

RNA Recognition and Recognition Control of α -Peptide Ribonucleic Acids Containing Arginine Residue by External Factors

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Introduction

We have reported the effect of adding borax on the nucleobase orientation and recognition behavior of novel oligomeric α - and γ -peptide ribonucleic acids (α - and γ -PRNAs) [1]. The base orientation of 5'-aminopyrimidine ribonucleosides in recognition moieties of PRNAs were shown by CD and NOE difference spectral studies to switch from *anti* to *syn* upon addition of borax. The origin of this phenomenon is elucidated to be the cooperative effect of the cyclic borate esterification of the ribose's *cis*-2',3'-diol and the hydrogen-bonding interaction between the ribose's 5'-amino proton and the base's 2-carbonyl oxygen. Therefore, it was unambiguously demonstrated that γ -PRNAs with an isopoly(L-glutamic acid) backbone can form a stable complex with DNA and further the recognition of DNA with γ -PRNAs is controlled by the borate added as an external factor. Nevertheless, the transfection efficiency of not only natural and modified DNA, but also artificial nucleic acids possessing amide backbones, such as PNA is often not satisfactory, primarily due to the low permeability of the molecules through cell membrane [2]. In the meantime, active cellular-uptake using membrane-permeable peptide vectors is a recently developed methodology. The efficient delivery of proteins and nucleic acids using the method by conjugating with basic peptide segments, such as arginine-rich peptides has been reported [2]. Thus, in this paper, we have designed and synthesized a series of α -PRNAs possessing alternative α -PRNA/lysine and α -PRNA/arginine sequences (Chart 1).

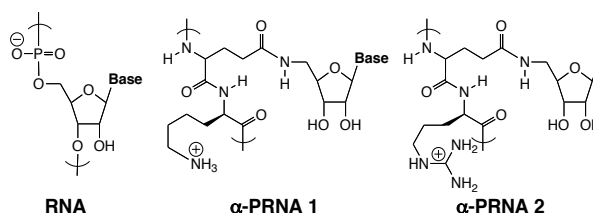


Chart 1. Structure of RNA and α -PRNAs.

Results and Discussion

Synthesis of α -PRNAs. - α -PRNA 1, and α -PRNA 2 are synthesized by Fmoc-solid phase peptide synthesis [3]. The PRNA oligomers obtained were purified by reversed phase preparative HPLC.

CD Spectral Study on α -PRNA Oligomers. - The CD spectra were measured at various concentrations of borax added to a phosphate buffer solution of α -PRNA 8-mers (1 and 2). In phosphate buffer, the $[\theta]_{\text{ext}}$ value of α -PRNA 1 obtained (6500 deg cm² dmol⁻¹) was nearly the same as that observed for 5'-amino-5'-dexoyuridine, and this is compatible with the preferred *anti* orientation in phosphate buffer.¹ The CD intensity continuously decreased as the concentration of borax increased, leveling off between 1.9 and 5 mM. The CD spectra observed at borax concentrations of borax >1.9 mM is almost superimposable upon that obtained in borate buffer, which contains 20 mM of borax. The gradual CD spectral changes, accompanied by the isosbestic points observed at *ca.* 230 and 250 nm, indicate clearly that the reversible *anti*-to-*syn* orientation switching process is caused by a cooperative cyclic borate ester formation and hydrogen bonding interaction, as observed for the 5'-aminouridine and the γ -PRNA oligomers [1]. A quantitative treatment of these CD spectral changes of α -PRNA 1, using the non-linear least squares fitting to the curve for 1:1 stoichiometric complexation, gave the equilibrium constant of 3000 M⁻¹ for the formation of borate ester for each ribonucleoside, which is exactly 40 times greater than that obtained for 5'-aminouridine under the comparable conditions.

Table 1. Melting temperatures (T_m) of α -PRNA 1 and 2 with oligonucleotide complexes

PRNA or DNA	complement	$T_m / ^\circ\text{C}$	
		borax /mM	0
α -PRNA 1 (UKUKUKUKUKUKUKUK)	d(A) ₈	25	< 2
α -PRNA 2 (URURURURURURUK)		40	< 2
d(T) ₈		2	5

[PRNA] = [Oligonucleotide] = 1.0×10^{-5} M (1 / 3000 M phosphate buffer, pH 7.0)

Control of Hybridization of α -PRNA with Complementary DNA by Borate Ester Formation -

The hybridization ability of α -PRNA 1 and 2 with complementary d(A)₈ was evaluated from the melting temperature, T_m . In order to elucidate the effects of borate on the formation and stability of the α -PRNA-DNA hybrid, the melting profiles for the α -PRNA 1 and 2 pairs and the reference compound d(T)₈ with the complementary d(A)₈ were measured independently in phosphate buffer with and without borax (20 mM). Table 1 summarizes the T_m values observed. The stoichiometry of the complex with d(A)₈ was determined to be 1:1 in each case (uracil or thymine: adenine unit ratio) using the Job plot of the hypochromic change upon mixing. Control runs using non-complementary d(T)₈ in place of d(A)₈ were also carried out under compatible conditions and found to show no hypochromicity or appreciable melting behaviour. This confirms the base-specific interaction of α -PRNA 1 and 2 with the complementary d(A)₈, and then also indicates that an inconsiderable contribution of a non-specific electrostatic interaction between basic amino residues of α -PRNA 1 and 2 and phosphate backbone of DNA's in the α -PRNA – DNA complexes.

As shown in Table 1, in borax-free buffer, the hybrid complex between α -PRNA 1 and d(A)₈ gave a considerably higher T_m of 25.0 °C than the complementary d(T)₈-d(A)₈ duplex (T_m = 2.0 °C) under the same condition, indicating a stronger interaction in the hybrid than in the natural pair. The complexation behaviour of α -PRNA 2, which possesses alternating uridine PRNA monomer and arginine residue, was studied under the same condition. The α -PRNA 2 complex with d(A)₈ gives a T_m of 40.0 °C with hypochromicity of 50%, which is appreciably higher than that obtained for the α -PRNA 1–DNA complex. This result may indicate that a guanidinium cation group of arginine residue of α -PRNA 2 efficiently binds to target DNA phosphate anions on the backbone, rather than that of an ammonium group of lysine residue of α -PRNA 1, although the non-specific electrostatic interaction between cationic residues of α -PRNA and negatively charged DNA backbone would be negligible.

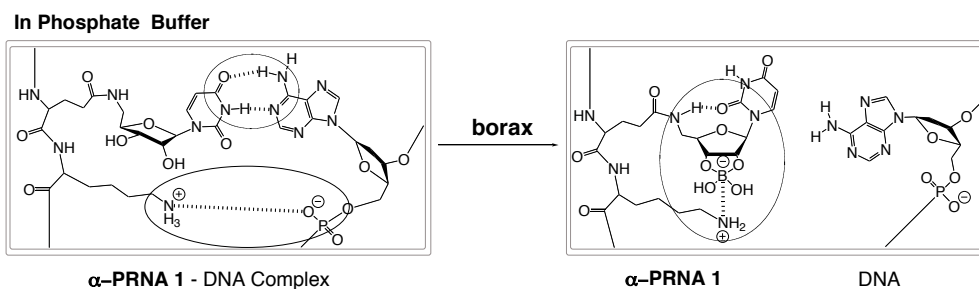
In contrast, in the borax-containing buffer solution, the hybrid complex of α -PRNA 1 and 2 with d(A)₈ did not exhibit any melting behavior above 2 °C, or hypochromic changes, while the complementary (T)₈-d(A)₈ duplex gave an appreciably higher T_m of 5.0 °C, presumably due to the slight increase in the ionic strength. This contrasting behavior between the natural and PRNA hybrid pairs in the presence/absence of borax is most likely attributable to the *anti*-to-*syn* orientation switching of the uracil base in α -PRNA, for which a cooperative borate ester formation at the *cis*-2',3'-diol and a hydrogen bonding interaction between the 5'-amide proton and the 2-carbonyl oxygen are responsible. However, the electrostatic repulsion between the adjacent anionic borate esters makes some contribution, as illustrated in Scheme 1.

Acknowledgments

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Scheme 1. Recognition and complexation behaviour control of α -PRNA1-DNA.