

Self-assembled nanotubes from single fluorescent amino acid

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Abstract Self-assembly of biomolecules has gained increasing attention as it generates various supramolecular structural assemblies having potential applications principally in biomedical sciences. Here, we show that amino acid like tryptophan or tyrosine readily aggregates as nanotubes via a simple self-assembly process. These were characterized by FTIR, scanning electron microscopy, and by fluorescence microscopy. Nanotubes prepared from tryptophan are having ~200 nm inner diameter and those from tyrosine are having the same around ~50 nm diameter.

Keywords Self-assembly · Tyrosine · Tryptophan · Nanotubes

Introduction

The molecular aggregation of amino acids in creating all the essential proteins are dictated by DNA using basic coded assemblies under RNA scaffold. Nevertheless, while continuing such faultless assemblies, on aging, sometimes the damaged DNA and also the RNA result in the production of non-functional protein aggregates. Such age-related or environmentally damaged proteins normally introduce

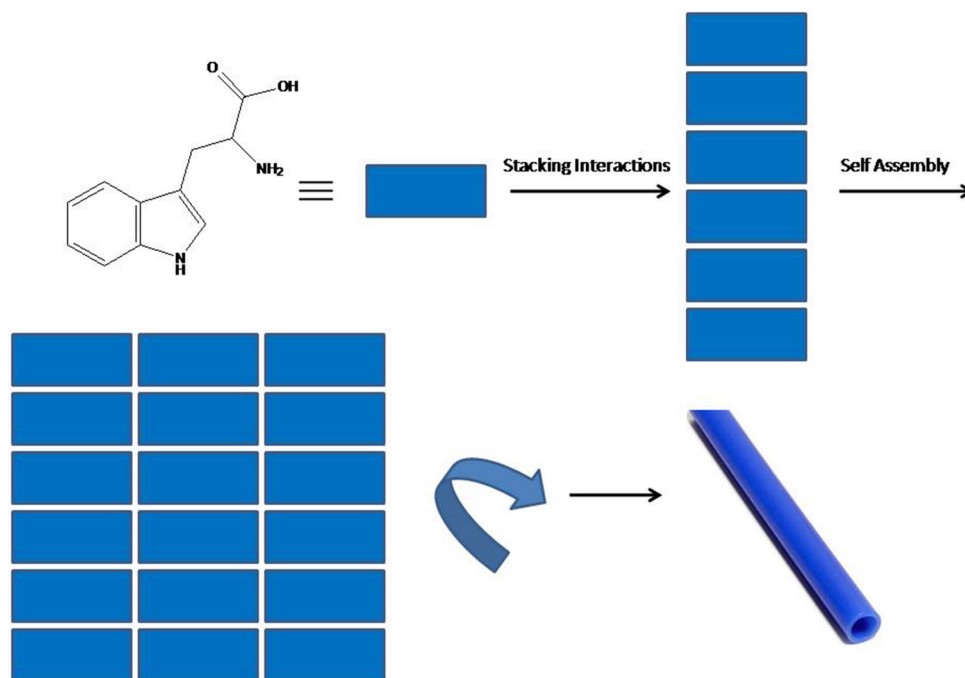
diseases with abnormal function by themselves and also influencing normal proteins by their antagonistic interaction. As specific shape is very much related to selective functioning of a complex biomolecule, the possibility of self-assembly of amino acids even under non-coded form is worth exploring. This could be related to exploring the bio world in relevance to its benign utilization as carriers for drug and for fluorescent species to be utilized for bio-imaging. Furthermore, such possible assemblies may address the role of amyloid proteins which invite various diseases, disrupting normal physiological functions leading to disrupt the healthy function of tissues and organs (Pulawski et al. 2012). General investigation where chances to explore various nano-shaped aggregates prepared by self-assembly of simple building blocks have attracted immense attention in recent years. Such aggregation have been shown to be extremely useful in the domain of nanoscience and nanotechnology because of their uses in casting metal nanowires (Carny et al. 2006; Reches and Gazit 2003), vessels for nanomaterials (Shimizu 2006; Zhao et al. 2006), nanofluidic devices (Sott et al. 2006) and for drug delivery (Masaru et al. 2011; Raviv et al. 2005). The various forces that control the molecular assembly or organization of biomolecules in forming nanoshapes are the hydrogen-bonding interactions, van der Waals attractions, steric repulsions, and capillary forces during solvent evaporation. In 1993, Ghadiri et al. (1993) prepared organic nanotubes from cyclic D,L-peptide rings. Other routes involved cyclic peptides of all β -amino acids for the formation of nanotubes (Clark et al. 1998; Seebach et al. 1997). Serine-based aromatic cyclodepsipeptide has been transformed into crystalline cylindrical assemblies (Ranganathan et al. 1998), and to cyclodextrin (Harada et al. 1993) or to cyclic D,L-rhamnopyranose (Ashton et al. 1996). Reches and Gazit (2003) showed a simple self-assembled system for the preparation of fiber structure using a dipeptide

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Scheme 1 Synthetic route for the formation of nanotubes from amino acids building blocks



(phenylalanine–phenylalanine, FF). Ryan et al. (2010) detailed the using of an Fmoc-protected simpler biomolecule phenylalanine (Fmoc-F5-Phe) to form hollow tubular structures. In 2012, scientist used unprotected phenylaniline-made fibrous structures (Adler-Abramovich et al. 2012). So far, only derivatized amino acids were used to synthesize nanostructured materials especially nanotubes via self-assembly through peptide formation. Perween et al. (2013) used single amino acid (phenylalanine, tyrosine and glycine) for the preparation of febrile structure under neutral, aqueous conditions. The nature of such interactions was simply dominated by non-covalent π – π interactions. A stable structure in nanodomain using single amino acid is rare. Therefore, in this communication, we describe the synthesis of stable nanotube type aggregates from individual amino acids like tryptophan or tyrosine retaining characteristic fluorescence.

Experimental

Materials

All chemicals used in the synthesis were purchased from Sigma-Aldrich and were used without any further purification.

Synthesis of nanotubes from tryptophan and tyrosine

In a typical sample preparation for nanotubes of tryptophan or tyrosine, a stock solution of each was prepared

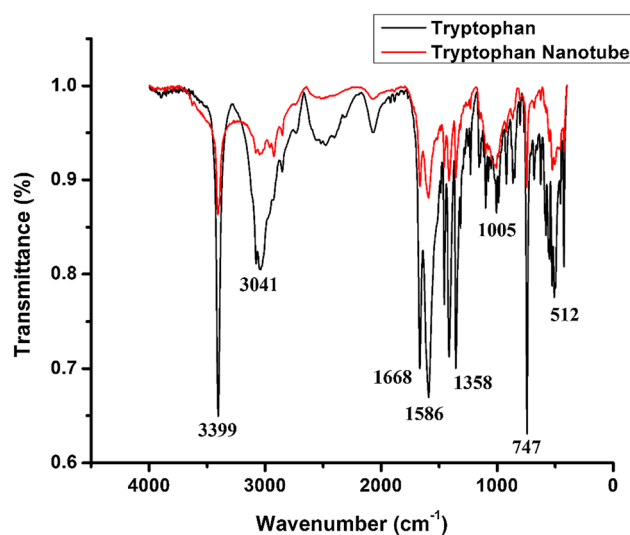


Fig. 1 FTIR spectra of tryptophan (black) and tryptophan nanotubes (red)

in ethanol with concentration of 50 mg/mL. Each stock solution was used fresh to avoid undesirable reactions in the aging process. The stock solution was diluted to the final concentration of 0.5 mg/mL before use. The solution was sonicated for 10 min and 5 μ L of such a solution was deposited onto brass stub in each case separately. The samples were dried for 3 h under a table lamp (60 W tungsten) and then subjected to field emission scanning electron microscopic (FESEM) analysis. Sequential gold sputtering was done before imaging.

Macroscopic and spectroscopic characterization

Infrared spectroscopy

The infrared spectroscopic measurements of nanotubes were recorded on a Bruker Vertex 70, FT-IR spectrophotometer in KBr phase.

Field emission scanning electron microscopy (FESEM)

SUPRA 40VP field emission scanning electron microscope (Carl ZeissNTS GmbH, Oberkochen, Germany) equipped with energy-dispersive X-ray (EDAX), in high vacuum mode at 10 kV, was used for the visualization of nanostructures of amino acids tryptophan and tyrosine.

Fluorescence microscopy

The fluorescence images of nanotubes of tryptophan and tyrosine were performed on a Leica inverted microscope (Leica DC200, Leica microscopy system Ltd., CH-9435, Heerbrugg) equipped with an RS PhotometricsSensys camera, KAF1401E G1.

Results and discussion

Amino acid nanotubes

Molecular interactions play very important role in self-assembly to form aggregate nanotubular structures. The polymer assembly process could be visualized as shown in Scheme 1. This is assumed on the basis of previous reports (Ghadiri et al. 1993; Khazanovich et al. 1994). Here, once stacking took place to get a flat structure then the drive for

minimum surface area spontaneously made it to a cylindrical shape. The formation of self-assembled structures, here, are favored by aromatic ring stacking (π - π) of amino acids as envisioned earlier with aromatic functionalities (Mishra and Chauhan 2011; Pandit et al. 2008). Along with such interaction, there are other possible interactions like van der Waals and hydrogen bonding like C-H- π as discussed earlier (Meital and Ehud 2006; Mishra and Chauhan 2011; Parween et al. 2014; Rechtes and Gazit 2006). In addition, hydrogen bonding of other type may occur via nitrogen of amine of tryptophan to the carboxylic hydrogen end of the adjoining molecule of tryptophan. Similar interactions may be invoked for the other amino acid like tyrosine. In tyrosine, the presence of -OH group may also participate in the formation hydrogen bonding. They may also involve the backbone-backbone-type, intermolecular hydrogen-bonding interactions. These interactions may be parallel or antiparallel (Granja and Ghadiri 1994; Hartgerink et al. 1996; Kobayashi et al. 1995). In addition, these molecules are self-assembled using co-operative force of water molecules present in the solvent like ethanol and those were used in the assembly utilizing hydrogen bonding. The role of water molecule in such self-assembly of nanotubes is reported (Fujibayashi et al. 2008; Begum et al. 2014). Water also plays important role in the stacking process. It interacts with formed nanotubes wall and also with the other water molecules with the help of hydrogen bonding that may lead to the stabilization of achieved structure (Andrade-Filho et al. 2016). By changing the concentration of water, Kim et al. (2010) showed such a variation may dictate the change in the shape of the final product from nanotube to nanowire. The self-assembly of peptides to nanotubes are fashioned via a network of hydrogen bonds between the backbone of the peptide and water molecules, which can enter the channels of polypeptide chain due to its hydrophilic nature (Mao et al. 2000). The use of amino acid side chain and charged termini also may play crucial role in initiating self-assembled processes. Therefore, in the present case stacking interaction followed by self-assembly readily drive to attain minimum energy on the surface leading to tube form of the amino acids under nanodomain (Scheme 1).

FTIR study

The FTIR spectra of the tryptophan and nanotubes of tryptophan are shown in Fig. 1. The black lined corresponds to the starting material tryptophan and red lined spectrum shown in Fig. 1, corresponds to the nanotubes. There is no makeable difference in both the spectra displaying that the integrity of the amino acid is retained. It is only to be noted that in nanotube the intensity of a particular vibration is damped and that could be due to the

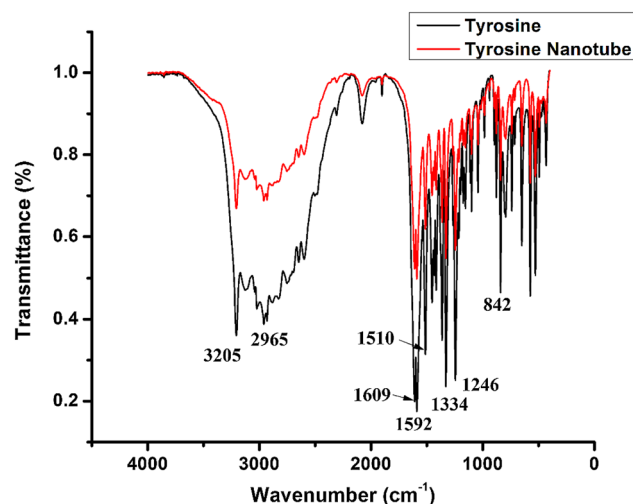


Fig. 2 FTIR spectra of tyrosine (black) and tyrosine nanotubes (red)

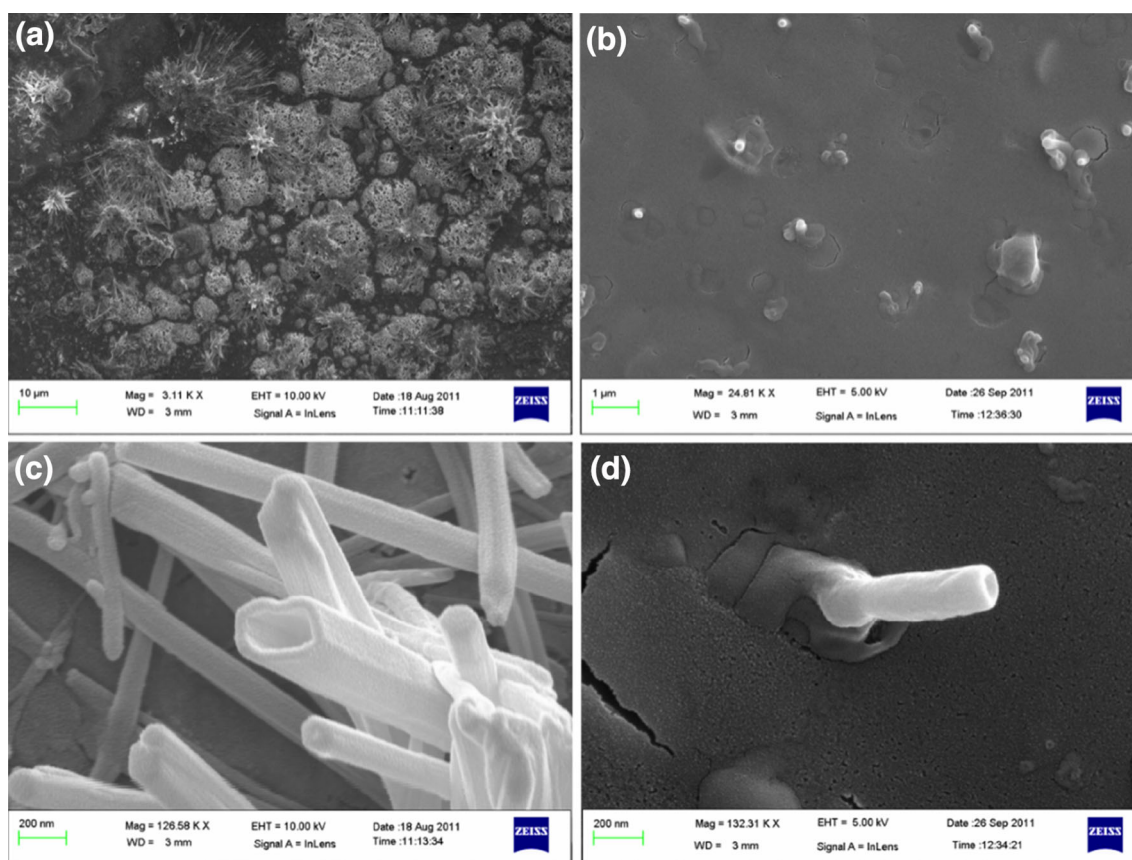


Fig. 3 FESEM images of tryptophan nanotubes (a) and (b) low resolution FESEM images (c) and (d) high resolution FESEM images

movement of big mass associated with the aggregated assembly in nanotube structure. The peak at 1668 cm^{-1} corresponds to the C=O stretching of the tryptophan (Tsuguo 1972) and the deformation vibrations lie in between 700 and 500 cm^{-1} (Carubelli et al. 1997). The peak at 1586 cm^{-1} is due to C=C stretching in the aromatic ring. The peak around 1358 cm^{-1} corresponds to the stretching vibration of C=C in indole ring (Cao and Fischer 1999). The peaks in between 1230 cm^{-1} and 1000 cm^{-1} are due to the in-plane deformation of C–H in indole ring (Ma et al. 2009). The intense peak at around 3399 cm^{-1} corresponds to the NH stretching vibrations of indole ring (Ivanova 2006). As per the present discussion, most of these vibrations of monomeric tryptophan get broadened with the loss in intensity. The vibrations around 3041 cm^{-1} which is related to aromatic CH vibrations of tryptophan responds to further splitting indicative of (π – π) and C–H– π interactions in tryptophan nanotube. The drastic change in the shape of deformation vibrations around 700 – 500 cm^{-1} supports such interactions.

Similarly, Fig. 2 shows the FTIR spectrum-free tyrosine (black line) and tyrosine nanotubes as shown by red line. Again, we observed that both the spectra are almost identical with respect to their relative peak intensities. The peak

at 3205 cm^{-1} corresponds to the OH stretching vibration. Similarly, the broad absorption centered at 2965 cm^{-1} is the composite vibrations arising of $\nu(\text{NH})$, $\nu(\text{CH})$ stretching. In the nanoaggregate of tyrosine, these peaks retained the profile but some distinctive changes occur in the ring deformation region around 840 – 500 cm^{-1} region suggesting similar π – π and C–H– π type interactions. The peak at 1609 cm^{-1} belongs to C=O stretching frequency and the peak at 1592 cm^{-1} corresponds to the C=C stretching in the aromatic ring (Arp et al. 2001; Encinar et al. 2002; Reid et al. 2003). Therefore, to attain a new complex shape these molecules almost retain their individual IR signature.

FESEM study

The spontaneous self-assembly of amino acids like tryptophan or tyrosine in ethanol can be viewed in the nanodomain by FESEM imaging. The FESEM analysis of nanotubes prepared from tryptophan or tyrosine are shown in Fig. 3. The low-resolution FESEM images of tryptophan and tyrosine nanotubes are presented in Fig. 3a, b, respectively, and high-resolution FESEM images of tryptophan nanotubes are shown in Fig. 3c, d. In Fig. 3a there are some fibre type structures along with the nanotubes.

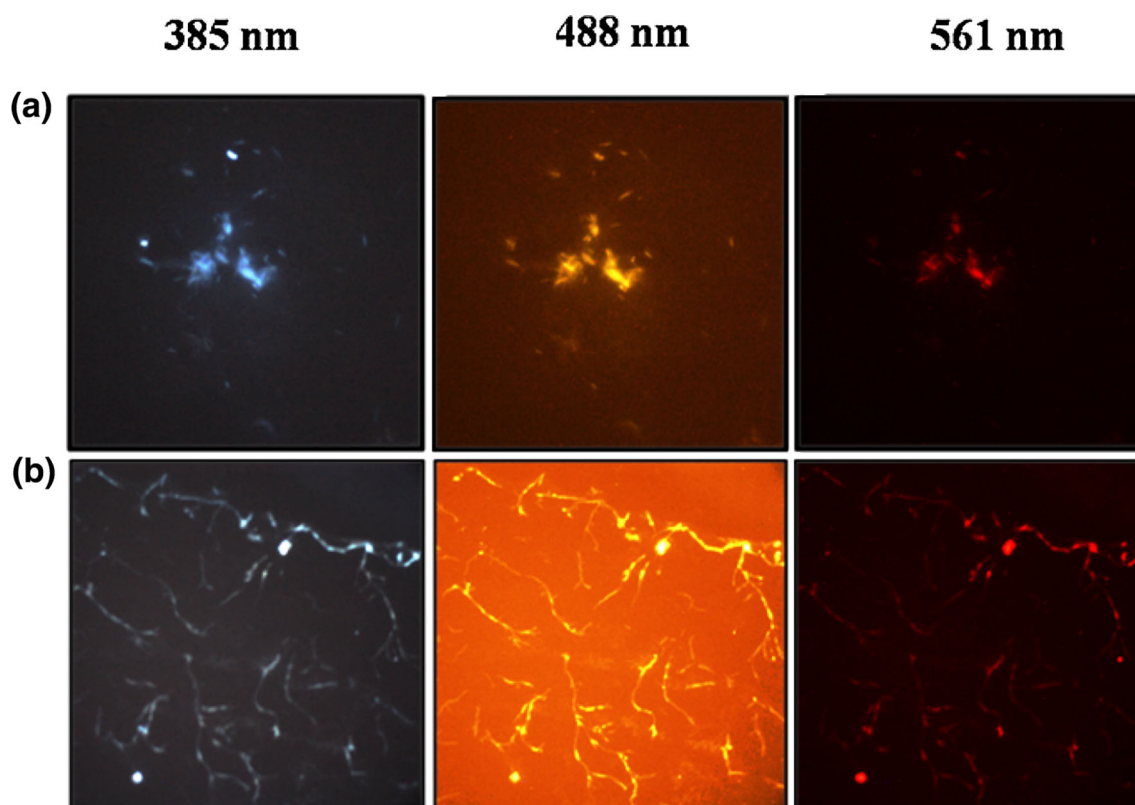


Fig. 4 Fluorescence images of nanotubes (a) formed from tryptophan, (b) formed from tyrosine each under three different excitations

The fibres may correspond to amyloid structures (Meital and Ehud 2006). Figure 3b shows only the tyrosine nanotubes, which are uniform in nature. These FESEM images clearly indicate that formed nanotube have wide diversity in their diameter in the case of tryptophan nanotubes. The inner diameter is ~ 200 nm and the length lies between 4 and 6 μm . High-resolution FESEM images clearly indicate that the formed nanotubes from tyrosine (Fig. 3b) are uniform, having nearly same length and diameter. The inner diameter of the nanotube is ~ 50 nm and outer is ~ 150 nm.

Fluorescence study

Tryptophan and tyrosine are amino acids having aromatic side chains which are fluorescent constituents in proteins. Tryptophan and tyrosine respond to fluorescence emission around 348 and 303 nm (Keleti 1970; Teale and Weber 1957). There are very fewer reports about the construction of fluorescent peptide nanotubes. Fluorescence images of tryptophan and tyrosine nanotubes are shown in Fig. 4. In this panel (a) corresponds to the tryptophan nanotubes and (b) for the tyrosine nanotubes. These nanotubes show fluorescence at different excitation wavelength viz. 385 (blue), 488 (yellow) and 561 (red) nm. This shows drastic changes in the fluorescence properties of these aggregates

which could be a measure for their aggregation. Such shift in fluorescence in the visible region is due to the quantum confined phenomenon in the nanotube assembly (Amdursky et al. 2009; Fan et al. 2016).

Conclusion

The amino acid nanotubes are synthesized using simple building block such as tryptophan and tyrosine through self-assembly using ethanol as a solvent. These nanotubes synthesized are homogeneous in nature. These nanotubes show good fluorescence in varied excitation lines used. This simple strategy described herein would allow synthesizing a varied range of nanotubes from single or mixture of amino acids and also to use these as drug carriers. The toxicity study of these aggregates may encourage such study in future.

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