Denaturation of nucleic acids, molecular hybridization

Hydrogen bonds in double-stranded DNA (double strand DNA, **dsDNA**) can be broken by extreme pH, urea or heat. The subsequent separation of the chains, i.e. **DNA denaturation**, can be monitored using the so-called **hyperchromic effect**. Due to the interaction of electrons in paired bases, dsDNA solutions absorb UV light (at 260 nm) less than the same concentration of bases in mononucleotides or in single-stranded DNA (single strand DNA, **ssDNA**). When the dsDNA solution is heated, the absorbance suddenly rises at a certain temperature. The temperature at which the increase in absorbance reaches its half is called **the melting point of DNA** (melting point, Tm). The more G=C pairs the examined dsDNA contains, the higher the Tm. Three hydrogen bonds in G=C require more thermal energy than two in A=T.

DNA denaturation is **reversible**. Complementary ssDNA can rejoin into dsDNA when, for example, the temperature drops or the concentration of urea decreases. Unfolding and folding of the double helix is a common phenomenon *in vivo* (e.g. in the biosynthesis of nucleic acids). Fusing of two complementary strands of nucleic acids *in vitro* into double helices is called **reassociation**. If different types of nucleic acids reassociate (DNA with RNA), it is called **molecular hybridization**. Methodically, these phenomena are used to separate a certain ssDNA or ssRNA from a mixture, to map the genome and to detect disorders of the gene structure (diagnosis of genetic defects).

The **technical arrangement** of reassociation of nucleic acids is different. First, ssDNA with a known sequence, complementary to the nucleic acid sought in the mixture, is attached to e.g. a membrane filter or a carbohydrate gel in the column. During the flow of the investigated mixture of nucleic acids through a filter or column modified in this way, the sought complementary ssDNA or ssRNA associates with the DNA fixed to the carrier. After washing, the captured nucleic acid can be released (eluted) with a suitable hydrogen-bonding agent.

For **diagnostic purposes**, the patient's DNA (from lymphocytes, amniotic cells, etc.) is first cleaved in defined places by one of the restriction endonucleases. The mixed fragments are separated by gel electrophoresis. It is then determined whether any of the DNA fragments contain a certain sequence of nucleotides, critical for the disease in question. In the procedure, the DNA fragments are transferred from the gel electrophorogram to a material more suitable for hybridization, i.e. a nitrocellulose filter. This filter needs be placed on dry filter paper, the electrophorogram needs to be printed on the filter, and filter paper moistened with a concentrated salt solution should be placed on top of this gel.

Electrophoretic fractions (DNA fragments) are **blotted** onto a nitrocellulose filter and denatured. This creates suitable technical conditions for the reassociation of complementary chains. The filter is poured with a solution of radioactively labeled and short artificial nucleic acid that contains the desired sequence. This so-called **probe** associates only with a DNA restriction fragment containing a complementary sequence, which will be shown by the attached photographic film (**autoradiography**) after rinsing the filter.

The described method of DNA restriction fragment analysis was developed by Eduard M. Southern. It bears his name (**Southern blotting**) and is widely used. If a complementary sequence is searched for in a mixture of blotted mRNAs in a completely analogous way, it is called **Northern blotting**. Mr. Southern's name became the victim of a nomenclature joke (northern). Immunochemists continued this joke: in **western blotting**, it is an analogous technique, but the antigen interacts with the antibody. However, blotting is an elegant, fast and routinely manageable method.

Links

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Source

- ŠTÍPEK, Stanislav. *Stručná biochemie : Uchování a exprese genetické informace.* 1. edition. Medprint, 1998. 92 pp. pp. 17–18. ISBN 80-902036-2-0.