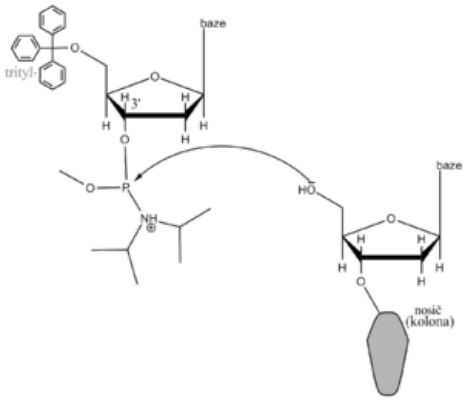
Synthesis of artificial DNA

The required DNA strand can be prepared in various ways. There are even **automated procedures** for synthesizing DNA with the desired sequence from individual nucleotides.

The basic principle is to suppress the reactivity of 3'-OH, 5'-OH and possibly also -NH_2 groups, sensitive to condensing agents, on nucleotides. Their protection is achieved by binding suitable organic groups, e.g. trityl, benzoyl, acetyl, etc. These groups can be easily removed, e.g. by changing the pH, which exposes the reactive group of the nucleotide for immediate reaction.

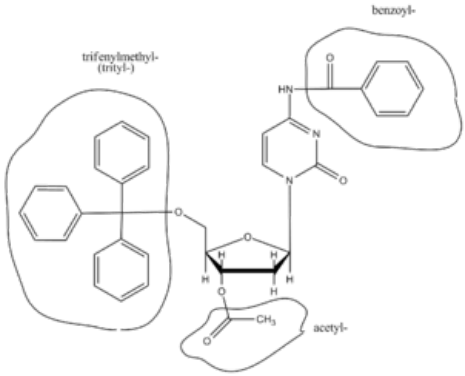
A typical example is this arrangement: the first nucleotide is attached to a column with a glass support (small particles), on which all groups except 5'-OH are protected in the described manner. The column is then washed with a solution of another, activated nucleotide. It is usually a **deoxyribonucleoside 3'-phosphoamidite**, that binds to the 5'-OH of the fixed nucleotide. After oxidation of trivalent phosphorus to pentavalent, a phosphodiester bond and a dinucleolide are formed.



The phosphoamidite formula (5'-OH is blocked with trityl) and its binding to the growing polynucleotide, fixed to the support in the column.

The protection at its 5'-end is removed and the column is washed with another activated nucleotide, etc. All protective groups and methyls from the phosphates are then removed by appropriate conditions, and the finished oligonucleotide is released from the column.

The description of the procedure is greatly simplified, the synthesis of the oligonucleotide takes hours. The speed of the process does not bear comparison with the speed of natural biosynthesis (e.g. in E. coli 16,000 bases per minute). However, the ability to make DNA of any primary structure is an immense advance. Synthetic DNA fragments can be enzymatically linked into longer chains of artificial DNA.



Protection of reactive groups in the synthesis of artificial oligonucleolide

Links

Related articles

- Biochemistry of genetic engineering
- DNA cleavage
- Separation of DNA fragments by electrophoresis
- Identification of restriction fragments
- Amplification and expression of the isolated gene in the host cell

References

- ŠTÍPEK, Stanislav. *Concise Biochemistry : storage and expression of genetic information.* 1. edition. Medprint, 1998. ISBN 80-902036-2-0.

Kategorie:Biochemie Kategorie:Genetika Kategorie:Molekulární biologie