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Genosensor on gold thin-films with enzymatic electrochemical detection of a SARS virus sequence

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36.1 OBJECTIVES

(a) To construct a hybridisation-based genosensor for a SARS (severe acute respiratory syndrome) virus sequence on a 100 nm sputtered gold film which works as immobilisation and transduction surface. (b) To test the sensitivity and the selectivity of the SARS genosensor using complementary strands of SARS virus and three-base mismatch strands.

36.2 MATERIALS AND INSTRUMENTS

36.2.1 Materials

A target sequence corresponds to a portion of SARS virus, bases exactly comprise between 29218 and 29247, both included. A three-base mismatch strand with mismatches located in bases number 5, 15 and 26. Both strands are biotinylated at the 3' end for allowing hybridisation detection.

- Biotinylated target (193 nmol): 5'-ACA-GAG-CCT-AAA-AAG-GAC-AAA-AAG-AAA-AAG-3'-biotin
- Biotinylated 3-base mismatch target (184 nmol): 5'-ACA-GCG-CCT-AAA-AAC-GAC-AAA-AAG-AGA-AAG-3'-biotin
- A thiolated complementary probe. The thiol group is separated to the first base by an aliphatic linker of three carbons; (103 nmol): 5'-CTT-TTT-CTT-TTT-GTC-CTT-TTT-AGG-CTC-TGT-3'-(CH₂)₃-SH

Oligonucleotide solutions are prepared in TE buffer pH 8 (0.1 M Tris-HCl buffer solution, 1 mM in EDTA). Aliquots are prepared and maintained at -20°C . Working solutions were conserved at 4°C . Hybridisation takes place in a $2 \times \text{SSC}$ (saline sodium citrate, 30 mM sodium citrate buffer with 300 mM sodium chloride and pH 7.0) buffer containing 50% of formamide.

1-hexanethiol is solved in ethanol.

Alkaline-phosphatase (AP) labelled streptavidin (ST-AP) is prepared in 0.1 M Tris-HCl buffer, 1 mM MgCl_2 , pH 7.2. Aliquots are prepared and maintained at -20°C . Working solutions are conserved at 4°C .

3 mM 3-indoxyl phosphate (3-IP) solutions are daily prepared in a 0.1 M Tris-HCl buffer, 10 mM MgCl_2 , pH 9.8. They are kept at 4°C and protected from light.

Ethanol, magnesium chloride, sodium citrate, Trizma base, sodium chloride, EDTA, formamide, hydrochloric and sulphuric (95–97%) acids are of analytical grade. Water is purified employing a Milli-Q plus 185 equip from Millipore.

A 0.125-mm-thick polyimide substrate named Kapton HN[®] (Goodfellow), an epoxy resin (CW2400) obtained from RS Components and self-adhesive washers of 5 mm internal diameter (19.6 mm^2 of internal area) are used to construct the working electrodes.

36.2.2 Instruments

Measurements are performed with an Autolab PGSTAT 10 (Eco-Chemie) potentiostat interfaced to an ADL Pentium 120 computer system and controlled by Autolab GPES software version 4.8 for Windows 98.

A Crison Micro-pH 2001 pH-meter and a Sanyo (MIR-162) incubator are also used.

An Emitech sputter coater model K550 is used to deposit the gold thin film working electrode.

36.2.3 Construction of the three-electrode potentiostatic system

The three-electrode potentiostatic system is schematised in Fig. 36.1. Working electrodes are made on $5 \times 5 \text{ cm}^2$ supports of 0.125-mm-thick polyimide substrate named Kapton HN[®] (Goodfellow). The kapton slide is cleaned with ethanol and after drying, it is covered with gold by

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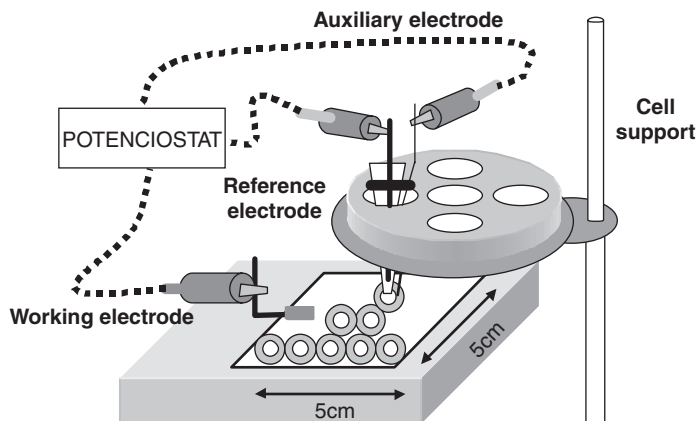


Fig. 36.1. Schematic diagram of the three-electrode potentiostatic system. Reprinted from Ref. [1], Copyright 2005, with permission from Elsevier.

a sputtering process using an Emitech sputter coater model K550. Gold atoms are deposited (from the cathode) over kapton (placed on the anode) in a vacuum chamber filled with argon. Gold layer thickness is controlled by the time and the intensity of the discharge. For a 100-nm-thick layer a 35 mA discharge is applied over 220 s. After that, a conductor wire is attached to the centre of one of the sides by means of an epoxy resin (CW2400) obtained from RS Components, that is cured at room temperature.

The working area is limited by self-adhesive washers of 5 mm internal diameter (19.6 mm^2 of internal area). The total area of the gold surface lets to stick 23 washers approximately. The gold film is placed on a support where a crocodile connection is fixed.

Reference and auxiliary electrodes are coupled in a micropipette tip. The reference electrode consists of an anodised silver wire introduced in a tip through a syringe rubber piston. The tip is filled with saturated KCl solution and contains a low-resistance liquid junction. The platinum wire that acts as auxiliary electrode is fixed with insulating tape. For measurement recording, the tip is fixed on an electrochemical cell Metrohm support allowing horizontal and vertical movement.

36.3 GENOSENSOR CONSTRUCTION

- Deposit a drop of $5 \mu\text{L}$ of $1.02 \mu\text{M}$ thiolated probe on the gold film and maintain at 37°C for 20 min or at 4°C for 12 h.
- Clean with 0.1 M Tris-HCl buffer pH 7.2.

- Deposit a 15 μL drop of a 2% 1-hexanethiol solution on the gold film and maintain for 10 min.
- Clean with a $2 \times$ SSC buffer solution pH 7.

36.4 HYBRIDISATION ASSAY AND RECORDING OF THE ANALYTICAL SIGNAL

- Deposit 20 μL drops of different concentrations of biotinylated target strand or biotinylated three-base mismatch target for 60 min at room temperature.
- Wash the film with 0.1 M Tris-HCl buffer, 10 mM of MgCl_2 , pH 7.2.
- Deposit a 20 μL drop of AP-labelled streptavidin in a 10^{-9} M concentration for 60 min.
- Wash the film with 0.1 M Tris-HCl buffer, 10 mM MgCl_2 , pH 9.8.
- Deposit a 20- μL drop of 3 mM 3-IP solution.
- Stop the reaction by adding 5 μL of concentrated H_2SO_4 , and add 5 μL of Milli-Q water for carrying out the electrochemical measurement (next step).
- Introduce the tip with reference and auxiliary electrodes on the 30 μL drop (20 μL coming from the 3-IP solution plus 5 μL of concentrated H_2SO_4 and 5 μL of Milli-Q water) deposited on the gold film. Apply a potential of -0.35 V for 30 s before scanning the potential between -0.15 and $+0.3$ V following a square wave format with 50 Hz of frequency and 50 mV of amplitude.

The main steps of Sections 36.3 and 36.4 are schematised in [Fig. 36.2](#).

36.5 DISCUSSION

The response curves for different concentrations of complementary target are shown in [Fig. 36.3](#).

There is a notorious difference between the analytical signal obtained for a 3.03 nM solution of the complementary target strand and three-base mismatch strand. In stringent experimental conditions ($2 \times$ SSC containing 50% formamide), the analytical signals are 47.61 and 3.96 μA , respectively, when hybridisation takes place for 1 h. Therefore, a 100% discrimination is achieved.

Repeatability is checked under these experimental conditions, 1 h of hybridisation and a $2 \times$ SSC buffer containing 50% of formamide. The value of the RSD was 11% for nine measurements.

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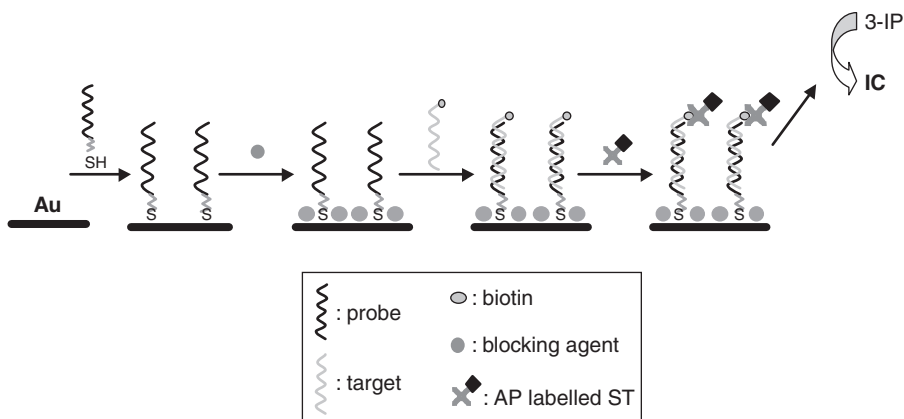


Fig. 36. 2. Genosensor construction and hybridisation assay. Reprinted from Ref. [1], Copyright 2005, with permission from Elsevier.

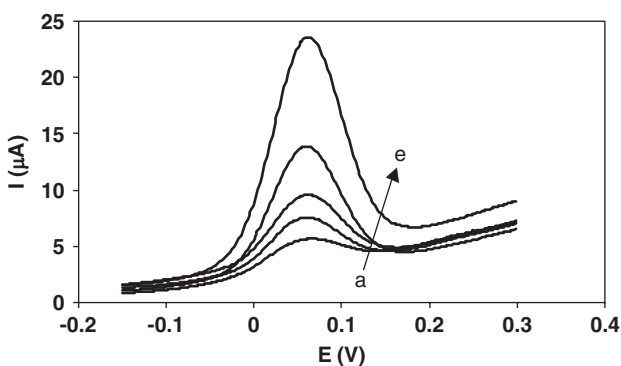


Fig. 36.3. Response curves for: (a) background; (b) 0.01 nM; (c) 0.10 nM; (d) 0.51 nM; (e) 1.01 nM complementary target concentration. $V_{\text{droptarget}} = 20 \mu\text{L}$, $t_{\text{hybrid}} = 60 \text{ min}$, hybridisation buffer = $2 \times \text{SSC}$ (pH 7) + 50% formamide. Reprinted from Ref. [1], Copyright 2005, with permission from Elsevier.

A linear calibration curve is obtained (see voltammograms in Fig. 36.3) between 0.01 and 1.01 nM. The linearity is represented by the equation:

$$i_p(\mu\text{A}) = 14.6[\text{target}](\text{nM}) + 3.97, r = 0.998, n = 5$$

The limit of detection, calculated as the concentration corresponding to a signal which is three times the standard deviation of the intercept, results to be 5 pM. This means an improvement of various orders of

magnitude when compared with limits of detection reported in the bibliography for DNA assays that detect α -naphthol on thick-film gold electrodes 0.25 nM for Carpini *et al.* [2] and 0.22 nM for Xu *et al.* [3]. 146 nM is the limit of detection obtained for an AP-based DNA assay that employs *p*-aminophenylphosphate as substrate [4]. Specific quantification of 1 nM is achieved with other enzymatic schemes [5]. A limit of detection of 0.20 nM [6] and 30 nM [7] are achieved for indirect detection using an electrochemical indicator and for direct electrochemical detection respectively.

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