Clear-cut photo-regulation of the formation and dissociation of the DNA duplex by modified oligonucleotide involving multiple azobenzenes

Hiroyuki Asanuma\textsuperscript{1,2}, Daijiro Matsunaga\textsuperscript{3} and Makoto Komiyama\textsuperscript{3}

\textsuperscript{1}Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Chikusa, Nagoya 464-8603, Japan, \textsuperscript{2}PRESTO, Japan Science and Technology Corporation (JST), Kawaguchi 332-0012, Japan and \textsuperscript{3}Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Chikusa, Nagoya 464-8603, Japan

ABSTRACT

Multiple azobenzenes were introduced into the oligonucleotide on 3-threoninol for the clear-cut photo-regulation of the formation and dissociation of DNA duplex. When azobenzenes took \textit{trans} form, introduction of multiple azobenzenes (azobenzenes : nucleobases = 1:2) did not interfere with duplex formation at all compared with their native duplex. In contrast, melting temperature ($T_m$) uniformly decreased with the number of azobenzenes when they took \textit{cis} form. As a result, clear-cut photo-regulation of the duplex formation was attained under physiological conditions. Furthermore, nearest-neighbor parameters for these azobenzene-tethered oligonucleotides were obtained. It was found that $T_m$s estimated from these parameters well coincided with those of measured ones.

INTRODUCTION

Modification of DNA is one of the growing fields in both synthetic and bio-oriented chemistries because of its potential application to gene-therapy, bio- and nanotechnology.\textsuperscript{1} Previously, we have synthesized various photo-responsive DNAs tethering azobenzene or spiropyran, for the photo-regulation of DNA functions.\textsuperscript{2-4} With these modified DNAs, formation and dissociation of the duplex or triplex is efficiently photo-regulated by irradiating with UV or visible light: planar \textit{trans}-azobenzene (visible light irradiation) stabilizes the duplex while non-planar \textit{cis}-azobenzene (UV light irradiation) destabilizes the duplex. Although melting temperature ($T_m$) of the duplex largely changes by photo-induced \textit{trans-cis} isomerization, photo-regulation was demonstrated only with short oligonucleotides (smaller than ten bases). For the practical application such as an antisense strategy, much longer DNA should be efficiently photo-regulated. In the present paper, an oligonucleotide involving twenty natural nucleobases was applied to the photo-regulation. Here, multiple azobenzenes were introduced into the oligonucleotide, and clear-cut photo-regulation of the duplex formation was attained under physiological conditions. Furthermore, nearest-neighbor parameters for

\begin{align*}
\text{Azo-} & : 0: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 1: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 2: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 3: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 4: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 5: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 6: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 7: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 8: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 9: 5'\text{-CTTTAAGGAAGGATATACCC-3'} \\
\text{Azo-} & : 10: 5'\text{-CTTTAAGGAAGGATATACCC-3'}
\end{align*}

Complementary Strand:

G: 3'\text{-GAAATCTTCTCCTATATGG-5'}

Scheme 1. Photo-responsive oligonucleotides synthesized in this study.

![Fig.1](https://example.com/fig1.png)

Fig.1. Effect of the number of \textit{X} residues on the $T_m$, and comparison of measured $T_m$s (circles) with those estimated from the parameter (squares) in the \textit{trans}- (open) and \textit{cis}-form (closed). [DNAs] = 2 \text{ \mu M}, [NaCl] = 1 \text{ M}, pH 7.0.

these azobenzene-tethered oligonucleotides were obtained to predict the $T_m$ of photo-responsive DNA.

RESULTS AND DISCUSSION

Modified oligonucleotides synthesized here are listed in Scheme 1. Azobenzenes were systematically tethered on 3-threoninol to the oligonucleotide involving twenty natural nucleobases (\textit{Azo-0} in Scheme 1), and $T_m$s of Azon/C duplexes (either in the \textit{trans}- or \textit{cis}-form) were measured. Interestingly, introduction of multiple azobenzenes did not interfere with duplex formation at all
when they took trans-form as shown by open circles in Fig.1: \( T_m \) of Azo-9/C involving nine azobenzenes with respect to twenty natural nucleobases was 63.5 °C, which was even higher than that of natural Azo-0/C (61.5 °C). In contrast, \( T_m \) uniformly decreased with the number of azobenzenes when they took cis-form (see closed squares in Fig.1) and minimum \( T_m \) (32.0 °C) was observed for Azo-9/C. Change of \( T_m \) induced by trans-cis isomerization for Azo-9/C duplex was as great as 31.5 °C. As estimated from the \( T_m \) curves of Azo-9/C in Fig.2, more than 80 % of the duplex was expected to be photo-regulated at around 40 °C. In fact, 85 % of the duplex could be photo-regulated at GCGYXZTCG-3' /3'-CGCY'Z'AGC-5' (Y,Y',Z,Z' = A,G,C,T) were first measured by varying their concentration, and then thermodynamic parameters of all these sixteen duplexes were calculated. Nearest-neighbor parameters of 5'-YXZ-3'/3'-Y'Z'-5' were obtained as Table 1 by substituting the parameters of the rest parts of the sequence that were already reported elsewhere. Then, \( T_m \) of all the duplexes listed in Scheme 1 were predicted from these new parameters, and were superimposed on Fig.1. In case of trans-azobenzene (closed squares), the estimated \( T_m \) coincided well with the measured ones (closed circles) irrespective of the number of azobenzenes. Even in cis-form, estimated \( T_m \) fairly well coincided as long as the number of azobenzenes were less than four (compare closed circles with squares in Fig.1). These parameters should be very useful for the thermodynamic analysis of the intercalator as well as for the sequence design of photo-responsive DNA.

**CONCLUSION**

By introducing multiple azobenzenes in the oligonucleotides, clear-cut photo-regulation of the DNA duplex was attained. Nearest-neighbor parameters of the azobenzene-tethered DNA were also obtained, and the \( T_m \) estimated from these parameters fairly coincided with the measured ones.

**REFERENCES**


**Table 1.** Nearest-neighbor thermodynamic parameters for the 5'-YXZ-3'/3'-Y'Z'-5' sequences.\(^a\)

<table>
<thead>
<tr>
<th>X(Z')</th>
<th>(\Delta H)</th>
<th>(\Delta S)</th>
<th>(\Delta G_{37})</th>
<th>(\Delta H)</th>
<th>(\Delta S)</th>
<th>(\Delta G_{37})</th>
<th>(\Delta H)</th>
<th>(\Delta S)</th>
<th>(\Delta G_{37})</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(T)</td>
<td>-13.0</td>
<td>-35.4</td>
<td>-2.1</td>
<td>-7.5</td>
<td>-19.0</td>
<td>-1.6</td>
<td>-10.4</td>
<td>-27.1</td>
<td>-2.0</td>
</tr>
<tr>
<td>cis</td>
<td>4.8</td>
<td>13.5</td>
<td>0.6</td>
<td>-4.2</td>
<td>-14.8</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>T(A)</td>
<td>-5.0</td>
<td>-13.2</td>
<td>-1.0</td>
<td>0.6</td>
<td>3.8</td>
<td>-0.6</td>
<td>1.5</td>
<td>7.6</td>
<td>-0.9</td>
</tr>
<tr>
<td>cis</td>
<td>21.5</td>
<td>63.7</td>
<td>1.6</td>
<td>25.8</td>
<td>78.0</td>
<td>1.6</td>
<td>22.7</td>
<td>67.6</td>
<td>1.7</td>
</tr>
<tr>
<td>G(C)</td>
<td>-10.2</td>
<td>-25.7</td>
<td>-2.3</td>
<td>-2.8</td>
<td>-3.0</td>
<td>-1.9</td>
<td>-5.5</td>
<td>-10.8</td>
<td>-2.3</td>
</tr>
<tr>
<td>cis</td>
<td>-8.9</td>
<td>-29.4</td>
<td>0.1</td>
<td>10.7</td>
<td>33.5</td>
<td>0.3</td>
<td>11.9</td>
<td>36.5</td>
<td>0.5</td>
</tr>
<tr>
<td>C(G)</td>
<td>-0.9</td>
<td>0.1</td>
<td>-1.1</td>
<td>-2.7</td>
<td>4.7</td>
<td>-1.3</td>
<td>-2.9</td>
<td>4.3</td>
<td>-1.6</td>
</tr>
<tr>
<td>cis</td>
<td>1.7</td>
<td>2.9</td>
<td>0.7</td>
<td>1.5</td>
<td>2.9</td>
<td>0.5</td>
<td>11.4</td>
<td>34.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\(^a\) \(\Delta H\), kcal mol\(^{-1}\); \(\Delta S\), cal mol\(^{-1}\) K\(^{-1}\); \(\Delta G_{37}\), kcal mol\(^{-1}\)