New thermodynamic characterization and transition mechanism of DNA duplex formation

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ABSTRACT
A new transition mechanism of DNA duplex association was proposed and a segregated transition model (STM) was further derived. The experimental results in various molar ratios showed that the duplex association transition is imperfect and the thermodynamic properties and self-transition behavior of single strands exert a significant influence on DNA duplex formation.

INTRODUCTION
With dramatic progress of the HGP (Human Genome Project), the gene sequences of many proteins are known but their structure and function are hardly understood, and current techniques for determining gene function cannot keep simply with rapid accumulation of the new sequence information. Therefore, thermodynamic data analysis becomes an effective approach in understanding and predicting the structural features and function of DNA, RNA and protein from sequences.¹ UV (UltraViolet) spectroscopy and DSC (Differential Scanning Calorimetry) are the widely applied methods to determine the thermodynamic parameters of oligomers.²,³ However, when van't Hoff enthalpies derived from UV method were directly compared with the calorimetric enthalpies derived from DSC method for longer oligomer sequences, the obvious deviations between the two quantities were often found.³,⁴

In this paper, we proposed the transition mechanism of DNA duplex formation based on the experimental data of UV, CD (Circular Dichroism) and DSC, and a segregated transition model (STM) was further derived. When the duplex association yield was considered, the transition enthalpies derived from UV and DSC were in excellent agreement.

MATERIALS AND METHODS
The single-stranded DNA oligomers were prepared and further purified by HPLC (High Performance Liquid Chromatography).⁵ Then the oligomers were aliquoted for UV, CD and DSC experiments.³,⁴ All the experiments were carried out in a buffer solution containing 1M NaCl/10mM Na₂HPO₄/1mM Na₂EDTA (pH 7.0). Single strand concentrations of oligomers were determined by measuring the absorbance at 260 nm at a high temperature and the total strand concentration was calculated by extinction coefficients and molar fraction of each strand.

RESULTS AND DISCUSSION
On the basis of the experimental results of UV, CD and DSC, we proposed the following transition mechanism of DNA duplex formation:
1) Association of two complementary single strands;
2) Self-transition of the single strands;
3) The interstrand stacking and weak cooperativity.
When the thermodynamic contributions of the inter-
strand stacking and weak cooperativity to the over-all transition can be neglected, we further obtain a segregated transition model (STM). Considering various molar ratios of two complementary single strands, there exists

\[ \frac{1}{h_m} = \frac{\varepsilon_D - (\varepsilon_A + \varepsilon_B) / h_B}{\varepsilon_A + m \varepsilon_B} + \frac{1}{h_B} \]  

(1)

where \( h_m \) and \( h_B \) indicate hyperchromicity factors of mixing solution in various molar ratio and single strand \( B \). \( \varepsilon_A \), \( \varepsilon_B \) and \( \varepsilon_D \) indicate extinction coefficients of single strands \( A \), \( B \), and duplex strand \( D \). \( m \) indicates the molar ratio of single strands \( B \) to \( A \). The plots of \( h_m^{-1} \) vs. \( (\varepsilon_A + m \varepsilon_B)^{-1} \) present a linear relationship, and extinction coefficient of the resulting duplex strands was estimated by the slope of the linear fitting of eq 1. Finally, the duplex association yield \( Y \) was obtained by eq 2

\[ Y = \frac{1}{h_D} - \frac{(x_A / h_A + x_B / h_B)}{1 / h_D - (x_A / h_A + x_B / h_B)} \]  

(2)

where \( h_A \) and \( h_D \) indicate hyperchromicity factors of single strand \( A \) and duplex strand \( D \). \( h_{obs} \) is the observed hyperchromicity factor of duplex formation. \( x_i \) is defined as \( \varepsilon_i / (\varepsilon_A + \varepsilon_B) \), (i = A or B). The calculated results were summarized in Table 1. These results reveal a fact that duplex association transition is imperfect, and the thermodynamic properties and self-transition behavior of single strands exert a significant influence on duplex formation. Consequently, the transition enthalpies derived from van’t Hoff analysis and calorimetric measurement, in essence, would be different. The “true” transition enthalpies of duplex formation can be obtained by introducing the duplex association yield,

\[ T_m^{-1} = \frac{R}{\Delta H_T} \ln \frac{1}{4Y} C_T + \frac{\Delta S_T}{\Delta H_T} \]  

(3)

and

\[ \Delta H_T = Y \Delta H_{DSC} \]  

(4)

where \( T_m \) is the melting temperature and \( R \) is the gas constant. \( \Delta H_T \) is the “true” transition enthalpy and \( \Delta H_{DSC} \) is the calorimetric enthalpy, respectively. \( C_T \) is the total species concentration.

<table>
<thead>
<tr>
<th>Sequencea</th>
<th>( Y^b ) (%)</th>
<th>( \Delta H^c ) (kcal/mol)</th>
<th>( \Delta H_T^d ) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1S2</td>
<td>0.991</td>
<td>-64.4</td>
<td>-65.1</td>
</tr>
<tr>
<td>S3S4</td>
<td>0.945</td>
<td>-105.9</td>
<td>-111.1</td>
</tr>
<tr>
<td>S5S6</td>
<td>0.919</td>
<td>-144.8</td>
<td>-157.2</td>
</tr>
</tbody>
</table>

a S1S2, d(GCTTGTTGC)/d(GCAACAAGC); S3S4, d(GCAGGTTGTTCCGC)/d(GCGGAAACAACCTG C); S5S6, d(GCAACAGGTTTCCGGTGTC)/d(GC AACGAAACAACCTGGTC). b The duplex association yield. c The transition enthalpies, where \( vH \) indicates the van’t Hoff enthalpy derived from the shape analysis of UV melting curves and \( cal \) indicates the calorimetric enthalpy directly derived by integrating DSC transition profile. d The corrected transition enthalpies calculated by eqs 3 and 4.

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REFERENCES