A novel strategy for reversible control of conformation and DNA/RNA recognition of peptide ribonucleic acid (PRNA) by external factors

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ABSTRACT

A novel nucleic acid model using peptide ribonucleic acid (PRNA), which contains 5'-amino-5'-deoxyribonucleoside as a recognition site for nucleic acids, has been designed, synthesized and applied to the external reversible control of recognition behavior of the complementary oligomeric DNA through the orientational switching of the nucleobase induced by borates. In case of PRNA 12-mers, efficient orientational change of nucleobases was observed. Furthermore, these oligomeric PRNAs form a stable complex with complementary DNA's and the recognition behavior of oligomeric PRNAs with DNA's is controlled by the borate added as an external factor.

INTRODUCTION

Following the achievement of the human genome sequencing,¹ gene therapeutic drugs in antisense and/or antigen strategy have been received much attention. The modified oligonucleotides, in which the furanose-phosphodiester backbone is replaced by furanose-phosphorothioate, -alkylyphosphonate, -phosphoramidate, and N-2-aminomethylglycine (peptide nucleic acid; PNA) have been proposed not only to improve the stability of the oligomers and/or hybrids in the presence of nuclease, but also to enhance the hybridization affinity.² Nevertheless, most of modified nucleotides and nucleic acid model compounds have been concentrated on sequence specific binding and increasing the complex stability with target DNA/RNA, and little effort has been devoted to the active control of the DNA/RNA recognition processes, possibly as a result of a lack of suitable fundamental strategy and the necessary practical tools. In other words, inherent and crucial drawback of these model systems is the lack of a direct method to actively control the function of these nucleic acids. If one can control the recognition and complexation behavior of the nucleic acid model compounds, the occasion of the function of nucleic acid can be controlled and it is expected to find various biochemical and pharmaceutical applications as a powerful and versatile tool for manipulating the relevant events (Chart 1). For this purpose, we wish to propose a new category of nucleic acid analogues, i.e. peptide ribonucleic acids (PRNAs, Chart 2), in which the ribonucleoside units are not directly incorporated into the main chain, as is the case with RNA, but are instead attached to the peptide backbone as pendant groups.

RESULTS AND DISCUSSION

It is well documented that in the recognition/binding process of nucleic acids, nucleobase orientation, such as anti/syn, plays crucial role. The anti-orientation of nucleobase is an essential factor for efficient recognition, since syn-orientated nucleobases are unfavorable for forming intermolecular hydrogen bonds with the complementary nucleobase. This means that, if the nucleobase orientation could be switched from anti to syn by some additives
and/or external factor, one can readily materialize external control of the recognition behavior. Unfortunately, no external agent or factor which affects the syn-anti orientation and therefore the recognition behavior of nucleic acids and analogues, has been found or proposed to date, although the unusual syn-orientation is known to be induced by several internal factors, such as increased steric hindrance and altered sugar puckering. Indeed, ketalization of the cis-2', 3'-diol of uridine enhances the syn/anti ratio through the imposed 2',3'-planar-O6-exo-furanose structure. However, the energetically disfavored syn orientation has not been induced by external agents.

It is also well known that boronic acids form cyclic esters with a variety of cis-1,2-diols, including sugars and ribonucleosides, and this esterification process is reversible in aqueous solutions at moderate pH. Recently, we have reported that the nucleobase orientation of 5'-aminopyrimidine nucleosides, such as 5'-amino-5'-deoxycytidine and 5'-amino-5'-deoxyuridine, is switched from anti to syn simply by using borates, as determined by circular dichroism (CD) and 1H NMR-NOE spectroscopy. This unprecedented orientational switching phenomenon is expected to provide us with a unique methodology for externally controlling nucleic acid recognition, if a nucleic acid analogue these functionalities are carrying a ribose unit with free 2',3'-diol and 5'-amino/amide proton can be prepared, since both of indispensable to the orientational switching process. These structural and functional requirements are not satisfied through conventional modifications of RNA/DNA or peptide nucleic acids (PNA). For this purpose, we wish to propose a new category of nucleic acid analogues, i.e. peptide ribonucleic acid (PRNA). We have demonstrated that this strategy works well with two kinds of PRNAs, named α-PRNA, which carries 5'-amino-5'-deoxynucleoside units appended to poly(L-glutamic acid), and γ-PRNA, in which 5'-amino-5'-nucleoside units are tethered to the isopropy(L-glutamic acid) backbone at the remaining α-carboxyl group of glutamic acid through the 5'-amino group of nucleoside derivatives. (Chart 2)

The CD spectra of γ-PRNA 12-mer with homo uracil nucleobase were measured in both borate and phosphate buffers. In phosphate buffer, the [θ]max value obtained (5000 deg cm² dmol⁻¹) was nearly the same as that observed for 5'-amino-5'-deoxycytidine, and this is compatible with the preferred anti orientation in phosphate buffer. In contrast, the [θ]max value of this oligomer was greatly reduced to 600 deg cm² dmol⁻¹ in borate buffer, which is significantly smaller than the value observed for 5'-amino-5'-deoxyuridine in borate buffer (1600 deg cm² dmol⁻¹). These results strongly indicate the predominant syn orientation of nucleobase in borate buffer. In order to elucidate the effects of borate on the formation and stability of the PRNA-DNA hybrid, the melting profiles for the γ-PRNA pairs and the reference compound (T)₁₂ with the complementary d(A)₁₂ were measured independently in phosphate buffer with and without borax (20 mM). The stoichiometry of the complex with d(A)₁₂ 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. In borax-free buffer, the hybrid complex between γ-PRNA and d(A)₁₂ gave a considerably higher Tm of 26.6 °C than the complementary (T)₁₂-d(A)₁₂ duplex (Tm = 23.4 °C), indicating a stronger interaction in the hybrid than in the natural pair. In contrast, in the borax-containing buffer solution, the hybrid complex did not exhibit any melting behavior above 0 °C, or hypochromic changes, while the complementary (T)₁₂-d(A)₁₂ duplex gave an even higher Tm of 25.2 °C, presumably due to the slight increase in the ionic strength. This contrasting behavior between the natural and hybrid pairs in the presence/absence of borax is most likely attributable to the anti-to-syn orientation switching of the uracil base in γ-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.

In the case of α-PRNA1, though, the efficiency of external control of recognition and complexation behavior was not very high, as a result of the mismatched repetition distance of the nucleobases. Thus, α-PRNA2 and 3, which alterate sequences consisted of monomeric α-PRNAs and L-alanine and L-lysine, have been synthesized. The excellent orientation switching through the addition of a small amount of borate was shown with any monomeric and oligomeric models. Finally, we demonstrated that the α-PRNA2 and 3 with an α-peptide backbone can definitely form tight complexes with DNA, α-PRNA3-DNA complex was more stable than that of corresponding DNA-DNA.

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REFERENCES