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PII: S0040-4039(18)31044-X
DOI: https://doi.org/10.1016/j.tetlet.2018.08.043
Reference: TETL 50218

To appear in: *Tetrahedron Letters*

Received Date: 21 July 2018
Revised Date: 21 August 2018
Accepted Date: 22 August 2018

Please cite this article as: Tomar, K., Kaur, G., Verma, S., Ramanathan, G., A Self-assembled tetrapeptide that acts as a "turn-on" fluorescent sensor for Hg^{2+} ion, *Tetrahedron Letters* (2018), doi: https://doi.org/10.1016/j.tetlet.2018.08.043

Graphical Abstract

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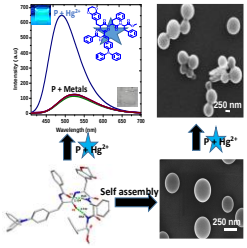

Tetrahedron Letters
journal homepage: www.elsevier.com

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Dehydrophenylalanine
Phenylalanine
Overlapping beta-turns
Mercury sensing
Self-assembly

ABSTRACT

The tetrapeptide (Bz-ΔPhe(*p*-NPh_2)-*L*-DOPA(protected)-*L*-Phe-*L*-Phe-OMe was designed to incorporate seven phenyl rings so that it's conformation, self-assembly and application in Hg^{2+} ions sensing could be studied. Peptide molecules adopted an overlapping β-turn of type III/III conformation in crystals. The peptide showed a highly selective turn-on response towards mercuric ion over other metal ions with a 10-fold enhancement in fluorescence intensity. This intensity change coupled with the selectivity of the peptide towards mercury allowed us to demonstrate simple colorimetric dip sensing of Hg^{2+} ions. The technique provides a highly selective and effective way to detect Hg^{2+} ions. The peptide also self-assembled into nanospheres with diameter ranges from 100-500 nm. Mercuric ion coordination enabled these peptide nanospheres to aggregate into well-defined nanoparticles. The enhanced fluorescence upon Hg^{2+} addition demonstrates that peptide scaffolds can be exploited in the development of different selective sensors.

Introduction

Rapid industrialisation in several parts of our world has led to contamination of fresh water with heavy metal ions.[1] Among these, mercury is a major pollutant with adverse effects on human health and environment. Small amounts of mercury in the human body can trigger long-term irreversible damage to vital organs and tissues such as kidney, liver, brain, nervous system etc. These mercuric ion exposures often induce cognitive and motion disorders.[2-4]

Spin-orbital couplings, energy transfer mechanisms or electron transfer mechanisms typically result in fluorescence quenching in the presence of many heavy metal ions (Cu^{2+}, Hg^{2+}, Pb^{2+}).[5,6] Thus, the detection of Hg^{2+} ions through fluorescence enhancement is relatively scarce.[7-11] Several peptide-based chemosensors for heavy metal ions are reported in the literature.[12,7,13-15] and these chemosensors have their advantages. Peptides can be easily synthesized to incorporate different fluorophores. Besides, a high affinity and specificity can be optimized by modulating the peptide backbone.[16,17] Peptides can be made water soluble. Peptides are biocompatible and also biodegradable. These features make peptides comparatively less toxic in comparison to other chemical sensors. [18-21] Moreover, peptides form variously ordered nanostructures[22] that have been exploited in various applications ranging from tissue engineering,[16] catalysis,[17] drug delivery etc.[23-26] Hydrogen bonding, hydrophobic interactions and other weak interactions are some of the major factors that regulate the self-assembly of peptides.[27,28] These soft molecular self-assembly also responds to external stimuli like temperature, pH, light, electric field, chemicals, ionic strength etc. The responses of soft structures to stimuli often leads to a predictable alteration in their physicochemical properties.[29,30] Consequently, external stimuli-sensitive self-assembling properties of peptides and specific metal-peptide interactions can be exploited to yield fruitful routes towards a more efficient bio-inspired metal peptide framework (MPF) [31,32]

Herein, we report a tetrapeptide that has been designed with a protected *L*-DOPA, two phenylalanine and a benzyl protected dehydrophenylalanine analogue (Scheme 1). From x-ray crystallography, the peptide shows an overlapping β-turn structure of a Type III/III conformation. This peptide further self-assembled into nanospheres. We describe the application of this peptide as a ratiometric "turn-on" fluorescence for Hg^{2+} ions in acetonitrile solvent.

We have crystallized the *N*-benzoyl protected peptide from dichloromethane (DCM) solution through slow evaporation. The structure was determined by X-ray diffraction. All the crystallographic parameters are given in Table ST1. The peptide adopted 3_{10}-helical conformation with an average value of all Φ, Ψ as -60° and -30° respectively except Φ of the first residue and Ψ of the last *L*-Phe residue. Torsion angles are listed in table ST2. In the asymmetric unit, one molecule of the tetrapeptide crystallized with two molecules of DCM. Crystal data revealed that the tetrapeptide adopted an incipient 3_{10}-helical structure. The overlapping β-turn of type III/III were stabilized through intra and intermolecular N-H…O-C bond. The first turn involved C21-O1...H4A-N4 hydrogen bond whereas other one involved C21-O1... H5A-N5 hydrogen bond (Fig. 1). Table ST3 reports all inter and intramolecular H-bonding found in the peptide crystal.

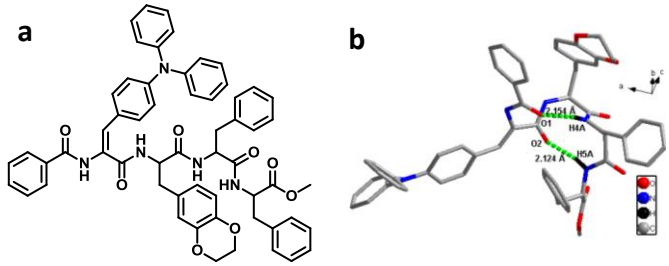


Fig. 1 (a) Molecular structure of peptide. (b) Crystal structure of peptide showing intramolecular hydrogen bonding. Only those hydrogens which are involved in H-bonding are shown for clarity.

Along the *c* axis, the molecules pack in a head to the tail manner that runs in antiparallel fashion (Fig. S2). Conventional CO...HN Hydrogen bond [N2H2A…O5 = 2.69Å] links two molecules to give a chain like structure as depicted in Figure S2. The stacking of the molecules in the crystal is eclipsed along *a* axis (Fig. S1). In addition to the conventional CO...HN hydrogen bonds, the molecules exhibit other types of intermolecular interactions. Intermolecular hydrogen bonding exists between the carbonyl group of amide bond and C-H of an aromatic ring [C39-O5…H23-C23 = 2.45Å and C48-O6…H32-C32 = 2.48Å] (Fig. S3) One intermolecular CO…HC (CHO) bonding exist between a carbonyl group of an amide bond and the $C^{\beta}H$ of *L*-dopa involving C48-O6…H32B-C32 (2.30 Å). (Fig. S3). The closest contact of one CH_2Cl_2 molecule to the peptide is to O2…H60-C60 with a distance of 2.34 Å while second CH group has an O4…H60A-C60 contact of 2.581 Å. It is noteworthy that Intramolecular average distances of CO…HN bonding are larger than intermolecular CO…HN hydrogen bonding (Table ST3).

We report the differential optical response of tetrapeptide towards Hg^{2+}. The fluorometric behaviour of the peptide was investigated in the presence of perchlorate salt of several metal

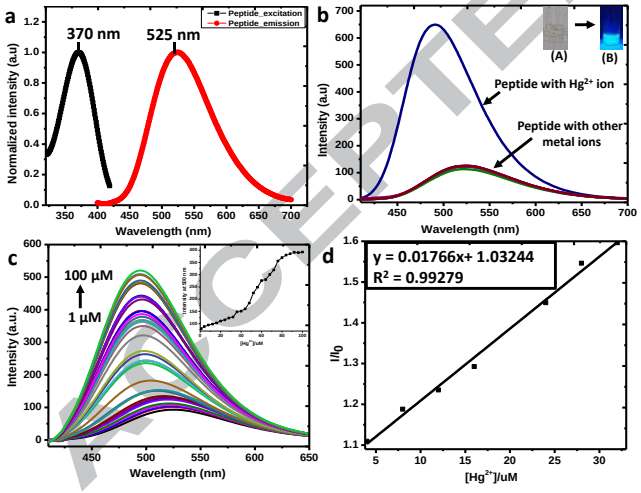


Fig. 2 (a) Excitation and emission spectra of the peptide (50 µM)). (b)Emission spectra of the peptide (50 µM) in the presence of 10 equiv. of metal ions in acetonitrile (λex = 370 nm). Inset: the light yellow colour of peptide with Hg^{2+} ion (A) turns cyan under UV light (B). (c) Fluorescence titration spectra (λex = 370 nm) of the peptide (50µM) in CH_3CN upon increasing concentration of Hg^{2+}. Inset: fluorescent intensity at 500nm against the concentration of the Hg^{2+} ion. (d) The stern-Volmer plot for a peptide with the Hg^{2+} ion.

ions such as Na^{+}, Ca^{2+}, K^{+}, Zn^{2+}, Ag^{+}, Mn^{2+}, Fe^{2+}, Cd^{2+}, Mn^{2+} in acetonitrile solvent (Fig. 2). The emission spectra of ligand have a weak emission band with maxima positioned around 525 nm when excited at 370 nm (Fig. 2a). The fluorophore in the peptide is a dehydrophenylalanine residue that combines an intramolecular push-pull electronic effect and a conjugative effect that results in a long wavelength excitation and a long wavelength emission of the peptide. The alkali and other metal ions exhibited no change relative to the emission spectra of the peptide. While Hg^{2+} shows enhancement in fluorescence intensity of peptide. However, upon addition of Hg^{2+}, the fluorescence emission band at 525 nm become blue shifted to 500 nm causing a color emission changes from light yellowish to cyan under UV light (Fig. 2b inset). Interestingly the colour change is not observed with other metal ions which manifest its selectivity as colorimetric sensor towards Hg^{2+} ions (Fig. S5). We investigated binding stoichiometry of peptide with the Hg^{2+} ion. Job's plot analysis from fluorescence data in acetonitrile at excitation wavelength 370 nm suggested a 1:1 stoichiometry of peptide with Hg^{2+} ion (Fig. S4b). As per the formation of a complex with 1:1 ratio, we carried out fluorescence titration experiment in CH_3CN (Fig. 2c). A 50µM solution of the peptide was titrated with different aliquots of the Hg^{2+} solution. However, on titration of the peptide with Hg^{2+} (up to 10 equiv.), the fluorescence intensity of peptide enhanced gradually (Fig. 2c). From the plot I/I_0 against $[Hg^{2+}]$/uM, the value of K extracted from slope was $1.7X10^4$ M^{-1}. (Fig. 2d) [33,34] These binding constant quantitatively suggest that peptide has a more potent binding affinity towards Hg^{2+} in CH_3CN. Based on fluorescence titration data, the detection limit for Hg^{2+} detection was calculated to be 10 µM by plotting the emission intensity at 500 nm against the concentration of Hg^{2+} ions (Fig. 2c inset). The versatility of sensing in the different solvent was checked by sensing in different solvents which shows that all solvent is showing the similar sensing except DCM (Fig. S6).

We also investigated secondary structure of peptide and peptide with mercury by CD spectroscopy. CD spectra of the peptide indicate a turn type of structure with bands at 204 nm.[35] On addition of one equivalent of Hg^{2+} ion, a general shifting of the band to 208nm is observed (Fig. S9) which further indicates that there is no significant effect of Hg^{2+} ions on peptide turn type of structure.

UV and fluorescence measurements for compound (4) and (6) were recorded to determine if the binding site of Hg^{2+} ion required the overlapping III/III beta-turn. UV and fluorescence spectra were recorded for comp (4) in acetonitrile [at λex = 367nm, λem = 546nm] which shows no enhancement in fluorescence intensity in the presence of Hg^{2+} ion (Fig. S7a, b). Similar results were obtained for comp (6) [λex = 365nm and λem = 507nm] (Fig. S8a, b). The emission results of these two compounds in the presence of Hg^{2+} ions suggested that mercuric ion specific binding requires the overlapping beta-turn scaffold.
The two tetrapeptides with dehydrophenylalanine analogue and Aib residue have recently been reported from our group having overlapping β-turns showing Hg^{2+} ion sensing in CH_3OH solvent.[36]

Peptide (1 mM, CH_3CN) was studied for its self-assembly behaviour using various microscopic techniques as shown in Figure 4. The peptide monomers self-assembled into highly mono-dispersed spherical nanoparticles with a smooth surface as observed in AFM images with an approximate diameter ranging from 100-500 nm (Fig. 3, Fig. S10). Further, these spherical particles were subjected to scanning electron microscopy (SEM) (Figure 3b,c) and transmission electron microscopy (TEM) studies (Fig. 3d,e). TEM studies revealed that the nanospheres consisted of a hollow inner core (Fig. 3d,e) with diameter ranges of 100-500 nm as shown in DLS spectrum (Fig. 3f). The interactions observed in crystal structure for the formation of these spherical structures were hydrophobic interactions and hydrogen bonding within peptide molecules and with solvent molecules.[37-40]

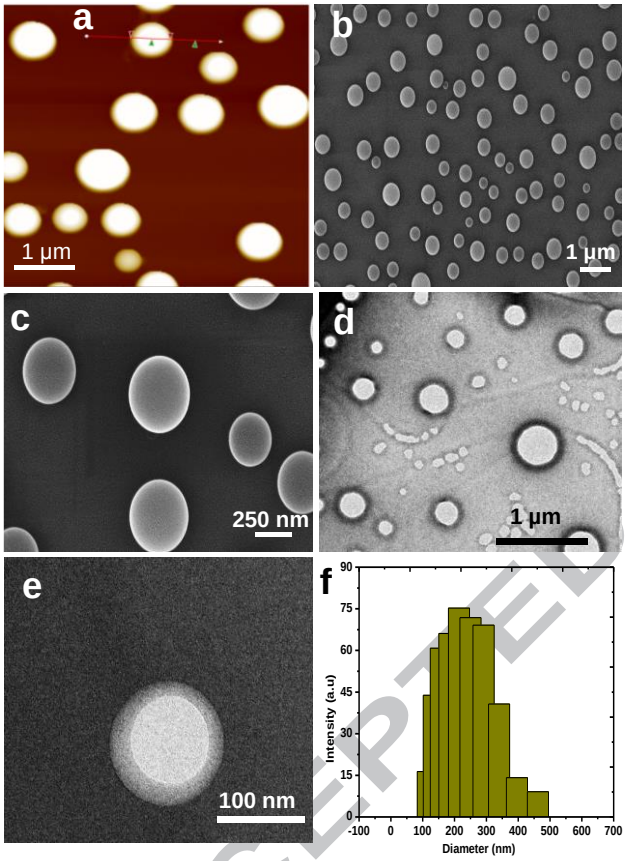
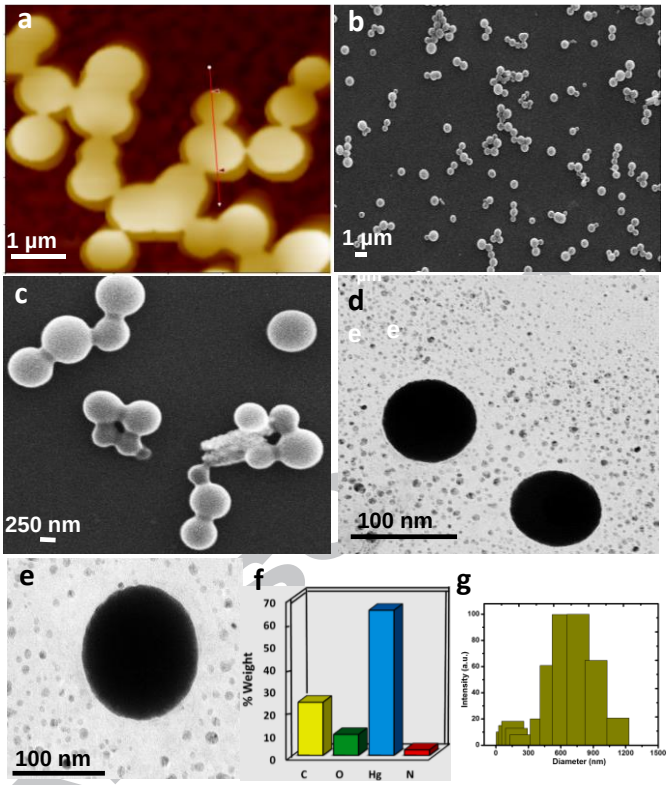


Fig. 3 Self-assembled structure of peptide (1mM, CH_3CN). (a,) AFM micrograph. (b,c) SEM images. (d,e) TEM images. (f) DLS spectrum is showing size distribution of peptide.

Peptide with metal binding sites is interesting biomaterials for MOF formation. Many reports of the metal-peptide framework are reported in the literature. In recent literature, Jose *et al.* reported a tripeptide based metal-organic framework for the enantioselective separation of methamphetamine and ephedrine.[31] However, in case of the peptide, the samples for metalation were prepared with the perchlorate salt of Hg^{2+} in CH_3CN (1:1 eq). The microscopic studies revealed the fusion of the spherical structures as shown by AFM, SEM and TEM images (Fig. 4). The presence of Hg^{2+} ions was further established with energy dispersive x-ray spectroscopy (EDX) analysis (Fig. 4f). The peptide solution (1 mM) was incubated for 12 h to enable self-assembly and then mixed with Hg^{2+} ions (1:1 eq). The fused spherical particles were confirmed by atomic force microscopy



images (Fig. 4a). The SEM images revealed the well-defined fusion of spherical nanoparticles of peptides due to metal ion

Fig. 4 Microscopic images of peptide(9) (1mM, CH_3CN) + Hg^{2+} (1mM, CH_3CN) complex in CH_3CN solvent with 1:1 ratio. (a) AFM image. (b,c) SEM images. (d-e) TEM images. (f) EDX analysis (Si and Au has been removed) (g) DLS spectrum.

interactions (Fig. 4b,c). Transmission electron microscopy images exhibited the accumulation of Hg^{2+} ions into hollow nanospheres of the peptide (Fig. 4d,e).

The fluorescence enhancement of peptide in the presence of Hg^{2+} may be due to aggregation of the peptide by complexation with mercury.[41-44] Dynamic light scattering revealed that the peptide did not aggregate (Fig. 3) by itself and showed negligible fluorescent emission. Upon addition of mercury, the average particle size increased (Fig. 4g) due to aggregation with an enhancement of fluorescence intensity (Fig 2b). This aggregation-induced emission (AIE) effect was further confirmed by a microscopic study of the peptide in the presence and absence of Hg^{2+} ions (Fig. 3,4).

In conclusion, our reported self-assembling fluorescent synthetic tetrapeptide with an overlapping turn of Type III/III leading to an incipient 3_{10} –helix is a viable probe for sensing of Hg^{2+}. The fluorescence intensity enhancement is a result of peptide aggregation in the presence of mercuric ions. We believe that improvement in the sensitivity of the peptide and engineering its solubility in water can make this molecule a promising tool for sensing mercury. Microscopic characterization revealed a spherical morphology. However, detailed microscopic studies reveal that interaction of the peptide with mercury ions result in fusion of nanospheres of different sizes.

Acknowledgement

KT thanks the council of scientific and industrial research (India) for a junior and senior research fellowship.

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Highlights

- We have synthesized dehydrophenylalanine containing tetrapeptide.
- Peptide folded as an overlapping beta-turn of type III/III conformation.
- The Peptide has been shown as a fluorometric sensor for Hg^{2+} metal ion.
- Peptide self-assembled into spherical nanoparticles.
- Addition of Hg^{2+} in peptide solution results in fusion of nanospheres.