Peptides with nucleobase moieties as a stabilizing factor for a two-stranded α-helix

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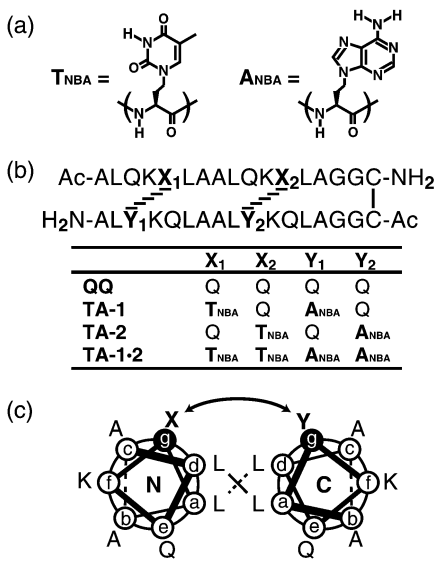
The designed α-helical coiled-coil peptides, which contain nucleobase moieties at the amino acid side chains, were found to take a stable α-helical form owing to the base-pair interaction.

α-Helical coiled-coil, found in many natural proteins, is one of the well-studied motifs aiming to understand protein sequence–structure relationships,[1] and also to develop novel proteins with functional properties by *de novo* protein design.[2] The initial studies have focused on the usage of hydrophobic and electrostatic interactions between amino acid side chains to assemble α-helical peptide chains.[3] In this study we, for the first time, provide another example utilizing hydrogen bonds between nucleobases at amino acid side chains to form an α-helical coiled-coil. In nature, DNA and RNA take advantage of hydrogen bonds in their structures and functions. Sequence-complementarity of antiparallel strands and high-fidelity information transfer including transcription, translation, and replication are performed owing to the nucleobase-molecular complementarity through hydrogen bonds. Thus, peptides equipped with the nucleobase-molecular complementarity should gain new useful characters in their structure and function. In order to explore the applicability of nucleobase-complementarity in a peptide structure, we have introduced nucleobase amino acids (NBAs)[4] in a two-stranded α-helix with the coiled-coil motif (Fig. 1), and examined the effects on the peptide secondary structure.

We designed an antiparallel, two-stranded coiled coil QQ as a basic framework, based on the peptide designed by Zhou *et al*.[5] QQ consists of two amphiphilic α-helices, each containing a two-heptad repeat unit with the amino acid sequence $A_cL_dQ_eK_fQ_gL_aA_b$. In the design of the peptide, Leu residues at the a and d positions are expected to form a hydrophobic face, which drives coiled-coil formation through its burial in the interface. Gln residues are selected at the e and g positions to prevent induction of inter- or intrahelical electrostatic interactions. To produce a disulfide-bridged coiled-coil in an antiparallel fashion, the sequence Cys-Gly-Gly- is added to the N-terminus of one helix, and the sequence -Gly-Gly-Cys is placed at the C-terminus of the other helix. Since the Cα-Cβ vectors of amino acid at the g and g′ positions are pointed toward each other in antiparallel coiled-coils in the wheel diagram (Fig. 1c), NBAs were incorporated at the g and g′ heptad positions instead of Gln. Thereby, we expected the specific interaction between thymine NBA (T_{NBA}) and adenine NBA (A_{NBA}) in the two-stranded α-helix. We designed three peptides with T–A pair(s) at the g and g′ heptad positions; TA-1 has the pair apart from the disulfide-bridged end, TA-2 has the pair near the disulfide linker, and TA-1·2 contains pairs at both positions (Fig. 1).



	X_1	X_2	Y_1	Y_2
QQ	Q	Q	Q	Q
TA-1	T_{NBA}	Q	A_{NBA}	Q
TA-2	Q	T_{NBA}	Q	A_{NBA}
TA-1·2	T_{NBA}	T_{NBA}	A_{NBA}	A_{NBA}

Fig. 1 (a) Structures of nucleobase amino acids (NBAs); (b) amino acid sequences; (c) helix wheel drawing of the designed peptides.

The designed peptides were synthesized manually by the solid-phase method using the Fmoc-strategy.[6] To introduce A_{NBA} and T_{NBA} to the Fmoc chemistry protocols, *N*-Fmoc-*L*-α-amino-γ-(6-*N*-benzyloxycarbonyl)adenine butanoic acid [Fmoc-A_{NBA}(Z)-OH] and *N*-Fmoc-L-α-amino-γ-thymine butanoic acid [Fmoc-T_{NBA}-OH] were prepared according to literature methods with some modifications.[7] All peptides were acetylated at the N-terminus and amidated at the C-terminus to avoid unfavorable helix–dipole interactions. To build an antiparallel heterodimer, an asymmetric disulfide bond formation between two peptides was performed *via* activation of the thiol function of one peptide by pyridinesulfenylation, and the reaction with the second peptide with the free thiol.[8] The peptides were purified by reversed-phase HPLC, and identified by matrix-assisted laser desorption time-of-flight mass spectrometry and amino acid analysis.[9] The concentrations of the peptides were determined by quantitative amino acid analysis using valine as an internal standard.

The circular dichroism (CD) spectra of the designed peptides in a buffer (pH 7.4) at 25 °C are shown in Fig. 2. All peptides showed spectra characteristic of an α-helical conformation with negative maxima near 208 and 222 nm and a positive maximum at 195 nm. The ratio of the molar ellipticities at 222 and 208 nm, $[\theta]_{222}/[\theta]_{208}$, was reduced in a solution containing trifluoroethanol, known as a solvent enhancing an α-helix form but isolating a helix-dimer, indicating two-stranded α-helical formation in the buffer. The $[\theta]_{222}$ values of TA-1 and TA-2, both having a T–A pair, were −20500 and −22100 deg cm^2 dmol^{-1}, respectively, showing their higher helical contents than the content of QQ ($[\theta]_{222}$ = −15100 deg cm^2 dmol^{-1}). Furthermore, TA-1·2 having two T–A pairs showed a higher α-helicity ($[\theta]_{222}$ = −23800 deg cm^2 dmol^{-1}) than TA-1 and TA-2. We assumed that this helix-inducing effect was not the result of the peptide aggregation, judged from the concentration independence of the $[\theta]_{222}$ values of designed peptides (ranging

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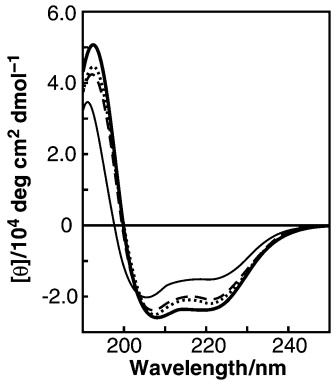

Fig. 2 CD spectra for the designed peptides in 2.0×10^{-2} mol dm^{-3} Tris·HCl buffer (pH 7.4) at 25 °C. [Peptide] = 1.0×10^{-5} mol dm^{-3}. QQ, (——); TA-1, (– – –); TA-2, (-----); TA-1·2, (▬).

from 7.5×10^{-7} to 1.0×10^{-4} mol dm^{-3}). The addition of an excess amount of exogenous adenine reduced the α-helical contents in the cases of peptides possessing T–A pair(s) ($\Delta[\theta]_{222}$ = 6500 deg cm^2 dmol^{-1} in TA-1·2), whereas no change occurred in the case of QQ.[10] This demonstration implied that the added interaction between A and T increases the α-helical content.

Denaturation studies were carried out to determine the stabilities of the peptides. With increasing temperature of the peptide solution, the bimodal spectra were gradually converted into those of a random conformation, indicating the decrease of the fraction of α-helix structure. The presence of an isodichroic point at *ca*. 202 nm was consistent with the helix-coil transition. From the temperature dependence of $[\theta]_{222}$ shown in Fig. 3, it was apparent that the thermal stabilities of the nucleobase-containing species, especially TA-1·2, were noticeably increased as compared to the stability of QQ. Furthermore, the guanidine hydrochloride (GuHCl) denaturation study also provided the observation of the stabilizing effect in nucleobase-containing peptides (Table 1). The incorporation of one T–A pair in the two-stranded α-helix (TA-1 and TA-2) made peptides with a higher stability than QQ, and the additional incorporation (TA-1·2) afforded further stability to the peptide. TA-2 is slightly more stable than TA-1, which is consistent with the α-helicity. An explanation may be the difference of the position of a T–A pair (Fig. 1). In TA-2 the pair was located near the disulfide-linker so that T and A might configure to interact at g–g′ positions, whereas in TA-1 the pair was at the end leading to fluctuation of T and A. Moreover, the effect of nucleobase-incorporation on the helix stability was not additive. The incorporation of one T–A pair into QQ increased ΔGu^{H_2O} by 0.56 (TA-1) and 0.90 kcal mol^{-1} (TA-2), whereas two T–A pairs increased greatly by 1.88 kcal mol^{-1}.

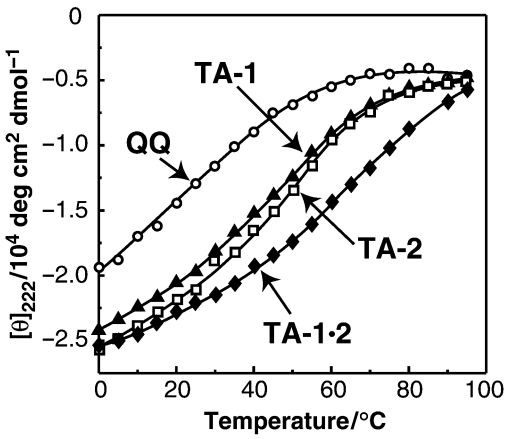

Fig. 3 Temperature dependence of the CD signal at 222 nm for QQ, TA-1, TA-2, and TA-1·2 in 2.0×10^{-2} mol dm^{-3} Tris·HCl buffer (pH 7.4).

Table 1 Stabilities of the designed peptides estimated by GuHCl denaturation

Peptide	QQ	TA-1	TA-2	TA-1·2
ΔGu^{H_2O}[a]/kcal mol^{-1}	0.74	1.30	1.64	2.62
$\Delta\Delta Gu^{H_2O}$[a]/kcal mol^{-1}	0	0.56	0.90	1.88
$[GuHCl]_{1/2}$[b]/mol dm^{-3}	1.7	2.0	2.3	3.2
$\Delta[GuHCl]_{1/2}$[b]/mol dm^{-3}	0	0.3	0.6	1.5

[a] ΔGu^{H_2O} is the free energy of unfolding in the absence of GuHCl at 25 °C, estimated according to the equation: $\Delta Gu = \Delta Gu^{H_2O} - m[GuHCl]$, where m is the slope term. ΔGu is calculated from the equation: $\Delta Gu = -RT\ln[(1 - fn)/fn]$.[11] [b] The $[GuHCl]_{1/2}$ values represent the concentration of GuHCl at which 50% of the peptide is unfolded.

We also examined the peptides having two T–T pairs (TT-1·2) or two A–A pairs (AA-1·2) at the g–g′ positions in the two-stranded α-helix. However, these mismatch pairs did not significantly improve the α-helical form unlike T–A pairs (TT-1·2, $[\theta]_{222}$ = −17800; AA-1·2, $[\theta]_{222}$ = −18100 deg cm^2 dmol^{-1}). These results strongly suggest that the specific interaction between the T–A pair is important in promoting the α-helical formation. In such a case of the specific pair(s), T and A in the two-α-helix might orient and interact in the appropriate fashion. One possible interaction might be base stacking (hydrophobic interaction), and another might be hydrogen-bonding. The former does not contribute greatly to the helix formation, because the α-helical content of AA-1·2, containing two A–A mismatch pairs, is not as large as that of TA-1 or TA-2. In respect to the latter interaction, the pH dependence of $[\theta]_{222}$ revealed that T–A-containing peptides took a higher helical form under neutral conditions than under acidic or basic conditions. The protonation of nucleobases had an influence on the interaction between T and A, suggesting a large contribution of the hydrogen-bonding interaction. Although detailed inspection is necessary to answer how these bases interact and induce the helical structure, the specific interaction, including the hydrogen-bonds, were successful at stabilizing two-stranded α-helix structures.

In conclusion, peptides containing NBAs at the g–g′ positions in the coiled-coil structure were synthesized, and the incorporated A–T nucleobase pairs were found to make an effective contribution to the formation of stable α-helical structures. The base-pair interaction can be utilized as a new tool for designing secondary or tertiary peptide structure, and also applied to development of novel functions based on the complementarity.

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9 QQ, *m/z* 3563.9 [$(M + H)^+$] (calc. = 3563.8); TA-1, *m/z* 3734.7 [$(M + H)^+$] (calc. = 3735.0); TA-2, *m/z* 3736.1 [$(M + H)^+$] (calc. = 3735.0); TA-1·2, *m/z* 3904.8 [$(M + H)^+$] (calc. = 3906.4).
10 [Peptide] = 7.0–9.0 × 10^{-6} mol dm^{-3}, [Adenine] = 1.0 × 10^{-3} mol dm^{-3}.
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