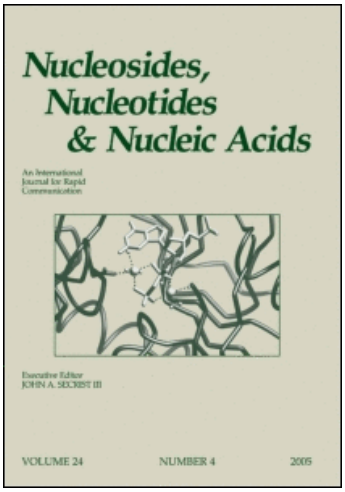

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Preparation of DNA Chips Using Polyamine-Oligonucleotide Conjugates

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Preparation of DNA Chips Using Polyamine-Oligonucleotide Conjugates

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ABSTRACT

A convenient and efficient method for three-dimensional immobilizing oligonucleotides on glass was developed using oligonucleotide derivatives bearing a polyamine linker (PA-oligo conjugates). Polyamine (polylysine, poly(lysine, phenylalanine), polyethyleneimine) residues stipulate durable fixation of such conjugates to the glass surface with a high yield (90–95%). A DNA fragment (414-mer) is hybridized specifically to an immobilized oligonucleotide.

Key Words: Polyamine-oligonucleotide conjugates; Immobilization; DNA chips.

INTRODUCTION

Most of the methods for immobilizing oligonucleotides to solid supports describe the covalent attachment of modified oligonucleotides bearing different functional groups to the pretreated derivatized solid support.[1–4] The dendrimeric linker system was used to multiply the coupling sites on glass slides.[5] Acrylamide gel pads[6] or agarose film[7] are examples of methods allowing three-dimensional attachment of substrate molecules that enhances the loading capacity and the sensitivity of the following hybridization study. Other methods propose the precoating of the solid phases with polylysine[8] or

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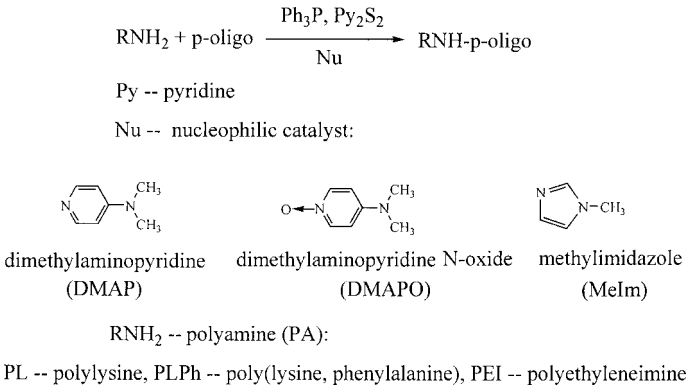
polyethyleneimine[9] followed by the covalent attachment of either amino or thiol containing oligonucleotides using bifunctional cross-linking agents. All the above methods require the pretreatment of the solid phase to introduce the functional groups as well as the use of cross-linking or coupling agents to provide the formation of covalent bonds between a solid surface and oligonucleotides.

RESULTS AND DISCUSSION

The present paper relates to a method for non-covalent three-dimensional immobilizing oligonucleotides carrying polyamine residues to unmodified glass slides. To prepare polyamine-oligonucleotide conjugates (PA ~ oligo), an oligonucleotide containing the 3′- or 5′-terminal phosphate is activated with the redox pair triphenylphosphine/dipyridyldisulfide (Ph3P/Py2S2) in the presence of nucleophilic catalyst (methylimidazole, dimethylaminopyridine, or dimethylaminopyridine N-oxide), and the formed active intermediate interacts then with the amino groups of polyamine.[10] Polylysine (PL), poly(lysine,phenylalanine) (PLPh), or polyethyleneimine (PEI), were used for the synthesis of PA-oligo conjugates (Scheme 1). The conjugates can be stored in solution for a long time (months) until utilizing.

PA-oligo conjugates can be immobilized to unmodified glass slides by spotting them on the surface directly from the reaction mixture without isolation. After drying slides, PA-oligo conjugates become attached to the surface by passive immobilization. They exhibit high adhesion to the surface and stability after several washing steps (K-phosphate buffer, water, ethanol). The additional washing with hot water (90°C) resulted in the loss of only 10–20% of the immobilized material.

The dependence of the amount of the immobilized PA conjugates on their concentration in the spotting solution is shown to be linear in a wide range of concentration (1 μM–1 mM) (Fig. 1). Thus, the surface capacity of immobilized PA conjugates can be easily monitored by changing their concentration in the spotting solution.



Scheme 1. The synthesis of PA-oligo conjugates.

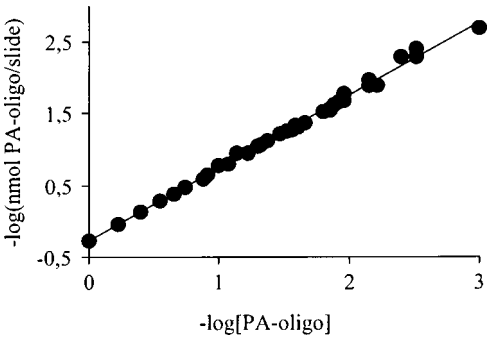


Figure 1. Dependence of the amount of immobilized PA-oligo conjugates on their spotting concentration (1 μM–1 mM).

The proposed method for DNA chips fabrication was shown to be applicable in nucleic acid hybridization assays. An oligonucleotide conjugate bearing the polylysine residue (GATTATTTGGAAAAGp-PL) was immobilized on glass slides from the spotting solutions of different concentration ($8 \cdot 10^{-7}$ M, and $4 \cdot 10^{-7}$ M). After blocking amino groups of the immobilized PA-oligo conjugates with acetic anhydride to avoid unspecific electrostatic interaction, slides were subjected to hybridization with DNA fragments. The specific binding occurs only in the case of using DNA fragment containing complementary region to the immobilized oligonucleotide (Fig. 2).

The advantages of the proposed method of immobilizing oligonucleotides are considered to be the following: the method provides ''three-dimensional'' immobilization of oligonucleotides on the surface; the conjugates contain a long linker providing enough distance between an oligonucleotide and the surface; more than one molecule of an oligonucleotide can be attached to one polyamine molecule, so that the affixture of one molecule of the conjugate ensures the attachment of several oligonucleotide molecules simultaneously that provides high efficiency and capacity of immobilization; there is no necessity of using cross-linking or coupling agents; unmodified glass slides can be used as a support, which simplifies significantly the fabrication of DNA chips.

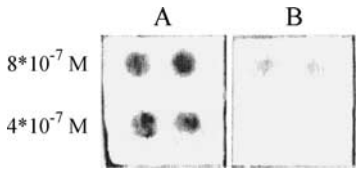


Figure 2. Hybridization of PCR fragments (414 bp) bearing the biotin label on glass slides containing PL-oligo conjugates immobilized from the spotting solution of different concentration. PCR fragments contained (A) or did not contain (B) the complementary site to the immobilized oligonucleotide. Streptavidin-alkaline phosphatase conjugate in the presence of chromogenic substrates was used for visualization of the formed duplexe.

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