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Insight into the binding of a non-toxic, self-assembling aromatic tripeptide with ct-DNA: Spectroscopic and viscositic studies

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ABSTRACT

The report describes the synthesis, self-association and DNA binding studies of an aromatic tripeptide H-Phe-Phe-Phe-OH (FFF). The peptide backbone adopts β—sheet conformation both in solid and solution. In aqueous solution, FFF self-assembles to form nanostructured aggregates. Interactions of this peptide with calf-thymus DNA (ct-DNA) have been studied using various biophysical techniques including ultraviolet (UV) absorption spectroscopy, fluorescence spectroscopy and circular dichroism (CD) spectroscopy. The value of mean binding constant calculated from UV and fluorescence spectroscopic data is $(2.914 \pm 0.74) \times 10^3\ M^{-1}$ which is consistent with an external binding mode. Fluorescence intercalator displacement (FID) assay, iodide quenching study, viscosity measurement and thermal denaturation study of DNA further confirm the groove binding mode of peptide, FFF with ct-DNA. MTT cell survival assay reveals very low cytotoxicity of the peptide toward human lung carcinoma cell line A549.

1. Introduction

In recent years, peptide-based biomaterials have drawn immense attention to the scientific community owing to their various functional activities in different areas including drug delivery [1a-f], tissue engineering [1g], bio-diagnostic tool [1h], antibiotic [1i-j] and regenerative medicine [1k]. The structural and chemical diversity of peptides, in addition to their ability to adopt the specific secondary structure, provide a unique bottom-up platform for the construction of nanomaterials [2]. A number of peptide-based building blocks, such as cyclic peptides [3], amphiphilic peptides [4], co-polypeptides [5], dendritic peptides [6], and aromatic dipeptides have been reported which can form various supramolecular structures having applications in biology and nanotechnology. Among these structural building blocks, an aromatic dipeptide diphenylalanine (H-Phe-Phe-OH, FF) is most extensively studied. Diphenylalanine produces nanotubes which could serve as casts for the formation of silver nanowires with the average diameter of 20 nm [7]. FF nanotubes have unique chemical and thermal stability, as well as exhibit excellent mechanical properties [8]. Synthesis of D-Phe-D-Phe based peptide-nanotube-platinum nanoparticles composite was reported by Song et al. [9] Fluorenylmethyloxycarbonyl (Fmoc) protected diphenylalanine (Fmoc-Phe-Phe-OH) has been used for the formation of nanofibrous hydrogel with notable physical and mechanical properties [10]. It can support the cell growth and release the entrapped small molecules in a controlled manner. Fmoc-Phe-Phe-OH has also been used for the formation of hydrogel nanoparticles (HNPs) which has the ability to deliver various drugs and bioactive molecules [10c]. Li et al. reported concentration dependent reversible shape transition between self-assembled cationic dipeptide (H-Phe-Phe-NH_2) nanotubes and vesicle-like structures [11]. Moreover, the self-assembled positively charged nanotubes can bind with fluorescently labeled ssDNA using electrostatic interactions and also enter into the cells readily. Furthermore, the glycopeptide conjugates (conjugation of FF dipeptide with several saccharides) adopt various structures and increased solubility in the aqueous medium compared to the original diphenylalanine building block. The increased solubility and different nanostructures pave the way for new applications in the aqueous environment [12]. The morphology of the FF assemblies could be fine-tuned and tailored by various factors such as pH, substrate roughness, hydrophobicity, temperature and ionic strength which make it a promising candidate for *in vivo* biological applications [13]. In 2013, Bosne et al. reported that FF peptide nanotubes exhibit piezoelectric effect [14a]. Nmoc-F/FF (Nmoc: naphthalene-2-methoxycarbonyl) system has been used to generate Nmoc-Phe-Phe-Phe-OH as a preferred product in the protease catalysed dynamic peptide library [14b]. The self-assembled tripeptide Boc-FFF based bionanospheres

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have been used in conventional device-fabrication processes using nanolithography [14c].

Here, we explore the aptitude of an aromatic tripeptide H-Phe-Phe-Phe-OH (FFF) as DNA molecular probe which deserves much attention for the development of DNA-targeting drugs and new therapeutics. Previously, it was reported that FFF has higher network propensities and FFF aggregates are more stable than FF networks [15]. Our results clearly show that the aromatic tripeptide FFF forms nanostructured aggregates upon self-association in aqueous solution. Moreover, ultraviolet (UV) absorption spectroscopy, fluorescence spectroscopy, circular dichroism (CD) study, viscosity measurement and thermal denaturation analysis of DNA indicate that FFF binds with ct-DNA presumably *via* groove binding manner. Finally, the cytotoxicity of this tripeptide has been tested by MTT cell survival assay using human lung carcinoma cell line A549.

2. Results and discussion

2.1. Conformational analysis

Information regarding the conformation of the peptide in solid state has been obtained from Fourier Transform Infrared (FT-IR) spectroscopy. FT-IR spectrum of the peptide in the solid state shows a well defined C=O stretching band (amide I) at 1648 cm^{-1} and N—H stretching band at 3380 cm^{-1} (Fig. S10), typical of intermolecularly hydrogen-bonded β—sheet structure in the solid state [16a]. Moreover, N—H bending frequency of this peptide appears at 1518 cm^{-1} suggesting also the formation of β-sheet structure in the solid state [16b]. Conformational analysis of the peptide in solution has been done by NMR spectroscopy. ^{1}H NMR coupling constants provide information about the conformation of the peptide main chain. The vicinal coupling constant between NH and $C^{\alpha}H$ of a peptide ($^3J_{HN\alpha}$) reflects the dihedral angle between these two protons, and hence the main chain φ—angle. Coupling constants ($^3J_{HN\alpha}$) greater than 7 Hz are consistent with β-sheet structure, while coupling constants less than 6 Hz are consistent with α-helical structure [17a]. From NMR study of the peptide in DMSO-d_6, it is found that the value of $^3J_{HN\alpha}$ for Phe(2) and Phe(3) residues is 8 Hz (Fig. S6), clearly indicating β-sheet conformation of the peptide in solution. Further to analyze the conformation of the peptide in solution, CD spectroscopy has been studied. Fig. S18 is the CD spectrum of the peptide in water. From the spectral data, it is estimated that, β-sheet content of the peptide in solution is 52.93% following the method reported by Perez-Iratxeta et al. [17b]

2.2. Self-association study

The formation of supramolecular structures through molecular self-assembly was primarily accessed by dynamic light scattering (DLS) study. It is frequently used to determine the size distribution profile of small particles or assemblies in solution. From the DLS study, it is found that nanostructures of various sizes are formed (Fig. S11) at a peptide concentration of 0.82 mg mL^{-1}. The average size (hydrodynamic diameter) of the aggregate is found to be 930 nm. This observation encouraged us to further study these structures by electron microscope. Transmission electron microscopic (TEM) study has been performed to reveal the morphology of these self-assembled structures. The experiment was done at 1.57 mg mL^{-1} concentration of peptide. The images (Fig. 1a and Fig. S12a) prove the formation of 'nanosheet' like aggregates which is very much similar with the report of Tamamis et al. [15] The width of the sheets are in the range from 1.8 to 26.1 nm. Moreover, the high resolution TEM images reveal that each 'nanosheet' consists of several small fibril-like aggregates having average diameter of 0.95 nm (Fig. 1b and Fig. S12b).

2.3. DNA binding study

DNA is one of the important intracellular targets for a wide range of anticancer and antibiotic drugs. The binding between small molecules and DNA is very much important for rational design of new drugs for clinical use. Recently, a growing emphasis has been placed on binding studies of small molecules [18a,b,c,d,e] and nanoparticles [18f,g,h,i,j,k] with DNA, but interactions specificity of small synthetic peptide containing consecutive aromatic amino acid residues is relatively less explored [19]. Here, we have studied the interactions of FFF with calf thymus DNA (ct-DNA) by various techniques including ultraviolet (UV) absorption spectroscopy, fluorescence spectroscopy, circular dichroism (CD) study, viscosity measurement, and thermal denaturation study of DNA.

2.4. UV absorption study

UV absorption measurement is a very efficient method to perceive the binding of small molecules with DNA by monitoring the changes of absorption properties of either interacting molecules or DNA. In general, when the binding mode is through intercalation then it results in both hypochromism and bathochromism [18e]. On the other hand, hyperchromism indicates the partial or non-intercalative binding modes, such as electrostatic forces, van der Waals interactions, dative bonds, hydrogen bonds and hydrophobic interactions [20]. The absorption spectra of ct-DNA upon gradual addition of peptide have been shown in Fig. S13. From the titration study, it was observed that, the intensity of absorption maxima at 258 nm increased gradually upon addition of peptide without any shift of absorption band which clearly indicates the non-intercalative binding of peptide with ct-DNA [18e]. The value of binding constant K_b, is calculated from the spectral data using double reciprocal plot following the equation:

$$\frac{1}{A_0 - A} = \frac{1}{A_0} + \frac{1}{A_0 C K_b}$$

where A_0 is the absorbance of ct-DNA at 258 nm in the absence of peptide and A is the recorded absorbance of ct-DNA in presence of different concentration of peptide, C is the concentration of peptide and K_b is the binding constant. The plot of $1/(A_0\text{-}A)$ against $1/C$ is found (Fig. S14) to be linear ($R^2 = 0.976$) and the value of K_b is calculated to be $3.801 \times 10^3\ M^{-1}$ from the slope of the plot.

2.5. Fluorescence study

The change in fluorescence spectral intensities is often employed to assess the binding of ligand with DNA. The fluorescence behavior of the peptide was monitored in presence of various concentration of DNA upon excitation at 257 nm. From the emission spectra (Fig. 2a), it was observed that the fluorescence intensity of peptide decreased gradually upon progressive addition of ct-DNA. Furthermore, fluorescence titration data have been used to determine the binding constant (K) and the binding stoichiometry (n) for the interaction of peptide with ct-DNA using the following equation:

$$\log \frac{F_0 - F}{F} = \log K + n \log [DNA]$$

where, F_0 and F are the fluorescence intensities of peptide in absence and presence of different concentrations of ct-DNA. K and n are the binding constant and binding stoichiometry respectively. Fig. 2b shows that the plot of $\log \{(F_0\text{-}F)/F\}$ *vs* log[DNA] is linear with R^2 value 0.96924. From the plot, calculated binding constant value is $2.028 \times 10^3\ M^{-1}$ which is in good agreement with the binding constant value obtained from UV absorption study. The value of n is found to be 1.26.