PNA Chips

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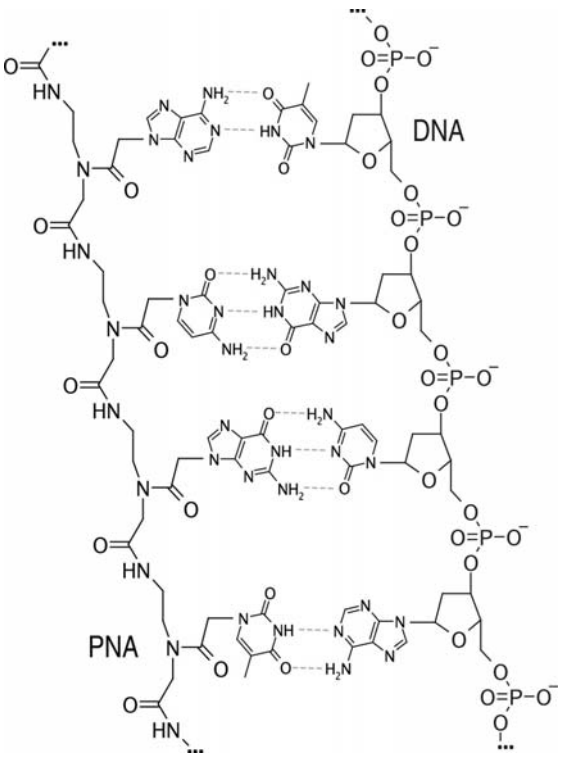
Definition

Peptide nucleic acid (PNA) is an artificial mimic of nucleic acid, which was invented more than a decade ago (7). The molecule combines many features that are similar to those of natural nucleic acids with characteristics that are rather different or even unique to PNA. First and foremost, N-(2-aminoethyl)-glycine units linked by ►amide bonds substitute the (deoxy)ribose-phosphate backbone and the nucleobases are attached *via* a methylene carbonyl link to the glycine moiety. Nevertheless, the distances and angles within PNA oligomers much resemble the conformation of nucleic acids (Fig. 1). One format of application is the microarrays that are made for a highly parallel analysis of nucleic acids. To this end, very many different PNA molecules are attached onto a small planar surface, e.g. a microscope slide. Upon addition of a DNA or RNA sample, hybridisation occurs on the chip at PNA-oligomers that are of complementary sequence to molecules in the sample. Recorded by detecting a label that is attached to the DNA or RNA, various forms of information can be deduced from such studies, such as for example, sequence variations or changes in the amount of an individual RNA molecule within a complex mixture.



PNA Chips. Figure 1 Structures of PNA and DNA.

Description

PNA binds to complementary single-stranded DNA or RNA in a sequence-specific manner. Because of the PNA's uncharged nature, the duplexes are substantially more stable than the equivalent double-strand molecules formed by DNA and RNA alone. The neutral amide backbone also allows PNA to hybridise to nucleic acids in the absence of salt, since no positive ions are required for counteracting the interstrand repulsion that occurs between two negatively charged nucleic acid molecules. As a result, the target DNA has fewer secondary structures and is therefore more accessible to a PNA probe than it would be to a DNA oligonucleotide. Being an artificial molecule, PNA is not degraded by nucleases or proteases. This makes it a versatile tool in assays that are subjected to environmental constraints.

Applications of PNA in biotechnology divide into reactions that occur in homogenous solution and analyses that take place on a solid support. Important examples of the former assay format are the use of PNA as an antisense reagent, as a DNA-opener and as a tool for PCR-clamping or the detection of single nucleotide polymorphisms. A comprehensive and up-to-date overview on the various applications of PNA is provided by Nielsen et al. (12). For all its advantages, however, PNA has not had quite the impact that was initially predicted. The limited solubility of PNA molecules is especially an obstacle that hampers many applications in solution. For the analysis of nucleic acids on microarrays, however, this limitation is not so restricting, since the PNA concentration is low and the molecules are constrained by their physical attachment to the solid support. ►DNA-microarrays, also named DNA chips, are an assay format that is widely used in biotechnology and biomedical research, since they permit molecular analyses of complex samples, such as genome-wide ►transcriptional profiling, typing of ►single nucleotide polymorphisms or epigenetic studies. Moreover, microarrays will become even more important in future, once the various types of analysis are established to a quality standard that will, for example, allow their use in routine medical diagnostics or food-processing control. Substituting PNA for DNA oligomers as the chip-bound capture molecules could

contribute substantially to achieving higher sensitivity and improved assay robustness. Also, taking advantage of the chemical differences between the PNA probe and the nucleic acid analyte, their interaction can be detected directly, with neither molecule carrying a label. In consequence, the processing is much simplified, facilitating automation. Simultaneously, the degree of bias caused by introduction of a label is avoided and the effort in sample preparation is reduced significantly.

Synthesis

As with DNA microarrays, the probes for PNA chips can either be pre-fabricated and attached to the chip surface by means of robotic devices or synthesised directly *in situ*. With respect to synthesis chemistry, PNA does not differ from ►peptides. Therefore, most procedures developed for the synthesis of peptides can be used for the production of PNA-oligomers and *vice versa*.

Parallel synthesis in 384-well filter-bottom microtiter plates has been established. This releases adequate numbers of PNA-molecules that are of good quality and in sufficient yield for the production of microarrays. In this process, the consumption of reagents per individual oligomer is reduced significantly compared to standard column-based synthesis protocols. Furthermore, procedures for the attachment to the chip surface exist, which select for the binding of full-length molecules (1). In the analysis of biological samples, this leads not only to better reproducibility but also to a higher dynamic range of detection and thus an improved accuracy.

Alternatively, *in situ* synthesis on the array by the SPOT method (8), in which the individual monomers are placed sequentially at the relevant positions of the chip in small volumes of liquid, has been described. Unfortunately, following this protocol, throughput is limited with current liquid handling systems. Also, only a very limited number of array copies can be produced simultaneously. However, drawing on expertise from ink jet technology, for example, handling of small volumes is improving and should soon allow the quick synthesis of many different molecules in many copies. In addition, the control of synthesis by ►photolithography was described for peptide arrays even before the technique was used for the production of DNA chips (3). In combination with PNA monomers that can be deprotected by light, procedures could be established that are equal in throughput to current production processes for DNA microarrays. All *in situ* synthesis formats, however, have the disadvantage that not only full-length molecules but also shorter derivatives that result from the incomplete yield during each synthesis step will be on the arrays.

Basic Hybridisation Characteristics

Overall, hybridisation to PNA chips is performed similarly to hybridisation to DNA microarrays. The main difference is the stability of the duplex formed. Comparable to results on PNA-DNA interaction in solution, the stability on a solid support of a duplex formed by a DNA molecule and a surface-attached PNA oligomer of mixed base composition is in general 1–1.5°C higher per base pair than that of the corresponding DNA-DNA hybrid. Therefore, both higher hybridisation and washing temperatures are used on PNA chips or the length of the PNA oligomers is reduced. The dissociation temperature of PNAs can be predicted from their sequence with reasonable accuracy and the sequence specificity of binding is high. In addition, PNAs that contain a 'chiral box' at their centre exhibited even higher mismatch recognition than normal molecules. ►Chirality can be introduced into PNA-monomers by substituting D-lysine for the usual N-aminoethylglycine backbone (6). ►Hybridisation to complementary molecules in a parallel rather than anti-parallel fashion, potentially possible because of the PNA's achiral nature, is strongly disfavoured on PNA chips.

Label-Free Diagnostics

Especially for routine applications in a clinical setting, it would be advantageous to avoid labelling of the probes and the target molecules altogether. Besides speeding up the analysis process and reducing cost, all labelling steps introduce an additional degree of variation. In addition, such procedures should assist in the integration of the assays into automated analysis systems, which are eventually required for the analysis of patient samples. From its hybrid nature – being chemically rather different from nucleic acids while nevertheless behaving very similarly – PNA offers some unique opportunities to achieve such ends.

►*Surface Plasmon Resonance*

One means for the direct detection of DNA-binding to microarrays is surface plasmon resonance, although it is not unique to PNA arrays. The microarray surface is illuminated with polarised light. Reflection of the light occurs at a conductive film at the interface between the chip and the fluid containing the sample. Upon hybridisation of DNA molecules to the chip-bound PNA oligomers, the refractive index changes, resulting in a resonance signal that can be detected. The refractive index is directly proportional to the mass change at the particular position. This allows a real-time monitoring of the hybridisation process. The variation in signal intensities for a single C to T conversion was found to be as high as 300-fold (2). To date, however, the sensitivity of the procedure is still

inadequate for the analysis of complex mixtures of analyte molecules.

Electrochemical and Direct Electronic Detection

Beside the obvious benefit of avoiding extra labelling steps, a direct electrical detection of binding events would have the additional advantage that the connection to the electronic data analysis could be simplified even further. In addition, the read-out could be much faster than with other systems and real-time measurement during the hybridisation process would be possible. Conductance measurement (4) or ►impedance-based detection (5) have been used for signal recording. As above, there are still limits in the sensitivity of the assay. In order to circumvent this dilemma, various electrochemical detection methods based on electrochemically active substances such as methylene blue or Hoechst 33258 are being investigated. Micro-fabricated PNA arrays were equipped with electrodes made of gold. Different PNA molecules that had a cysteine at their N-termini were bound to the electrodes *via* the thiol groups. The advantage of using PNAs is the smaller adhesion of the positively charged electrochemical compound to the neutral backbone, thereby reducing the background signal. Subsequent to the hybridisation of a DNA-fragment and appropriate washing steps for the removal of unspecific binding, the array is incubated in a solution that contains the electrochemical substance. Hoechst 33258, for example, binds to the minor groove of PNA-DNA duplexes. This produces an anodic peak current that is measured by linear sweep voltametry. In all systems based on electrical measurements, the complexity of the array is still limited at present, thereby turning the advantage of continuous and parallel measurement at all array features into a handicap as far as arrays with very many spots are concerned. However, the number of array features required for many routine assays in the microarray format will anyway be relatively small.

Secondary Ion Mass Spectrometry

A third process seems sensitive enough to circumvent the above-mentioned limitation on sample complexity. In addition, the hardware required for the detection process could be adapted to deal with large feature numbers on the chip, although detection is performed sequentially rather than in parallel as with electrical measurement. A combination of PNA microarrays and detection of bound nucleic acids by time-of-flight secondary ion ►mass spectrometry (TOF-SIMS) could permit a direct analysis of genomic DNA (1). SIMS is very sensitive in detecting fragments (PO_2^- and PO_3^-) of the phosphate ions, which are an integral part of nucleic acids but missing entirely in PNA. In addition, a DNA fragment contains very many phosphates. Since both the detectable marker and signal amplification are therefore intrinsic to the target molecule, analyses could be performed without any prior amplification by means such as PCR and without labelling. In this respect, it is also of advantage that relatively long fragments can be hybridised to PNA-oligomers under low salt or no salt conditions and that the duplex stability is high.

Subsequent to a hybridisation of nucleic acids to a PNA microarray and the removal of non-specifically bound molecules by appropriate washing steps, the binding of nucleic acids is identified by mass spectrometry. A primary ion beam is directed at the microarray surface, causing the fragmentation and release of molecule fragments present at this position. However, just a monolayer of molecules is actually affected. Concurrently with fragmentation, the released molecules are ionised by the primary ion beam. Negatively charged ions are accelerated into the flight tube of the mass spectrometer, in which they are separated according to their mass-to-charge ratios. Phosphates and therefore the respective signals should only be present when nucleic acid has hybridised to the complementary PNA molecule at a given position.

From the difference in backbone structure of PNA and nucleic acids, other fragments, such as parts of the sugar molecules, could also be used as an indicator for the binding of nucleic acids. Since the sugar molecules of RNA and DNA are different at the 2′-position, the procedure could even distinguish between the two molecular classes. This process could even allow discrimination of living cells or organisms, producing a signal from both DNA and RNA, from dead ones, which should exhibit a signal that only represents the relatively long-lived DNA.

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►Chip Technologies, Basic Principles

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PNET

Definition

PNET stands for Primitive Neuroectodermal Tumours.

►Medulloblastoma

Pneumothorax

Definition

A pneumothorax is an accumulation of air in the pleural cavity.

►Marfan Syndrome

PNS

►Peripheral Nervous System

Podophyllotoxins

Definition

Podophyllotoxins are natural anticancer products, acting as chemotherapeutics by inhibition of DNA synthesis in a cell cycle dependent manner. They form a complex with topoisomerase II and DNA, thus inducing double stranded DNA breaks, and preventing repair by topoisomerase II binding and the entry phase of cell division. Commonly used podophyllotoxins include etoposide and teniposide.

►Multi-Drug Resistance

Point Mutation

Definition

Point Mutation defines a change or deletion/insertion of a single base in a gene, which changes the nucleotide composition of the resulting RNA. In protein encoding genes, it can lead to a single amino acid replacement in the protein with or without loss or change of function.

►Mouse Genomics
►tRNA

Point Spread Function

Definition

An optical system produces a complex three-dimensional light distribution of an ideal point light source in the image space in and near the image plane. This light distribution represents the point spread function of the optical system. For an aberration-free, diffraction-limited optical system, the slice of the diffraction pattern in the image plane is designated as Airy function, which is the mathematical description of the diffraction pattern of a circular aperture. Above and below the image plane, the diffraction pattern changes periodically along the optical axis, so that bright and dark Airy-disk-like patterns appear alternately with diameters increasing proportional to the distance from the image plane.

►Fluorescence Microscopy: Single Particle Tracking

Poisson-Boltzmann Electrostatics

Definition

Poisson-Boltzmann electrostatics describes classical treatment of electrostatic interactions in solution using