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Self-assembly of aromatic α-amino acids into amyloid inspired nano/micro scaled architects

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ABSTRACT

In the pursuit for design of novel bio inspired materials, aromatic α-amino acids (phenylalanine, tyrosine, tryptophan and histidine) have been investigated for the generation of well-ordered self-assembled architects such as fibrils, rods, ribbons and twisted nanosheets in varying solvent systems. These nano/micro scaled architects were thoroughly characterized using FE-SEM, confocal microscopy, optical microscopy, ^{1}H NMR, FTIR, XRD and TGA. These self-assembled architects were histologically stained with Congo red and thioflavin T dyes for investigation of amyloid morphology which revealed that the deposited state of ordered assemblies exhibit specific characteristic of amyloid deposits. The self-assembly of aromatic amino acids was observed to be driven by non-covalent forces such as π-π stacking, van der Waals and electrostatic interaction.

1. Introduction

Molecular self-assembly of monomeric units into distinct three dimensional supramolecular patterns at nanoscale is an area of growing interest in the last few years. Natural systems consisting of biomolecules like DNA, ribosomes, proteins, microtubules, membranes, hemoglobin, phycobilisomes have been exhibiting self-assembly based on predominantly non-covalent interactions such as hydrogen bonding, π-π stacking, van der Waals and electrostatic interactions [1–3]. Exploring a set of natural self-assembled structures [4–5], efforts have been made in the past to mimic and create new assemblies outside the natural systems by changing solvo-thermal interactions, pH or by modification of interacting sites [6–8]. The motive of generating such novel assemblies has been to express the biological and surface properties in a better manner [9–10]. Specifically, peptide based self-assembled systems including peptide amphiphiles have been studied to form a range of nanostructures including vesicles [11], tubes [12,13], belts [14] and fibrils [15] displaying applications in drug delivery, gene delivery [16], hydrogel formation for tissue engineering [17], antibacterial [18] antimicrobial [19,20], 3D cell cultures and regenerative medicines [21].

There has been immense interest recently in self-assembly driven peptide and protein aggregation, including formation of ordered amyloid fibrils, as it plays an important role in amyloidosis and neurodegenerative diseases. In this context, short aromatic dipeptide (FF) has been shown to play a significant role in the formation of amyloid aggregates. The first report in this regard discussed the formation of peptide nanotubes using FF dipeptide which was further used as nanoscale molds to synthesize Ag nanowires by reducing Ag ions [22]. Further, these peptide nanotubes have been used for different applications which include formation of bio-inspired ceramic nanostructural material showing strong piezoelectric activity, electrochemical biosensing, modifying carbon electrodes for supercapacitor devices, precise patterning for controlling wettability of surfaces and demonstrating quantum confinement phenomena with optical absorption characteristics of quantum dots [23–27].

The self-assembly pattern generation of some of the aromatic amino acids has been reported to be crucial for various biological processes. In this context, phenylalanine has been reported to self-assemble into fibril like structures which is known to play a key role in many pathological disorders especially arousing interest in exploration of single metabolite molecular self-assembly [28–32]. Detailed molecular dynamics and ion-mobility mass spectrometry reveals that phenylalanine self-assembles into layers of four molecules with hydrophilic core and hydrophobic exterior [33,34]. Apart from α-amino acids, non-coded

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amino acids such as γ-amino butyric acid derivative has also been recently reported to form tunable nano/micro scale architects under varying experimental conditions [35]. The striking ability of aromatic peptides and amino acids to self-assemble suggests that aromatic residues play a crucial role in the formation of the nanostructures [36]. It is pertinent to mention here that among all naturally occurring amino acids, self-assemble structure generation of only phenylalanine and tyrosine, in water as solvent system, has been reported so far [37,38].

Recently, it has been reported that non-proteinaceous metabolites (which include some naturally occurring amino acids) may also form toxic assemblies in biological environment [30].

Therefore, we are reporting a new paradigm of single amino acid self-assembly for tryptophan and histidine into well defined structures. Moreover, the control over well ordered amino acid structures using dielectric has been done for first time. Further, detailed study on the nature of self-assembly and their amyloid characterization has been performed for all naturally occurring aromatic α-amino acids. All the generated self-assembly based patterns have been thoroughly investigated using techniques such as field emission scanning electron microscopy (FE-SEM), optical microscopy and confocal microscopy. The intermolecular interactions responsible for self-assembly of aromatic amino acids have been elucidated using solvent variation studies, nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infra-red (FTIR) spectroscopy. Further, thermogravimetric analysis (TGA) and X-ray diffraction (XRD) studies have been carried out to examine the differences in the stability and packing of the self-assembled and nonself-assembled amino acids. Congo Red (CR) and thioflavin T (ThT) histological dyes were used to investigate the amyloidal morphology of self-assembled structures. To the best of our knowledge, such an in-depth analysis and characterization of self-assembled architects of aromatic amino acids has not been reported earlier.

2. Experimental section

2.1. Materials

Amino acids (L-phenylalanine, L-tyrosine, L-tryptophan and L-histidine) were purchased from Sigma-Aldrich and were used as such. Milli-Q water (resistivity ~18.2 MΩ) used for all the self-assembly experiments. Methanol used for the self-assembly experiments was of analytical grade having >99% purity.

2.2. FE-SEM analysis

FE-SEM was used to investigate the morphology of the self-assembled structures. In general, 10 μl solutions of freshly prepared aromatic amino acids at concentration 1 mg/ml were evaporated slowly on the smooth surface of Ag film pasted on FE-SEM step. Pt coating was applied on the samples to make it conductive and followed by FE-SEM analysis on a Hitachi, SU8010 electron microscope, operating at 10–15 kV.

2.3. Optical and confocal microscopy

Optical images were captured by depositing 10 μl aromatic amino acid solution on microscopic glass slide. Fresh solutions of aromatic amino acid were prepared at 1 mg/ml and samples were prepared by directly depositing 10 μl of solution on microscopic glass slide and images were captured using confocal laser scanning microscope Zeiss, LSM 510.

2.4. NMR spectroscopic studies

^{1}H NMR spectra were recorded using Bruker Ascend 500 MHz NMR spectrometer. ^{1}H NMR spectra were recorded at 1 mg/ml, 5 mg/ml 10 mg/ml concentration for phenylalanine and histidine, whereas for tryptophan it was taken at 1 mg/ml and 5 mg/ml concentration in D_2O as well as in 50% D_2O:methanol as solvent system.

2.5. FTIR spectroscopy

Thermo Scientific Nicolet iS50 FT-IR spectrophotometer was employed to collect infrared spectra (4000–400 cm^{-1}) using amount of 5 mg for both self-assembled and nonself-assembled amino acids in the attenuated total reflection mode.

2.6. X-ray diffraction studies

XRD studies were performed to investigate polymorphism in self-assembled and nonself-assembled aromatic amino acid. 5 mg of dried self-assembled and nonself-assembled sample was characterized by Shimadzu XRD 6000 diffractometer with Cu Ka radiation (k = 1.54 Å)

2.7. Thermogravimetric analysis (TGA)

TGA for the self-assembled and nonself-assembled aromatic amino acid deposits was performed on SDT Q 600, TA.

2.8. Histological test for amyloid morphology using CR and ThT binding studies

Absorbance spectra of CR dye was recorded using UV–Vis spectrophotometer Agilent Technologies Cary 60. For this study, Congo red staining solution was prepared according to Putchler's method and 200 μl of it was mixed with 2 ml of fresh and 2 ml of ten days aged amino acid solutions (1 mg/ml) in water as well as in water:methanol (1:1). Fluorescence spectra were recorded on Agilent Technologies Cary Bundle and 200 μl of ThT (2 mM) solution with was mixed with 2 ml of ten days aged solution of phenylalanine, tyrosine, tryptophan and histidine. ThT binding studies were carried out by directly depositing mixture of 20 μl amino acid solution (1 mg/ml) with 10 μl of ThT 2 mM solution). These self-assembled architects were characterized by using confocal laser scanning microscope Zeiss, LSM 510.

3. Results and discussion

3.1. Morphological studies.

The L-phenylalanine self-assembly pattern was investigated under varying solvent systems. Initially, when water was used as solvent, the extended fibril based morphology was found to be similar to the one reported earlier [37] [Fig. 1(a–b)]. The role of varying dielectric constant of the solvent system on the self-assembly pattern of phenylalanine was further ascertained by addition of methanol in water.

Interestingly, it was observed that phenylalanine molecules self-assembled into more distinct and well defined fibrils with an average width of 300–400 nm in the case of water:methanol (1:1) solvent system (with dielectric constant of 54.9) [Fig. 1(c) and (d)]. In comparison, water as solvent leads to the formation of thick fibrils of 1 μm (approximately) width with visible discontinuous patterns in (with comparatively higher dielectric constant of 83). However, formed fibril morphology was found in line with previous report on phenylalanine [37], whereas dielectric based control over self-assembly of amino acid has been reported for first time. Further, increase in the relative proportion of methanol by using water:methanol (1:9) solvent system (with dielectric constant of 31.5) led to the formation of fused fibrils with comparatively lower width (100–200 nm) as compared to the higher dielectric solvent systems [Fig. 1(e–f)]. These observations may be explained using the hypothesis that more π-π stacking is possible with slower evaporation of solvent from amino acid solution in higher dielectric constant based solvent systems leading to the formation of thicker fibrils. On the other hand, the lower dielectric solvent systems on evaporation will allow more electrostatic interactions between zwitter ions deriving the fusion of fibrils as in case of water:methanol (1:9) solvent system in comparison to water. Thus, it may be suggested