Water soluble synthetic dieptide-based biodegradable nanoporous materials†

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Two water-soluble synthetic dipeptides, β-alanyl-L-phenylglycine and its retro analogue L-phenylglycyl-β-alanine form a new class of dipeptide-based nanoporous materials, which are composed of a hybrid of α and β-amino acids. These materials adsorb N_2 gas with adsorption capacity of 173 cc g^{-1} and 71 cc g^{-1} and BET surface area of 56.76 m^2g^{-1} and 41.73 m^2g^{-1} for these dipeptides, respectively. Moreover, these nanoporous materials are found to be biodegradable towards soil bacterial consortium making them an interesting class of eco-friendly dipeptide-based nanoporous materials.

Introduction

Nanoporous materials based on purely inorganic materials such as zeolites[1] (aluminosilicates) and hybrid materials, consisting of both organic and inorganic counterparts such as metal–organic frameworks (MOFs),[2] have been extensively studied. These porous materials can be used in gas storage, chiral recognition, molecular separation, ion exchange, catalysis, and in sensors.[3] However, zeolites and MOFs are not based on purely organic molecules. There are a few examples of nanoporous materials based on purely organic molecules and these are termed as organo zeolites.[4–6] These include cyclic *bis*-urea based porous materials that bind reversibly with guest molecules,[4] Gorbitz's Val-Ala and Phe-Phe class structures formed from hydrophobic dipeptides[5] and Ripmeester's dipeptide-based microporous (nanoporous) materials that can adsorb inert gas such as Xe.[6] Recently Sozzani and co-workers have reported dipeptide-based nanoporous materials and investigated their absorption, separation, and storage of gases such as methane, carbon dioxide, and hydrogen.[7] Some dipeptide-based compounds are able to host small organic molecules.[5f,8] A recent report describes that a polymeric sorbent has been used for very efficient removal of H_2S from a mixture of gases for hydrogen purification.[9] Nanoporous materials based on water soluble peptide-based molecules have advantages over purely inorganic and hybrid materials, because they are of biological origin and generally non-toxic, eco-friendly and may be used as nanobiomaterials. Environmentally benign nanomaterials are an emerging field of current research.[10] However, none of the previously mentioned organic nanoporous materials are experimentally proven as biodegredable nanomaterials.

It is also very challenging to degrade non-protein amino acid based peptides. Seebach and coworkers have reported that soil bacterial consortium[11] can grow with β-amino acid containing peptides as the sole carbon and energy source.[12] Moreover, two aminopeptidases capable of degrading N-terminal β-alanine containing peptides, namely, L-aminopeptidase-D-alanine-esterase/amidase (DmpA) from *Ochrobactrum anthropi* and β-Ala-Xaa dipeptidase BapA from *Pseudomonas* sp. MCI3434 (Ps BapA), have been identified and characterized.[13] In this paper we report, a new class of water soluble dipeptide-based nanoporous materials, which are composed of a hybrid of β,α-amino acids having sequences β-alanyl-L-phenylglycine (β-Ala-L-Phg, **1**) and its retro analogue L-phenylglycyl-β-alanine (L-Phg-β-Ala, **2**). β-Alanine (β-Ala) is a non-coded naturally occuring β-amino acid and it has a role in a variety of biological processes.[14] It is believed to be a neurotransmitter in the central nervous system, binding to receptor sites common to glycine and γ-amino butyric acid (GABA), and acting in the visual system.[14a] β-Ala is one of the constituents of the naturally occuring dipeptides anserine and carnosine.[14c,d] α-Phenylglycine (Phg) is a non protein α-amino acid. Its molecular structure is more rigid than naturally occuring α-phenylalanine (Phe) with a lower degree of rotational freedom, as it has one CH_2 unit less than phenylalanine. Nanoporous materials formed by dipeptides **1** and **2** are structurally different from the previously reported dipeptide-based nanoporous materials,[5,6] as they are composed of both α and β-amino acids instead of just α-amino acids. Moreover, these hybrid β,α-amino acid based nanoporous materials are found to be biodegradable towards a consortium of bacteria,[11] making them a novel, ecofriendly, new class of nanoporous materials. These compounds also adsorb nitrogen gas.[2a–e,j]

Results and discussion

Two water soluble dipeptides, where β-alanine and L-phenylglycine are used as constituents, β-Ala-L-Phg (**1**), and its retro analogue L-Phg-β-Ala (**2**) (Fig. 1a and b) have been synthesized by conventional solution-phase methodology,[15,16]

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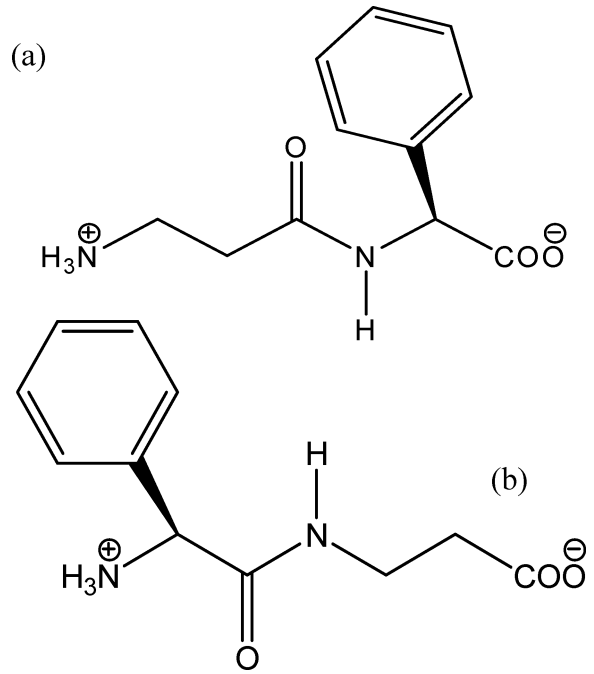

Fig. 1 The molecular structures of the dipeptides (a) **1** and (b) **2**.

purified, characterized, and studied. Gorbitz's hydrophobic dipeptide-based microporous materials[5] are based exclusively on α-amino acids with pore diameters in the range of 3–10 Å. In contrast to the previous work, two reported dipeptide-based microporous materials are composed of a hybrid of β and α-amino acids, where one of the components is a β-amino acid (β-alanine) and the other component is an α-amino acid (L-phenylglycine) with varying pore size. These pore sizes are in the range of 6.4–3.2 Å indicating that they are comparable to the previously reported dipeptide-based nanoporous materials.

X-ray powder diffraction (XRPD) studies

The synthesized dipeptides **1** and **2** were separately grounded to a powder and examined by X-ray powder diffraction (XRPD) (Fig. 2). Dipeptide **1** has peaks at $2\theta = 7°$, 10.6°, 11.6°, 12.1°, 14.2°, 16.5°, 20°, 21.3°, 21.9°, 24°, 24.4°, 28° and dipeptide **2** has peaks at $2\theta = 5.6°$, 7.6°, 11.5°, 16°, 20.8°, 21.9°, 23°, 24.3°, 24.7°, 26.8°, 28.5°, 29.7°. These two dipeptides are retro analogues with each other and their XRPD patterns are slightly different. The sharp lines in the XRPD patterns (Fig. 2) suggest the crystalline behavior of these two compounds. The above mentioned peaks give an indication that nanopores may be present in these structures.[2h,17] To confirm the nanoporous structures we further investigated using TEM and gas adsorption experiments.

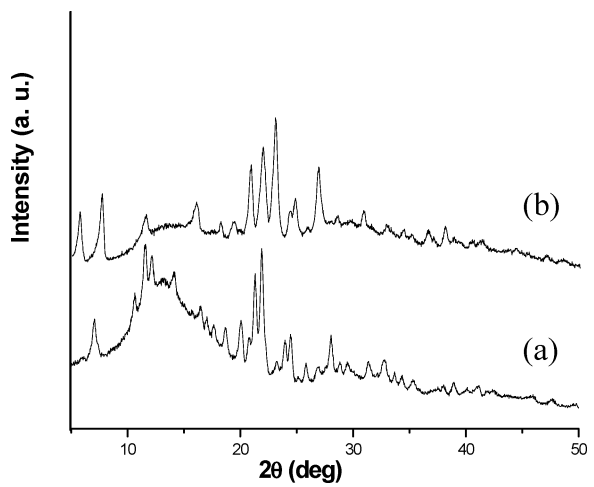

Fig. 2 XRPD patterns of the dipeptides (a) **1** and (b) **2** synthesized (ground at room temperature).

Transmission electron microscopic (TEM) studies

To prove their porous architecture we have studied Transmission electron microscopy (TEM). TEM studies of dipeptides **1** and **2** were carried out using an aqueous solution of the corresponding compounds (3 mg mL^{-1}) on a carbon coated copper grid (300 mesh) by slow evaporation and vacuum drying at 30 °C for 2 days. The TEM images and electron diffraction patterns clearly indicate that the formation of nanoporous architecture with dimensions of 5.8 Å × 6.4 Å and 3.2 Å × 3.2 Å for dipeptides **1** and **2** respectively (Fig. 3 and Fig. 4). TEM images clearly show that dipeptides **1** and **2** form an ordered porous framework. Selected area electron diffraction pattern of these porous architectures show sharp spots. This suggests that these porous structures are crystalline in nature. Energy dispersive X-ray (EDX) analyses of nanoporous structures indicate the presence of C, N and O (ESI† Fig. S1a and b). C, N and O come from the dipeptide.

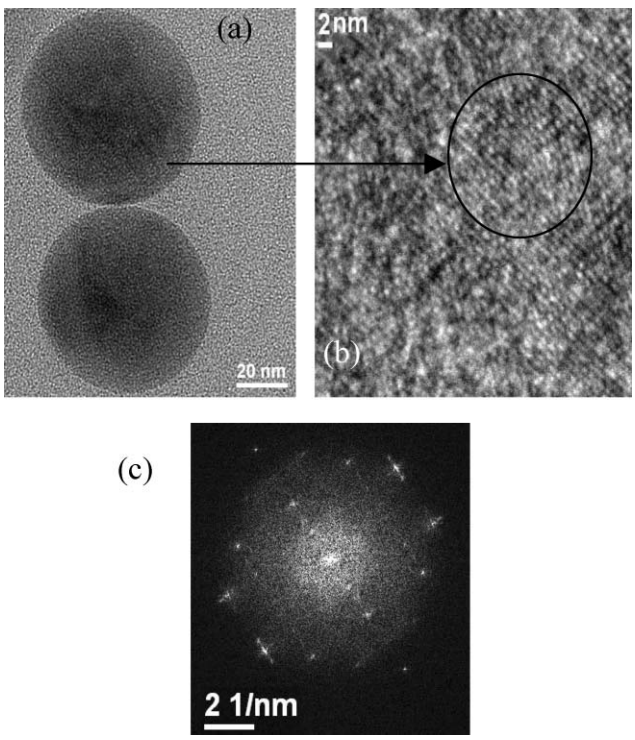

Fig. 3 TEM image of dipeptide (a) **1** showing the formation of an ordered nanoporous structure; enlarged portion of the figure (a) is shown in figure (b). (c) Electron diffraction pattern of the porous structure formed by dipeptide **1** shows sharp spots, indicating its crystalline behaviour.

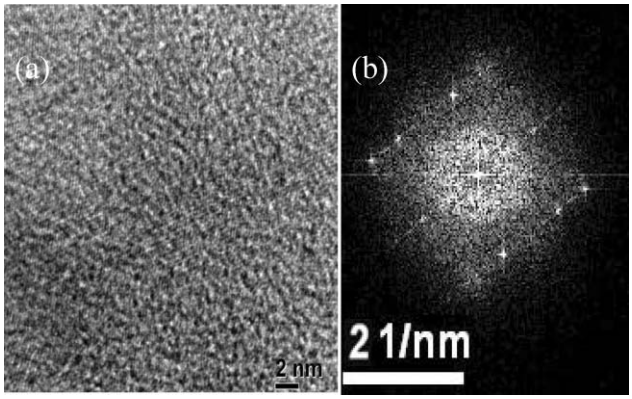

Fig. 4 TEM image of (a) dipeptide **2** showing the formation of an ordered nanoporous structure. (b) Sharp spots in the selected area electron diffraction pattern of the porous structure formed by dipeptide **2** indicate its crystallinity.

Nitrogen gas adsorption experiments

To prove the porous architecture of dipeptides **1** and **2** we also carried out N_2 gas adsorption experiments. The N_2 adsorption–desorption isotherms (77 K)[2a–e,j] of dipeptides **1** and **2** are shown in Fig. 5 and are close to the type I adsorption isotherm typical for a nanoporous material.[2a–e,j,4c] Gas adsorption data show that the adsorption capacities of dipeptides **1** and **2** are about 173 cc g^{-1} and 71 cc g^{-1}, respectively. Pore sizes of dipeptides **1** and **2** were also confirmed by the N_2 gas adsorption studies. From the nitrogen adsorption at low P/P_0 we observed the following pore size distribution of the sample using the Horvath–Kawajoe (H–K) method (micropore analysis). The pore size distribution curve of dipeptide **1** showed two peaks centered at 5.8 Å and 6.4 Å (inset Fig. 5a) indicating that dipeptide **1** forms a nanoporous architecture. The pore size distribution curve of dipeptide **2** showed one peak centered at 3.2 Å (inset Fig. 5b) also indicating that dipeptide **2** forms a nanoporous architecture. From the Brunauer–Emmett–Teller (BET) equation, the BET surface areas were calculated as 56.76 m^2g^{-1} and 41.73 m^2g^{-1} for dipeptides **1** and **2**, respectively (Fig. 6). Prior to the analyses these samples were degassed at 40 °C for 5 h.

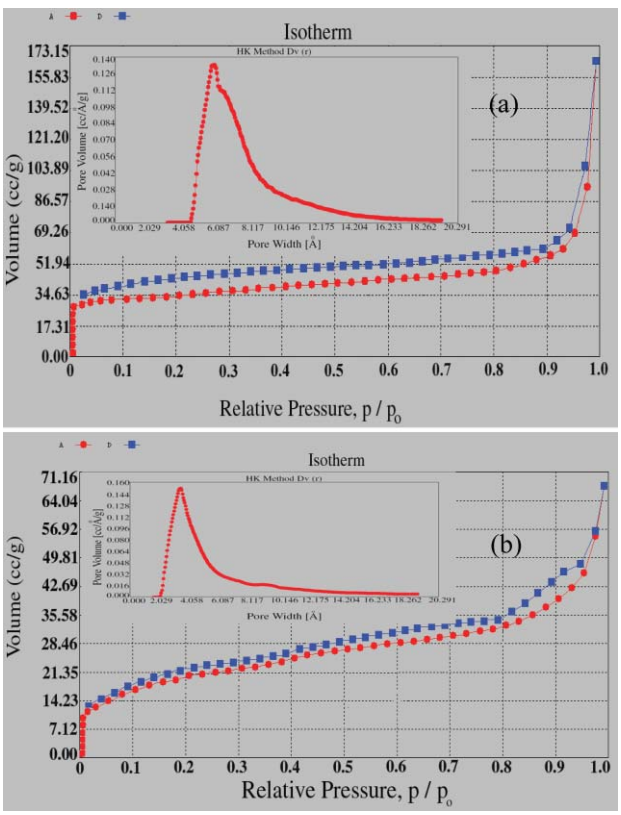

Fig. 5 N_2 gas adsorption isotherms (type I), adsorption ■, desorption ■ for dipeptides (a) **1** and (b) **2**. Adsorption capacities are 173 cc g^{-1} and 71 cc g^{-1} for **1** and **2**, respectively. Insets represent the pore size distribution curves showing pore sizes of 5.8 Å × 6.4 Å and 3.2 Å × 3.2 Å of dipeptides **1** and **2**, respectively.

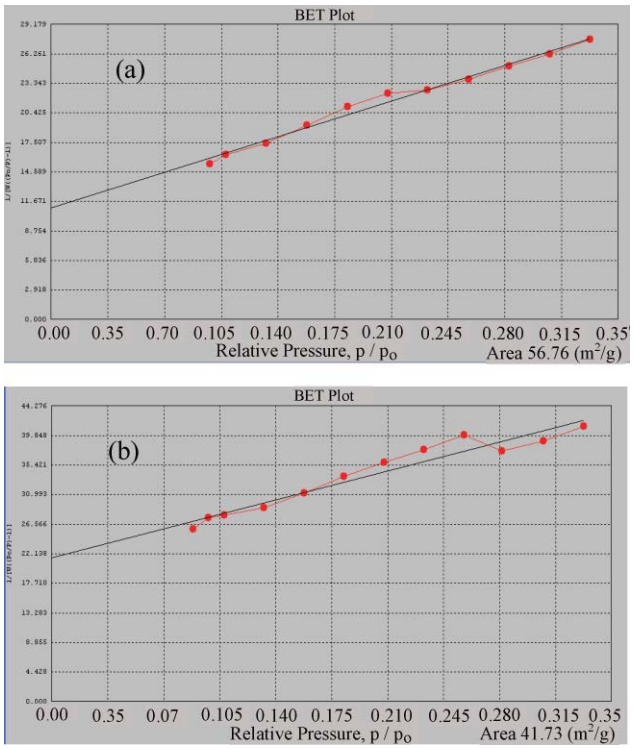

Fig. 6 BET equation shows that the BET surface areas of dipeptides (a) **1** and (b) **2** are 56.76 m^2g^{-1} and 41.73 m^2g^{-1}, respectively.

From TEM images, XRPD patterns and N_2 gas adsorption data it can be said that these reported dipeptides form crystalline nanoporous structures. However, the pore sizes of these two dipeptides varied slightly.

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) experiments

Dipeptides **1** and **2** showed significant thermal stability in the solid state, as demonstrated by TGA-DTA experiments. The TGA-DTA experiments showed that no solvent molecules are present in the crystal lattice, which is also confirmed from their elemental data analyses. These dipeptides showed no decomposition, phase transitions or mass loss up to their melting points of ~270 °C and ~240 °C, respectively (Fig. 7). The high melting points of these dipeptides indicate that the nanoporous frameworks are highly stable at a high temperature.

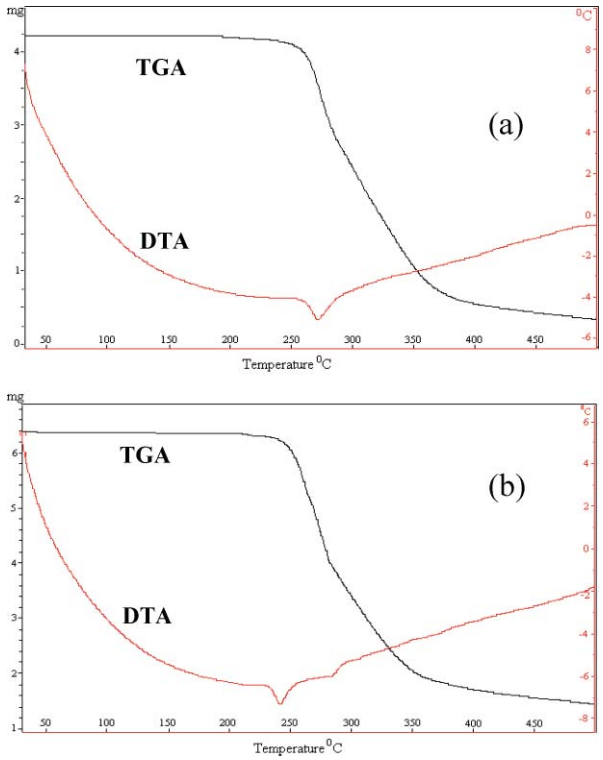

Fig. 7 TGA-DTA curves of the dipeptides (a) **1** and (b) **2**. It is clear from these figures that no solvent molecule is present within the crystal lattice.

Biodegradability test

Soil bacterial population can utilize and degrade many natural and synthetic materials, and they often do it as a social network, or community. Such communities can be selected from a pre-existing population. We wanted to test this hypothesis against the dipeptide nanoporous materials described here because it is challenging to degrade these non-protein amino acid based dipeptides. Biodegradation of the dipeptide nanoporous materials was performed using a soil derived culturable bacterial consortium.[18] The bacterial consortium was selected in two steps. First, in a low level non-specific peptide as nutrient and then in the specific dipeptide in same medium. After selection on a specific dipeptide, the growth of the selected bacterial population in the presence of a dipeptide as the major carbon and nitrogen source was measured in comparison with a control without bacteria. The growth was monitored for a period of eight weeks at 30 °C. Growth was measured from optical density (OD) taken at 595 nm. The degradation of the nanoporous materials based on dipeptides **1** and **2** have been detected using mass spectrometric analysis of these nanomaterials containing soil bacterial consortium with respect to different time intervals (0 h, 24 h, 7 d, 14 d). It was observed that intensity of the peak corresponding to the $[M + H]^+$ (where M = molecular ion of dipeptides **1** and **2**) gradually decreased with respect to time and after 14 d the intensity was diminished to a great extent leading to the appearance of a tiny peak of $[M + H]^+$ in the spectrum (Fig. 8) (ESI† Fig. S2 and S3). This clearly indicates that dipeptide nanomaterials have been used as food for soil bacterial consortium. The growth was further confirmed

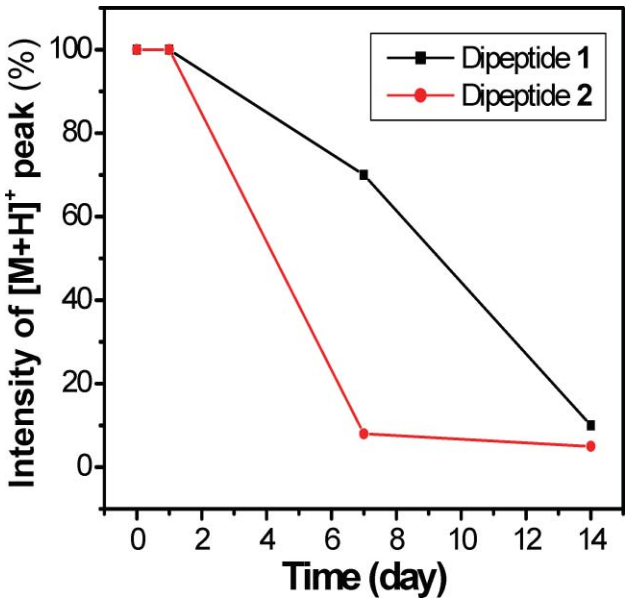

Fig. 8 Time dependent biodegradation of dipeptides **1** and **2** at 0 h, 24 h (1 d), 7 d and 14 d after treatment with bacterial consortium quantified through ESI-MS data. Intensity of the molecular ion peak for dieptides **1** and **2**, $[M + H]^+$ gradually decreases with respect to time.

by colony formation assay on agar plates (ESI† Fig. S4). The growth of bacteria in the dipeptide containing medium was observed in parallel with the depletion of each dipeptide in the medium, and indicated biodegradation as well as utilization of the nanomaterial for subsistence (Fig. 9). Dipeptides **1** and **2** were utilized easily by the bacterial consortium, which is also confirmed from their respective mass spectra. Viability was tested with 1% peptone in M9 media[19] where the bacteria give good growth. However, bacterial consortia from other sources may be tried to challenge the recalcitrant nature of this material. The population of bacteria utilizing dipeptide was found to be

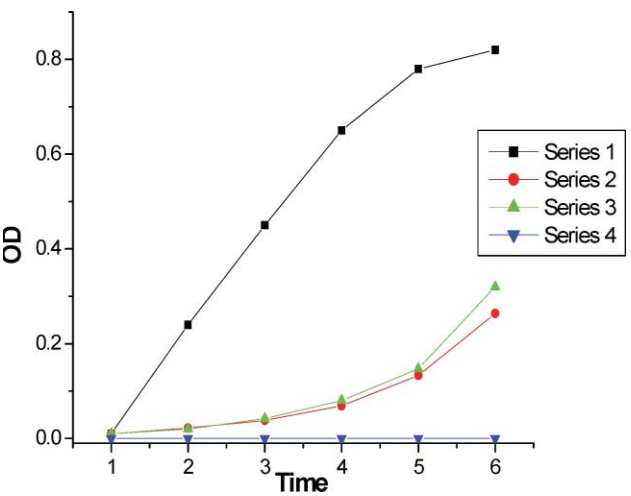

Fig. 9 The growth of bacterial consortium on dipeptides, where series 2 and 3 represent dipeptides **1** and **2**, respectively. Series 1 represents the same consortium grown on 1% peptone in M9 medium and series 4 is the same as series 2, but uninoculated to serve as background control. OD_{595} was plotted against time, (1, 2, 3, 4, 5, 6 time scale represents time 0 h, 6 h, 12 h, 24 h, 7 d, 14 d). Growth remained stalled and did not decline much for at least 4 weeks.

of a mixed variety, and the dominant *Pseudomonas* sp. isolated from the population failed to utilize or degrade the dipeptide in isolation see Fig. 10. Apparently, the mixed population is needed for its utilization. However, further studies will be needed to prove this point conclusively. The solubility and uptake of dipeptide may not be the decisive factor in these bacterial subsistence and degradation phenomena. The two dipeptides are water soluble. This result indicates that some bacterial populations can subsist on the peptide–nanomaterial, which makes these nanomaterials environmentally safe. It is expected that a soil bacterial population would be able to degrade such material once disposed to the environment. Recently, bacteria subsisting on antibiotics have been isolated from soil, and shown to be phylogenetically diverse.[20] The same may hold for nanomaterials formed from peptides, carbohydrates, hydrocarbon and nucleic acids.

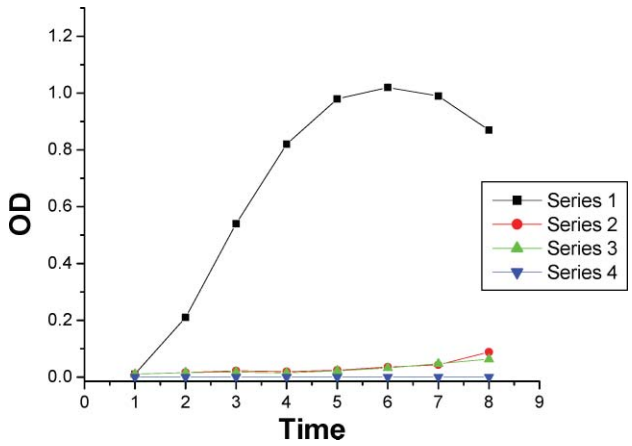

Fig. 10 The growth of a clonally selected *Pseudomonas* sp., a dominant member of the same bacterial consortia failed to utilize or degrade the dipeptide, where series 2 and 3 represent dipeptides **1** and **2**, respectively. Series 1 represents the same consortium grown on 1% peptone (control) in M9 medium and series 4 is the same as series 2, but uninoculated to serve as the background control. OD_{595} was plotted against time, (1, 2, 3, 4, 5, 6, 7, 8 time scale represents time 0 h, 6 h, 12 h, 24 h, 7 d, 14 d, 30 d, 60 d).

Comparison between hydrophobic micropous materials, Ala-Val, Val-Ala with these reported dipeptides

The reported dipeptides are structurally different from the previously reported Ala-Val and Val-Ala dipeptides[6] because the reported dipeptides are composed of both α and β-amino acid residues instead of just α-amino acids. However, all these compounds are nanoporous materials. Moreover, the reported dipeptides **1** and **2** are experimentally proven to be biodegradable nanoporous materials.

Conclusions

In conclusion this study indicates a new class of self-assembling water soluble dipeptide-based nanoporous environmentally benign materials in which the constituent dipeptides are made up of a hybrid of α and β-amino acid residues, instead of conventionally used dipeptides containing only α-amino acid residues.[5,6] These nanoporous materials have been used for adsorption and desorption of N_2 gas. Moreover, the biodegradibility of these nanobiomaterials towards soil bacterial consortium and water solubility makes them interesting candidates in green nanobiotechnology. Our results clearly indicate that the reported dipeptides composed of a hybrid of α and β-amino acids are amenable to biodegradation and that a soil bacterial consortium was able to utilize these dipeptides as sole carbon and energy sources. Water soluble, biodegradable short peptide-based nanoporous materials can be regarded as a new entry in the plethora of nanoporous materials, which are environmentally safe and can be used as eco-friendly nanobiomaterials.

Experimental section

Dipeptide synthesis

The dipeptides **1** and **2** were synthesized by conventional solution-phase methods using racemization free fragment condensation strategy. The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). Methyl ester deprotection was performed *via* the saponification method, and the Boc group was deprotected by 98% formic acid. All the intermediates were characterized by 300 MHz ^{1}H NMR and mass spectrometry. The final compounds were fully characterized by 300 MHz ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, DEPT 135, mass spectrometry.

(a) Boc-β-Ala-OH. See reference 16.

(b) Boc-Phg-OH. A solution of L-phenylglycine (4.53 g, 30 mmol) in a mixture of dioxane (60 mL), water (30 mL), and 1 N NaOH (30 mL) was stirred and cooled in an ice-water bath. Di-*tert*-butylpyrocarbonate (7.2 g, 33 mmol) was added, and stirring was continued at room temperature for 6 h. Then the solution was concentrated in a vacuum to about 20–30 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (about 50 mL) and acidified with a dilute solution of $KHSO_4$ to pH 2–3 (Congo red). The aqueous phase was extracted with ethyl acetate, and this operation was carried out repeatedly. The ethyl acetate extracts were pooled, washed with water, dried over anhydrous Na_2SO_4, and evaporated in a vacuum. The pure material was obtained.

Yield: 6.58 g (26.2 mmol, 87%).

Anal. Calcd for $C_{13}H_{17}NO_4$ (251): C, 62.14; H, 6.82; N, 5.57. Found: C, 62.19; H, 6.75; N 5.59%.

(c) Boc-β-Ala(1)-Phg(2)-OMe. 3.78 g (20 mmol) of Boc-β-Ala-OH was dissolved in 10 mL of DMF in an ice-water bath. H-Phg-OMe was isolated from 8.07 g (40 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and ethyl acetate extract was concentrated to 10 mL. It was then added to the reaction mixture, followed immediately by 4.12 g (20 mmol) of dicyclohexylcarbodiimide (DCC) and 2.7 g (20 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stirred for 3 d. The residue was taken up in ethyl acetate (40 mL) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1 N HCl (3 × 30 mL), brine (1 × 30 mL), 1 M sodium carbonate (3 × 30 mL),

brine (2 × 30 mL), dried over anhydrous sodium sulfate, and evaporated in a vacuum. A white material was obtained.

Yield: 5.99 g (17.8 mmol, 89%).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.31–7.36 (m, 5H, Phg(**2**) phenyl ring protons), 6.64 (d, J = 7.1 Hz, 1H, Phg(**2**) NH), 5.55 (d, J = 7.1 Hz, 1H, Phg(**2**) C$^{\alpha}$ H), 5.16 (br, 1H, β-Ala(1) NH), 3.73 (s, 3H, -OCH_3), 3.39 (q, J = 6.3 Hz, 2H, β-Ala(1) C$^{\beta}$ Hs); 2.47 (t, J = 6.3 Hz, 2H, β-Ala(1) C$^{\alpha}$ Hs), 1.42 (s, 9H, Boc CH_3).

HRMS m/z 359.3267 [M + Na]$^+$

Anal. Calcd for $C_{17}H_{24}N_2O_5$ (336): C, 60.70; H, 7.19; N 8.33. Found: C, 60.76; H, 7.11; N, 8.39%.

(d) Boc-Phg(1)-β-Ala(2)-OMe. 5.02 g (20 mmol) of Boc-Phg-OH was dissolved in 10 mL of DMF in an ice-water bath. H-β-Ala-OMe was isolated from 5.58 g (40 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and ethyl acetate extract was concentrated to 10 mL. It was then added to the reaction mixture, followed immediately by 4.12 g (20 mmol) of dicyclohexylcarbodiimide (DCC) and 2.7 g (20 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stirred for 3 d. The residue was taken up in ethyl acetate (40 mL) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1 N HCl (3 × 30 mL), brine (1 × 30 mL), 1 M sodium carbonate (3 × 30 mL), brine (2 × 30 mL), dried over anhydrous sodium sulfate, and evaporated in a vacuum. A white material was obtained.

Yield: 5.68 g (16.9 mmol, 84.5%).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30–7.34 (m, 5H, Phg(**1**) phenyl ring protons), 6.57 (br, 1H, β-Ala(**2**) NH), 5.86 (d, J = 6.5 Hz, 1H, Phg(**1**) NH), 5.12 (d, J = 6.5 Hz, 1H, Phg(**1**) C$^{\alpha}$ H), 3.59 (s, 3H, -OCH_3), 3.46 (q, J = 5.9 Hz, 2H, β-Ala(**2**) C$^{\beta}$ Hs), 2.46 (t, J = 6 Hz, 2H, β-Ala(**2**) C$^{\alpha}$ Hs); 1.40 (s, 9H, Boc CH_3).

HRMS m/z 337.0668 [M + H]$^+$, 359.0295 [M + Na]$^+$

Anal. Calcd for $C_{17}H_{24}N_2O_5$ (336): C, 60.70; H, 7.19; N 8.33. Found: C, 60.61; H, 7.23; N, 8.29%.

(e) Boc-β-Ala(1)-Phg(2)-OH. To 5.05 g (15 mmol) of Boc-β-Ala(**1**)-Phg(**2**)-OMe were added 35 mL MeOH and 25 mL 2 N NaOH, and the progress of saponification was monitored by thin-layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under a vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2 × 50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 N HCl, and it was extracted with ethyl acetate (3 × 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in a vacuum. A white crystalline material was obtained.

Yield: 3.99 g (12.4 mmol, 83%).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.32 (br, 1H, -COOH), 8.54 (d, J = 7.4 Hz, 1H, Phg(**2**) NH), 7.25–7.32 (m, 5H, Phg(**2**) phenyl ring protons), 6.62 (br, 1H, β-Ala(**1**) NH), 5.25 (d, J = 7.4 Hz, 1H, Phg(**2**) C$^{\alpha}$ H), 3.02–3.08 (q, 2H, J = 6.7 Hz, β-Ala(**1**) C$^{\beta}$ Hs); 2.28 (t, J = 6.7 Hz, 2H, β-Ala(**1**) C$^{\alpha}$ Hs); 1.30 (s, 9H, Boc CH_3).

HRMS m/z 345.3268 [M + Na]$^+$

Anal. Calcd for $C_{16}H_{22}N_2O_5$ (322): C, 59.61; H, 6.88; N, 8.69. Found: C, 59.52; H, 6.93; N, 8.61%.

(f) Boc-Phg(1)-β-Ala(2)-OH. To 5.05 g (15 mmol) of Boc-Phg(**1**)-β-Ala(**2**)-OMe were added 35 mL MeOH and 25 mL 2 N NaOH, and the progress of saponification was monitored by thin-layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under a vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2 × 50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 N HCl, and it was extracted with ethyl acetate (3 × 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in a vacuum. A white crystalline material was obtained.

Yield: 4.13 g (12.8 mmol, 85%).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.38 (br, 1H, –COOH); 8.20 (br, 1H, Phg(**1**) NH), 7.24–7.37 (m, 5H, Phg(**1**) phenyl ring protons), 7.19 (br, 1H, β-Ala(**2**) NH), 5.13 (d, J = 7 Hz, 1H, Phg(**1**) C$^{\alpha}$ H), 3.21 – 3.23 (m, 2H, β-Ala(**2**) C$^{\beta}$ Hs); 2.34 (t, J = 6 Hz, 2H, β-Ala(**2**) C$^{\alpha}$ Hs); 1.34 (s, 9H, Boc CH_3).

HRMS m/z 345.0036 [M + Na]$^+$, 667.0282 [2M + Na]$^+$.

Anal. Calcd for $C_{16}H_{22}N_2O_5$ (322): C, 59.61; H, 6.88; N, 8.69. Found: C, 59.54; H, 6.83; N, 8.75%.

(g) H_3N^+-β-Ala(1)-Phg(2)-COO^- (Dipeptide 1). To 3.22 g (10 mmol) of Boc-β-Ala(**1**)-Phg(**2**)-OH was added 4 mL of 98% formic acid, and the removal of the Boc group was monitored by TLC. After 8 h, formic acid was removed under a vacuum. The residue was taken in water (20 mL) and washed with diethyl ether (2 × 30 mL). The pH of the aqueous solution was then adjusted to 8 with 30% aqueous NH_3. The aqueous portion was evaporated in a vacuum to yield dipeptide **1** as white solid.

Yield: 1.64 g (7.4 mmol, 74%). m.p. 270 °C

^{1}H NMR (300 MHz, D_2O): δ = 7.24–7.33 (m, 5H, Phg(**2**) phenyl ring protons), 5.08 (s, 1H, Phg(**2**) C$^{\alpha}$ H), 3.13 (t, J = 6.7 Hz, 2H, β-Ala(**1**) C$^{\beta}$ Hs); 2.61 (t, J = 6.7 Hz, 2H, β-Ala(**1**) C$^{\alpha}$ Hs).

^{13}C NMR (75 MHz, D_2O): δ = 177.123 (C of COO^-), 171.570 (C of CONH), 138.427 (tertiary C of phenyl ring), 129.357 (m-C's of phenyl ring), 128.542 (p-C of phenyl ring), 127.783 (o-C's of phenyl ring), 60.129 (α-C of Phg), 36.023 (α-C of β-Ala), 32.214 (β-C of β-Ala).

DEPT 135 (D_2O): δ = 177.123, 171.570 and 138.427 (disappear); 129.357, 128.542, 127.783, 60.129 (positive); 36.023 and 32.214 (negative).

HRMS m/z 223.0015 [M + H]$^+$, 244.9728 [M + Na]$^+$, 466.9553 [2M + Na]$^+$.

Anal. Calcd for $C_{11}H_{14}N_2O_3.H_2O$ (222): C, 59.45; H, 6.35; N, 12.61.

Found: C, 59.39; H, 6.41; N, 12.72%.

(h) H_3N^+-Phg(1)-β-Ala(2)-COO^- (Dipeptide 2). To 3.22 g (10 mmol) of Boc Phg(**1**)-β-Ala(2)-OH was added 4 mL of 98% formic acid, and the removal of the Boc group was monitored by TLC. After 8 h, formic acid was removed under a vacuum. The residue was taken in water (20 mL) and washed with diethyl ether (2 × 30 mL). The pH of the aqueous solution was then adjusted to 8 with 30% aqueous NH_3. The aqueous portion was evaporated in a vacuum to yield dipeptide **1** as white solid.

Yield: 1.60 g (7.2 mmol, 72%). m.p. 240 °C

^{1}H NMR (300 MHz, D_2O): δ = 7.36–7.43 (m, 5H, Phg(**2**) phenyl ring protons), 4.99 (s, 1H, Phg(**2**) C$^{\alpha}$ H), 3.27–3.39 (m, 2H, β-Ala(**1**) C$^{\beta}$ Hs); 2.21–2.27 (m, 2H, β-Ala(**1**) C$^{\alpha}$ Hs).

^{13}C NMR (75 MHz, D_2O): $\delta = 180.117$ (C of COO^-), 168.748 (C of CONH), 134.480 (tertiary C of phenyl ring), 130.717 (m-C's of phenyl ring), 129.813 (p-C of phenyl ring), 128.375 (o-C's of phenyl ring), 58.899 (α-C of Phg), 37.339 (α-C of β-Ala), 36.624 (β-C of β-Ala).

DEPT 135 (D_2O): $\delta = 180.117$, 168.748 and 134.480 (disappear); 130.717, 129.813, 128.375, 58.899 (positive); 37.339 and 36.624 (negative).

HRMS m/z 222.9990 $[M + H]^+$, 244.9732 $[M + Na]^+$, 445.0010 $[2M + H]^+$, 466.9638 $[2M + Na]^+$.

Anal. Calcd for $C_{11}H_{14}N_2O_3.H_2O$ (222): C, 59.45; H, 6.35; N, 12.61.

Found: C, 59.49; H, 6.42; N, 12.58%.

NMR experiments. All 300 MHz NMR studies were carried out on a Brüker DPX 300 MHz spectrometer at 300 K.

Mass spectrometry. Mass spectra were recorded on a Qtof Micro YA263 high-resolution mass spectrometer.

Transmission Electron Microscopy. Transmission Electron Microscopy (TEM) was carried out to investigate the morphology of the nanoporous materials. TEM images were recorded on a JEOL JEM 2010 instrument.

X-Ray Powder Diffraction. XRPD were recordedon a X Pert PRO high resolution X-ray diffractometer instrument.

Gas Adsorption Experiment. Nitrogen adsorption/desorption isotherms were obtained using a Quantachrome Autosorb Automated Gas Sorption System at 77 K. Before the analysis the sample was degassed at 40 °C for 5 h.

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