MOLECULAR RECOGNITION AND SELF-ASSEMBLING PROPERTIES OF PEPTIDE-BASED SYSTEMS

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Molecular Recognition and Self-assembling Properties of Peptide-Based Systems

by

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Dedicated to my dear parents

(P. K. Prabhakaran and K. V. Sunandha)

CERTIFICATE

This is to certify that the thesis entitled, **"Molecular Recognition and Self-assembling Properties of Peptide-Based Systems"**, being submitted by **Mr. Praveen Kumar P. P**, to the Indian Institute of Technology, Delhi, for the award of degree of '**Doctor of philosophy in Chemistry**', is a record of bonafide research work carried out by him. **Mr. Praveen Kumar P. P** has worked under my guidance and supervision and has fulfilled all the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard. The results embodied in this thesis have not been submitted in part or in full, to any other University or Institute for award of any degree or diploma.

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ABSTRACT

This thesis, **"Molecular recognition and self-assembling properties of peptide-based systems"** deals with the design and synthesis of various peptide-based molecules. We have demonstrated the utility of these molecules for binding various ions and as self-assembled materials for various applications. Molecular recognition of cations, anions and neutral molecules play a significant role in biology and chemistry. Cation transport is an important biological event that keeps the ionic balance in the body. Anions regulate the flux of key metabolites into and out of cells while maintaining osmotic balance. By keeping this idea in mind, we have designed and synthesized various peptide-based molecules and their sensing abilities has been studied in detail by using various spectroscopic techniques and by DFT studies. The self-assembling properties of these peptide-based molecules were studied in detail by various microscopic techniques.

Chapter 1 deals with a brief history and scope of the application of peptide-based fluorescent systems in molecular recognition, and as self-assembling materials for various applications.

Chapter 2 describes the design and synthesis of cysteine-based "turn-on" sensors for metal ions. In this chapter, we have designed three cysteine based molecules **B3-B5**, and their metal binding ability was studied in detail by UV-visible, fluorescence spectroscopy, ITC, NMR and by DFT calculations. Experimental results showed that **B3** can sense Cu^{2+}, **B4** can sense Ag^{+} and **B5** can sense Hg^{2+}. The self-assembling properties of these molecules and their stimuli response with metal ions were also studied by using various microscopic techniques.

Chapter 3 addresses the design and synthesis of cystine based fluorescent sensors (**C2-C4**) for metal ions. Detailed spectroscopic studied revealed that **C2** can sense Cu^{2+}, **C3** can sense Hg^{2+}, and **C4** can sense Cd^{2+} colorimetrically. We have demonstrated the formation of a

supramolecular heterodimer (**C2**:**C3**) from **C2** and **C3**, and which detects Pb^{2+} by a fluorescent resonance energy transfer mechanism (FRET). The heterodimer formation was supported by UV-visible, fluorescent spectroscopy, H NMR, ITC, DSC and by DFT calculations.

Chapter 4 discusses about design, synthesis, self-assembly and anion binding properties of tryptophan based molecules (**D2**, **D4**, **D6**, **D8**, and **D10**) which were arranged at different positions on a benzene aromatic core. Self-assembling properties of these molecules were studied in detail by various microscopic techniques. The binding property of these peptide molecules towards various anions were studied in detail.

Chapter 5 deals with the design, synthesis, self-assembly and mechanical properties of lipidated Asp/Glu- based dendrons and dendrimers were studied. The results showed that most of these dendrons and dendrimers can form excellent organogels and the guanidinylated dendrimer **E16** can form hydrogel. The rheological studies showed that the higher lipidated Glutamic acid based dendrimer **E13'** possess high mechanical strength of the order 10^7 Pa.

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NOTES

1. All amino acids used were of L-configuration. Unless otherwise stated, all reagents were used without further purification.
2. All solvents employed in the reaction were distilled or dried from appropriate drying agent prior to use.
3. Melting points were recorded in a Fisher-Johns melting point apparatus and were uncorrected.
4. IR spectra were recorded on a Nicolet, Protégé 460 spectrometer as KBr pellets.
5. ^{1}H NMR spectra were recorded on Brucker-DPX-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer using tetramethylsilane (^{1}H) as an internal standard. Coupling constants are in Hz and the ^{1}H NMR data are reported as s (singlet), d (doublet), br (broad), br d (broad doublet), t (triplet), q (quartet), m (multiplet).
6. HRMS were recorded with Bruker MicrO-TOF- QII model and AB Sciex, 1011273/A model using ESI-technique.
7. Reactions were monitored wherever possible by thin layer chromatography (TLC). Silica gel G (Merck) was used for TLC and column chromatography was done on silica gel (100-200 mesh) columns, which were generally made from slurry in hexane, hexane/ethyl acetate or chloroform.
8. UV-Visible spectra were recorded in Shimadzu double beam spectrophotometer, UV-2400.
9. Emission spectra were recorded using HORIBA JOBIN YVON Scientific, fluoromax-4 spectrophotometer, with slit width of 5 nm.
10. Time-resolved fluorescence spectra were recorded using an IBH picosecond single-photon-counting system with an adjustable nano-LED excitation source.

11. Isothermal Calorimetric Titration (ITC) experiments were performed by MicroCal iTC_{200} system at 298 K.
12. SEM measurements were done using ZEISS EVO® and JEOL-JSM-5600 LV, instrument with an EHT of 20 kV.
13. AFM measurements were performed using Nanoscope Multimode AFM operating in tapping mode in air.
14. HR-TEM images were recorded using Philips CM 12 electron microscope.
15. ATR-FTIR measurements were performed on Agilent-Cary 660 series FTIR spectrometer.
16. Rheological measurements were done on MODULAR COMPACT RHEOMETER (MCR-150, Anton Par Ltd, India, PAAR PHYSICA, Germany).
17. Differential Scanning Calorimetric (DSC) experiments were carried out using Perkin Elmer Diamond DSC

LIST OF ABBREVIATIONS

%	percent
δ	chemical shift
^{o}C	degree centigrade
τ	decay-time
eV	electron volt
kV	Killo volt
aq.	aqueous
AFM	Atomic force spectroscopy
Boc	t-butyloxycarbonyl
br	broad
Conc.	Concentrated
d	doublet
dd	double doublet
DCC	N,N'-dicyclohexylcarbodiimide
DIEA	N,N'-Diisopropylethylamine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
ESI-MS	Electrospray ionization mass spectroscopy
equiv.	Equivalents
F	Fluorescence intensity
g	gram

G'	Storage modulus
G"	Loss modulus
h	hour
Hz	Hertz
HRMS	High resolution mass spectra
HR-TEM	High resolution transmission electron microscope
IR	infrared
J	coupling constant
K	kelvin
Kcal/mol	Kilocalories per mol
KJ/mol	Kilo joules per mol
M	molar
μM	micromolar
mM	millimolar
m	multiplet
mg	milli gram
mL	milli liter
min	minutes
mmol	milli moles
mol	mole
mp	melting point
m/z	mass/charge
NHS	N-hydroxysuccinimide

NMR	Nuclear magnetic resonance
ns	nano seconds
nm	nanometer
ppm	parts per million
q	quartet
RT	Room temperature
s	singlet
SEM	Scanning electron microscope
TBAF	Tetrabutylammonium fluoride
TBACl	Tetrabutylammonium chloride
TBABr	Tetrabutylammonium bromide
TBAI	Tetrabutylammonium iodide
$TBAH_2PO_4$	Tetrabutylammonium phosphate
$TBAHSO_4$	Tetrabutylammonium sulfate
TEM	Transmission electron microscope
t	triplet
TLC	Thin layer chromatography
UV-vis.	Ultraviolet-visible

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