Covalent binding of oligonucleotides and hybridization using amine-substituted conducting polymer films

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1. Introduction

Amine-substituted polymers are often used as immobilization materials. The amine group can form covalent bonds with carboxylic acid or phosphate. This is useful for immobilization of biomolecules for instance.

Polytyramine (PTyr) presents one primary aliphatic amine per tyramine moiety, i.e. a very high surface concentration of reactive sites.

PTyr films obtained by electrooxidation of tyramine (4-hydroxyphenylethylamine) in alkaline methanolic solution are not very conductive [1]. In this work, the films are electrosynthesized in aqueous acid leading to more conducting and thicker films.

We focus particularly on the ability of phosphate-modified ODN (pODN) to bind on PTyr film. ODN binding is discussed in terms of physical adsorption including electrostatic interactions and covalent binding. Hybridization experiments and measurements of the discrimination capacity of native versus mutations containing sequences of ODN-PTyr films are also included.

2. Results

2.1. Poly(tyramine) film characterization

FT-IR spectrum of a PTyr film shows bands from primary amine, indicating that the amino group remains free after electropolymerisation.

PTyr film was analyzed by XPS. The N_{1s} spectrum presents two peaks, at 400.2 eV and 402.2 eV, which can be attributed respectively to neutral amine ($-NH_2$, 20 % of the total peak surface) and protonated amine ($-NH_3^+$, 80 %). The strong protonation of the amine group corroborates the presence of ClO_4^- ions in the Cl_{2p} spectrum.

2.2. Oligonucleotide binding

ODN can easily bind to a polymer by non-specific adsorption. The chemical nature of PTyr film (protonated amino groups at neutral pH) enhances this phenomenon. Therefore, it could be difficult to distinguish between adsorbed and covalently bound ODN. We paid a particular attention to this point.

Immobilized pODN have been separated in weakly adsorbed, strongly adsorbed and covalently bound ODN. To block strong adsorption sites, films have been pre-treated with salmon DNA.

A series of salmon DNA-pretreated films were reacted on the 20-mer thymine homonucleotide pT20. Under these conditions, probes are immobilized by covalent bond (T20-C) as well as adsorbed on weak adsorption sites (T20-W), for an average value of 880 pmol.cm^{-2}. After elimination of T20-W, a mean value of 510 ± 100 pmol.cm^{-2} of probes are still bound to the films, considered as covalently linked (T20-C).

For an untreated film, immobilized ODN are mainly composed of adsorbed ODN on strong sites (T20-S, 45 %), adsorbed ODN on weak sites (T20-W, 35%), and covalently bound ODN (T20-C, 20%). When films are pre-treated with salmon DNA, T20-S disappear and T20-C represent around 60% of the total amount. After an appropriate washing, only covalently bound ODN remain.

2.3. Hybridization

Tests consist in soaking probes-modified PTyr films in a solution containing the target ODN. In order to mimic real conditions of detection, we used the GEM oligonucleotide, a sequence complementary to this derived from the gag protein of HIV. The single point mutation sequence (MHIV) was derived from a sequence found in a patient. Films containing an average value of 800 pmol.cm^{-2} of immobilized pGEM were used to challenge their capacity to discriminate complementary (HIV) and mismatches containing sequences (MHIV and MMHIV). The discrimination capacity is defined as the ratio *f1* = HIV/MHIV or *f2* = HIV/MMHIV where HIV, MHIV and MMHIV represent the quantity of remaining ODN after hybridization and washing.

For 30 min hybridization, the ratio *f1* is about 3 at 50°C. In the same conditions, *f2* is around 9, with an hybridized HIV concentration of 90 pmol.cm^{-2}.

3. Conclusion

The synthesis of PTyr films as described in this work lead to high surface concentration of reactive primary amines. This results in high yield of covalent attachment of ODN. However, adsorption is also a dominant process. We demonstrated that adsorption of the strong type can be blocked by DNA while those of the weak type can be easily removed by a washing procedure. A study of sequence recognition and discrimination showed discrimination factors which allow to envisage future applications of this system in electrochemical detection devices.

[1] M.C. Pham, P.C. Lacaze and J.E. Dubois, J. Electrochem. Soc. 131 (1984) 777