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Self-Assembling Synthetic Oligopeptide-Based Gelators

*Apurba K. Das, Arindam Banerjee**

Summary: Synthetic self-assembling oligopeptide gelators are an important class of compounds which form thermoreversible gels in various organic solvents as well as in aqueous medium. These gels are soft, viscoelastic materials which are envisaged for useful applications in biological and material sciences. The terminally protected self-assembling synthetic tripeptide Boc-Ala-Aib-β-Ala-OMe **1** (Aib: α-aminoisobutyric acid *i.e.* dimethyl glycine and β-Ala: β-Alanine) forms gels in various organic solvents, whereas its structural analog *i.e.* the peptide Boc-Ala-Gly-β-Ala-OMe **2** (another self-assembling synthetic tripeptide) fails to form gels under similar conditions and this issue has been addressed. The terminally protected tripeptide Boc-Ala-Val-Ala-OMe **3** has been found to form gels in different aromatic organic solvents. Several structural analogs of peptide **3** [using small structural changes either in protecting groups (at the N or C-terminal position) or in amino acid side chains] have been synthesized, characterized and studied for gelation to address the question how structural changes can regulate the gelation property. Results of the gelation studies indicate that some structural changes are useful to make new peptide gelators with some variations in gelation property and efficiency, while a few structural changes in the protecting groups are really detrimental, leading to abolition the gelation property. These gels are studied by scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier-Transform Infrared (FT-IR) spectroscopy and ^{1}H NMR studies.

Keywords: Aib; gels; self-assembly; synthetic peptide

Introduction

Gels are soft, pervasive viscoelastic materials, which form entangled network structures. Polymer gels are known for about a century and their applications in diverse fields like food, medicine, cosmetics, material science, sanitation *etc.* have been well recognized. However, gels obtained from low molecular mass organic compounds have started getting enormous attention for about last fifteen years and their diverse applications have been envisaged.[1] Low molecular mass organogels can be regarded as supramolecular gels as low molecular mass compounds are self-assembled using various non-covalent physical interactions to provide a suitable supramolecular network structure for gelation.[2] These non-covalent interactions include intermolecular hydrogen bonding, π···π, C–H···π, N–H···π and O–H···π interactions, van der Waals' interactions, metal ion coordination, donor-acceptor interactions and solvophobic interactions (hydrophobic for gels in water). Since network formation actually involves non-covalent, weak interactions, they can be easily transformed from gel state to sol state simply by heating and are therefore thermoreversible. Though the discovery of low molecular mass gelator occurred in early nineteenth century,[3] their supramolecular nature was very poorly understood and is neglected till the late 20-th century. Low

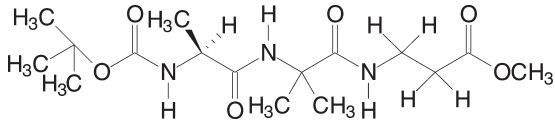
Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700032, India
Fax: +91 3324732805
E-mail: bcab@mahendra.iacs.res.in

molecular mass organogelators are structurally very diverse, as for example simplest alkane to giant porphyrin or third generation dendrons or complex phthalocyanins have been discovered to form gels.

Peptide gelators are regarded as hydrogen bonding gelators as they self-associate mainly by using intermolecular hydrogen bonding interactions to provide the gel forming network structure, which encapsulate a large volume of solvent molecules under suitable conditions. There are several examples of oligopeptide based hydrogelators that form responsive gels whose viscoelastic behavior can be regulated by using chemical (hydrogen ion concentrations) or physical (shear) influences.[4] The interesting examples of oligopeptide based hydrogelators include the formation of hydrogels by a fragment 1-28 of Alzheimer's β-peptide,[5] formation of pH and Ca^{2+} ion responsive gels by peptide based amphiphiles,[6] thermally and photochemically triggered hydrogel formation by a 16-residue peptide with alternating hydrophilic and hydrophobic residues.[7] Interesting applications of oligopeptide based hydrogels have been documented in the literature. Zhang and his coworkers have demonstrated that a self-assembling dodecapeptide consisting of L-lysine, L-leucine and L-aspartic acid residues forms hydrogel which acts as a 3-D scaffold to encapsulate primary chondrocytes construct, a 3-D piece of cartilage tissue which shows close resemblance with an isolated tissue from animals. This self-assembling hydrogel forming peptide scaffold can be used for culturing cells and repairing tissues in regenerative medicine.[8] However, the examples of oligopeptide based organogelators are relatively much less than the hydrogelators obtained form oligopeptides. Early examples of oligopeptide based organogelators include the formation of organogels in various solvents like DMF, DMSO, nitrobenzene *etc.* by oligomers of L-valine, L-isoleucine, L-phenylalanine and L-glutamate esters.[9] One classic example of short peptide based organogelator includes the oxidized form of glutathione, a biologically active tripeptide.[10] Cyclodipeptides can also be used as gelling agents for hardening various organic fluids such as benzene, toluene, chlorobenzene, methoxybenzene and nitrobenzene.[11] For last few years our group is actively involved in discovery and study of short peptide based organogelators. During the study of self-assembly of a terminally protected tripeptide Boc-β-Ala(1)-Aib(2)-β-Ala(3)-OMe, the organogels are serendipitously discovered. This peptide forms gels in benzene, monochlorobenzene, 1,2-dichlorobenzene and gelation mechanism was studied at length.[12] This gives an impetus to search for organogelators obtained from self-assembling short peptides. The discovery and study of short peptide based organogelators from our laboratory will be presented here. Although most discoveries of low molecular mass organogelators are serendipitous, still some of the factors that promote successful gelation can be gleaned from the systematic syntheses of structural analogs of a known gelator molecule. Tuning of the gelation property for a series of tripeptide gelators by changing the molecular structures of known gelator compounds will be discussed in the succedding section of this paper.

Results and Discussion

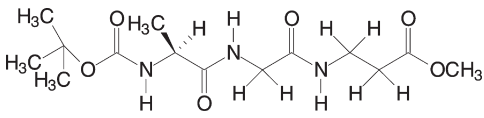
In course of our investigation for self-assembling synthetic short peptide, it has been discovered that the terminally protected tripeptide Boc-Ala(1)-Aib(2)-β-Ala(3)-OMe (Boc: *tert*-butyloxy carbonyl, Aib: α-aminoisobutyric acid, β-Ala: β-Alanine) **1** forms thermoreversible gels in different organic solvents including benzene, toluene, 1,2-dichlorobenzene, cyclohexane at very low concentrations.



Peptide **1**

Scanning electron microscopic investigation of the dried gel of the peptide **1** obtained from the solvent benzene have shown that the morphology of the dried gel exhibits an entangled network consisting of regular shaped long rod-like fibers.[13] The thermal behavior of 5% (w/v) peptide **1** gel in benzene was examined by differential scanning calorimetry (DSC) and both heating and cooling thermogram suggest the reversible first order transition. Self-association of the gelator peptide **1** in benzene was examined by FT-IR spectroscopy. At low concentration (<0.3% w/v of the gelator, where the gel formation does not occur), the gelator peptide exhibits a sharp single peak at 3419 cm^{-1} with a shoulder at 3377 cm^{-1} corresponding to NH stretching frequencies. Both the peaks are independent of concentration at low concentration range. The sharp peak at 3419 cm^{-1} is characteristic of non-hydrogen bonded NH while the shoulder centered at 3377 cm^{-1} is typical for hydrogen bonded amide NHs. With the increase in concentration of that gelator peptide **1**, a gradual decrease in intensity of the peak corresponding to non-hydrogen bonded NHs and an increase in intensity of the peak corresponding to hydrogen-bonded NHs have been observed. However, at higher concentration (at 3% w/v) only two peaks with strong intensity corresponding to hydrogen bonded NHs at 3346 cm^{-1} and 3282 cm^{-1} have been found. The self-assembly of peptide **1** is mediated by intermolecular hydrogen bonds, as it is evident from the above mentioned concentration dependent FT-IR studies.

Various ^1H NMR studies (concentration dependent, temperature dependent, solvent perturbation experiments) have been performed to investigate detailed conformational features of the gel forming peptide **1** in C_6D_6. The results of the solvent perturbation experiments by the addition of small amount of the strongly hydrogen bonding solvent $(CD_3)_2SO$ in C_6D_6 solution at 0.16% w/v concentration, where the gelation and peptide aggregation does not occur, indicate that out of three peptide NHs, only the β-Ala(3) NH is intramolecularly hydrogen bonded. The result of the concentration dependent ^1H NMR experiment within the concentration range 0.16% (w/v) to 2% (w/v) suggests the strong involvement of β-Ala(1) NH and Aib(2) NH in intermolecular hydrogen bonding and β-Ala(3) NH may also form a very weak intermolecular hydrogen bond apart from its engagement in intermolecular hydrogen bonding, to form the aggregated gel state. The results of temperature dependent chemical shifts of the gelator peptide **1** in C_6D_6 (1% w/v) within the temperature range 25 °C (gel state) to 70 °C (sol state) indicates that β-Ala(1) and Aib(2) NH are engaged in intermolecular hydrogen bonding to form the aggregated gel state. The crystal structure of the gel-forming peptide **1** can provide an useful insight into the supramolecular structure that might be responsible for gel fiber network. The crystal structure of this peptide indicates the adoption of an intramolecular hydrogen bonded β-turn molecular structure.[13] This molecular structure then self-assembles to form a semi-cylindrical ribbon structures along the crystallographic *b* axis *via* one intermolecular hydrogen bond. These ribbon structure are then joined *via* two intermolecular hydrogen bonds to form a two dimensional cage forming double-columner sheet-like structure (Figure 1).



Peptide **2**

The terminally protected peptide Boc-β-Ala(1)-Gly(2)-β-Ala(3)-OMe **2** is a structural analog of the gel forming peptide **1**,

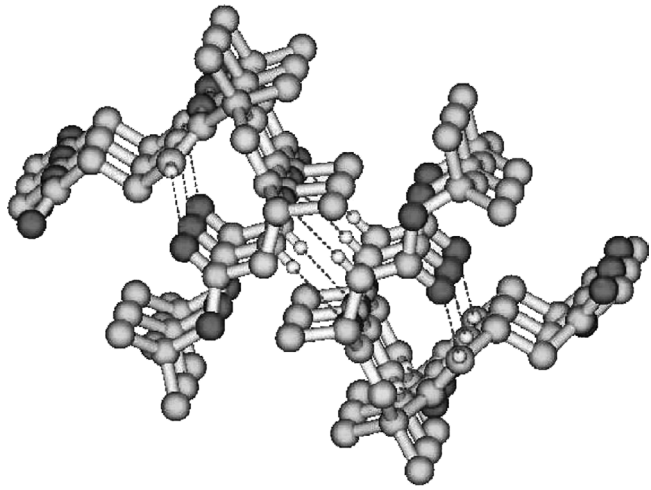


Figure 1.
Higher order packing of peptide Boc-Ala-Aib-β-Ala-OMe **1** showing the double columnar sheet-like structure. Non-hydrogen bonded hydrogen atoms are omitted for clarity. Hydrogen bonds are shown as dotted lines.

which does not form gel even in high concentration under similar conditions. In peptide **2**, the central position is occupied by the glycine residue in substitution of dimethyl glycine residue which is present in peptide **1**. Figure 2 represents the molecular packing diagram of peptide **2** in crystals showing intermolecularly hydrogen bonded antiparallel β-sheet structure. The molecular arrangement of the gelator peptide **1** is entirely different from that of the structurally analogous non-gelator peptide **2** indicating that conditions and interactions responsible for gelation is very specific and non-gelating property of the peptide **2** is envisaged as the different molecular arrangement of the peptide **2** from that of peptide **1** in crystals.

From the above mentioned example, it is clear that minor structural change in molecular structure can abolish the gelation property. In this connection, we can make an attempt to tune the gelation properties of terminally protected short peptide based gelators by making minor structural changes of a known oligopeptide gelator molecule. A self-assembling terminally protected tripeptide Boc-Ala-Val-Ala-

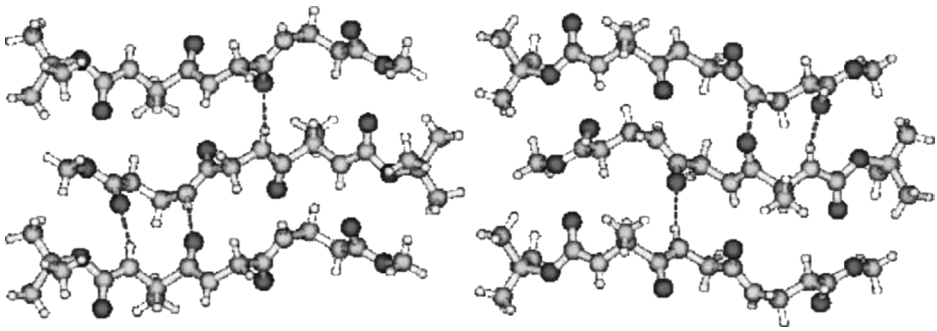
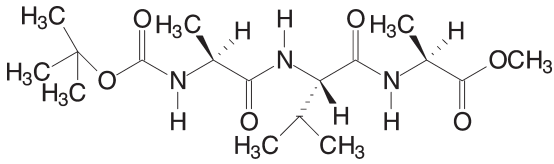


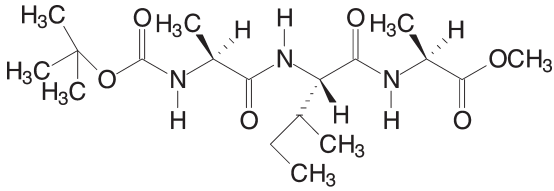
Figure 2.
Higher order packing diagram of peptide Boc-Ala-Gly-β-Ala-OMe **2** showing the intermolecular antiparallel β-sheet structure.

OMe **3** was first discovered to be a gelator. The tripeptide **3** gelates various organic solvents including benzene, toluene, *o*-dichlorobenzene, *m*-xylene, chlorobenzene. There are two ways to change the molecular structure of the gelator peptide **3**: (a) by changing the amino acid residues in the side chain and (b) by altering the N- and C-terminal protecting groups. Three structurally analogous peptides of the lead gelator peptide **3** have been synthesized, purified and characterized by changing the amino acid side chain residues. These peptides are Boc-Ala-Ile-Ala-OMe **4**, Boc-Ala-Leu-Ala-OMe **5** and Boc-Ala-Phe-Ala-OMe **6**. Chemical structures of peptides **3**–**6** are represented as follows:

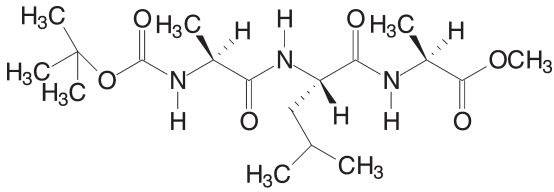
All these peptides **4**–**6** form gels. However, their gelation properties and efficiencies are different and it is listed in Table 1. By analyzing Table 1, it has been observed that both peptides **4** and **5** form gels in various aromatic organic solvents including benzene, toluene, *o*-dichlorobenzene (*o*-DCB), *m*-xylene and *p*-xylene showing similarity with the lead gelator peptide **3**. The efficiency of gelation *i.e.* minimum amount of a particular gelator required to gelate a given volume of the solvent (as expressed in % w/v) is varied among the gelator peptides **3**, **4** and **5**. However, the peptide **6** can only gelate toluene at high concentration (12.5% w/v). These results indicate that minor structural changes in



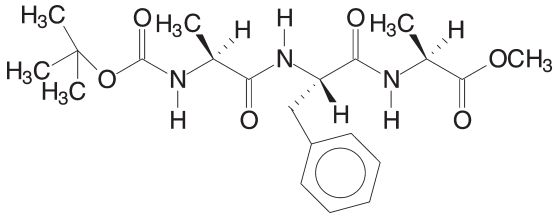
Peptide **3**



Peptide **4**



Peptide **5**



Peptide **6**

Table 1.
Gelation properties of peptides **3–6** in organic solvents[a].

Solvent	Peptide **3**	Peptide **4**	Peptide **5**	Peptide **6**
(i) Methanol	S	S	S	S
(ii) Ethanol	S	S	S	S
(iii) 1-Butanol	S	S	S	S
(iv) *o*-DCB	G (2.5)	G (8.5)	G (1.25)	S
(v) Toluene	G (5.5)	G (6.4)	G (4.5)	G (12.5)
(vi) Benzene	G (1.5)	G (7)	G (3.3)	I
(vii) *m*-Xylene	G (7.5)	G (8)	G (12)	I
(viii) *p*-Xylene	G (8)	G (15)	G (12)	S
(ix) Ethyl acetate	S	S	S	S
(x) Chloroform	S	S	S	S
(xi) DMF	S	S	S	S
(xii) Cyclohexane	I	I	I	I
(xiii) *n*-Hexane	I	I	I	I
(xiv) *n*-Decane	P	P	P	P

[a] G: stable gel formed at room temperature, in parentheses: minimum gel concentration (% w/v); S: soluble; I: insoluble; P: precipitate.

the amino acid side chains can tune the gelation property for this tripeptide series. The peptide **5** gelates *o*-DCB and toluene more efficiently than its structural analogs (gelator peptides **3** and **4**). Peptide **3** gelates benzene most efficiently (1.5% w/v) than the gelator peptides **4** and **5**. It also gelates *m*- and *p*-xylenes more efficiently than its structurally analogous gelators **4** and **5**.

The morphology of the dried gels obtained from gelator peptides **3** and **4** were studied using scanning electron microscope (SEM). Figure 3 shows the SEM image of the dried gel of peptide Boc-Ala-Val-Ala-OMe **3** obtained from benzene and similarly Figure 4 exhibits the SEM picture of the dried gel of peptide Boc-Ala-Ile-Ala-OMe **4** obtained from toluene.

Both gelator peptides **3** and **4** have cross-linked fibrillar network structure. DSC (differential scanning calorimetry) studies of the gels of the gelator peptide **3** in benzene show reversible first order phase transition indicating the thermoreversible nature of gels. FT-IR studies of the gelator peptide **3** were carried out in three different states (a) solid state, (b) dried gel obtained from *o*-DCB and (c) wet gel state obtained from *o*-DCB. For wet gel solvent subtracted FT-IR spectrum was obtained. Amide I band corresponding to C=O stretching frequency varies from 1634 cm^{-1} to 1639 cm^{-1} indicating β-sheet conformation with very minor variations in above mentioned three states.[14] However, a significant shift ~27 cm^{-1} (from 3292 cm^{-1} to

Figure 3.
SEM picture of dried gel of peptide Boc-Ala-Val-Ala-OMe **3** obtained from benzene.

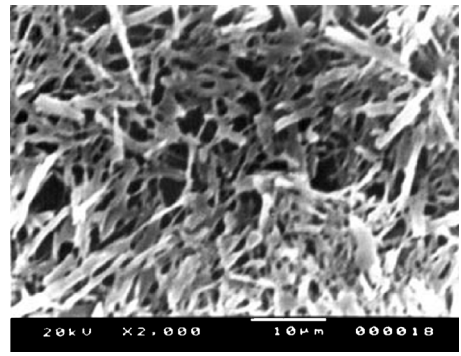


Figure 4.
SEM picture of dried gel of peptide Boc-Ala-Ile-Ala-OMe **4** obtained from toluene.

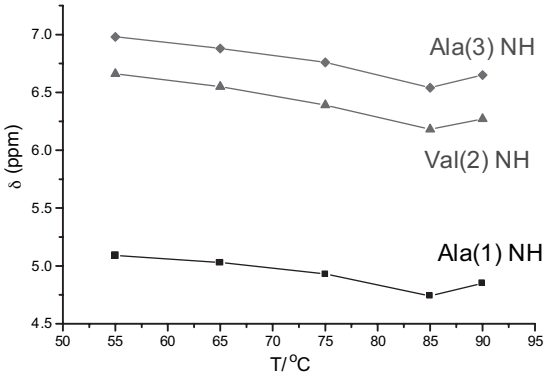


Figure 5.
Plot of chemical shifts of three NHs of Boc-Ala(1)-Val(2)-Ala(3)-OMe **3** in C_6D_6 (2.2% w/v) *vs.* temperature ranging from 55 °C (gel state) to 90 °C (solution state).

3265 cm^{-1}) in NH stretching frequency has been observed in the peptide **3**/benzene gel (wet) state structure from that of the solid/xerogel state. Results of the FT-IR studies clearly suggest that solid state and dried gel structures are closely similar. However, they are distinctly different from the wet gel structure. Temperature dependent ^1H NMR studies of the peptide **3** gel in C_6D_6 (at 2.2% w/v) have been performed between the temperature range 55 °C (gel state) to 90 °C (solution state). The result shows that there is a steady and significant upfield chemical shift for all amide NHs of the gelator peptide **3** upto 85 °C (gel state) and at 90 °C (sol state) all NHs exhibit a small down field chemical shifts. Figure 5 represents a diagram showing the chemical shifts of all three NHs of the gelator peptide Boc-Ala(1)-Val(2)-Ala(3)-OMe **3** in C_6D_6 (at 2.2% w/v) *vs.* temperature ranging from 55 °C (gel) to 90 °C (solution).

Gelator peptides **3**, **4** and **5** crystallize from methanol-water system. Crystal packing arrangements of these peptides reveal that their supramolecular arrangements are similar in the solid state. They all form intermolecular hydrogen bonded antiparallel β-sheet structures in crystals. So, by making a small structural change (at the molecular level), we are able to make new gelator molecules which gelates same type of organic solvents with some variations of the efficiency of the gelation property.[15]

So far we have discussed about the structural changes of the lead gelator molecule **3** by altering amino acid side chain at the central position *i.e.* by substituting Val by Leu/Ile/Phe residues keeping the N-and C-terminal protecting group intact. Regulating the gelation properties of the terminally protected tripeptides (Pr^n-Ala-Val-Ala-Pr^c, Pr^n and Pr^c are the N- and C-terminal protecting groups) by only changing the N- and C-terminal protecting groups one by one of the lead gelator peptide **3** have also been investigated.[16] The following peptides have been synthesized, purified, characterized and studied for gelation (Table 2).

Boc-Ala-Val-Ala-OEt [-OEt: ethyl ester, Boc: *tert*-butyloxy carbonyl]	Peptide **7**
Boc-Ala-Val-Ala-O^{n}Pr [-O^{n}Pr: *n*-propyl ester]	Peptide **8**
Boc-Ala-Val-Ala-O^{i}Pr [-O^{i}Pr: *iso*-propyl ester]	Peptide **9**
Boc-Ala-Val-Ala-OBz [-OBz: benzyl ester]	Peptide **10**
Piv-Ala-Val-Ala-OMe [Piv: pivaloyl (*tert*-butyl carbonyl)]	Peptide **11**
Ac-Ala-Val-Ala-OMe [Ac: Acetyl, OMe: methyl ester]	Peptide **12**
Z-Ala-Val-Ala-OMe [Z: benzyloxy carbonyl]	Peptide **13**

Table 2.
Gelation properties of peptides **7–13** in organic solvents[a].

Solvent	7	8	9	10	11	12	13
o-DCB	G	G	S	S	P	S	G
Toluene	G	P	S	P	P	S	G
Benzene	G	S	I	P	S	I	P
m-Xylene	P	P	S	P	P	S	G
Tetralin	G	P	P	S	P	S	G
Nitrobenzene	G	S	S	S	S	P	G
Chlorobenzene	G	S	S	G	P	S	G
Methanol	S	S	S	S	S	S	S
Ethanol	S	S	S	S	S	S	S
DMF	S	S	S	S	S	S	S

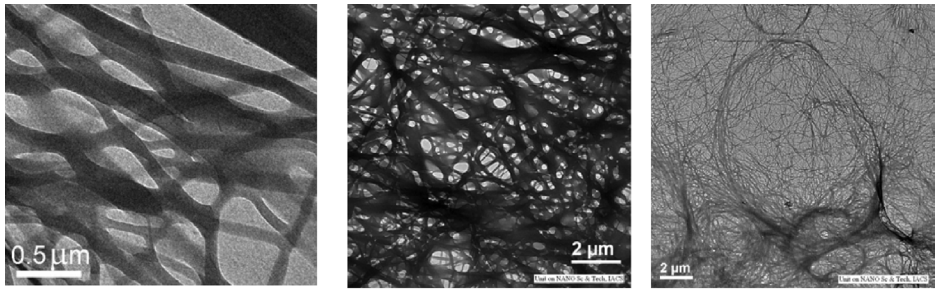
[a] G: stable gel formed at room temperature, S: soluble; I: insoluble; P: precipitate.

The peptide **7** [in which only C-terminal protecting group changes from methyl ester (in peptide **3**) to ethyl ester] gelates various organic solvents including benzene, toluene, *o*-dichlorobenzene (*o*-DCB), nitrobenzene, chlorobenzene and tetralin. The lead gelator peptide **3** gelates solvents like *o*-DCB, benzene, and toluene with better efficiency than that of peptide **7**. However, it fails to gelate nitrobenzene and gelates tetralin as well as chlorobenzene with less efficiency than that of of peptide **3**. Peptide **13** [in which only N-terminal protecting group has been changed from Boc (*tert*-butyloxy carbonyl) in peptide **3** to benzyloxy carbonyl] gelates several organic solvents including *o*-DCB, toluene, nitrobenzene, chlorobenzene more efficiently than the gelator peptide **3**, while it gelates *o*-DCB less efficiently than the peptide **3**. Each of the peptides **8** and **10** gelates only one solvent. The peptide **8** gelates only *o*-DCB and the peptide **10** gelates only chlorobenzene. The minimum gelation concentration required for gelating *o*-DCB is 2.5% w/v for peptide **3** and 10% w/v for peptide **8** showing less efficiency of peptide **8** in gelation. Peptides **9**, **11** and **12** are unable to gelate the above mentioned solvents under the investigation even at very high concentration. It is interesting to note that the substitution of methyl ester group (C-terminal protecting group, abbreviated as OMe) in the gelator peptide **3** by *iso*-propyl ester in peptide **9** results in complete abolition of gelation property. Similar is the case for peptides **11** and **12**. This indicates small structural change in the protecting group can not only tune the gelation property of the lead gelator but also can be able to abolish the gelation properties.

Figures 6 a, b and c show the TEM images of the dried gels obtained from peptide **7** in toluene (in 6% w/v), peptide **10** in chlorobenzene (in 7% w/v) and peptide **13** in *m*-xylene (in 5% w/v) respectively. These transmission electron micrographs indicate entangled nano-fibrillar network formation.

Conclusion

Though the discovery of self-assembling short peptide based organogelators is made



(a) (b) (c)

Figure 6.
Transmission electron micrographs of the dried gel derived from (a) peptide Boc-Ala-Val-Ala-OEt **7** in toluene (in 6% w/v), (b) peptide Boc-Ala-Val-Ala-OBz **10** in chlorobenzene (in 7% w/v) and (c) peptide Z-Ala-Val-Ala-OMe **13** in *m*-xylene (in 5% w/v) showing nano-fibrillar network formation.

by chance, here a successful attempt has been made to probe how the small structural change at the molecular level of a lead gelator (newly discovered gelator molecule) can affect the gelation property. The gels are characterized by various spectroscopic techniques (FT-IR, ^{1}H NMR) and morphological studies of the gels have been accomplished by SEM/TEM. Substitutions at the amino acid side chains can regulate the gelation property of the known gelator peptide **3**. However, structural alteration of the protecting groups (N-terminal or C-terminal) can not only regulates the gelation property but also destroys the gel-forming tendency in a given terminally protected tripeptide series. Some structural changes are welcomed leading to the restorations of gelation properties and others are detrimental to gel formations. This may lead to design new short peptide based gelators with better efficacy in future.

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