Promotion mechanism of triplex DNA formation by comb-type polycations: Thermodynamic analyses of sequence specificity and ionic strength dependence

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ABSTRACT
We have previously reported that in the presence of poly (L-lysine)-graft-Dextran (PLL-g-Dex) co-polymer, the binding constant of the pyrimidine-motif triplex formation at neutral pH is about 100-times higher than that observed without any triplex stabilizer. Here, to explore the mechanism of the promotion effect of the PLL-g-Dex copolymer at neutral pH, the sequence specificity and the ionic strength dependence of the pyrimidine-motif triplex formation was examined in the absence or presence of the copolymer. The sequence specificity of the pyrimidine-motif triplex formation at neutral pH in the presence of copolymer was almost similar to that at acidic pH without the copolymer. As the concentration of magnesium cation increased, the binding constant of the pyrimidine-motif triplex formation without the copolymer increased. On the other hand, the binding constant of the pyrimidine-motif triplex formation in the presence of the copolymer decreased upon the increase in the concentration of magnesium cation. Considering these results in light of counterion condensation (CC) theory, we conclude that the copolymer does not hinder the sequence specificity of the triplex formation, and isolates the triplex formation from the CC effect, which may lead to a net increase in entropy change upon the triplex formation, providing a favorable component to binding constant of the triplex formation.

INTRODUCTION
In recent years, triplex DNA has attracted considerable interest because of its possible biological function in vivo and its wide variety of potential applications, such as regulation of gene expression, site-specific cleavage of DNA, and mapping of genomic DNA (1). Triplex is usually formed through sequence-specific interaction between a homopurine-homopyrimidine stretch of duplex and a homopyrimidine or homopurine single strand, called a triplex-forming oligonucleotide (TFO). In the pyrimidine-motif triplex formation, a homopyrimidine TFO binds to the major groove of the duplex in parallel with the homopurine strand via Hoogsteen hydrogen bonding. Stable base triplets, T*AT and C**GC, are formed between thymines (T) in TFO and adenine* thymine (A:T) base pairs of duplex, and between protonated cytosines (C*) in TFO and guanine*cytosine (G:C) base pairs of duplex, respectively. As protonation of the cytosine base in TFO is required to bind with the guanine base of the G:C duplex, the pyrimidine-motif triplex is usually formed at acidic pH and unstable at neutral pH. However, stabilization of the pyrimidine-motif triplex at neutral pH is quite necessary for its applicability as an antigenic drug in vivo. We have previously shown that in the presence of poly (L-lysine)-graft-Dextran (PLL-g-Dex) copolymer (Figure 1), the binding constant of the pyrimidine-motif triplex formation at neutral pH between a homopurine-homopyrimidine stretch of a 23-bp target duplex and a 15-mer specific TFO was about 100 times higher than that observed without any triplex stabilizer (2-4). Here, we have further extended our study to explore the mechanism of the promotion effect of the PLL-g-Dex copolymer at neutral pH. The sequence specificity and the ionic strength dependence of the pyrimidine-motif triplex formation was examined in the absence or presence of the PLL-g-Dex copolymer by isothermal titration calorimetry (ITC).

MATERIALS AND METHODS
We synthesized complementary 23-mer DNA oligonucleotides, 5'-GCCGAGAAGAAAAAGACGGC-3' (denoted as Pur23A) and 5'-CCGGTGTGTGTGTGTGTGT-3' (denoted as Pyr23T), a 15-mer DNA oligonucleotide with perfect match to target duplex, 5'-CTCTTTCTTCTTCT-3' (denoted as Pyr15T), and a 15-mer DNA oligonucleotide with one base mismatch to target duplex, 5'-CTCTTTCTTCTTCT-3' (denoted as Pyr15A), on a DNA synthesizer and purified them with a reverse-phase HPLC. We prepared the PLL-g-Dex copolymer by a reductive amination reaction between poly (L-lysine) and Dextran T-10 (2). ITC experiments were carried out on a MCS ITC system (Microcal Inc., U. S. A.) (4, 5).

Figure 1. Structural formula of PLL-g-Dex copolymer.
RESULTS AND DISCUSSION

Table 1 summarizes the thermodynamic parameters for the binding of a perfect match TFO, Pyr15T, or a one-base mismatch TFO, Pyr15A, with the target duplex, Pur23A•Pyr23T, at 25 °C, obtained from ITC measurements. At pH 4.8 without the PLL-g-Dex copolymer, the binding constant for Pyr15T (denoted as Ka (perfect)) and for Pyr15A (denoted as Ka (mismatch)) was 9.08 x 10^7 and 4.42 x 10^7 M^-1, respectively, and the ratio of Ka (mismatch)/Ka (perfect) was 0.49. Furthermore, Ka (perfect) at pH 6.8 without the copolymer was 1.97 x 10^5 M^-1, which was much smaller than that at pH 4.8 without the copolymer. Ka (mismatch) in the same experimental condition was out of the optimum range for the ITC measurements, and could not be determined. Thus, we could not estimate the ratio of Ka (mismatch)/Ka (perfect) at pH 6.8 without the copolymer. On the other hand, Ka (perfect) and Ka (mismatch) at pH 6.8 in the presence of the copolymer was 1.89 x 10^7 M^-1 and 1.83 x 10^6 M^-1, respectively, and the ratio of Ka (mismatch)/Ka (perfect) was 0.10, which was a little smaller than that observed at pH 4.8 without the copolymer. We conclude that the copolymer does not hinder the sequence specificity of the triplex formation, which may be favorable for the application of the copolymer to the antigenic strategy in vivo.

Next, we have examined the ionic strength dependence of the pyrimidine-motif triplex formation at neutral pH in the absence or presence of the PLL-g-Dex copolymer. The triplex formation between Pyr15T and Pur23A•Pyr23T was investigated in 10 mM sodium cacodylate-cacodylic acid buffer (pH 6.80) containing different concentrations of magnesium chloride in the absence or presence of the copolymer. The binding constant of the pyrimidine-motif triplex formation at 20 mM and 160 mM magnesium chloride without the copolymer was 4.9 x 10^5 M^-1 and 3.4 x 10^6 M^-1, respectively, indicating that, as the concentration of magnesium cation increased, the binding constant without the copolymer increased. On the other hand, the binding constant of the pyrimidine-motif triplex formation at 20 mM and 160 mM magnesium chloride in the presence of 9 μM copolymer was 1.6 x 10^7 M^-1 and 3.0 x 10^6 M^-1, respectively. As the concentration of magnesium cation increased, the binding constant in the presence of the copolymer decreased. Record et al. (6) has proposed that the salt concentration dependence of the binding constant for the complex formation between DNA and ligand is a function of both the extent of counterion condensation (CC) and a net charge of ligand, which is based on the CC theory originally proposed by Manning (7). According to this proposal, the increase in the binding constant without the copolymer upon the increase in the magnesium cation concentration indicates that the extent of the CC is increased upon the triplex formation without the copolymer. Because the binding of TFO with the duplex to form the triplex results in an increase in the linear charge density of DNA, and the extent of CC is a function of the linear charge density of DNA (6, 7), an increase extent of the CC upon the triplex formation without the copolymer is quite reasonable. In contrast, the decrease in the binding constant in the presence of the copolymer upon the increase in the magnesium cation concentration indicates that the triplex formation in the presence of the copolymer is accompanied with counterion release and isolated from the CC effect. Because the stable association of the polycationic material, such as the PLL-g-Dex copolymer, with DNA is a counterion release process (8), the counterion release upon the triplex formation in the presence of the copolymer is understandable. The counterion release causes a net increase in the entropy change of the system, providing a favorable component to the binding constant. We conclude that the counterion release upon the triplex formation and the isolation of the triplex formation from the CC effect is a major driving force for the promotion effect of the copolymer.

REFERENCES