DNA-Templated Organic Synthesis

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Outline

- Introduction

- DNA-Templated Oligonucleotide Ligation
  - Selenium-Mediated Autoligation
  - Photoreversible Ligation

- DNA-Templated Small Molecule Synthesis
  - One-Step Synthesis
  - Multistep Synthesis
  - One-Pot Reaction

- Summary
Nature’s Approach to Discovery

DNA → RNA → Protein → Natural product

Selection, amplification, diversification
Rate Acceleration of Glycoside Hydrolysis

Acid-catalyzed hydrolysis

\[ K_{\text{obs}} = 1.9 \times 10^{-6} \text{ s}^{-1} \text{ in } 0.1 \text{ M HCl} \]

Intramolecular catalysis

\[ K_{\text{uni}} = 1.4 \times 10^{-3} \text{ s}^{-1} \]

\( \beta \)-galactosidase

\[ K_{\text{cat}} = 40 \text{ s}^{-1} \]

Bugg, T. *An Introduction to Enzyme and Coenzyme Chemistry*;
How Do Enzymes Work?

Enzyme + Substrate → Products
The Secret to the Rate Enhancement

-----Proximity Effect

- Proximity effect: enzymes bind their substrates so that active functional groups are brought close together and stay in place long enough for the reaction to proceed.
What Can We Learn from Nature?

- Is it possible to mimic enzyme-catalyzed chemical reactions?
- Is it possible to use Nature’s approach to devise the desired product?
Effective molarity (M) for various metal cations as templates for the synthesis of benzo-18-crown-6 at 25 °C.

<table>
<thead>
<tr>
<th></th>
<th>EM\textsubscript{Temp}</th>
<th></th>
<th>EM\textsubscript{Untemp}</th>
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<tr>
<td>Na\textsuperscript{+}</td>
<td>14</td>
<td>K\textsuperscript{+}</td>
<td>123</td>
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<tr>
<td>Rb\textsuperscript{+}</td>
<td>48</td>
<td>Cs\textsuperscript{+}</td>
<td>22</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>0.08</td>
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</table>

Molecular Template: Definition

“A chemical template organizes an assembly of atoms with respect to one or more geometric loci, in order to achieve a particular linking of atoms”.

D. H. Busch

Essential Features of Molecular Template

- Organizes an assembly of atoms in a **specific** spatial arrangement.
- Favors the formation of a **single** product.
- **Promotes** attractive interaction.
What Makes DNA a Good Template?
PCR: Polymerase Chain Reaction

30 - 40 cycles of 3 steps:

**Step 1: Denaturation**
1 minute 94 °C

**Step 2: Annealing**
45 seconds 54 °C
forward and reverse primers !!!

**Step 3: Extension**
2 minutes 72 °C
only dNTPs

PCR: Polymerase Chain Reaction

PCR: Polymerase Chain Reaction

Exponential amplification

2^1 = 2 copies
2^2 = 4 copies
2^3 = 8 copies
2^4 = 16 copies
2^5 = 32 copies
2^6 = 64 copies
2^7 = 128 copies
2^8 = 256 copies
2^9 = 512 copies
2^{10} = 1024 copies
2^{11} = 2048 copies
2^{12} = 4096 copies
2^{13} = 8192 copies
2^{14} = 16384 copies
2^{15} = 32768 copies
2^{16} = 65536 copies
2^{17} = 131072 copies
2^{18} = 262144 copies
2^{19} = 524288 copies
2^{20} = 1048576 copies
2^{21} = 2097152 copies
2^{22} = 4194304 copies
2^{23} = 8388608 copies
2^{24} = 16777216 copies
2^{25} = 33554432 copies
2^{26} = 67108864 copies
2^{27} = 134217728 copies
2^{28} = 268435456 copies
2^{29} = 536870912 copies
2^{30} = 1073741824 copies
2^{31} = 2147483648 copies
2^{32} = 4294967296 copies
2^{33} = 8589934592 copies
2^{34} = 17179869184 copies
2^{35} = 34359738368 copies
2^{36} = 68719476736 copies

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➢ DNA-Templated Small Molecules Synthesis
  ➢ One-Step Synthesis
  ➢ Multistep Synthesis
  ➢ One-Pot Reaction

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Why is Ligation Important?

Two reasons:

1. All reactions in the cycles are ligation.
2. Need to develop new and efficient way to detect disease in nucleotides.

The central dogma of molecular biology as formulated in 1958 with probable (solid arrows) and possible (broken arrows) reactions indicated.

Minimal Scheme for a Catalytic Template Directed Ligation

Scheme of template-directed ligation with steps 1, molecular recognition; 2, ligation; 3, product dissociation.
Selenium-Mediated Autoligation

Highly sensitive to the sequence of the target nucleic acid.

Selenium-Mediated Autoligation

1. Selenium reaction proceeded 2 times faster than sulfur counterpart
2. All-oxygen phosphate showed no ligation.
3. May be useful for direct analysis of RNAs.

Photoreversible Ligation

ODN 1

5’
T G T G C

3’

ACACGGACGCAC 5’

ODN 2

5’
G C G T G

3’

3’ nm 366 nm 96 %, 12 h

ODN 3

ODN 4

5’
T G T G C

3’

A G A C G G

A C G G

A C G G

A C G G

5’

Photoreversible Ligation

Lane 1: control 12-mer;
Lane 2: control 6-mer;
Lane 3: ODN 1 + ODN 2, irradiation at 366 nm;
Lane 4: ODN 1 + ODN 2 + ODN 3, irradiation at 366 nm, 80% yield 3 h;
Lane 5: irradiation of lane 4 at 302 nm, 1 h;
Lane 6: irradiation of lane 5 at 366 nm, 3 h.

Summary of DNA Ligation

- Highly efficient.
- Sequence specific.
- First DNA-templated carbon-carbon bond forming reaction.
- Possible application in ligation of other nucleic acids.

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DNA-Templated Small Molecule Synthesis

Preparation of DNA-Linked Reagents

\[ \text{DNA-5NH}_2 + \text{R} \to \text{DNA-5NHCO}_R \]

\[ \text{R} = \begin{array}{c} \text{benzyl iodide} \\ \text{4-iodophenylsulfone} \\ \text{N-bromoacetamide} \\ \text{4-nitroimidazole} \end{array} \]

pH 7.2, 25 °C, 1 h

## One-Step Synthesis

<table>
<thead>
<tr>
<th>Reaction Conditions</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sn2 Reaction</strong></td>
<td></td>
</tr>
<tr>
<td>pH 8.5, 0.25 M NaCl</td>
<td><img src="image1" alt="Product 1" /></td>
</tr>
<tr>
<td>37 °C, 16 h, 60 nM template and reagent</td>
<td></td>
</tr>
<tr>
<td>pH 7.5, 0.25 M NaCl,</td>
<td><img src="image2" alt="Product 2" /></td>
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<tr>
<td>37 °C, 16 h, 60 nM template and reagent</td>
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<tr>
<td><strong>Conjugate addition</strong></td>
<td><img src="image3" alt="Product 3" /></td>
</tr>
<tr>
<td>pH 7.5, 0.25 M NaCl</td>
<td><img src="image4" alt="Product 4" /></td>
</tr>
<tr>
<td>25 °C, 10 min, 60 nM template and reagent</td>
<td></td>
</tr>
<tr>
<td>pH 8.5, 0.25 M NaCl,</td>
<td><img src="image5" alt="Product 5" /></td>
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<tr>
<td>25 °C, 75 min, 60 nM template and reagent</td>
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</tr>
<tr>
<td>pH 7.5, 0.25 M NaCl,</td>
<td><img src="image6" alt="Product 6" /></td>
</tr>
<tr>
<td>25 °C, 10 min, 60 nM template and reagent</td>
<td></td>
</tr>
</tbody>
</table>

## Generality of DNA-Templated Reaction

<table>
<thead>
<tr>
<th>Reaction Type</th>
<th>Reaction Condition</th>
<th>Product</th>
<th>Yield (%)</th>
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</thead>
<tbody>
<tr>
<td>Reductive Amination</td>
<td>NaBH₃CN, pH 6.0, 0.5 M NaCl, 25 ºC, 1.5 h</td>
<td><img src="image" alt="Reductive Amination Product" /></td>
<td>81</td>
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<tr>
<td>Nitro -Aldol</td>
<td>pH 8.5, 0.3 M NaCl, 25 ºC, 12 h</td>
<td><img src="image" alt="Nitro -Aldol Product" /></td>
<td>45</td>
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<tr>
<td>Wittig Olefination</td>
<td>pH 8.0, 1 M NaCl, 55 ºC, 1.5 h</td>
<td><img src="image" alt="Wittig Olefination Product" /></td>
<td>&gt;97</td>
</tr>
<tr>
<td>1,3-Dipolar Cycloaddition</td>
<td>pH 7.5, 2.8 M NaCl, 25 ºC, 22 h</td>
<td><img src="image" alt="1,3-Dipolar Cycloaddition Product" /></td>
<td>53</td>
</tr>
<tr>
<td>Heck Reaction</td>
<td>1) Na₂PdCl₄ with 2 eq. P(p-SO₂C₆H₄) 2) 0.075 M NaCl, 25 ºC, 2 h</td>
<td><img src="image" alt="Heck Reaction Product" /></td>
<td>54</td>
</tr>
</tbody>
</table>

(a) Hairpin templates linked to α-iodoacetamide group were reacted with thiol reagents containing 0, 1, or 3 mismatches at 25 °C.

(b) Reactions in (a) were repeated at the indicated temperature for 16 h.

Multistep Synthesis?

Challenges:

- How to remove DNA used to direct reagents in the former steps?
- How to purify and isolate intermediates and final product?
Linker Strategies

The solution to remove reagent-directing DNA:

- Scarless linker
- Useful scar linker
- Autocleaving linker
Scarless Linker

Useful Scar Linker

Autocleaving Linker


Chemical structure and reaction scheme.
Purification by Biotin-Avidin

- Biotinylated molecules will bind to the streptavidin magnetic beads.
- Non biotinylated molecules can be removed by washing with buffer.
Multistep Small Molecule Synthesis Programmed by DNA Templates

Multistep Small Molecule Synthesis Programmed by DNA Templates

Multistep Small Molecule Synthesis Programmed by DNA Templates

Synthesis of Non-Natural Tripeptide

EDC, Sulfo-NHS DNA-templated amide formation (step 1, 82%)

capture with avidin-inked beads, elute with pH 11.8 buffer

Template bases 21-30

Template bases 11-20

Synthesis of Non-Natural Tripeptide

1) EDC, Sulfo-NHS (step 2, 52%)
2) avidin beads, then pH 11.8 buffer

15

anneal third reagent

Multistep Small Molecule Synthesis Programmed by DNA Templates

- 3% overall yield was achieved for three bond-forming reactions, three purification steps and three linker cleavages.

- The final tripeptide linked to the template was characterized by MALDI mass spectrometry. (expected mass 10069 vs observed mass 10059-10075)
New Architecture Enables Two Reactions on One Template in One Step

One-Pot Reaction

One-Pot Reaction

1) in vitro selection with streptavidin beads
2) PCR amplification of selected products

DNA encoding selected and amplified molecules characterized by DNA sequencing and digestion

1025 presumed products out of 1,050, 625 theoretical products

primary product (1000 fold)

One-pot reactions containing one biotinylated template (15, 16, 17, 18, 19, or 20) + five non-biotinylated templates (out of 15-20) + six reagents (21-26)

One-Pot Reaction

Summary

DNA-Templated Synthesis

Present

Multiple

One-Pot

Past

Ligation

Future

?

Generality

Base pair

Proximity
Limitations

- Need to prepare DNA-linked reagents.
- Final product is still bound to DNA.
- Restricted to aqueous, DNA-compatible chemistry.
- PCR cannot amplify the desired small molecule.
Proposed Solutions to Limitations

Cleavage Final Product from DNA by Photolabile Linker

Proposed Solutions to Limitations

Multiple-Release

Biotin - Photocleavable linker - Product 1

Biotin - Mild acid cleavable - Product 2

Biotin - Strong acid cleavable - Product 3

Biotin - Enzyme cleavable - Product 4

Release A: Photolysis
Release B: 0.5% TFA/ CH₂Cl₂
Release C: 50% TFA/ CH₂Cl₂
Release D: Enzyme

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  - Mapitso Dongming
  - Sing Heather

- Wife: Zhiqiu
Thank You for Your Attentions!