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Computational Study on Helical Structure of α,α–Disubstituted Oligopeptides Containing Chiral α-Amino Acids

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Computational simulation using conformational search calculations with AMBER force field is most useful for conformational analysis of oligopeptides containing α,α-disubstituted α-amino acids. The results were in agreement with those of x-ray and were most stable conformation evaluated by molecular orbital calculation.*

Keywords: α,α-disubstituted α-amino acid, oligopeptide, conformational search, *MacroModel*, molecular orbital calculation

Introduction

We have studied on the conformational search of ligands in receptor or enzyme with AMBER* force field. [1-2] Prediction of the conformation of oligopeptides using computational simulation presents an interesting challenge to design functionalized and bioactive peptides. We have shown that the Monte Carlo conformational search method using MacroModel is useful to predict helical structures (α-helix, 3_{10}-helix) of oligopeptides prepared from α,α-disubstituted α-amino acids. Moreover, we have studied conformational analysis of oligopeptides containing chiral α,α-disubstituted α-amino acids to predict the helical screw sense of helical structures. [3-6]

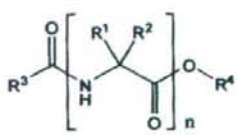
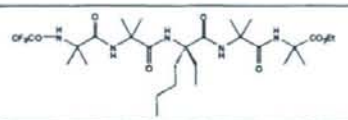
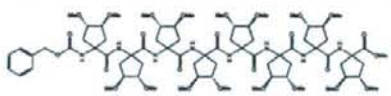
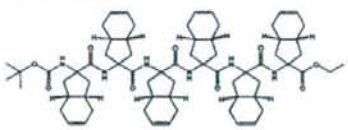


Fig. 1. Structures of α,α-disubstituted oligopeptides.

Results and Discussion

We calculated α,α-disubstituted peptide using MCMM conformational search with various force fields (AMBER*, MMFF, OPLS) and showed the results in Table 1. In the case of using AMBER* force field the results were in agreement with those of x-ray and were most stable conformation evaluated by 3-21G level molecular orbital calculation. These results indicated that computational simulation using conformational search calculations with AMBER* force field is most useful for conformational analysis of oligopeptides containing α,α-disubstituted α-amino acids.

Table1. conformational analysis of oligopeptides containing α,α-disubstituted α-amino acids

Peptides	Global Minimum By *MacroModel* MCMM Conformational Search			X-ray
	AMBER*	MMFF	OPLS	
	(*P*)-3_{10} -helix	Random coil	Random coil	(*P*)-3_{10} -helix
	3-21G by *Spartan*			
	0 (kcal/mol)	+14.09	+16.83	
	(*M*)-α -helix	(*M*)-α,3_{10} -helix	(*M*)-α -helix	(*M*)-α -helix
	3-21G by *Spartan*			
	0 (kcal/mol)	+13.52	+9.32	
	(*P*)-3_{10} -helix	(*P*)-α,3_{10} -helix	(*M*)-α -helix	(*P*),(*M*)-3_{10}-helix
	3-21G by *Spartan*			
	0 (*M*:+1.14)	+14.28	+20.67	

Acknowledgement

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Design and Synthesis of Chiral Cyclic α,α-Disubstituted Amino Acid Having Azido Functions, and Its Oligopeptides

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We designed and synthesized a chiral cyclic α,α-disubstituted α-amino acid having azido functions: {(3R,4R)-1-amino-3,4-diazidocyclopentanecarboxylic acid; (R,R)-Ac$_5$c^{dN3}}, and studied the preferred secondary structure of its homopeptides. Furthermore, the azido functions in the cyclic amino acid (R,R)-Ac$_5$c^{dN3} could be converted into various functional groups.

Keywords: azido functions, cyclic amino acid, α,α-disubstituted α-amino acid, peptide conformation, chiral center

Introduction

Helices in proteins almost always show a right-handed (*P*) helical screw, which is believed to result from the asymmetric center at the α-position of L-α-amino acids. We have previously reported that the side-chain chiral centers of amino acid affect the secondary structures of their peptides [1]; that is to say, we synthesized a chiral cyclic α,α-disubstituted α-amino acid; (*S,S*)-Ac$_5$c^{dOM}, in which the α-carbon atom is not a chiral center but asymmetric centers exist at the side chain. The (*S,S*)-Ac$_5$c^{dOM} homopeptides preferentially formed a left-handed helical screw from the side-chain chiral centers (Fig. 1).

Herein, we designed a chiral cyclic amino acid with azido functions; (3*R*,4*R*)-1-amino-3,4-diazidocyclopentanecarboxylic acid {(*R,R*)-Ac$_5$c^{dN3}}, in which azido functions were thought to be easily converted into various functional groups. Moreover, we prepared homopeptides composed of (*R,R*)-Ac$_5$c^{dN3}, and studied the preferred conformation of peptides.

Fig. 1. Helical structure of (S,S)-Ac_5c^{dOM} octapeptide.

Results and Discussion

We synthesized a chiral cyclic amino acid (*R*,*R*)-Ac_5c^{dN3} starting from dimethyl L-(+)-tartrate (Fig. 2). After conversion of dimethyl L-(+)-tartrate to diiodide (**1**), dimethyl malonate was bisalkylated with diiodide **1** to give a cyclic diester (**2**). Deprotection of the MOM ether, conversion of diol to diazido function, monohydrolysis of diester, and subsequent Curtius rearrangement afforded the chiral cyclic amino acid (*R*,*R*)-Ac_5c^{dN3}. Azido functions in the amino acid could be easily converted into amino functions by reduction, triazole functions by the "click" reaction, and amide functions by reduction and acylation.

We prepared homopeptides composed of (*R*,*R*)-Ac_5c^{dN3} by solution-phase methods, and studied the preferred secondary structure of peptides in solution. FT-IR and the ROESY ^{1}H NMR spectra indicated that (*R*,*R*)-Ac_5c^{dN3} homopeptides preferentially formed a helical structure in $CDCl_3$ solution. Now we are studying the preferred secondary structure of peptides in the crystal state by X-ray crystallographic analysis.

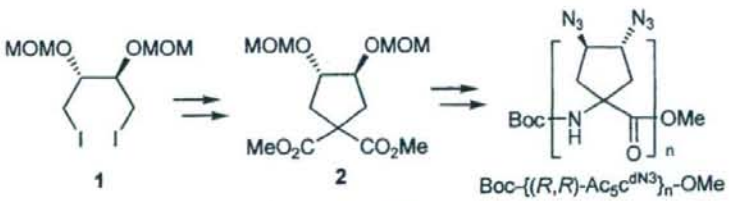

Fig. 2. Synthesis of (R,R)-Ac_5c^{dN3} and its homopeptides.

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Cooperative Strand Invasion by Peptide Nucleic Acid

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Peptide nucleic acid (PNA) is a synthetic DNA/RNA mimic in which the sugar-phosphate backbone is replaced by a peptide backbone. A remarkable feature of PNA is its ability to recognize sequences within duplex DNA by strand invasion. In order to improve DNA binding properties of PNA, we tested the effect of cooperativity on triplex invasion. We here demonstrate that a PNA targeting six bases cooperatively strand invades into duplex DNA with excellent sequence specificity.

Keywords: antigene, cooperativity , peptide nucleic acid

Introduction

Among the numerous methods for DNA recognition, of particular interest is strand invasion by peptide nucleic acid (PNA). PNA is one of the most successful analogues of oligonucleotides with potential applications in antisense and antigene strategy [1]. Strand invasion can occur via several distinct mechanisms: triplex invasion, double-duplex invasion, and duplex invasion. Among them most studies have focused on triplex invasion by using homopyrimidine PNAs, because a number of oligonucleotide-dependent enzymatic reactions are inhibited by PNA, including transcription and translation. Recognition of a unique site in the human genome requires discrimination of a specific sequence of 15-16 base pairs from all other possible sequences. However, the affinity of even relatively short bis-PNAs (8-10 bases) to their target sites is so high that PNA binding to correct and even to mismatched sites is virtually irreversible. In this regard, sequence-specificity of PNA triplex invasion is limited and this limitation hinders its application in living cells. As a possible mechanism for improving the specificity of strand invasion, we tested whether PNAs could cooperatively invade into a duplex DNA.

Results and Discussion

A hexameric bis-PNA, PNA **1**, was used to test the effect of cooperative binding interaction on triplex invasion (Fig. 1). The purine target sequence

5'-$GA_{10}G$-3' can be considered as two contiguous target sites, 5'-GA_5-3' and 5'-A_5G-3' (Fig. 2).

Figure 2 shows the results of the gel mobility shift assay. PNA **1** incubated with D^M generated no detectable band. A similar result was observed for $D^D(4)$. This suggests that this short pyrimidine PNA **1** has low binding affinity for their target sites that are isolated or separated by 4 nucleobases. The PNA molecules are rapidly displaced by the internal competing complementary DNA strand during electrophoresis. In contrast, when PNA **1** was incubated with $D^D(0)$, clear bands corresponding to triplex invasion complexes were observed. This marked improvement of the binding efficiency indicates a positive binding interaction between contiguous PNAs, aligned head to head in the invasion complex (Fig. 1).

The sequence specificity of PNA **1** was examined by comparing its affinity for fully matched 12 base pair target to that for a sequence containing a single-base mismatch, D^Dmis. PNA **1** displayed excellent specificity in an all-or-none manner. Since each PNA molecule is bound to a short DNA target, a single mismatched base pair destabilizes such a complex to a large extent, thus destabilizing the whole invasion complexes. Consequently, high specificity was realized.

PNA 1

H-Cys(SBu^t)-eg-TTTTTC-Lys_3-eg_3-CTTTTT-eg-Cys(SBu^t)Lys-$CONH_2$

Fig. 1. Schematic representation of cooperative strand invasion of dsDNA by PNA (left) and the sequence of PNA used in this study (right). eg denotes 8-amino-2,6-dioxaoctanoic acid unit.

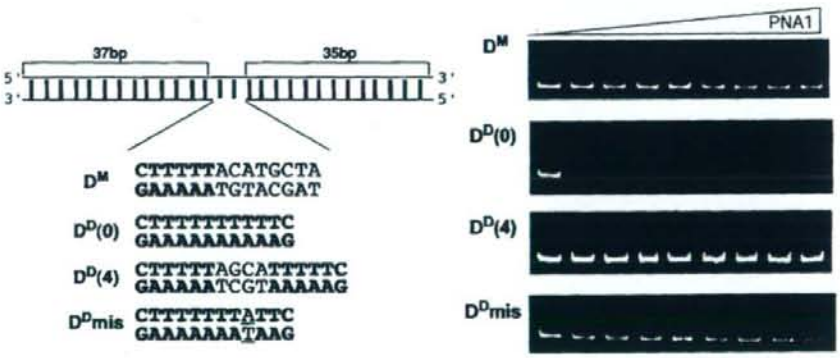

Fig. 2. An illustration of dsDNA used in this study (left) and the results of polyacrylamide gel mobility shift assay (right). PNA concentrations were 0, 1, 1.3, 1.8, 2.4, 3.2, 4.2, 5.6, and 7.5 μM, respectively.

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