

Active Control by External Factors of DNA Recognition Behavior of α -Peptide Ribonucleic Acids Containing Basic Amino Acid Residues

Takehiko Wada,^{*1} Nobuya Sawa,² Hirofumi Sato,² Mayuko Kikkawa,² Keiko Onodera,¹ Seiji Sakamoto,¹ and Yoshihisa Inoue^{*2}

¹Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Aoba-ku, Sendai 980-8577

²Department of Applied Chemistry, Osaka University, 2-1 Yamada-oka, Suita 565-0871

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A series of novel α -peptide ribonucleic acid (α PRNA) oligomers, possessing alternative α PRNA-Gly/Lys sequences, were newly designed, synthesized, and evaluated as the second-generation PRNA. As expected, the α PRNA with Lys formed stable sequence-specific complex with the complementary DNA, for which both the hydrogen-bonding interactions between the complementary nucleobase pairs and the electrostatic interactions between the ammonium cation on the lysine side chain and the DNA phosphate anion on the backbone are jointly responsible.

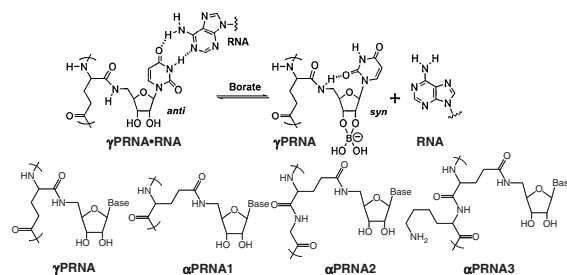


Chart 1. Structure of γ PRNA and α PRNAs.

Recently gene therapeutic methods for selectively down regulating the expression of the genetic information of cancer growth and disease related proteins by specific recognition and binding to the target mRNA through the siRNA and antisense RNA strategies have become a target of intensive studies.¹ In particular the down regulation of genetic information by siRNA is considered one of the most powerful tools not only for molecular biology and genetic engineering but also for oligonucleotide therapeutics.¹ Nevertheless, the currently used siRNA molecules have some drawbacks, such as poor reproducibility, resulting probably from intracellular instability, low cell membrane permeability, and low binding affinity.¹ Most of the other gene therapeutic methods share some of these problems, and hence these are regarded as the most crucial research targets to be attacked.² To remedy these drawbacks, a variety of modified nucleotides and nucleic acid model compounds have been proposed for a better resistance to nucleases and an enhanced hybridization affinity of the oligomer.²

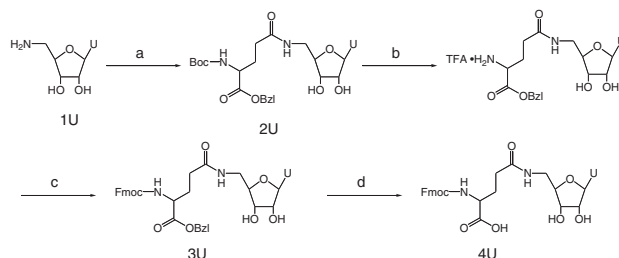
Peptide nucleic acid (PNA) with a peptide-based backbone structure is one of the most successful nucleic acid models.³ Inspired by the unique backbone structure and properties of PNA, several attempts to improve and/or functionalize existing gene therapeutic compounds have been done through the modification of the oligonucleotide backbone² as well as the development of nucleic acid model compounds with different backbone structures.² However, these hitherto reported nucleic acid derivatives and their model compounds have an intrinsic restriction or disadvantage, lacking the ability to *actively* control the function of oligonucleotide therapeutics by internal physiological and/or external physical/chemical stimuli.

We have recently proposed a new category of nucleic acid model compound; named peptide ribonucleic acid (PRNA), in which a 5'-amino-5'-deoxypyrimidine ribonucleoside (**1**) moiety is appended to an oligo(γ -L-glutamine) backbone through the ribose 5'-amino group (Chart 1).⁴ γ PRNAs form stable complexes with complementary DNAs (cDNAs) and RNAs with high nucleobase sequence specificities. Furthermore, the recognition and complexation behavior of γ PRNAs with target RNAs can be controlled by borax added as an external factor through

the anti-to-syn switching of the nucleobase orientation, which is attributable to the synergetic effect of the borate ester formation with the 2',3'-*cis* diol of the ribose moiety and the intramolecular hydrogen-bond formation between the nucleobase and the furanose moiety of PRNA.⁴ Despite the unique features useful for gene therapeutics, PRNA has a potential burden; namely, the relatively slow hybridization, the low cell membrane permeability, and/or the moderate water solubility critically depend on the nucleobase sequence in the side chain.

In the present study, we propose a new strategy not only to improve these potential disadvantages of PRNA but also to add a functionality to PRNA by introducing derivatizable amino acid residues. Thus, we have designed and synthesized a series of α PRNAs, in which the moiety **1** is appended to the α -(L-glutamine) backbone through the 5'-amino group, and also several α PRNA derivatives, which have a mixed backbone composed of α PRNA and glycine or basic amino acids, such as lysine (Chart 1). By using this strategy, both the water solubility and hybridization ability of PRNA are expected to be improved.

In the target α PRNA synthesis, monomer **4** was prepared in four steps starting from *N*-Boc-L-Glu-OBzl and **1** (Scheme 1). *N*-Boc-L-Glu-OBzl and **1** was reacted with BOP reagent and HOBt in DMF to give **2U**. Subsequently, **2U** was treated with TFA to remove Boc, and then the free N-terminus was protected with Fmoc by reaction with Fmoc-OSu to give Fmoc-Glu(5'U)-



Scheme 1. Reagents: (a) *N*-Boc-L-Glu-OBzl, Bop reagent, HOBt, DIEA, DMF, 83%; (b) TFA; (c) Fmoc-OSu, DIEA, DMF, 90%; (d) H₂, Pd/C, DMF/methanol, 96%.

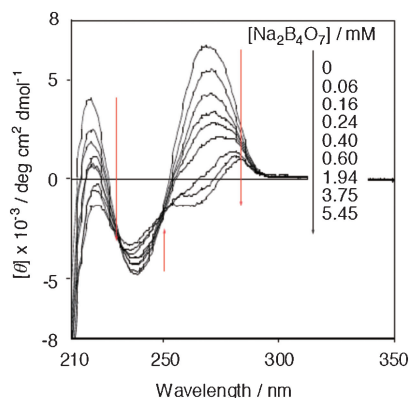


Figure 1. CD spectral changes of α PRNA3 with increasing concentrations of $\text{Na}_2\text{B}_4\text{O}_7$ in phosphate buffer (0.033 M KH_2PO_4 –0.033 M Na_2HPO_3 , pH 7.2); $[\alpha\text{PRNA3}] = 1.0 \times 10^{-4}$ M.

OBzl (**3U**). Finally, the C-terminus benzyl ester was removed by hydrogenation over 10% Pd/C in dry DMF/methanol mixed solvent to give Fmoc-Glu(5'U)-OH (**4U**).⁵ The α PRNA 12mers of homo-uracil sequence with end-capping Lys₃ at the N- and C-termini, i.e., α PRNA1, α PRNA-glycine (α PRNA2), and 8mer of homo-uracil sequenced α PRNA-lysine (α PRNA3), for the use in a first examination of the recognition behavior with DNA, were prepared from **4U** by a Fmoc solid-phase peptide synthesis, which was employed also in a PRNA synthesis reported previously.^{4,7} The α PRNA oligomers thus obtained were purified by reversed phase preparative HPLC and finally confirmed by analytical HPLC and MALDI-TOF MS measurements.⁶ Good yields of up to 98% at each step were obtained under the coupling condition.

Since the anti-to-syn switching of nucleobase orientation upon cyclic borate ester formation is an essential factor for achieving the “on-demand” gene therapeutics, we first examined the CD spectral behavior of α PRNA1–3. The gradual addition of borax to a phosphate buffer solution of α PRNA3 (pH 7.2) induced significant CD spectral changes, as shown in Figure 1. The isodichroic points observed at ca. 220 and 240 nm indicate that a single step is responsible for these changes. In the phosphate buffer, the $[\theta]_{\text{ext}}$ value at 270 nm ($6600 \text{ deg cm}^2 \text{ dmol}^{-1}$) was comparable to that observed for **1** and larger than that for original γ PRNA, which is compatible with the preferred anti orientation. In contrast, the $[\theta]_{\text{ext}}$ value of this oligomer was greatly reduced to $2800 \text{ deg cm}^2 \text{ dmol}^{-1}$ by increasing the borate concentration, which is similar to the value observed for the original γ PRNA in a borate buffer. These results unambiguously reveal the occurrence of the anti-to-syn switching of the nucleobase orientation upon addition of borax. A quantitative analysis of the CD spectral changes, using the nonlinear least-squares fit to the curve for 1:1 stoichiometric complexation, gave the equilibrium constant of 3000 M^{-1} for the reversible esterification of α PRNA3 with borax. This value is three times higher than those of the original γ PRNAs, indicating that the electrostatic repulsion of the positively charged ammonium groups of Lys residues may extend the peptide backbone, and therefore the steric hindrance between the nucleoside moieties in the side chain is reduced. Similar borax-induced anti-to-syn nucleobase orientation switching was also observed with α PRNA1 and -2.⁷

The complexation behaviors of α PRNA1, α PRNA2 (12mers), and α PRNA3 (8mer) with cDNA, d(A)₁₂, and d(A)₈ were examined in pH 7.2 phosphate buffers at normal 33 mM and very dilute 0.33 mM (where the electrostatic interactions are encouraged) concentrations. The structural difference in the two α PRNA 12mers is the Gly backbone introduced to α PRNA2, which led to a critical difference in complex stability. Thus, α PRNA2 forms a complex with d(A)₁₂ that melts at 22 °C, which is similar to the T_m (23 °C) obtained for the corresponding natural DNA pair. In keen contrast, the complexation of α PRNA1 with d(A)₁₂ was not observed ($T_m < 5$ °C). This clear distinction indicates that the nucleobase repeating distance plays one of the most crucial and important roles upon complexation. Interestingly, α PRNA3 8mer, in which Lys residues are introduced to the backbone, gave a stable complex ($T_m = 25$ °C) with d(A)₈ immediately after mixing, while no complex formation was observed for natural d(T)₈ and d(A)₈ under the same conditions, probably due to electrostatic repulsion. In contrast, in the borax-containing buffer, the hybrid complex did not exhibit any melting behavior above 0 °C, or hypochromic changes, and hence the on-off switching of nucleic acid recognition was achieved indeed. Moreover, no complex formation was observed for α PRNA3 with not only d(T)₈ but also d(A₃TTA₃), revealing that the introduction of basic amino acid residues into PRNA backbone can stabilize the PRNA–DNA complex, without losing the nucleobase sequence selectivity, and further accelerates the rate of complexation, both of which are thought to be caused by the electrostatic interaction. Therefore, a promising strategies to overcome the potential disadvantage of relatively slow hybridization, low cell membrane permeability, and moderate water solubility is to incorporate basic amino acid into α PRNA backbone. Studies along this line are currently in progress.

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References and Notes

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- ¹H NMR (270 MHz, DMSO-*d*₆): δ 1.69–2.07 (m, 2H, β -CH₂), 2.20 (t, 2H, γ -CH₂, $J = 7.8$ Hz), 3.11–3.50 (m, 2H, 5'-CH₂), 3.72–3.87 (m, 2H, 3'- and 4'-H), 3.87–4.09 (m, 1H, α - and 2'-H), 4.15–4.32 (m, 3H, Fuluorenyl-CH-CH₂), 5.19 (d, 1H, 3'-OH), 5.42 (d, 1H, 2'-OH), 5.62 (d, 1H, 5-H, $J = 7.8$ Hz), 5.74 (d, 1H, 1'-H, $J = 5.9$ Hz), 7.26–7.78 (m, 8H, Fmoc-H, Fmoc-NH, and 6-H), 7.90 (d, 2H, Fmoc-H), 8.05 (t, 1H, 5'-NH, $J = 5.9$ Hz), 11.37 (s, 1H, 3-NH); HR-FAB MS. m/z : found 595.2048 ($M + 1$), calcd 595.1962 ($M + H$).
- NH₂-Lys₃-(Glu(5'U))₁₂-Lys₃-OH (α PRNA1): MALDI-TOF MS (α -CHCA), m/z : found 5038.97 ($M + H$)⁺, calcd 5038.83; NH₂-(Glu(5'U)-Gly)₁₁-Glu(5'U)-Lys₃-OH (α PRNA2): MALDI-TOF MS (α -CHCA), m/z : found 5025.22 ($M + H$)⁺, calcd 5025.54; NH₂-(Lys-Glu(5'U))₈-Lys₃-OH (α PRNA3): MALDI-TOF MS (α -CHCA), m/z : found 4008.16 ($M + H$)⁺, calcd 4007.09.
- Supporting Information is available electronically on the CSJ-Journal Web site, <http://www.csj.jp/journals/chem-lett/index.html>.