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Tetrahedron Letters 44 (2003) 4103–4107

TETRAHEDRON LETTERS

A synthetic tripeptide as a novel organo-gelator: a structural investigation

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Received 9 January 2003; revised 19 March 2003; accepted 28 March 2003

Abstract—A self-associating synthetic tripeptide [Boc-Ala(1)-Aib(2)-β-Ala(3)-OMe (Aib: α-amino-isobutyric acid, β-Ala: β-alanine)] forms thermoreversible transparent gels in various organic solvents and this offers the first example of a peptide gelator whose molecular self-assembly afforded for gelation has been characterised by single-crystal X-ray diffraction and FT-IR and NMR spectroscopic studies. The crystal structure of an analogous synthetic non-gelator tripeptide [Boc-Ala(1)-Gly(2)-β-Ala(3)-OMe] is also discussed in light of the self-assembly of the gelator tripeptide.

There has been considerable recent interest in the development of new organogelators based on low molecular weight compounds[1] as gels have many potential applications in material and biological sciences.[1a,2] Various types of organogels and even hydrogels formed by different groups of low molecular weight organic compounds have been established in recent years.[3] However, the majority of organogelators are discovered by serendipity rather than by design. Many synthetic peptides self-assemble to form various types of supramolecular structures such as hollow tubes,[4] tapes,[5] and fibres.[6] When these superstructures encapsulate the bulk solvent under specific conditions, they form gels. There are several reports of synthetic and engineered β-sheet-forming peptides,[5,7,8] which form gels. Many amyloidogenic peptides can also form gels.[5]

The single-crystal X-ray diffraction study of a gelator molecule is important as it establishes how the gelator molecules self-assemble into a particular macroscopic network at atomic resolution. There are very limited reports of the crystal structures of gelator molecules[9] and to the best of our knowledge, there is no report of a crystal structure of a peptide gelator. We report here a new peptide gelator (with non-coded amino acids) whose crystal structure has been established and which gelifies a variety of organic solvents including benzene, toluene, cyclohexane and *o*-dichlorobenzene.

Recently, we have established that a short peptide containing non-coded amino acids can self-assemble to form a supramolecular β-sheet in the crystal and amyloid like fibrils in the solid state.[6c] In the course of our continuing keen interest to search for various conformationally diverse short synthetic peptides which form amyloid-like fibril forming supramolecular β-sheets, peptides Boc-Ala(1)-Aib(2)-β-Ala(3)-OMe **1**[10] and Boc-Ala(1)-Gly(2)-β-Ala(3)-OMe **2**[11] have been synthesised.

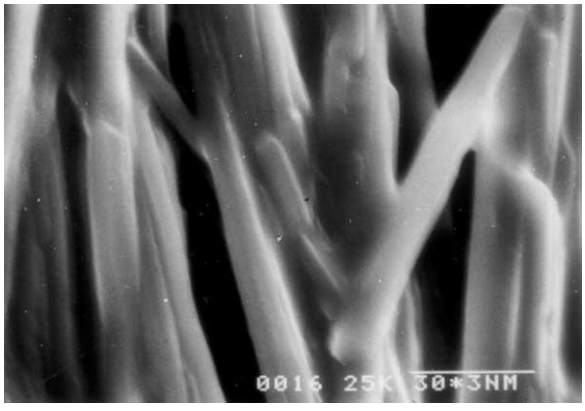

Figure 1. SEM micrograph of the dried gel of peptide **1** prepared from benzene.

Keywords: organogelator; Aib; synthetic peptide.
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0040-4039/03/$ - see front matter
doi:10.1016/S0040-4039(03)00839-6

While performing other structural studies, we have found that the peptide **1** forms thermoreversible transparent gels in many organic solvents such as cyclohexane and benzene.

The morphology of the dried gel (Fig. 1), investigated by scanning electron microscopy (SEM), exhibits an entangled network made of long rod-like fibres. The regular shape and morphological aspect of these fibres must arise from a strong anisotropic growth process, indicating that the fibres have well-ordered molecular packing.

The thermal behaviour of the gel has been studied by differential scanning calorimetry (DSC-7) and both heating and cooling thermograms (Fig. 2) indicate the reversible first order phase transition, i.e., peptide **1** produces a thermoreversible gel.[12,13] This gelation is observed even as low as 0.5% (w/v) so it may be termed a novel organo-gelator.

Aggregation of peptide **1** in benzene has been studied by FT-IR spectroscopy. At low concentration (<0.3% w/v gelator) peptide **1** shows a single sharp peak, at 3419 cm^{-1} and a shoulder at 3377 cm^{-1}, both independent of concentration, corresponding to NH stretching frequencies. The sharp peak is characteristic of the non-hydrogen bonded NH and the shoulder at 3377 cm^{-1} is assigned to hydrogen bonded NHs. There is a gradual decrease in intensity of the peak corresponding to non-hydrogen bonded NHs and an increase in intensity for hydrogen bonded NH peaks with an increase in concentration of the gelator peptide **1**. However, in 5% w/v gelator, only the strong intensity peaks at 3346 and 3282 cm^{-1} corresponding to hydrogen bonded NHs appear. Concentration dependent FT-IR studies clearly indicate that self-association of the peptide **1** is mediated by intermolecular hydrogen bonds.

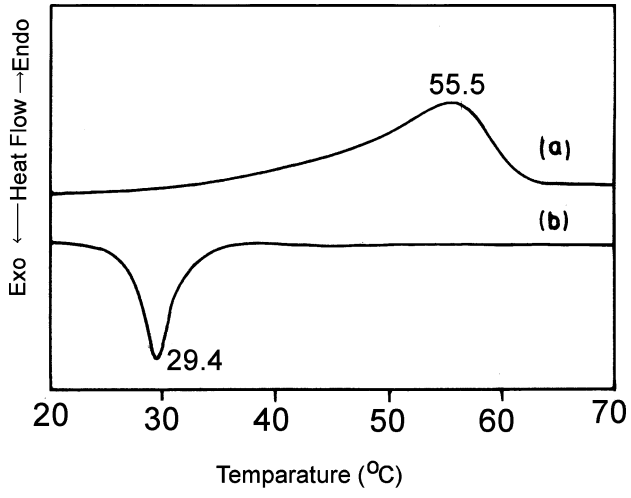

Figure 2. DSC thermograms of 5% (w/v) peptide **1** gel in benzene: (a) heating, at the rate of 10°C/min; (b) cooling, at the rate of 5°C/min.

NMR studies have been performed to probe further the conformational features of the gel-forming peptide **1** in C_6D_6. Solvent perturbation experiments have been performed by adding a small amount of the strongly hydrogen bonding solvent $(CD_3)_2SO$ in C_6D_6 solution at 0.16% (w/v) concentration, where gelation does not take place, to delineate, intramolecularly hydrogen bonded NHs.[14] For this reported peptide only two NH groups, viz. Ala(1)NH, and Aib(2)NH exhibit chemical shifts which are appreciably dependent upon solvent composition. The other NH group (β-Ala(3)NH) does not exhibit significant changes upon the addition of $(CD_3)_2SO$ and it is therefore solvent-shielded, suggesting its involvement in an intramolecular hydrogen bond. Concentration-dependent NMR experiments have been carried out in order to investigate which NH groups are involved in the self-assembly of the gelator molecule mediated by intermolecular hydrogen bonding. The results of these studies within the concentration range 0.16% (w/v) to 2% (w/v) of the gelator molecule reveal that the δ value change for NH(3) is only 0.21, whereas for NH(1) and NH(2) the δ value changes are 0.45 and 0.38, respectively. This indicates that NH(1) and NH(2) are involved in intermolecular hydrogen bonding and that the NH(3) may form a weak intermolecular hydrogen bond, apart from its participation in intramolecular hydrogen bonding, to form the aggregated gel state. The temperature dependence of the chemical shifts for the gelator peptide **1** in C_6D_6 (1% w/v) has been measured and the temperature range was from 25°C (gel state) to 70°C (solution state). Significant changes in chemical shifts have been observed for NH(1) and NH(2). However, the shift for NH(3) does not alter appreciably. This result supports the previous experiments indicating the involvement of NH(1) and NH(2) in intermolecular hydrogen bonding to form the aggregated gel state.

The crystal structure of the gelator peptide **1**[15] provides a valuable, insight into the molecular arrangement of this self-assembling molecule that might be responsible for forming the gel-fibre network. We were able to obtain single crystals of the gelator molecule, peptide **1** from an ethanol–dichloromethane mixture. The crystal structure of the peptide **1** (Fig. 3) reveals that it adopts a 10-atom intramolecular H-bonded β-turn, positioning Ala(1) and Aib(2) as the corner residues. The corresponding ϕ, ψ values of these residues (Table 1) indicate that peptide **1** possesses the Type II β-turn conformation in the solid state. Each 10-membered ring (β-turn) then self-assembles via one intermolecular hydrogen bond to form a semi-cylindrical ribbon structure along the short crystallographic *b* axis. These ribbons are connected by two unique intermolecular hydrogen bonds to form a two-dimensional double-columnar sheet-like structure (Fig. 4). The hydrogen bonding parameters are listed in Table 2. There is only one intramolecular hydrogen bond (N4–H···O11) to lock the molecule into a β-turn conformation (Fig. 3). There are two intermolecular hydrogen bonds for peptide **1** (N7–H7···O5, N10–H10···O8) to connect individual molecules to form and stabilise the supramolecular network assembly.

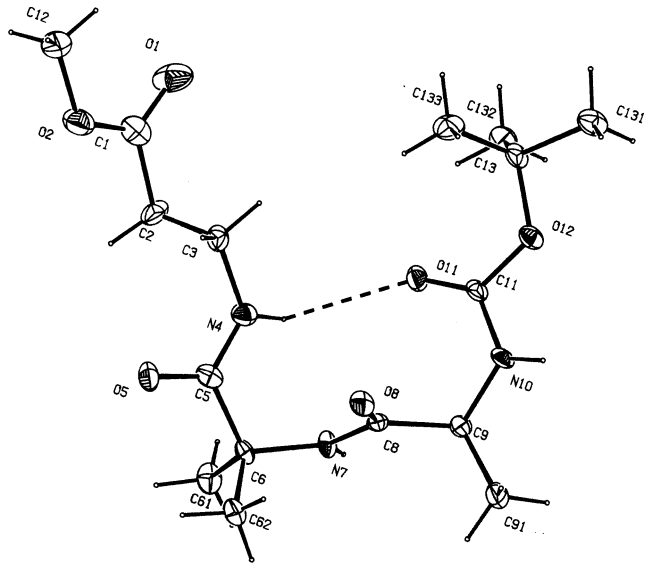

Figure 3. The ORTEP diagram of peptide **1** showing the atomic numbering scheme. Ellipsoids at 20% probability. The intramolecular hydrogen bond is shown as a dotted line.

Table 1. Backbone torsion angles of peptides **1** and **2** in the crystal state

Torsion angle		Peptide **1**	Peptide **2**
C9–N10–C11–O12	ω_0	−171.2(5)	−174.8(5)
C8–C9–N10–C11	$\phi 1$	−58.0(8)	−137.1(5)
N7–C8–C9–N10	$\psi 1$	134.7(6)	118.7(5)
C6–N7–C8–C9	ω_1	173.3(6)	−179.7(5)
C5–C6–N7–C8	$\phi 2$	62.9(9)	−68.1(7)
N4–C5–C6–N7	$\psi 2$	23.9(9)	154.7(5)
C3–N4–C5–C6	ω_2	−177.8(6)	179.9(5)
C2–C3–N4–C5	$\phi 3$	−81.4(10)	−79.3(8)
C1–C2–C3–N4	$\theta 3$	−173.0(9)	−60.4(8)
O2–C1–C2–C3	$\psi 3$	−99.3(12)	−177.9(6)

From previous studies it is evident that a small change in structure of the gelator and gelating conditions can shift the balance in favour of the crystalline state.[9] Thus, for example, peptide **2** which is identical to peptide **1** apart from the replacement of Aib(2) by Gly(2) does not form any gel under similar conditions. This indicates that the conditions and interactions responsible for gelation of the peptide **1** are very specific. The peptide **2** is highly crystalline in toluene and benzene. We were able to obtain single crystals of peptide **2**[15] from an ethyl acetate–toluene mixture. The torsion angles and dimensions of the hydrogen bonds of peptide **2** are listed in Tables 1 and 2, respectively. The crystal structure of the non-gelator peptide **2** (Fig. 5) reveals that it is extended in nature and forms an intermolecular-hydrogen bonded β-sheet structure (Fig. 6). The absence of gelation properties (under similar conditions) in peptide **2** when compared to peptide **1** may be due to the loss of the solvophobic-surface of the peptide strand as it has a centrally located Gly residue instead of the Aib (di-methyl Gly) residue present in peptide **1**.

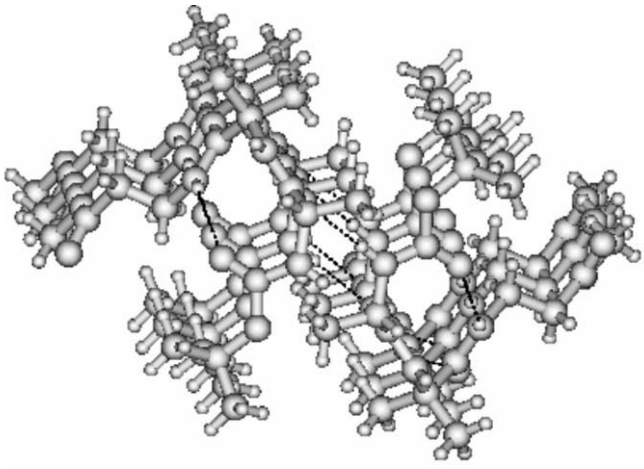

Figure 4. Crystal packing diagram of peptide **1** showing the double-columnar sheet-like structure.

We have discovered a novel and highly efficient peptide gelator, using non-coded amino acids, which shows aggregation propensities in organic solvents through hydrogen bonding. By applying various techniques including single-crystal X-ray diffraction studies, FT-IR and NMR we have established that the self-assembled peptide supramolecular structure responsible for gelation is formed by hydrogen bonds. From this study, it is evident that a turn-forming peptide provides the unique supramolecular fibrous network for gelation.

Table 2. Hydrogen bonds in peptide **1** and peptide **2**

Molecule	Type	Donor	Acceptor	N···O (Å)	H···O (Å)	N–H···O (°)
Peptide **1**	Intra	N4	O11	3.17	2.48	138
	Inter[a]	N7	O5	3.08	2.24	159
	Inter[b]	N10	O8	2.19	3.06	177
Peptide **2**	Inter[c]	N4	O8	2.97	2.12	175
	Inter[d]	N7	O5	2.89	2.05	167
	Inter[e]	N10	O1	3.02	2.23	153

[a] x, $y-1$, z.
[b] $1-x$, $y-0.5$, $1-z$.
[c] $0.5+x$, $0.5-y$, $-z$.
[d] $0.5+x$, $1.5-y$, $-z$.
[e] $-0.5+x$, $0.5-y$, $-z$.

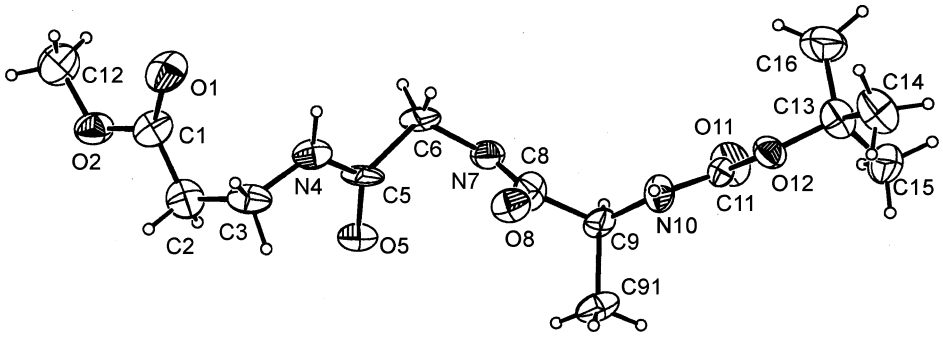

Figure 5. The ORTEP diagram of peptide **2** showing the atomic numbering scheme. Ellipsoids at 20% probability.

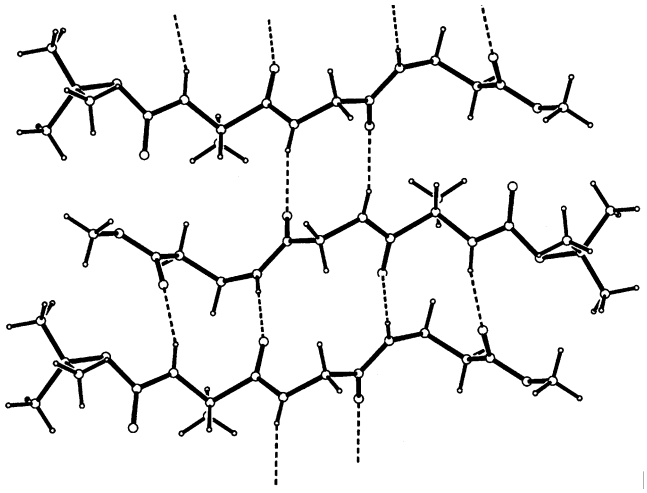

Figure 6. Crystal packing diagram of peptide **2** showing the intermolecular antiparallel β-sheet structure.

Acknowledgements

We thank EPSRC and the University of Reading, UK, for funds for the Image Plate System. This research is also supported by a grant from the Department of Science and Technology (DST), India. S. K. Maji wishes to acknowledge the Council for Scientific & Industrial research (C.S.I.R), New Delhi, India for financial assistance.

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10. The peptide Boc-Ala(1)-Aib(2)-β-Ala(3)-OMe **1** ($C_{16}H_{29}N_3O_6$) was synthesised by conventional solution phase methodology (Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: New York, 1984; pp. 1–282).

300 MHz ^{1}H NMR ($CDCl_3$, δ ppm): 6.99 [β-Ala(3) NH, 1H, $J=5$ Hz, t]; 6.59 [Aib(2) NH, 1H, s]; 5.02 [Ala(1) NH, 1H, $J=6.1$ Hz, d]; 4.02 [$C^{\alpha}H$ Ala(1), 1H, m]; 3.68 [-OCH_3, 3H, s]; 3.47–3.53 [$C^{\beta}H$ β-Ala(3), 2H, m]; 2.52–2.56 [β-Ala(3) $C^{\alpha}Hs$, 2H, m]; 1.50, 1.51 [$C^{\beta}Hs$ Aib, 6H, s]; 1.45 [Boc-CH_3s, 9H, s]; 1.32–1.34 [$C^{\beta}Hs$, Ala(1), 3H, $J=5.6$ Hz, d]; MS ($M+Na^+$)=382, $M_{calcd}=359$. Anal. calcd for $C_{16}H_{29}N_3O_6$ (359): C, 53.48; H, 8.08; N, 11.69. Found: C, 53.32; H, 7.99; N, 11.82. Mp 112°C.

11. The peptide Boc-Ala(1)-Gly(2)-β-Ala(3)-OMe **2** ($C_{14}H_{25}N_3O_6$) was synthesised by conventional solution phase methodology (Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: New York, 1984; pp. 1–282).
300 MHz ^{1}H NMR ($CDCl_3$, δ ppm: 7.35 [β-Ala(3) NH, 1H, $J=4.8$, t]; 6.87 [Gly(2) NH, 1H, $J=5.1$, t]; 5.18 [Ala(1) NH, 1H, $J=5.8$, d]; 4.14 [$C^{\alpha}H$ Ala(1), 1H, m]; 3.90–3.94 [$C^{\alpha}Hs$ Gly(2), 2H, m]; 3.69 [-OCH_3, 3H, s]; 3.45–3.59 [β-Ala(3) $C^{\beta}Hs$, 2H, m]; 2.52–2.57 [β-Ala(3) $C^{\alpha}Hs$, 2H, m]; 1.44 [Boc-CH_3s, 9H, s]; 1.38–1.39 [$C^{\beta}Hs$ Ala(1), 3H, $J=7.1$, d]; MS ($M+Na^+$)=354, $M_{calcd}=331$. Anal. calcd for $C_{14}H_{25}N_3O_6$ (331): C, 50.75; H, 7.55; N, 12.68. Found: C, 50.48; H, 7.76; N, 12.72. Mp 99°C.
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15. Intensity data were collected with MoKα radiation using the MAR Research Image Plate System The crystals were positioned 70 mm from the Image Plate. 100 frames were measured at 2° intervals with a counting time of 5 min to give 2207, 3255 independent reflections, respectively. Data analysis was carried out with the XDS program.[16] The structures were solved using direct methods with the Shelx86 program.[17] The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were included in geometric positions and given thermal parameters equivalent to 1.2 times those of the atom to which they were attached. The structures were refined on F^2 using Shelxl[18] to give final R values of R_1 and wR_2 of 0.0748, 0.1969 and 0.0849, 0.1799 for 1007, 1645 data with $I>2\sigma(I)$, respectively. The largest peak and hole in the final difference Fourier were 0.12, −0.18 and 0.15, −0.16 e $Å^{-3}$, respectively. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 183406 and CCDC 183407 for peptides **1** and **2**, respectively. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223336-033; e-mail: deposit@ccdc.cam.ac.uk).
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