Artificial Site-Selective DNA Cutters

Tuomas Lönnberg
Research Center for Advanced Science and Technology
University of Tokyo

DNA Structure

Base Pairing in DNA
Genetic information is stored in the base sequence of DNA. There are four bases: A, C, G and T. Base pairing (A:T and G:C) is a key process in reading the code.

Hydrogen bond donor
Hydrogen bond acceptor
Hydrogen bond

Site-Selective DNA Cleavage
- For manipulation of DNA, site-selective cleaving agents are needed
- Natural restriction enzymes can do this, but they only recognize a 4- or 6-base-pair sequence (in other words, a 4- or 6-letter "word")
- Human genome, e.g., is $3 \times 10^9$ base-pairs long, meaning that cleavage would occur at $3 \times 10^9 / 46 = 732,421$ sites!

Sequence-Specificity Example
Search by a 6-letter word
2,250,000 search results
At least the first 1,000 hits (probably more) are wrong – obviously the search criteria were not limiting enough

Search by a 14-letter word
10,100 search results
The first 10 hits are correct – in other words, the selectivity was considerably improved
DNA Cleavage by Ce(IV)

• Certain complexes of Ce(IV) cation are able to cleave single-stranded, but not double-stranded DNA

With complementary strands, single-stranded gaps may be formed in either single- or double-stranded DNA

Concentration of Ce(IV) by Phosphate

• Phosphate groups next to the gap accelerate the reaction by concentrating Ce(IV) ions close to the gap site

More Effective Alternatives to Phosphate?

• If a single phosphate group is effective at concentrating Ce(IV) close to the gap, then the following groups should be even better:

Aim of the Project

• The aim of my research in Tokyo has been to develop a method to covalently link NTP (and EDTP) to DNA

Solid Phase Synthesis of DNA

• The only practical way to chemically prepare long biopolymers (like DNA) is solid phase synthesis
• In solid phase synthesis, the growing DNA chain remains attached to a solid particle, usually a small glass bead, until the end of the synthesis
• This way, reagents can be used in a great excess, because after each step they can be simply washed off ⇒ high step-wise yield
• Typical scale for DNA synthesis is 1 µmol, a few milligrams depending on the length of the DNA
Solid Phase Synthesis of DNA

• The importance of a high step-wise yield is illustrated by the following graph:

An Automated DNA Synthesizer

The Chemistry of DNA Synthesis

Building Block Synthesis

• NTP itself is not very reactive, so a more reactive group has to be attached first
• One possibility to do this is to convert NTP to an internal anhydride, which can then react with an alcohol:

Building Block Synthesis

• Ideally, the reactive group should
  – be easily introduced to NTP (and EDTP)
  – react very efficiently with another reactive group attached to one end of the DNA (and only that)
  – not react with water
  – be compatible with the standard solid phase synthesis strategy
• Several different reactive groups were tried, but the results were not satisfactory
Finally, after some successful preliminary experiments, benzaldehyde was chosen as the reactive group. Aldehydes are known to react with amines to form imines. Although the reaction worked very well in solution with a large excess of the amine, only a small amount of the desired product was obtained using a solid phase bound DNA with an amino linker. The reaction between an aldehyde and an amine is actually a dynamic equilibrium, where the starting materials are favored. Unless water is carefully removed, the yield will be low. Unfortunately, the NTP building block is strongly hygroscopic, making removal of water almost impossible. But by changing the amino group to an aminooxy group, the equilibrium can be shifted to favor the products, even in water. Using a linker with an aminooxy group, the NTP building block could finally be attached to DNA with a high yield. In other words, a practical method to covalently link NTP to DNA has now been developed.
The next step of the project will be to synthesize a few aminooxy linkers usable in the new method:

EDTP will also be transformed to a similar benzaldehyde building block as the one prepared from NTP:

DNA strands modified with the new NTP and EDTP building blocks will be used in sequence-specific cleaving experiments. If the results of these experiments are promising enough, more elaborate DNAs having multiple NTP or EDTP groups as well as other modifications will be prepared for even more efficient and specific activity.

Where Is It?

Finland and Japan in Numbers
Climate

- In the more populated Southern part of the country, the climate is temperate
- In the North, a subarctic climate dominates
- The summers are fairly warm but short, whereas the winters are long and cold, especially in the North
- The effects of global warming are already clearly visible in Finland – the summers are getting longer and the winters shorter

Extreme Temperatures

- Highest: +35.9 °C (Turku, 1914)
- Lowest: -51.5 °C (Kittilä, 1999)

Polar Day

- In North Finland, the sun doesn’t set for two months in June – July

Polar Night

- Likewise, the sun doesn’t rise for two months in December – January

Wildlife

- Forests cover 75% of Finland’s land area, providing habitat for a large number of wild animals
- In late 19th and early 20th century, many species, especially predatory animals, were hunted to almost extinction
- Thanks to careful conservation and the establishment of big national parks, the populations are nowadays growing steadily

Wildlife – Large Herbivores
A Short History of Finland

- **8500 BC**
  - The last ice age ends in Northern Europe.
- **2560 BC**
  - The first people come to Finland.
- **~2000 BC**
  - Agriculture starts in Finland.
- **1099 AD**
  - The First Crusade ends with the conquest of Jerusalem by the Christian army.
- **1155 AD**
  - The First Swedish Crusade to Finland.
- **1249 AD**
  - The Second Swedish Crusade. Finland becomes part of the Swedish Kingdom.
- **1279 AD**
  - The Mongol Empire reaches its biggest expansion, dominating most of Asia as well as parts of Eastern Europe.
- **1279 AD**
  - The Great Pyramid is completed in Egypt.
- **1279 AD**
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- **1492 AD**
  - Christopher Columbus sails to America.
- **1543 AD**
  - The first book in Finnish is printed.
- **1640 AD**
  - The first university is founded in Finland.
- **1687 AD**
  - Newton publishes laws of mechanics.
- **1809 AD**
  - As a result of a war between Sweden and Russia, Finland is made an autonomous grand duchy of the Russian Empire.
- **1815 AD**
  - Napoleon loses his final battle at Waterloo.
- **1835 AD**
  - Publication of the epic poem Kalevala marks the dawn of Finnish nationalism.
- **1835 AD**
  - The Meiji Restoration in Japan.
- **1897 AD**
  - The Russian Empire collapses in the Bolshevik Revolution.
- **1907 AD**
  - A parliament is formed in the Grand Duchy of Finland. 19 women are elected – the very first ones in the world.
- **1914 AD**
  - The First World War begins.
- **1917 AD**
  - The First World War ends.
- **1917 AD**
  - Taking advantage of the chaotic situation in Russia, Finland declares independence.
- **1917 AD**
  - The Russian Empire collapses in the Bolshevik Revolution.
1939 AD
Nazi Germany invades Poland, starting the Second World War.

1939 AD
Soviet Union attacks Finland. After three months of intense fighting, peace is concluded. Although Finland has to give up some territory, it still remains a sovereign, democratic country.

1940 AD
Germany occupies The Netherlands, Belgium, Luxemburg, Denmark, Norway and France. Soviet Union occupies Estonia, Latvia and Lithuania.

1941 AD
Germany attacks Soviet Union. Hoping to regain lost territories, Finland joins the German invasion of Soviet Union.

1944 AD
Allied invasion of Normandy.

1945 AD
The Second World War ends.

1945 AD
A strategic Soviet attack forces Finland to make peace. Once again, occupation is only barely avoided.

1946 AD

1952 AD
The XV Olympic games are held in Helsinki.

1952 AD

1969 AD
Neil Armstrong walks on the Moon.

1969 AD

1977 AD
Finland’s first nuclear reactor is completed.

1977 AD

1989 AD
The Berlin Wall falls.

1989 AD

1991 AD
The Soviet Union collapses.

1991 AD

2001 AD
Terrorists strike in New York City.

2001 AD

2002 AD
12 countries, including Finland, adopt a common currency.

2002 AD

Economy
• Until about 60 years ago, Finland was a poor, agricultural country
• Since then, Finland has experienced an enormous growth in economy, very much like Japan or South Korea
• Nowadays, Finland’s economy is one of the strongest in the EU
• The economy relies heavily on industry and export of manufactured goods

Main Exports – Forest Industry
Main Exports – Metal Industry

Main Exports – Info Technology

Education

Education – Differences from Japan

Why Am I Here?

• Adventure
  – I wanted to experience a culture different from Europe or America

• Language
  – Learning East-Asian languages is considered more and more important in Europe

• Money
  – The JSPS scholarship is one of the most generous in the world