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A New Molecular Scaffold for the Formation of Supramolecular Peptide Double Helices: The Crystallographic Insight

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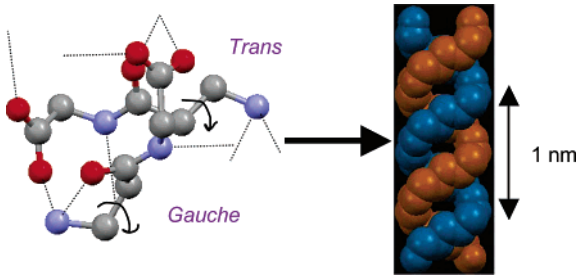
ABSTRACT



A series of water-soluble synthetic dipeptides (1–3) with an N-terminally located β-alanine residue, β-alanyl-L-valine (1), β-alanyl-L-isoleucine (2), and β-alanyl-L-phenylalanine (3), form hydrogen-bonded supramolecular double helices with a pitch length of 1 nm, whereas the C-terminally positioned β-alanine containing dipeptide (4), L-phenylalanyl-β-alanine, does not form a supramolecular double helical structure. β-Ala-Xaa (Xaa = Val/Ile/Phe) can be regarded as a new motif for the formation of supramolecular double helical structures in the solid state.

Helicity is a very important structural feature present in biological systems including α-helical structures in proteins, the DNA double helical structure, and the collagen triple helix. Among these biological systems, the naturally occurring DNA double helix is the most interesting because life is encoded within this double helical structure. A special class of well-defined synthetic supramolecular double-stranded helical metal complexes were pioneered by J. M. Lehn and characterized as "double-stranded helicates".[1] Hydrogen-bond-driven self-assembly is another common approach for constructing supramolecular duplexes.[2] Although hydrogen bonds are readily available and prove to be a versatile tool for constructing supramolecular assemblies, it is still difficult and challenging to design and construct supramolecular double helices[3] with suitable molecular building blocks. Recently, the formation of the hetero stranded double helix was demonstrated, in which different but complementary binding sites were introduced in two different molecular strands that interact by salt bridges between complementary amidinium and carboxylate units.[4] Only a few examples of peptide-based and peptide nucleic acid (PNA)-based double

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helices are reported in the literature.[5,6] In this report, we present the formation of supramolecular double helical structures in crystals from a series of water-soluble synthetic dipeptides (**1**−**3**), each containing an N-terminally positioned β-alanine (β-Ala) residue while the C-terminus is occupied by a bulky hydrophobic Xaa residue (Xaa = Val/Ile/Phe). Gorbitz has made a seminal contribution in structures of hydrophobic dipeptides[6] that are based exclusively on α-amino acids. The left-handed dipeptide double helices of Val-Ala class structures are formed through hydrogen bonds, and these hydrophobic dipeptides are self-assembled to form microporous organic materials.[6a] Though Gorbitz's dipeptides and our reported dipeptides (**1**−**3**) form supramolecular double helices through hydrogen bonds, our reported dipeptides are somewhat different from Gorbitz's dipeptides chemically and structurally, and they can be termed as the β-Ala-Xaa class.

A series of water-soluble dipeptides, where β-alanine is used as a constituent, β-Ala-L-Val (**1**), β-Ala-L-Ile (**2**), β-Ala-L-Phe (**3**), and its retro analogue L-Phe-β-Ala (**4**), have been synthesized by conventional solution-phase methodology,[7] purified, characterized, and studied. Colorless monoclinic crystals of peptides **1**, **3**, and **4** and colorless triclinic crystals of peptide **2**, suitable for X-ray diffraction studies,[8] were obtained from their aqueous solutions by slow evaporation. Peptides **1** and **3** crystallize with two peptide molecules in the asymmetric unit, named A and B, and peptide **2** crystallizes with four peptide molecules in the asymmetric unit, named A, B, C, and D (Figure 1a−c). However, peptide **4** has only one molecule in the asymmetric unit (Figure 1d). Interestingly, the central (θ) torsion angle around the −C(β)−C(α)− bond of the conformationally flexible β-Ala residue appears to play a critical role in dictating the overall distinct structural features. For peptides **1** and **3**, one of the conformers present in the asymmetric unit adopts a folded *gauche* conformation ($\theta \sim 60°$) whereas the other conformers favor an extended *trans* conformation ($\theta \sim 180°$) around the −C(β)−C(α)− bond of the β-Ala residue (Supporting Information, Table S1). For peptide **2**, two of the four

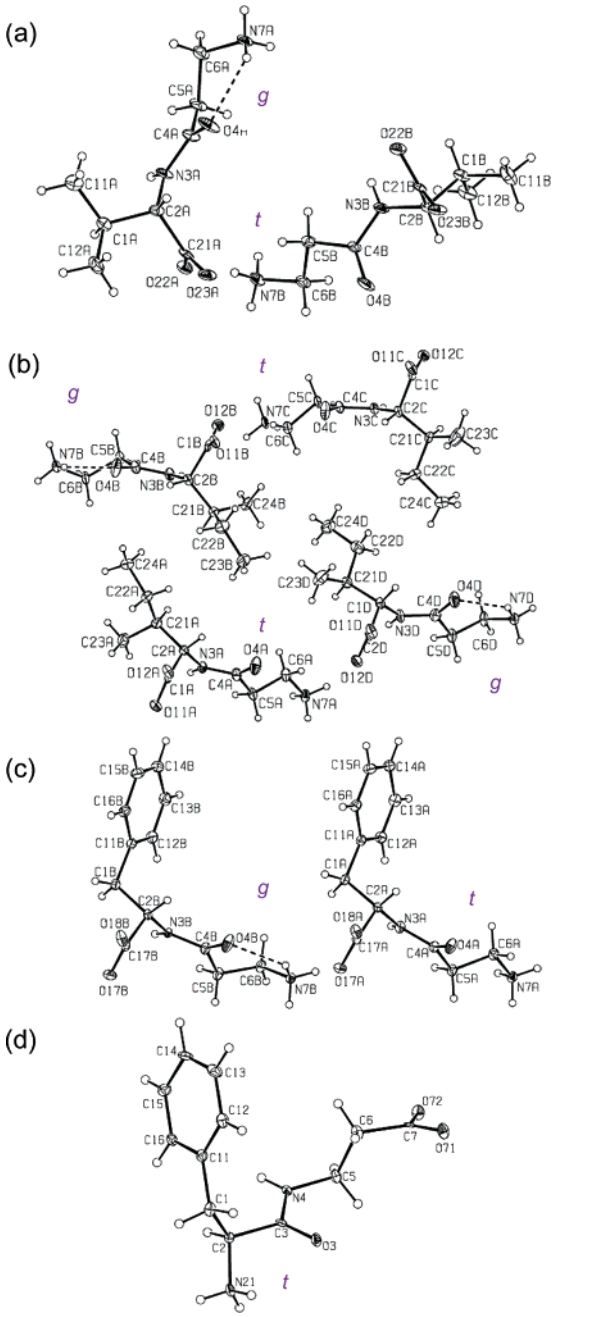

Figure 1. (a)−(d) ORTEP diagrams with atomic numbering scheme of peptides **1**−**4**, respectively. Ellipsoids are at 30% probability. There are, respectively, two, four, two, and one molecules in the asymmetric units of peptides **1**, **2**, **3**, and **4**. All independent molecules are shown. Intramolecular hydrogen bonds are shown as dotted lines (*g* = *gauche* and *t* = *trans*).

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(8) Crystal data for peptide **1**: $C_8H_{16}N_2O_3$, FW = 188.23, monoclinic, space group $P2_1$, a = 11.1153 (15) Å, b = 5.4908 (8) Å, c = 16.1643 (17) Å, β = 91.345 (10)°, Z = 4, d_{calcd} = 1.268 g cm^{-3}. Crystal data for peptide **2**: $C_9H_{18}N_2O_3$, FW = 202.25, triclinic, space group $P1$, a = 12.5522 (14) Å, b = 5.5160 (5) Å, c = 15.9248 (19) Å, α = 89.816 (9)°, β = 87.074 (10)°, γ = 89.234 (8)°, Z = 4, d_{calcd} = 1.220 g cm^{-3}. The structure is only slightly distorted from monoclinic $P2_1$. Crystal data for peptide **3**: $C_{12}H_{16}N_2O_3$, FW = 236.27, monoclinic, space group $P2_1$, a = 13.9345 (12) Å, b = 5.5848 (5) Å, c = 15.4060 (13) Å, β = 92.408 (7)°, Z = 4, d_{calcd} = 1.310 g cm^{-3}. Crystal data for peptide **4**: $C_{12}H_{16}N_2O_3$, FW = 236.27, monoclinic, space group $P2_1$, a = 8.1292 (11) Å, b = 5.7648 (8) Å, c = 12.434 (2) Å, β = 97.293 (13)°, Z = 2, d_{calcd} = 1.358 g cm^{-3}. Diffraction data were measured with Mo K$_\alpha$ (λ = 0.71073 Å) radiation at 150 K using an Oxford Diffraction X-Calibur CCD system. Data analyses were carried out with the Crysalis program.[9] The structures were solved by direct methods using the SHELXS-97[10] program. Refinements were carried out with a full matrix least squares method against F^2 using SHELXL-97.[11] 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 final R values were R1 = 0.0683, 0.1028, 0.0317, 0.0733 and wR2 = 0.1548, 0.2926, 0.0876, 0.1255 for 1297, 7405, 3691, 1183 data with $I > 2\sigma(I)$ for peptides **1**−**4**, respectively. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre with reference numbers CCDC 634356−634359.

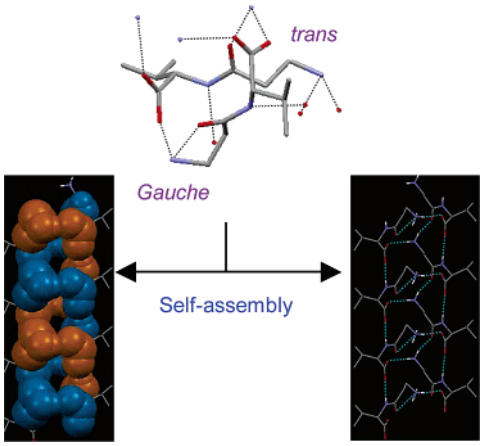

Figure 2. Space-filling and sticks model of peptide **1** showing a hydrogen-bonded supramolecular double helical structure using both the *gauche* and *trans* conformations.

conformers adopt *gauche* conformations and the other two adopt *trans* conformations along this −C(*β*)−C(α)− bond of the *β*-Ala residue. Interestingly, peptide **4** which contains only one molecule in the asymmetric unit adopts the *trans* conformation along the −C(*β*)−C(α)− bond of the *β*-Ala residue. In our reported peptides, six-membered intramolecular hydrogen bonds (NH_3^+···O═C(amide)) are formed when the *β*-Ala residue exists as a folded *gauche* conformation. However, no intramolecular hydrogen bond is formed when the *β*-Ala residue exists as an extended *trans* conformation. In peptides **1**−**3**, two different types of molecules (A-B for peptides **1** and **3** and A-B and C-D for peptide **2**) in the asymmetric unit further self-assemble to form supramolecular double helices along the crystallographic *c*-axis through three types of hydrogen bonds. These are head to tail NH_3^+···$^-$OOC hydrogen bonds between two different molecules (A-B for peptides **1** and **3** and A-B and C-D for

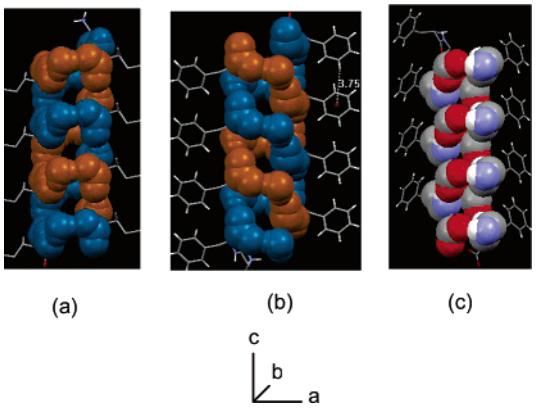

Figure 3. (a) and (b) Space-filling model of the supramolecular double helical architecture of peptides **2** and **3**. CH−*π* interaction is also shown. (c) Supramolecular single helix formation of peptide **4** along the crystallographic *c*-axis.

Table 1. Hydrogen-Bond Parameters of Peptide **1**[a]

D−H···A	H···A (Å)	D···A (Å)	D−H···A (deg)
N3A−H3A···O23A (a)	2.05	2.913(5)	176
N3B−H3B···O23B (a)	2.02	2.874(5)	173
N7A−H71A···O4A	2.24	2.885(5)	129
N7A−N71A···O22B (b)	2.40	2.898(5)	116
N7B−H71B···022A (a)	1.83	2.716(5)	172
N7A−H72A···O23B (c)	1.91	2.783(4)	168
N7B−H72B···O23A (d)	1.93	2.803(4)	167
N7A−H73A···O22B	1.85	2.735(5)	169
N7B−H73B···O4B (a)	2.00	2.809(5)	150

[a] Symmetry elements: (a) *x*, −1 + *y*, *z*; (b) 1 − *x*, 0.5 + *y*, 1 − *z*; (c) 1 − *x*, −0.5 + *y*, 1 − *z*; (d) 1 − *x*, −1.5 + *y*, 2 − *z*.

peptide **2**), NH_3^+···O═C (amide) hydrogen bonds between the same types of molecules (in the *gauche* conformation of the *β*-Ala residue, NH_3^+···O═C (amide) six-membered intramolecular hydrogen bonds are formed, and in the *trans* conformation of the *β*-Ala residue, NH_3^+···O═C (amide) intermolecular hydrogen bonds are formed), and amide NH···$^-$OOC intermolecuar hydrogen bonds between the same types of molecules for peptides **1**−**3** (Figures 2, 3a and b) (Tables 1, 2, and 3). Peptides **1**−**3** form hydrogen-bonded supramo-

Table 2. Hydrogen-Bond Parameters of Peptide **2**[a]

D−H···A	H···A (Å)	D···A (Å)	D−H···A (deg)
N3A−H3A···O12A (a)	2.06	2.894(6)	164
N3B−H3B···O11B (a)	2.07	2.925(6)	170
N3C−H3C···O11C (a)	2.06	2.909(6)	169
N3D−H3D···O11D (a)	2.14	2.983(6)	165
N7A−H71A···O12B	1.86	2.724(6)	165
N7B−H71B···O4B	2.14	2.825(7)	133
N7B−H71B···O12C	2.39	2.902(6)	117
N7C−H71C···O12D (b)	1.84	2.720(6)	170
N7D−H71D···O12C (c)	1.86	2.737(6)	167
N7A−H72A···O11D (a)	1.94	2.801(7)	163
N7B−H72B···O11C (a)	1.90	2.765(7)	165
N7C−H72C···O11B (b)	1.89	2.775(6)	178
N7C−H72C···O12B (b)	2.53	3.084(7)	121
N7D−H72D···O4D	2.20	2.841(7)	129
N7D−H72D···O11A(c)	2.51	2.989(6)	114
N7A−H73A···O4A (a)	1.95	2.774(7)	152
N7B−H73B···O11A (a)	1.86	2.740(6)	168
N7C−H73C···O4C (a)	1.95	2.802(7)	159
N7D−H73D···O12A (d)	1.90	2.777(7)	167

[a] Symmetry elements: (a) *x*, 1 + *y*, *z*; (b) *x*, 1 + *y*, 1 + *z*; (c) *x*, *y*, −1 + *z*; (d) *x*, 1 + *y*, −1 + *z*.

lecular double helices with a helical pitch length of 10.98, 11.03, and 11.17 Å, respectively (∼1 nm). The supramolecular double helical structure of peptide **3** is further stabilized by an aromatic CH−*π* interaction[12] with average

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Table 3. Hydrogen-Bond Parameters of Peptide **3**[a]

D−H···A	H···A (Å)	D···A (Å)	D−H···A (deg)
N3A−H3A···O18A (a)	2.06	2.849(2)	153
N3B−H3B···O18B (a)	2.07	2.903(2)	163
N7A−H71A···O17B (b)	1.82	2.712(2)	179
N7B−H71B···O4B	2.20	2.859(2)	131
N7B−H71B···O17A (c)	2.48	2.961(2)	115
N7A−H72A···O17B (a)	2.58	3.149(2)	122
N7A−H72A···O18B (a)	1.87	2.754(2)	171
N7B−H72B···O18A (d)	1.90	2.753(2)	159
N7A−H73A···.O4A (a)	1.96	2.810(2)	159
N7B−H73B···O17A (b)	1.85	2.724(2)	165

[a] Symmetry elements: (a) x, $1 + y$, z; (b) $1 - x$, $0.5 + y$, $-z$; (c) x, y, $-1 + z$; (d) x, $1 + y$, $-1 + z$.

CH−π distance of 3.75 Å (Figure 3b), but its retro analogue L-Phe-β-Ala (**4**) did not form a supramolecular double helix. Peptide **4** is self-assembled to form a supramolecular single helix through $NH_3^+\cdots{}^-OOC$ and NH(amide)$\cdots{}^-OOC$ intermolecular hydrogen bonds along the crystallographic *c*-axis (Figure 3c). The crystal structure further revealed that individual supramolecular double helices are regularly aligned via intermolecular hydrogen bonds between the third amino $NH_3^+\cdots{}^-OOC$ and other noncovalent interactions to form higher-order supramolecular arrays around the crystallographic *b* axes (Supporting Information, Figure S17). Reported dipeptides (**1**−**3**) share some crystal packing similarities with the Gorbitz dipeptide Val-Ala class structures. In both cases, head-to-tail hydrogen bonds ($NH_3^+\cdots{}^-OOC$) and other hydrogen bonds, namely, $NH_3^+\cdots O{=}C$ (amide) and amide NH$\cdots{}^-OOC$, are responsible for the formation of supramolecular double helices. However, the structures of our reported dipeptides (**1**−**3**) constitute a unique class of dipeptide structures where hydrogen bonds are formed between two distinctly different conformers present in the asymmetric unit unlike Gorbitz's dipeptide double helices.[6] The supramolecular double helical compounds (**1**−**3**) showed significant thermal stability, and it has been demonstrated by TGA−DTA (TGA, thermogravimetric analysis; DTA, differential thermal analysis) experiments (Supporting information, Figure S18). These dipeptides showed no decomposition, phase transitions, or mass loss up to their melting points of 255, 240, and 244 °C for peptides **1**, **2**, and **3**, respectively. This indicates that the double helical frameworks are very stable. Moreover, it is evident from X-ray crystal structure analyses, TGA−DTA data analyses, and elemental data analyses that no solvent molecules are present within the double helical framework.

We present here the water-soluble short peptide-based supramolecular double helices with a common motif in which one of the conformers present in the asymmetric unit adopts the *gauche* conformation while the other conformer adopts the *trans* conformation along the −C(β)−C(α)− bond of the β-Ala residue. Simultaneous existence of *gauche* and *trans* conformations along the −C(β)−C(α)− bond of the β-Ala residue can favor the formation of supramolecular double helical structures upon self-assembly. Our result clearly demonstrates that the position of the β-Ala residue is important in supramolecular double helix formation for this dipeptide series, as the peptide **4** does not form the supramolecular double helical structure. β-Ala-Xaa (Xaa = Val/Ile/Phe) can be considered as a new molecular scaffold for supramolecular double helix formation in crystals. The role of C-terminally positioned hydrophilic/polar amino acid residues (β-Ala-Xaa, Xaa = hydrophilic/polar amino acid residue) in supramolecular double helix formation is yet to be explored.

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Supporting Information Available: Syntheses, spectral characterization data, figures, tables, and CIF files of peptides **1**−**4** are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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