Characterization of a Water-Soluble, Helical β-Peptide

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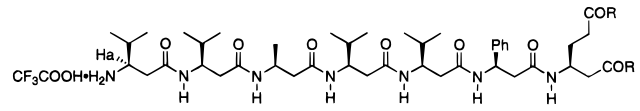
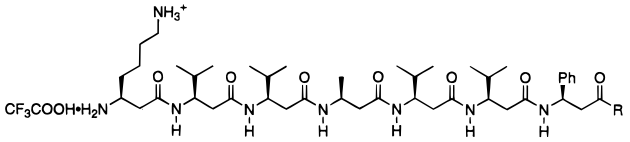
The synthesis and characterization of artificial biopolymers with specific secondary structures have evolved into a new area of chemistry, which is directly related to the chemistry of molecular recognition, catalysis, and protein folding.[1−9] Thus, it is of considerable interest that the oligomers of β-amino acids appear to form predictable, stable, helical conformations.[8−11]

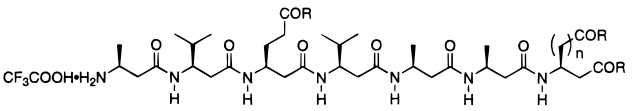
Initially, the high tendency of β-peptides to form helices was somewhat surprising because it has been shown that some β-alanine-containing triamides are resistant to intramolecular hydrogen bonding.[12] To investigate the origin of the high helix-forming propensity of β-peptides, we recently carried out a study of the conformational preference of pentameric β-peptides in chloroform and in methanol. The study showed that for short β-peptides built from monosubstituted β-amino acids bulky side chains promote the 3_1 helix. In addition, we studied the conformations of polyamides in organic solvents.[14] However, it is desirable to study the conformations of polar flexible molecules in water since most biological events occur in aqueous solution. We have now designed, synthesized, and characterized a few water-soluble β-peptides.[15]



1a (R = OMe), **1b** (R = OH)



2a (R = OMe), **2b** (R = OH)



R = OMe: **3a** (n = 2), **4a** (n = 0); R = OH: **3b** (n = 2); **4b** (n = 0)

Four heptameric β-peptides with varying groups of polar side chains were synthesized using solution-phase chemistry with modified procedures of Seebach.[8] A combination of both convergent and directional approaches (starting from the C-terminus and ending at the N-terminus) was employed in our synthesis. The preassembly of the peptide fragments that do not contain a polar residue allowed efficient synthesis of the polar β-peptides by minimizing the exposure of the polar side chains in the reaction sequence. Specific procedures are included in the Supporting Information.

Initially, one polar residue was placed in each of the first two heptamers. β-Peptide **1** contains a homoglutamate (β-HGlu) and **2** contains a homolysine (β-HLys) residue.[13] After a solubility test of **1** and **2**, the two polar residues were incorporated into peptides **3** and **4**. Peptide **3** contains two β-HGlu residues, and peptide **4** contains one β-HGlu and one D-Asp (at the C-terminus). The chirality of the D-Asp residue matches the rest of the β-amino acid residues if it is viewed as a β-amino acid with a carboxylate as its side chain. A β-HGlu residue is intentionally placed at the C-terminus of peptide **1**, and a β-HLys is intentionally placed at the N-terminus of peptide **2**, which encourages the formation of helical conformations by stabilizing the macrodipole of the helix.[16−18] Three nonpolar residues are placed between the two polar amino acids in peptides **3** and **4**. Therefore, the polar side chains in peptides **3** and **4** are pointed 120° apart from each other in a helical conformation avoiding electrostatic repulsion between the two polar side chains.

The solubility of peptides **1b** and **2b** in water is <0.1 mM, which is too small a concentration to use ^{1}H NMR spectroscopy to study their conformation in water. However, the water-solubility of peptide **3b** is about 5 mM and that of **4b** is greater than 15 mM. The ^{1}H NMR spectra of **4b** shows a minimal change in NH chemical shifts when recorded at various concentrations (<0.2 ppm from 0.5 mM to 10 mM) in water, which indicates no serious aggregation in this

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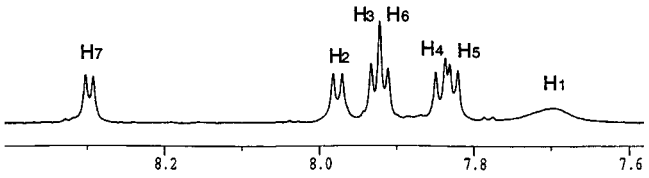

Figure 1. Amide region of the ^{1}H NMR spectrum of β-peptide **4b** in a solution of 10% D_2O/90% H_2O recorded on an 800 MHz spectrometer. The assignment of the NHs to specific residues is made on the basis of a TOCSY experiment.[20]

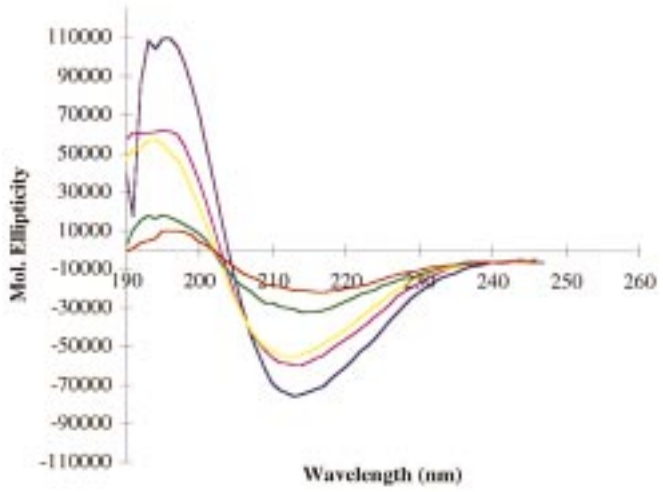

Figure 2. CD spectra of β-peptide **4b** in CH_3OH/H_2O mixture. Concentration is 0.2 mM, molar ellipticity [Q] in 10 deg cm^2 mol^{-1}. Ratio of CH_3OH/H_2O: red, 0:100; green, 25:75; yellow, 50:50; purple, 75:25; blue, 100:0.

concentration range. Significant aggregation is usually indicated by insolubility and broadening of proton NMR signals. The amide NH proton NMR signals of **4b** in 10% D_2O/90% H_2O remain distinct at 15 mM concentration as shown in Figure 1.[19,20]

The circular dichroism (CD) spectra of **4b** in aqueous methanol (Figure 2) and aqueous trifluoroethanol (not shown) indicate an increasing degree of helical conformations with an increasing amount of alcohol concentration. The typical CD signature of a helical β-peptide persists for **4b** even in 100% water. However, the helical content in water is not enough to produce cross NOE peaks typical of a 3_1 helix. Therefore, we have concentrated on the identification of the ester form of these β-peptides (**1a**−**4a**) in chloroform solution. Two-dimensional NMR methods, COSY, TOCSY, NOESY, and ROESY on an 800 MHz machine, allowed us to assign all signals in the ^{1}H NMR spectra to specific residues. The ^{13}C chemical shifts are assigned using the NMR methods DEPT and hydrogen−carbon correlation (HMQC) experiment.[20]

The peptide sequence is assigned by using the NOE cross-peaks between the NH(i) proton and the H−C (α, $i - 1$). A complete set of $\delta_{\alpha N}$ (i, $i + 1$) NOE cross-peaks are identified for peptide **4a** (see Table 1 in the Supporting Information). NOE cross-peaks are also observed in the NOESY spectrum for sequential neighboring NHs (the NHs of residue i and $i + 1$, δ_{NN}; see Figure 1 in Supporting Information). There are strong NOE cross-peaks between the side chain CH_x groups of the ith residue and the side chains of the $i + 3$ residue. Strong NOE cross-peaks are also observed between the H-Cβ of the ith residue and the side chains of the $i - 3$ residue. Those are strong indications of a 3_1 helical conformation.

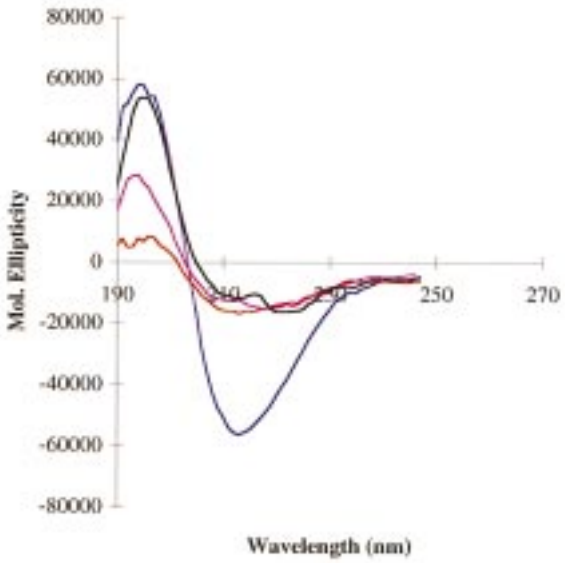

Figure 3. CD spectra of β-peptides **1b**−**4b** in 50% MeOH/50% H_2O. Concentration is 0.2 mM, molar ellipticity [Q] in 10 deg cm^2 mol^{-1}. β-Peptide: **1**, black; **2**, purple; **3**, red; **4**, blue.

The CD spectra of β-peptides (**1b**−**4b**) are collected on a JASCO 715 CD spectrometer. In Figure 2, the CD spectra of peptide **4b** in a mixture of CH_3OH and H_2O (0:100, 25:75, 50:50, 75:25, and 100:0) are displayed. The highest population of helices is observed when methanol is the solvent. As water is added to the solution, the molar ellipticity is proportionally decreased. Trifluoroethanol is known to enhance helix formation by natural peptides.[18] However, the origin of the enhancement is not clear. It is interesting to note that methanol also enhances the formation of helix in water by a β-peptide.

An overlay of the CD absorptions from **1b** to **4b** in 50% methanol/50% H_2O solutions at 0.2 mM concentration is displayed in Figure 3, which suggests a varying degree of helical conformations. It is noteworthy that **4b** assumes a considerably greater amount of the helical conformation than **3b** despite the fact that the difference between their structures is only two methylene units. It is not entirely clear what is the origin of this difference. However, this observation should be helpful in the design of helical, water-soluble β-peptides.

In summary, to achieve a reasonable water-solubility for β-peptides there should be one polar residue for every three nonpolar residues. For future studies in this area, a water-soluble peptide with a predictable helical conformation should be useful in the designing of molecular scaffolding for studies of other secondary structures. Our current work is aimed at synthesizing water-soluble β-peptides with a higher helix-forming propensity.

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Supporting Information Available: Experimental procedures and NMR data.

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