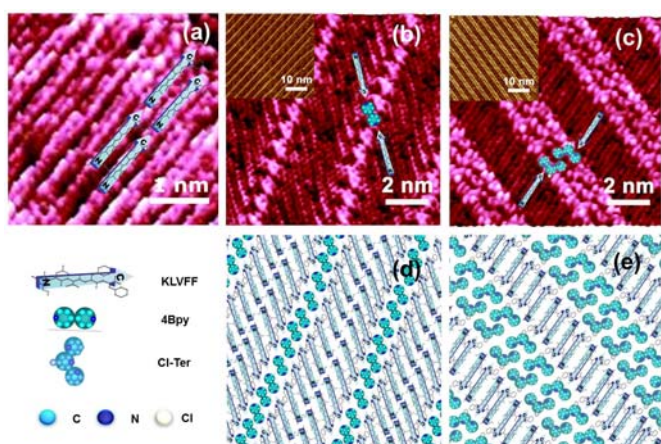


Fig. 1 High-resolution STM images (with schematic models) demonstrate the modulation of KLVFF secondary structures from parallel (d) to anti-parallel (e) β -sheet with pyridine molecules: (a) KLVFF, (b) KLVFF/4Bpy and (c) KLVFF/Cl-Ter. The molar ratios of KLVFF to pyridine molecules are 1:1. (d) Tentative model of KLVFF/4Bpy co-assembly on HOPG surface. (e) Tentative model of KLVFF/Cl-Ter co-assembly on HOPG surface. The corresponding tunneling conditions: (a) $I = 420.0\text{ pA}$, $V = 617.8\text{ mV}$. (b) $I = 321.4\text{ pA}$, $V = 699.8\text{ mV}$ and (c) $I = 321.4\text{ pA}$, $V = 699.8\text{ mV}$.

We firstly demonstrated 2D assembling behaviour of this key A β segment KLVFF on HOPG by using STM. The assembly structure of peptide KLVFF on HOPG surface was observed to be typical continuous lamella structures similar to the previously reported pentaalanine peptide (Fig. 1a).⁹ KLVFF has been known as an important segment for amyloid formation and inhibition by recognizing the identical sequence within full-length A β via

hydrophobic and electrostatic interactions.²⁰ When KLVFF molecules were deposited from the solution to the HOPG surfaces, it is a dynamic synergistic process with all kinds of interaction modes. From the close examination of the STM image, KLVFF molecules assembled into continuous lamella structures flexibly, without regular strips and clear junction points, grooves or boundaries. However, the separation between two neighboring peptide strands is approximately $4.7 \pm 0.1\text{ \AA}$, which could be attributed to the β -sheet formation according to the similar inter-stand distances in A β and other amyloid peptides with β -sheet secondary structures.^{7d,7e,8} We could not identify the β -sheet is parallel or anti-parallel because of the continuity and diversity of the lamella structures, while the assembly structure diversity facilitates the assembly modulation because of the flexibility. The secondary structure and its modulation of KLVFF could be closely related to the understanding of assembling pathways and related inhibition mechanism of A β peptides.

In order to examine the modulator effects on the peptide assemblies, we have introduced pyridine-containing species (4Bpy and Cl-Ter in this work) (Scheme 1b and 1c) that are expected to interact preferentially with the C-terminal of the peptides. As the

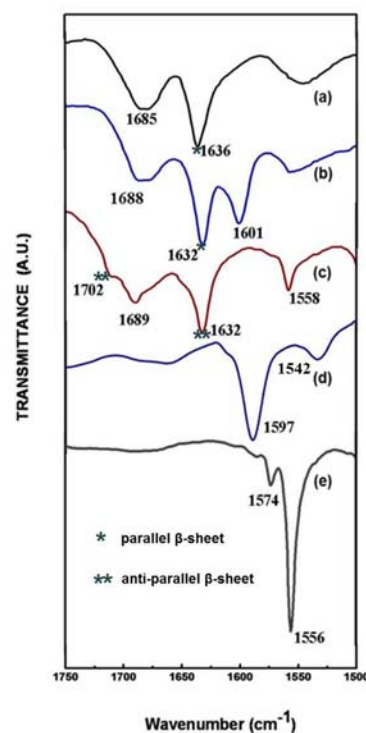


Fig. 2 FTIR spectra indicate the modulation of beta-sheet secondary structure with introduction of pyridine molecules: (a) KLVFF(parallel), (b) KLVFF/4Bpy(parallel), (c) KLVFF/Cl-Ter(anti-parallel), (d) 4Bpy, (e) Cl-Ter.

results, diverse peptide-organic assembly configurations with different pyridine modulators have been observed by STM with molecular level resolutions. Structure multiplicity of these pyridines is reflected in the multiple assembling characteristics of peptides that otherwise have specific β -sheet secondary structure.

Uniform molecular assembly structures of KLVFF co-assembled with 4Bpy can be observed in the STM image in Fig. 1b, in which KLVFF and 4Bpy are arranged as sandwich-like striped structures. The bright spot-like features are attributed to 4Bpy and the stripes with slightly reduced contrast to KLVFF. As a conjugated π -electron system, 4Bpy appears as high contrast in the STM images. The formation of the sandwich-like structure can be attributed to the formation of hydrogen bonds between 4Bpy and peptide molecules. 4Bpy molecule has two nitrogen atoms, which can form hydrogen bond with the -COOH terminal of KLVFF, *i.e.* one 4Bpy molecule binds with two peptide molecules. A proposed structural model is superimposed in Fig. 1b. The angle between the KLVFF axis and the direction of lamellae is $33 \pm 2^\circ$ which could be considered as a reflection of the interaction between terminal functional group and modulating species of 4Bpy. The average length of KLVFF and 4Bpy is measured to be 1.6 ± 0.2 nm and 0.7 ± 0.1 nm, respectively, which is in agreement with the expected extended molecular lengths. The separation between two neighbouring peptide strands is still 4.7 ± 0.1 Å, attributed to the β -sheet formation according to the similar inter-strand distances in KLVFF self-assembly structure. In addition, it could be proposed that the lamellae observed by STM are arranged in parallel β -sheet-like configuration. A detailed proposed structural model is presented in Fig. 1d. There are two KLVFF lamellae between the 4Bpy bright spot lines. Each KLVFF molecule forms backbone and said chain binds to its two neighboring molecules into parallel β -sheet-like configuration in the same KLVFF lamella. At the same time, it also interacts with the molecule in the neighboring KLVFF lamella by a face-to-face mode.

Different type of co-assembly structures of Cl-Ter and KLVFF is observed (as shown in Fig. 1c). A large-scale image of a well-ordered arrangement is exhibited in Fig. 1c, a new close-packed ladder-like structure, instead of the KLVFF-4Bpy co-assembly sandwich-like stripe one. The neighbouring terpyridine molecules are aligned in long uniform double rows. One may notice that, in Fig. 1c, it is discernible that adjacent terpyridine groups form a dimer-like structure. The peptide chains of neighbouring rows were changed from tail-to-tail arrangement to interdigitated pattern. This result indicates that, with the modulation of the terpyridine, the peptides are transformed to anti-parallel β -sheet structures. The average length of KLVFF and terpyridine is 1.6 ± 0.2 nm and 1.1 ± 0.1 nm, respectively. A unit cell is outlined in the model with the parameters of $a = 1.0 \pm 0.1$ nm, $b = 3.5 \pm 0.1$ nm, and $\alpha = 75 \pm 2^\circ$.

The transformation of the secondary structure for KLVFF assemblies was examined by using FTIR spectroscopy. The solution preparation procedures for FTIR experiments are similar to STM samples, and the mixed solutions were dropped onto the BaF₂ surface, followed by air drying prior to FTIR measurements. FTIR spectra were recorded on a PerkinElmer FTIR spectrometer at a resolution of 4 cm⁻¹. FTIR spectroscopic measurements were performed in the transmission mode with BaF₂ windows. The FTIR results could provide the information about the secondary structure of the peptides in the supramolecular peptides-modulator assembly structure.¹¹ Figure 2 presents the FTIR spectra of KLVFF and the KLVFF-modulator co-assembly structures.

The FTIR spectra of the peptide and organic modulators are shown in Curve a, Curve d and Curve e in Figure 2. The presence of one major

band at 1636 cm⁻¹ ± 2 cm⁻¹ indicates the parallel β -sheet conformation of KLVFF aggregates which has been shown in Curve a. The FTIR spectra of co-assembly structures of the peptides mixed with 4Bpy and Cl-Ter are shown in Curve b and Curve b in Figure 2, respectively. The typical bands for the pyridine molecules are nearly identical with the free molecules in Curves d and e. Parallel β -sheet conformation is maintained with introduction of 4Bpy molecule, as illustrated by the one major band at 1632 cm⁻¹ ± 2 cm⁻¹ in Curve b. However, in Cl-Ter/KLVFF system, the presence of two major bands at 1632 cm⁻¹ ± 2 cm⁻¹ and 1702 cm⁻¹ ± 2 cm⁻¹ indicate the presence of an anti-parallel β -sheet conformation of the peptide aggregates which has been shown in Curve c of Fig.2.^{5a}

It should be noted that several contributing factors could be considered that lead to the observed parallel to anti-parallel transformation of the secondary structures. Since the pyridine modulators are interacted with the peptide terminals, it is plausible to include the additional interactions associated with these small organic molecules, such as steric hindrance, hydrogen bond, aromatic interactions. These could contribute to the formation of the secondary structures which is sensitive to intermolecular interactions as revealed from the sequence-dependence XRD investigations.¹¹ In addition, the directional interaction between the pyridines with the peptide C-terminal would inevitably introduce tilted orientation of peptide chains with respect to the peptide lamellae direction, therefore changes to the relative registration of amino acid residues along the neighbouring peptide chains. This is different from the pristine peptide assemblies in which the peptide chains are qualitatively perpendicular to the lamellae direction. Both effects associated with the introduction of Cl-Ter into the peptide assemblies lead to varied inter-chain interactions that result in the observed transformation of secondary structures.

We studied the self-assembly behavior of a critical amyloid peptide fragment KLVFF and the co-assembly behavior of KLVFF with pyridine modulators. By introducing different molecular modulators to KLVFF assembly system, the co-assemblies can be transformed from stripe to ladder-like structures and the secondary structure of KLVFF could be transformed from parallel to anti-parallel β -sheet conformation, based on the conformation, configuration and interaction sites of these modulators. Modulating effects on peptide assemblies by chaperone species can be attributed to a wide range of inter-molecular interactions. This effort could help gain molecular level insight of amyloid formation and inhibition pathways relating to neurodegenerative diseases.

This work was supported by National Natural Science Foundation of China (Grant Nos. 21273051, 91127043, 21261130090). the National Basic Research Program of China (2011CB932800) are also gratefully acknowledged.

Notes and references

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[§]Acknowledgements: This work was supported by the National Natural Science Foundation of China (Grant no. 91127043, 21261130090). Financial support from the National Basic Research Program of China (2011CB932800) is also gratefully acknowledged.

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