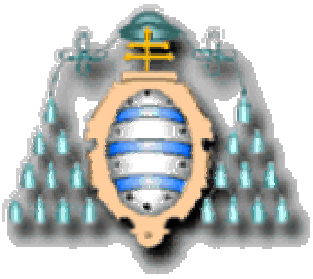

Enzyme-amplified electrochemical detection of *Legionella pneumophila* using hairpin DNA probes

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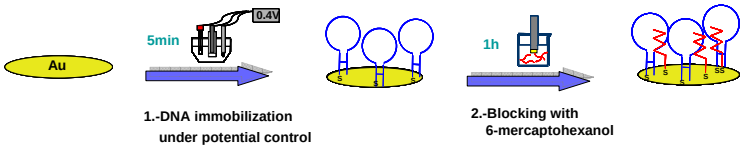
INTRODUCTION

Electrochemical detection of DNA based on the hybridization event is receiving considerable attention. Although linear single-stranded oligonucleotides are usually employed as probes, the use of hairpin DNA could result advantageous.

An electrochemical genosensor using DNA with a hairpin structure as molecular recognition element has been developed for the detection of specific sequences of pathogens. Mixed self-assembled monolayers (SAMs) of thiolated hairpin DNA and a spacer thiol were constructed on gold electrodes and used as sensing platform for a linear single stranded DNA sequence specific of *Legionella pneumophila* by hybridization.

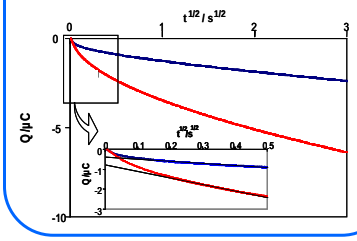
The hybridization event was detected in a sandwich assay format using a biotinylated 30-mer oligonucleotide as a tracer. Both streptavidin-horseradish peroxidase (Strep-HRP) and streptavidin-alkaline phosphatase (strep-AP) conjugates were assayed in the detection step and the analytical characteristics of the corresponding sensors were comparatively evaluated.

MONOLAYER FORMATION



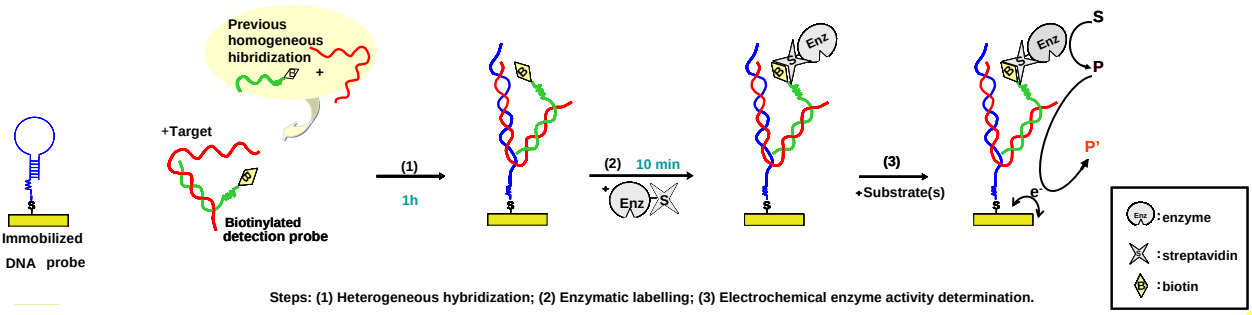
Chronocoulometry of $Ru(NH_3)_6^{+3}$ was employed to quantify DNA surface density via measuring redox charges of $Ru(NH_3)_6^{+3}$ at modified surface — without $Ru(NH_3)_6^{+3}$ — with 100μM $Ru(NH_3)_6^{+3}$

Quantification of surface densities of DNA



$\Gamma_{DNA} = (1.5 \pm 0.5) \cdot 10^{12}$ molecules of hairpin/cm^2

DETECTION SCHEME

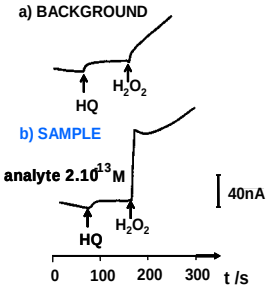


Steps: (1) Heterogeneous hybridization; (2) Enzymatic labelling; (3) Electrochemical enzyme activity determination.

Enzyme	Alkaline phosphatase (ALP)	Horseradish peroxidase (HRP)
Substrates	1-naphthylphosphate	Hydroquinone(HQ) H_2O_2
Detection technique	Differential pulse voltammetry	Amperometry
LOD	4.10^{-10}M	$<2.10^{-13}$M
Lineal range	2.10^{-10}-2.10^{-6}M	2.10^{-13}-2.10^{-9}M
Main advantage	Selectivity	Sensitivity

ENZYME : HRP

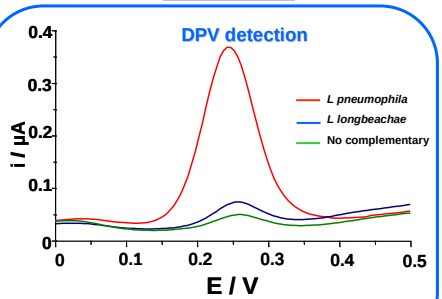
Amperometric detection



0.1MKCl 0.1M phosphate buffer pH7 E=-200mV

ENZYME : ALP

DPV detection



0.5 M Tris-HCl buffer pH9.8; 4.10^{-3} M 1-naphthylphosphate

Discrimination between two species of *legionella* at 200nM

CONCLUSIONS

Both sensors are suitable to detect *Legionella pnemophila.* If the streptavidin-horseradish peroxidase conjugate is used, high sensitivity is achieved in 2.5 h assay time; whereas with streptavidin-alkaline phosphatase a good discrimination between complementary and non-complementary sequences is found.

ACKNOWLEDGMENT

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