Thermodynamic analyses of triplex formation with homopurine oligonucleotide

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ABSTRACT
We analyzed the thermodynamics of purine motif triplex formation by isothermal titration calorimetry. The signs of calorimetric enthalpy change, ΔHcal, and entropy change, ΔS, of the triplex formation were negative in the temperature range between 15 and 35 °C. Since an observed negative ΔS was unfavorable for the triplex formation, the triplex formation was driven by a large negative ΔHcal. ΔHcal decreased with increasing temperature, yielding a negative heat capacity change, ΔCp, of approximately -1.2 kcal mol⁻¹ K⁻¹. We found that the binding constant, Kₐ, increased with increasing temperature, leading to an apparent positive van’t Hoff enthalpy change, ΔHvH, which was in sharp contrast with the large negative ΔHcal. The analyses of the observed temperature dependence of Kₐ and ΔHcal and the negative ΔCp suggest that the purine motif triplex formation near room temperature is not a simple two-state binding process but exhibits multiple states, which was previously observed for the pyrimidine motif triplex formation near room temperature.

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). A triplex is usually formed through the sequence-specific interaction of a single-stranded homopurine or homopyrimidine triplex-forming oligonucleotide (TFO) with the major groove of homopurine-homopyrimidine stretch in duplex DNA (1). In the pyrimidine motif triplex, a homopyrimidine TFO binds parallel to the homopurine strand of the target duplex by Hoogsteen hydrogen bonding to form A•••T (or T•••A) and G•••C triplets (1). On the other hand, in the purine motif triplex, a homopurine TFO binds antiparallel to the homopurine strand of the target duplex by reverse Hoogsteen hydrogen bonding to form A•••T (or T•••A) and G•••C triplets (1). We have previously reported that the pyrimidine motif triplex formation near room temperature (15-35 °C) is not expressed by a simple two-state model but exhibits multiple states, and also suggested that a self-structure of a homopyrimidine TFO may affect the thermodynamics of the pyrimidine motif triplex formation (2). Here, we have further extended our study to explore the thermodynamic properties of the purine motif triplex DNA formation near room temperature using a 30 bp homopurine-homopyrimidine target duplex from human Ki-ras gene promoter and its specific 22 mer homopurine TFO (Fig. 1). The thermodynamic analyses were performed at neutral pH in the temperature range between 15 and 35 °C by isothermal titration calorimetry (ITC) (2-4). We have found that the purine motif triplex formation is not a simple two-state binding process but exhibits multiple states, which was also observed for the pyrimidine motif triplex formation.

MATERIALS AND METHODS
We synthesized a 22-mer TFO, Pur22T (Fig. 1), and complementary 30-mer DNA oligonucleotides, Pur30 and Pyr30 (Fig. 1), on a DNA synthesizer and purified them with a reverse-phase HPLC. Thermodynamic experiments were carried out on a MCS ITC system (Microcal Inc., U. S. A.) (2-4).

RESULTS AND DISCUSSION
Table 1 summarizes the thermodynamic parameters of the purine motif triplex formation between TFO (Pur22T) and a target duplex by reverse Hoogsteen hydrogen bonding to form A•••T (or T•••A) and G•••C triplets (1). We have previously reported that the pyrimidine motif triplex formation near room temperature (15-35 °C) is not expressed by a simple two-state model but exhibits multiple states, and also suggested that a self-structure of a homopyrimidine TFO may affect the thermodynamics of the pyrimidine motif triplex formation (2). Here, we have further extended our study to explore the thermodynamic properties of the purine motif triplex DNA formation near room temperature using a 30 bp homopurine-homopyrimidine target duplex from human Ki-ras gene promoter and its specific 22 mer homopurine TFO (Fig. 1). The thermodynamic analyses were performed at neutral pH in the temperature range between 15 and 35 °C by isothermal titration calorimetry (ITC) (2-4). We have found that the purine motif triplex formation is not a simple two-state binding process but exhibits multiple states, which was also observed for the pyrimidine motif triplex formation.

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RESULTS AND DISCUSSION
Table 1 summarizes the thermodynamic parameters of the purine motif triplex formation between TFO (Pur22T) and a target duplex (Pur30•Pyr30) at pH 6.8 in the temperature range between 15 and 35 °C, obtained from ITC. The signs of enthalpy change, ΔH, and entropy change, ΔS, of the triplex formation were negative under all the conditions.

<table>
<thead>
<tr>
<th>Target</th>
<th>TFO</th>
<th>ΔH (kcal mol⁻¹)</th>
<th>ΔS (kcal mol⁻¹ K⁻¹)</th>
<th>ΔCp (kcal mol⁻¹ K⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pur30</td>
<td>Pur22T</td>
<td>-3.1</td>
<td>-2.3</td>
<td>-0.8</td>
</tr>
<tr>
<td>Pyr30</td>
<td>Pur22T</td>
<td>-3.0</td>
<td>-2.4</td>
<td>-0.6</td>
</tr>
</tbody>
</table>

Fig. 1: Oligonucleotide sequences of the target duplex and its specific homopurine TFO
Table 1: Thermodynamic parameters for the triplex formation between a 22-mer TFO (Pur22T) and a 30-bp target duplex (Pur30-Pyr30) at pH 6.8, obtained from ITC.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>$K_a$ (M$^{-1}$)</th>
<th>$\Delta G$ (kcal mol$^{-1}$)</th>
<th>$\Delta H$ (kcal mol$^{-1}$)</th>
<th>$\Delta S$ (cal mol$^{-1}$ K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>$2.17 \times 10^6$</td>
<td>-8.35</td>
<td>-29.3</td>
<td>-72.5</td>
</tr>
<tr>
<td>20</td>
<td>$4.32 \times 10^6$</td>
<td>-8.90</td>
<td>-33.5</td>
<td>-84.0</td>
</tr>
<tr>
<td>25</td>
<td>$6.04 \times 10^6$</td>
<td>-9.25</td>
<td>-37.9</td>
<td>-95.9</td>
</tr>
<tr>
<td>30</td>
<td>$2.74 \times 10^7$</td>
<td>-10.3</td>
<td>-43.9</td>
<td>-111</td>
</tr>
<tr>
<td>35</td>
<td>$7.29 \times 10^7$</td>
<td>-11.1</td>
<td>-54.1</td>
<td>-140</td>
</tr>
</tbody>
</table>

*10 mM sodium cacodylate-cacodylic acid and 20 mM magnesium chloride (pH 6.8).

Since an observed negative $\Delta S$ was unfavorable for the triplex formation, the purine motif triplex formation was driven by a large negative $\Delta H$. The negative $\Delta H$ decreased with increasing temperature, $T$, indicating that the purine motif triplex formation was accompanied by a negative heat capacity change, $\Delta C_p$. The plot of $\Delta H$ vs $T$ shows nearly linear dependence with a slope of negative sign in this temperature range (Fig. 2). The plot of $\Delta H$ vs $T$ is fit with a straight line, the estimated value of $\Delta C_p$ was -1.2 kcal mol$^{-1}$ K$^{-1}$.

The binding constant, $K_a$, increased with increasing temperature (Table 1). Assuming that $\Delta H$ and $\Delta S$ are independent of $T$, the van't Hoff enthalpy change, $\Delta H_n$, is calculated by

$$\Delta H_n = -R \partial \ln K_a / \partial (1/T) \quad (eq.1)$$

$\Delta H_n$ was estimated to be 31.2 kcal mol$^{-1}$ from a linear fit of $\ln K_a$ vs $1/T$ plot. The apparent positive $\Delta H_n$ was in sharp contrast with the negative calorimetric enthalpy change, $\Delta H_{cal}$, measured directly by ITC (Table 1). The discrepancies between $\Delta H_n$ and $\Delta H_{cal}$ have been recognized, and possible causes of the discrepancies have been discussed (5, 6). Weber (5) emphasized the importance of including the temperature dependence of $\Delta H$ and $\Delta S$, and in several model calculations on protein subunit association, the calculated enthalpy changes differed significantly from the simple $\Delta H_{th}$. In the present study, the assumption that $\Delta H$ and $\Delta S$ are independent of $T$ is not valid (Table 1), because a large negative $\Delta C_p$ was observed. Thus, we cannot use a linear fit to eq. 1 to calculate the true enthalpy change for the purine motif triplex formation. If $\Delta C_p$ is assumed to be independent of $T$ and to originate in a simple two-state binding process, we would expect the temperature dependence of $K_a$ to be (6)

$$\ln(K_a/K_{a0}) = R^1(\Delta H_T-T_0 \Delta C_p)(1/T_0-1/T) + R^1 \Delta C_p \ln(T/T_0) \quad (eq.2)$$

Inserting the observed $\Delta C_p$ (-1.2 kcal mol$^{-1}$ K$^{-1}$) and the values of $K_{a0}$ ($6.04 \times 10^6$ M$^{-1}$) and $\Delta H_0$ (-37.9 kcal mol$^{-1}$) at $T_0 = 25$ °C into eq. 2, the predicted temperature dependence of $\ln K_a$ would be the dashed curve shown in Fig. 3. The expected temperature dependence of $\ln K_a$ deviates significantly from the experimental data. The discrepancy suggests that the triplex formation may be more complex than a simple two-state binding process. We conclude that the purine motif triplex formation near room temperature is not a simple two-state binding process but exhibits multiple states, which was previously observed for the pyrimidine motif triplex formation near room temperature (2).

![Fig. 2: Temperature dependence of $\Delta H$, $\Delta G$, and $-T\Delta S$](https://academic.oup.com/nass/article-abstract/44/1/61/1019232/110232

![Fig. 3: Temperature dependence of experimentally observed $K_a$ (closed circles). The dashed curve is the temperature dependence predicted by eq. 2](https://academic.oup.com/nass/article-abstract/44/1/61/1019232/110232

REFERENCES