DNA-Based Asymmetric Catalysis

Presentation Outline

Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

- Example of DNAzyme that cleaves RNA
- In vitro selection approach to synthesize DNAzymes
- Example of DNAzyme that ligates RNA
- Other reactions catalyzed by DNAzymes
- DNAzymes catalytic parameters, mechanism and structures

DNA-based Asymmetric Catalysis

- Concept
- Synthesis of ligands
- Application to a copper-catalyzed Diels-Alder reaction
- Perspectives
Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

- Relatively few studies focus on nucleic acids as catalysts for bioorganic chemistry
  - The study of DNAzymes is only about a decade old, whereas that of RNAzymes goes back over 20 years

- Reasons for lack of development in this field
  - Compared with proteins, there are much less functional groups available

<table>
<thead>
<tr>
<th>Proteins</th>
<th>RNA/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 diverse sidechains</td>
<td>4 similar monomers</td>
</tr>
<tr>
<td>H-bonding capability of the polyamide backbone</td>
<td>H-bonding, π-stacking and metal-coordination</td>
</tr>
</tbody>
</table>

- DNA appears even less catalytically competent (lacks the 2'-hydroxyl group present in RNA)
- Exists almost entirely in double-helical form (single-stranded conformations are probably required for catalysis)

- No DNAzymes are known in nature (are they out there?)

Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

First examples of catalytic DNA: deoxyribozymes that cleave RNA

This reaction is the same as that promoted by most protein ribonucleases such as R Nase A

An in vitro selection approach can be used to identify RNA-cleaving DNAzymes

Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

In vitro selection approach to synthesize DNAzymes that cleave RNA

Start

1. Connect RNA strand to random DNA

(1) Connect RNA strand to random DNA

Biotin tag

B = Biotin tag

(3) PCR-amplify active DNA sequences
(Isolate single strand)

inactive DNA (discard)

3' 5'

active DNA (separate using streptavidin column or PAGE)

(2) Incubate to allow cleavage

(1) Attache more RNA to begin next round

(1) Attache more RNA to begin next round

3' 5'

clone and sequence

Finish (DNAzyme)

Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

Other examples of catalytic DNA: deoxyribozymes that ligate RNA

Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

➤ Deoxyribozyme catalytic parameters, mechanism and structures

• Quantitative assessment of a DNAzyme's catalytic activity can be made by comparing its rate constant to that of an appropriate background reaction

\[ \text{rate enhancement} = \frac{k_{\text{obs}}}{k_{\text{bkgd}}} \]

rate enhancements of $10^6$ to $10^7$ for RNA ligation reactions
rate enhancement as high as $10^{10}$ observed for other DNAzymes

• Little is known about the structures and mechanisms of any DNAzymes

➤ Why study DNAzymes instead of RNAzymes?

• If DNA and RNA have similar catalytic potential, practical concerns favor the use of DNA;

  1) DNA less expensive to make by solid-phase synthesis (ca 7 times less)
  2) DNA can generally be made in longer sequence lengths and higher purity
  3) Relative chemical and biochemical stability (ubiquitous ribonucleases)

Deoxyribozymes (DNAzymes): DNA Catalysts for Bioorganic Chemistry

Other reactions catalyzed by DNAzymes that covalently modify nucleic acids

- Change in the phosphorylation status of an RNA or DNA strand
  
  DNA phosphorylation
  DNA adenylation (capping)

- DNA deglycosylation
- Porphyrin metalation
- Thymine dimer photoreversion
- DNA cleavage

What do all these processes have in common?

The use of single-stranded DNA for catalysis

Is it possible to use duplex-DNA to catalyze a specific reaction?

**DNA-Based Asymmetric Catalysis**

Can the chirality of the DNA double helix be transferred directly to a metal-catalyzed reaction?

- Exploit the propensity of small aromatic molecules to interact with DNA in a noncovalent, yet kinetically stable way

The reaction...

DNA-Based Asymmetric Catalysis

Synthesis of the ligands

Boc\(\text{NH}_2\)_\(\text{NH}_2\) \(\rightarrow\) \(\text{Boc}\text{N}_\text{NH}_\text{NH}\)

1. 2-pyridinecarboxaldehyde \(\text{MeOH, rt}\)
2. \(\text{NaBH}_4, \text{rt}\)

\[\text{ClHN} \text{NH}_\text{N}_\text{N}_\text{R}\]

1. \(\text{RCl, K}_2\text{CO}_3, \text{MeCN, }\Delta\)
2. TFA, \(\text{CH}_2\text{Cl}_2, \text{PhSH, rt}\)
3. 9-chloroacridine \(\text{PhOH, 100 °C}\)

- \(n = 3\)
  - R = Me, \(t\)-Bu, Benzyl, 1-Naphthylmethyl, 2-Naphthylmethyl
  - 4-MeOC\(\text{C}_6\text{H}_4\text{CH}_2\), 3,5(\text{MeO})\(\text{C}_6\text{H}_3\text{CH}_2\)

- \(n = 2,4,5\)
  - R = 1-Naphthylmethyl, 3,5(\text{MeO})\(\text{C}_6\text{H}_3\text{CH}_2\)

**DNA-Based Asymmetric Catalysis**

![Chemical Structure and Reaction Scheme]

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>n</th>
<th>Dienophile (X)</th>
<th>Endo/Exo</th>
<th>Endo (%ee)</th>
<th>Exo (%ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>3</td>
<td>H</td>
<td>98:2</td>
<td>49</td>
<td>18</td>
</tr>
<tr>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>3</td>
<td>H</td>
<td>97:3</td>
<td>49</td>
<td>23</td>
</tr>
<tr>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>3</td>
<td>H</td>
<td>98:2</td>
<td>47</td>
<td>23</td>
</tr>
<tr>
<td>4&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>3</td>
<td>NO₂</td>
<td>96:4</td>
<td>37</td>
<td>16</td>
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<tr>
<td>5&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>3</td>
<td>Me</td>
<td>98:2</td>
<td>48</td>
<td>24</td>
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<tr>
<td>6</td>
<td></td>
<td>4</td>
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<td>98:2</td>
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<td>19</td>
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<tr>
<td>7</td>
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<td>&lt;5</td>
<td>&lt;5</td>
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<tr>
<td>8</td>
<td></td>
<td>2</td>
<td>H</td>
<td>96:4</td>
<td>-48</td>
<td>-37</td>
</tr>
</tbody>
</table>

<sup>a</sup> Catalyst (0.18 mM), dienophile (4 mM), cyclopentadiene (34 mM)

<sup>b</sup> Calf thymus DNA <sup>c</sup> ca. 50% conversion

• No significant ee when R = 2-Naphthylmethyl

**DNA-Based Asymmetric Catalysis**

![Chemical Structure](image)

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<tr>
<th>Entry</th>
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<tr>
<td>1</td>
<td></td>
<td>3</td>
<td>H</td>
<td>98:2</td>
<td>-37</td>
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<tr>
<td>2</td>
<td></td>
<td>2</td>
<td>H</td>
<td>92:8</td>
<td>-37</td>
<td>-78</td>
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<tr>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2</td>
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<td>-74</td>
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<tr>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>6&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>2</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt;</td>
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<tr>
<td>7&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>2</td>
<td>OMe</td>
<td>91:9</td>
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<td>-90</td>
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</tbody>
</table>

<sup>a</sup> Catalyst (0.18 mM), dienophile (4 mM), cyclopentadiene (34 mM)

<sup>b</sup> Calf thymus DNA

<sup>c</sup> DNA = synthetic duplex d(GACT)<sub>2</sub>-(AGTC)<sub>2</sub> (0.39 mM), cyclopentadiene (21 mM)

<sup>d</sup> ca. 50% conversion

DNA-Based Asymmetric Catalysis

\[
\text{Reactor} \xrightarrow{\text{rt}} \text{Product}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>ligand</th>
<th>Conv. (%)</th>
<th>Endo/Exo</th>
<th>Endo (%ee)</th>
<th>Exo (%ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cu(NO₃)₂/DNA</td>
<td>--</td>
<td>50-60</td>
<td>95:5</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>DNA</td>
<td>--</td>
<td>&lt;5%(^a)</td>
<td>n.d.</td>
<td>&lt;5</td>
<td>n.d.</td>
</tr>
<tr>
<td>3</td>
<td>Cu(NO₃)₂/DNA/9-aminoacridine</td>
<td>--</td>
<td>100</td>
<td>94:6</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>Cu(NO₃)₂/ligand</td>
<td>A</td>
<td>50-70</td>
<td>95:5</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>Cu(NO₃)₂/ligand</td>
<td>B</td>
<td>50-70</td>
<td>93:7</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\) Reaction performed at 5 \(^\circ\)C

A: \(n = 3, R = \text{2-Naphthylmethyl}\)
B: \(n = 2, R = 3,5(\text{OMe})C_6H_4CH_2\)

**Perspectives**

- The chirality of duplex DNA *can* be transferred directly to a catalytic reaction
- *Both* enantiomers of the Diels-Alder adduct are accessible by a judicious choice of ligand
- Rapid structural variation and optimization of catalysts for new reactions
- Ease of purification (Cu-ligand-DNA complex remains in aqueous solution)

Futur work should focus on

- The possibility to address specific DNA sequences by using a selective DNA binding moiety tethered to the catalyst
- Extending to other reactions that can be performed in buffered aqueous solutions (metal-catalyzed reactions, organocatalysis)