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Genome diagnostics with TOF-SIMS

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Abstract

A novel deoxyribonucleic acid (DNA) sequencing method is described that uses peptide nucleic acid (PNA) biosensor chips. PNA is a synthesized DNA analog in which both the phosphate and the deoxyribose of the DNA backbone are replaced by polypeptides. The hybridization properties of PNA to DNA are comparable to those of DNA to DNA. PNA can thus be used in the same applications as DNA. We have used TOF-SIMS to investigate its ability to detect DNA fragments hybridized to complementary PNAs. The data show that hybridized DNA could be readily identified by detecting the PO_2^- and PO_3^- masses. A good discrimination between complementary and non-complementary sequences could be achieved. It can be concluded that TOF-SIMS is a very useful technique for investigating the complexities of the immobilization and hybridization processes and that SIMS has the potential for providing a rapid method for genetic diagnostics.

Keywords: TOF-SIMS; Mass spectrometry; DNA; PNA; Biosensor; Hybridization

1. Introduction

Nucleic acid chip technology has been a subject of growing interest for clinical diagnostics as well as for sequencing cDNAs, partial sequencing of clones, deoxyribonucleic acid (DNA) sequencing, gene polymorphism studies, and identification of expressed genes. Nucleic acid chips are based on the method of sequencing by hybridization, where unknown DNA fragments are hybridized to known complementary nucleic acid sequences which are immobilized on a solid surface in an array format. The main variables in this process are the attachment of the nucleic acid sequences to a solid surface, the conditions for hybridization, and the detection of the hybridized DNA sequences.

One novel approach is the use of a peptide nucleic acid (PNA) biosensor chip [1–4]. PNA is a synthetic DNA analog in which both the phosphate and the deoxyribose of the DNA backbone are replaced by polypeptides (see Fig. 1). This DNA analog possesses the ability to hybridize to complementary DNA sequences. Thus, PNA chips can be used in the same way as DNA chips. Two major advantages of PNA over DNA are the neutral backbone and the increased strength of the PNA/DNA pairing. Since the PNA backbone is free of phosphate as opposed to that of DNA, which contains phosphates, a technique that identifies the presence of these phosphates in a molecular surface layer would allow the use of unmodified genomic DNA for hybridization on a biosensor chip rather than using amplified DNA fragments labeled with radioisotopes, stable isotopes, or fluorescent substances.

In this paper we investigated the possibility of using TOF-SIMS for detecting unlabeled DNA fragments hybridized to complementary PNAs.

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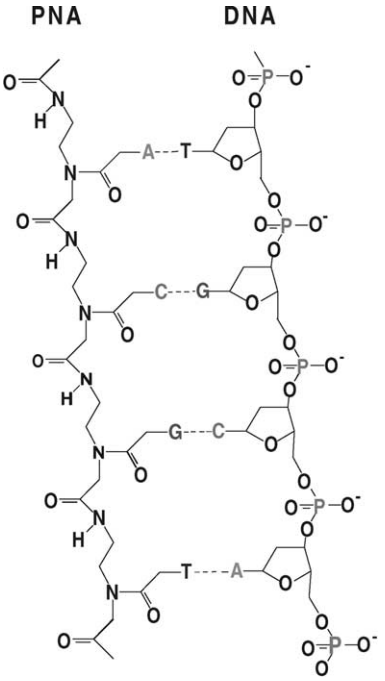

Fig. 1. Structure of PNA and DNA.

2. Experimental and sample preparation

For the analysis, a 10 keV primary Ar^+ ion beam was used for bombarding the sample (total ion dose density of 1×10^{12} cm^{-2}), a low-energy pulsed electron source (20 eV) for charge compensation, and a reflectron-type time-of-flight analyzer for analyzing the secondary ions.

For the experiments, PNAs were bound to a thiol-SA-layer on either Ag or Au surfaces (Fig. 2). To produce a thiol-SA-layer, the Ag- and Au-surface, respectively, was modified using a 1 mM solution of DTSP (3′3-dithio-bis(propionic acid-*N*-hydroxysuccinimide ester)) in DMSO (dimethyl sulfoxide). PNA was then immobilized to the DTSP thiol layer. To immobilize the PNA, a 10 μM solution of the 13-mer PNA sequence 5′-TTT TCC CTC TCT C-3′ in triple deionized water was pipetted onto the surfaces resulting in an immobilized PNA spot of approximately 1 mm diameter. To prevent unspecific binding of DNA to DTSP during hybridization, the DTSP was saturated using concentrated propylamine with a saturation time of 5 h.

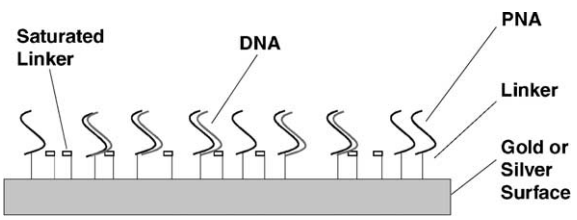

Fig. 2. Conceptional diagram of the make-up of the biosensor.

For hybridization, a buffer solution composed of a 1:5000 dilution of concentrated DNA (1 mM) in SSSarc buffer was used. Hybridization took place in an oven over 15 h at 313 K. The complementary DNA sequence used was 5′-GAG AGA GGG AAA A-3′, the non-complementary DNA sequence was 5′-CAC AGC ACA GAC A-3′. Non-complementary DNA sequences were removed from the biosensor by appropriate washing techniques.

3. Results and discussion

Fig. 3 depicts parts of negative TOF-SIMS spectra obtained from immobilized DNA (top figure) and

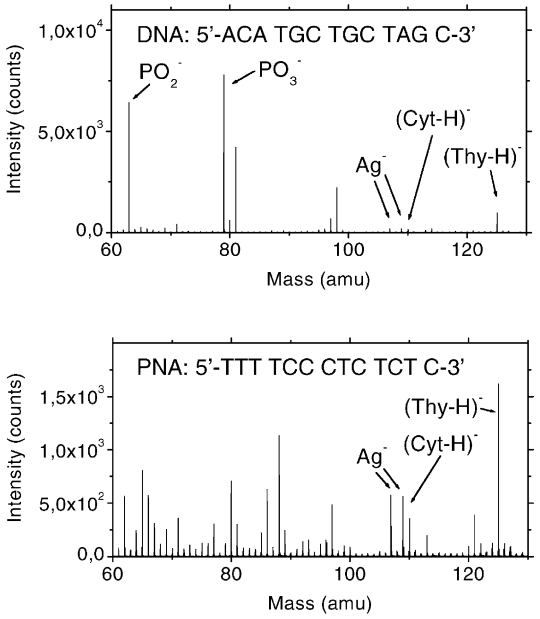

Fig. 3. Negative TOF-SIMS spectra (60–130 amu) obtained from immobilized DNA and PNA layers.

PNA (bottom figure). Besides the substrate Ag^- signal, the deprotonated $(M–H)^-$ signals of the bases cytosine and thymine are visible with only a slight variation in signal between DNA and PNA. Note, however, the two major DNA-specific phosphate peaks (PO_2^- and PO_3^-). A comparison between the PNA and the DNA spectra demonstrates that the masses corresponding to PO_2^-, PO_3^- provide the best way for detecting the presence of DNA.

After optimizing the immobilization steps [5], hybridization experiments were carried out. Single-stranded DNA can hybridize to single-stranded PNA according to the rules of Watson and Crick base pairing, exactly as in the hybridization of two DNA single strands. The Watson and Crick rules of base pairing state that only certain bases can hybridize together via hydrogen bonding. These base pairs are adenine–thymine, and guanine–cytosine.

Fig. 4 shows mass spectra of a hybridization experiment. The data show that the hybridized DNA can be unambiguously distinguished from PNA by the dominant peaks of PO_2^-, PO_3^- and $(Ade–H)^-$. Adenine can be used for identification of a positive hybridization reaction because the sequences were selected in such a way that adenine occurs only in the complementary and non-complementary DNA sequences but not in the immobilized PNA sequence. Further peaks visible are the substrate signal Ag^- and the deprotonated base signals of the PNA sequence which have similar intensities in both spectra. Some ion peaks caused by contaminants such as iodine are also observed. However, these do not cause any interferences. Minor peaks occurring at approximately the same mass as PO_2^-, PO_3^- and $(Ade–H)^-$ might cause mass interferences. However, they can be simply separated out by using a high-resolution mass spectrometer with a mass resolution of $m/\Delta m > 7000$.

Several hybridization experiments with unlabeled complementary and non-complementary DNAs were carried out to test the reproducibility. The column plots (Fig. 5) depict averaged substrate-specific signals obtained from four immobilized PNA samples on Ag-covered surfaces (Fig. 5a), of which two were hybridized with complementary and two with non-complementary DNAs, and from 10 immobilized PNA samples on Au-covered surfaces (Fig. 5b), of which five were hybridized with complementary and five

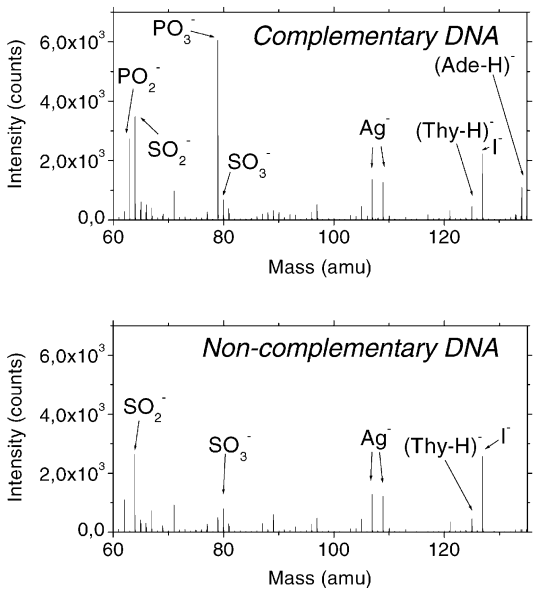

Fig. 4. Negative TOF-SIMS spectra (60–130 amu) obtained from hybridization experiments, in which complementary and non-complementary DNA sequences were hybridized to a PNA sequence.

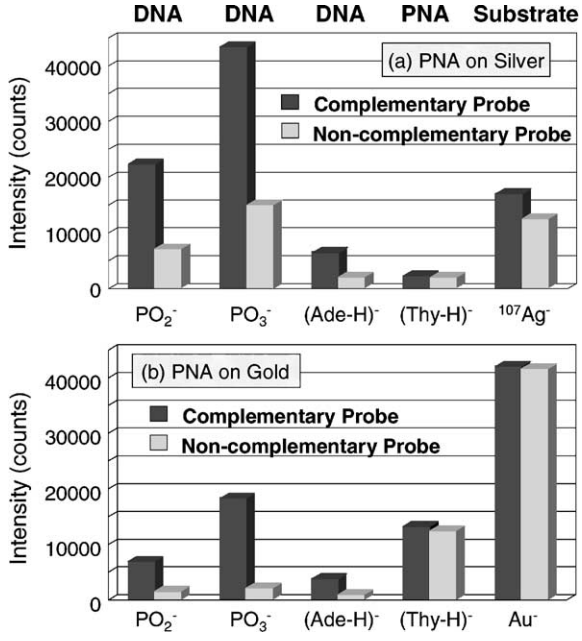

Fig. 5. Column plot of averaged substrate-specific signals obtained from several PNA samples, to which complementary and non-complementary DNAs were hybridized. (a) PNA on Ag and (b) PNA on Au.

with non-complementary DNAs. On each PNA spot, three to five different locations were analyzed with an analysis area of 100 μm × 100 μm each. For each individual measurement, the complementary signal was higher than the highest non-complementary signal. The ratio between complementary and non-complementary signals varied, e.g., from 28:1 to 5:1, for PO_3^-. A comparison between the data shows that a higher signal could be obtained on the Ag-covered sample. The observed deviation between PO_2^- and PO_3^- might be due to different ion formation behavior during the sputtering process for complementary and non-complementary DNAs as well as to phosphate surface contaminants.

4. Conclusion and outlook

We have successfully shown that TOF-SIMS can readily identify hybridized DNA on PNA biosensor chips by detecting the phosphates present in the DNA. The absence of a labeling procedure and the increase in the number of phosphates with increasing sequence length will be particularly advantageous for sequencing genomic DNA. In future experiments, we will further evaluate the potential of TOF-SIMS for providing a method for rapid genomic diagnostics and use it to examine in detail the complexity of the hybridization process.

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