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# Construction of HIV Rev Peptides Containing Peptide Nucleic Acid That Bind HIV RRE IIB RNA

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**Abstract**—Peptides containing peptide nucleic acid (PNA) have been designed and synthesized to construct molecules recognizing a bulge or a loop structure of RNA. Such peptides were here designed from the HIV Rev protein that can bind the stem-loop IIB of the Rev responsive element (RRE) RNA. Variations of PNA modulated the binding affinities of the peptides to RRE IIB RNA.


## Introduction

RNA-protein interaction plays important roles in nature. Many cellular functions, including transcription, RNA splicing, and translation, depend on the specific interaction of proteins and RNA. RNA-binding proteins seldom target fully double-stranded tracts for recognition, but interact secondary structural domains such as hairpin loops, internal loops, and bulges of RNA.[1] In most cases, an RNA-binding domain of protein forms a suitable conformation such as α-helix and β-strand to recognize a structured RNA, and amino acids orientated exactly in the protein structure make specific contacts to RNA bases, resulting in the high affinity and high specificity in the RNA binding. Construction of the molecules that specifically recognize not only RNA sequences but also 2D or 3D structures of RNA is useful for giving the information of designing highly specific drugs against RNA. On the other hand, peptide nucleic acid (PNA) is a DNA mimic with the nucleobases on a pseudopeptide backbone composed of *N*-(2-aminoethyl)glycine units.[2,3] A PNA molecule has an ability of efficient and sequence specific binding both single-stranded DNA and RNA as well as double-stranded DNA. These properties allow PNAs to be used for therapeutical agents as antisense or antigene strategy.[4] However, a simple PNA molecule may not recognize a highly structured RNA with high specificity, because PNA alone would not have a high ability of forming various conformations as proteins or peptides do. In order to develop such a novel RNA-binding molecule, we have attempted to combine peptide molecules that form a structure such as an α-helix and PNAs that specifically recognize bases in RNA. We thereby report here the design and synthesis of PNA-conjugated peptides, derived from the regulatory protein of virion expression (Rev) of human immunodeficiency virus type-1 (HIV-1),[5–8] and the effect of introduction of a PNA on the peptide conformation and the RNA-binding ability to check the possibility of using a PNA into RNA-binding peptides.

## Results and Discussion

The arginine-rich domain of the Rev (34-50) peptide (17-residues) binds specifically to the stem-loop IIB region of Rev-responsive element (RRE) RNA by forming an α-helix conformation.[9,10] The α-helix potential of the $Rev_{34-50}$ peptide affects the binding affinity and specificity of the peptide with RRE IIB RNA.[9,11] Because PNA is a flexible molecule, it may perturb the α-helix conformation when the peptide binds the RNA. Therefore, for the combination of the Rev-peptide with a PNA, the C-terminal Arg residue was selected, because the terminal residues may not influence the conformational property of the peptide. It

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is proposed that the C-terminal Arg residue at the 50 position interacts with U72 in the bulge of RRE RNA.[10] For the complementary interaction with U72, the Arg50 residue was replaced by $A_{PNA}$ ($R50A_{PNA}$). Moreover, $C_{PNA}$ and $T_{PNA}$ were also introduced in place of the Arg residue ($R50C_{PNA}$, $R50T_{PNA}$). R50RG, which has the same sequence of the $Rev_{34-50}$ peptide, and R50GG, which contains a glycine at the 50 position instead of arginine, were synthesized as reference compounds. Since the PNA monomer has the length equivalent to two amino acids in the main chain, R50RG and R50GG were combined with a glycine at the C-terminal. To increase the stability of the α-helix structure, N-terminal amino acids and C-terminal carboxyl groups of the peptides were succinylated and amidated, respectively. The PNA-peptides were synthesized by the solid-phase method using Fmoc-strategy.[12] Synthesized peptides were purified with HPLC, and identified by matrix assisted laser desorption ionization time-of-flight mass spectrometry[13] and amino acid analysis. RRE IIB RNA (47 nts) was prepared in vitro using T7 RNA polymerase and a synthetic oligonucleotide template, and then purified by polyacrylamide gel electrophoresis (Fig. 1).[14]

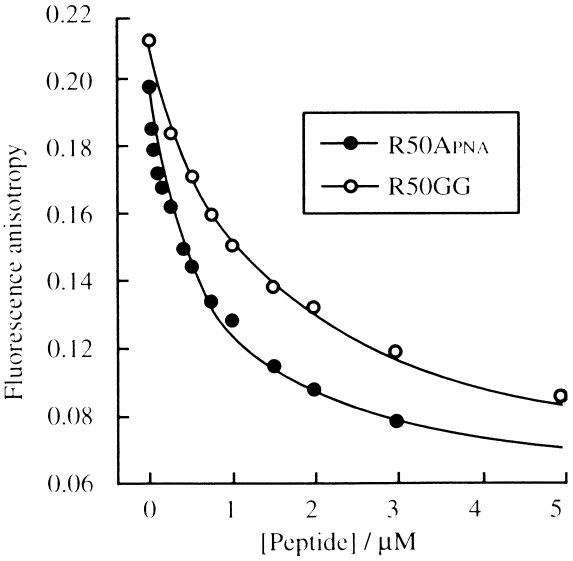


**Figure 2.** Fluorescence anisotropy of Rhod-Rev (10 nM) in the presence of RRE IIB RNA (25 nM) and $R50A_{PNA}$ or R50GG in various concentrations in buffer (10 mM Tris–HCl, pH 7.5, 100 mM KCl, 1 mM $MgCl_2$, 0.5 mM EDTA) at 25 °C ($\lambda_{ex} = 540$ nm, $\lambda_{em} = 580$ nm).

The binding affinities of the peptides with RRE IIB RNA were determined by the competition assay[15] using the 5-carboxytetramethylrhodamine-labeled Rev peptide (Rhod-Rev) as a fluorescence tracer. An initial experiment to determine the dissociation constant ($K_d$) between Rhod-Rev and the RNA was carried out in a buffer (pH 7.5). The fluorescence anisotropy of Rhod-Rev was increased with increasing the RRE IIB RNA concentrations. The significant increase of anisotropy was attributed to the difference in molecular size and conformation between the free and bound Rhod-Rev with RRE IIB RNA. The dissociation constant of 2.1 nM was obtained from the anisotropy change for Rhod-Rev using an equation with 1/1 stoichiometry. On the basis of this result, competition experiments were performed to determine the binding affinities of the designed peptides with RRE IIB RNA. In the mixture of Rhod-Rev (10 nM) and RRE IIB RNA (25 nM), fluorescence anisotropy values were decreased by the addition of the peptides as a competitor, affording the free Rhod-Rev (Fig. 2). The dissociation constants of the peptides with RNA were calculated by the equation assumed as a 1/1 stoichiometry. These competition experiments revealed that R50RG bound RRE IIB RNA strongly with a dissociation constant of 8.0 nM (Table 1). The lack of the Arg side chain (R50GG) diminished the binding affinity to RRE RNA ($K_d = 42$ nM). In contrast, the addition of $A_{PNA}$ at the 50 position ($R50A_{PNA}$) increased the binding affinity ($K_d = 10$ nM), comparable to the value of R50RG with the native sequence. These results suggest that the adenine nucleobase interacts with U72 in the bulge structure in a complementary manner. On the other hand, the binding affinities of $R50C_{PNA}$ and $R50T_{PNA}$ were moderate ($K_d = 21$ and 25 nM), supporting the above assumption. The addition of $C_{PNA}$ and $T_{PNA}$ affects the RNA binding, probably due to some hydrophobic interactions with the RNA.

Circular dichroism (CD) studies revealed that the PNA-peptides possessed the ability of forming an α-helix structure. The $R50A_{PNA}$ peptide showed a typical α-helix pattern with double negative maxima at 208 and

(a)

| | |
|---|---|
| R50APNA | suc-TRQARRNRRRRWRERQ**APNA**-NH2 |
| R50CPNA | suc-TRQARRNRRRRWRERQ**CPNA**-NH2 |
| R50TPNA | suc-TRQARRNRRRRWRERQ**TPNA**-NH2 |
| R39CPNA | suc-TRQAR**CPNA**NRRRRWRERQR-NH2 |
| R44CPNA | suc-TRQARRNRRR**CPNA**WRERQR-NH2 |
| R50RG | suc-TRQARRNRRRRWRERQRG-NH2 |
| R50GG | suc-TRQARRNRRRRWRERQGG-NH2 |
| Rev34-50 | $^{34}$TRQARRNRRRRWRERQR$^{50}$ |

(b)

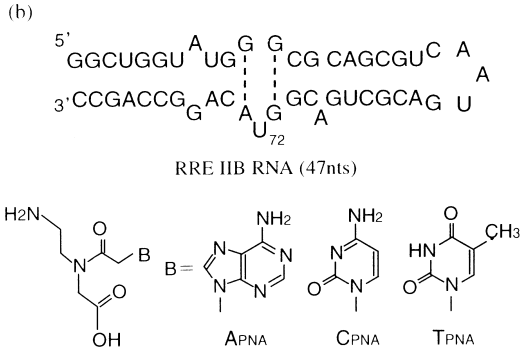


**Figure 1.** (a) The sequence of PNA-peptides, and (b) 2D structure of RRE IIB RNA and PNA structures.

**Table 1.** Dissociation constants ($K_d$) with RRE IIB RNA and α-helicities of the PNA-peptides

| Compounds | $K_d$ (nM) | α-Helicity (%) |
|---|---|---|
| $R50A_{PNA}$ | 10 | 56 |
| $R50C_{PNA}$ | 21 | 58 |
| $R50T_{PNA}$ | 25 | 54 |
| $R39C_{PNA}$ | 420 | 27 |
| $R44C_{PNA}$ | 240 | 29 |
| R50RG | 8.0 | 58 |
| R50GG | 42 | 57 |

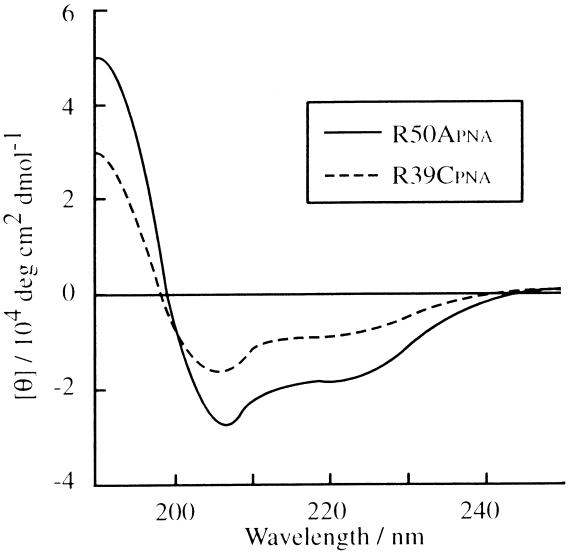


**Figure 3.** CD spectra of $R50A_{PNA}$ and $R39C_{PNA}$ [Peptide] = 20 μM in trifluoroethanol at 25 °C.

222 nm at 25 °C in trifluoroethanol (Fig. 3). From the ellipticity at 222 nm, the α-helicity of $R50A_{PNA}$ was estimated as 56%[16] (Table 1), which is comparable to the α-helicities of R50RG (58%) and R50GG (57%). These results indicate that the addition of PNA at the C-terminal of the peptide does not influence the α-helix conformation. In fact, $R50C_{PNA}$ and $R50T_{PNA}$ showed the comparable α-helicity values to $R50A_{PNA}$. In contrast, the introduction of flexible PNA into the middle sequence of the peptide disturbed the α-helix conformation. The replacement of Arg39 or Arg44 by $C_{PNA}$ significantly reduced the α-helix content (27 and 29%, respectively). As a consequence, the binding abilities of $R39C_{PNA}$ and $R44C_{PNA}$ to RRE IIB RNA were extremely lowered as 420 and 240 nM, respectively (Table 1). The disordered nature in conformation might decrease the possibility of the contact of $C_{PNA}$ to the complementary G in the RNA (Fig. 3).

In conclusion, the peptides containing PNA have been prepared successfully. Introduction of PNA at the terminal of the peptide does not interrupt the α-helix formation. An appropriate PNA at the C-terminal of peptide could be useful to design molecules with a modified binding property to RNA. This strategy combining PNA at terminal extension of the peptide may lead the development of artificial molecules that recognize RNA with high affinity and high specificity.

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