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DEVELOPMENT OF AN AMPEROMETRIC IMMUNOSENSOR FOR THE DETECTION OF LPS USING HORSERADISH PEROXIDASE LABELLED ANTI-RABBIT Ig-G

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ABSTRACT

An immunosensor based on indirect amperometric immunoassay was developed for the determination of LPS. The immunosensor employed horseradish peroxidase labelled antirabbit Ig-G. Peroxidase is a heme containing enzyme that catalyzes the oxidation of a variety of organic and inorganic compounds by hydrogen peroxide [1,2]. In this work the reaction between hydrogen peroxide and hydroquinone was catalyzed by HRP and the electrochemical reduction of the oxidised benzoquinone[3] yielded a current that was related to the concentration of LPS used.

LPS was immobilized on an IMMobilon AV membrane, incubated with anti-LPS rabbit serum and, in a second incubation step, with anti rabbit Ig-G - HRP. Capping and washing procedures were made in between, similarly to what is done in ELISA. The membranes thus prepared were applied to an US biosensor electrode base, similar to what has been used before[4]. A potential of -150mV was applied from an Amperometric Biosensor Detector and the current corresponding to the reduction of benzoquinone was recorded. Different incubation times and antibody dilutions were tested. The immunosensor was found to be sensitive to LPS and have good reproducibility.

Key Words: Immunosensor; amperometric; LPS; HRP, Hydroquinone.

INTRODUCTION

Amperometric immunosensors combine the high selectivity of antibody antigen reaction with the versatility and low detection limits of modern electrochemical techniques. They are cheaper than the alternative techniques for the detection of the analyte of interest. Besides that, they do not suffer from problems of sample turbidity, quenching and interferences from the many absorbing and fluorescing compounds that usually exist in samples and hamper spectroscopic techniques.

In this work, an amperometric immunosensor for the detection of LPS (Lipopolysacharide) from the outer membrane of a strain of a sulphate reducing bacteria (SRB) was developed.

EXPERIMENTAL

Materials

Na_2HPO_4, KH_2PO_4, $NaHCO_3$, KCl and NaCl pro analysi, Hydroquinone zur synthese and Perhydrol, 30% H_2O_2 zur Analyse were from Merck; Na_2CO_3 was BDH, AnalaR; Ethanolamine was Fluka, purum.
Tween: Polyoxyethylenesorbitan monolaurate (Tween-20) and bovine serum albumin BSA A-7906 were Sigma.

LPS was a gift from Prof. Ana Rosa L. Lino

Rabbit serum anti-LPS and the conjugate Imunoglobulin-G - Horseradish peroxidase (Ig-G - HRP) were a gift from Prof. Margarida Meireles

Immobilon -AV membranes were Millipore

Apparatus

An Amperometric Biosensor Detector (ABD-3001) and 4006 electrode, both from Universal Sensors (US) and a LKB 2210 BROMMA recorder were used.
A batch amperometric mode of operation was used throughout, with a magnetic stirrer and bar providing the convective transport.

Procedures

Preliminary tests of the ability of the US electrode to sense the enzymatic catalyzed reaction were done as follows: Ig-G - HRP was immobilized on Immobilon-AV membrane discs, which were applied on an US electrode. The electrode thus prepared was dipped in 5 ml of freshly prepared $2x10^{-5}$ mol dm^{-3} hydroquinone solution in PBS (Phosphate buffered saline). A potential of -150 mV was applied from the ABD and a base current was allowed to reach a steady value. Subsequently 50 µl of 1mmol dm^{-3} H_2O_2 were added and a corresponding stepwise increase in current was observed and recorded. The current corresponds to the reduction of the benzoquinone at the cathode according to:

$$H_2O_2 + H_2Q \xrightarrow{HRP} 2H_2O + Q$$
$$Q + 2H^+ + 2e^- \rightarrow H_2Q$$

Since the electrode was found to be sensitive to the enzyme catalysed reaction, we proceeded with the development of the LPS immunosensor.Thus, LPS solution in coupling buffer was dotted on Immobilon membrane discs at two concentrations: 5 and 10 µg/ml, for varying incubation times. Blocking of excessive active groups was done and incubation with anti LPS rabbit serum followed. In a second incubation step, anti-rabbit Ig-G - HRP was allowed to react. Membranes were washed of excess uncoupled ligands between each step, according to the membrane manufacturers' instructions. Blanks which had no LPS were also used. Membranes thus prepared were applied on the electrodes and the consequent immunosensor was dipped in a cell containing 5 ml of freshly prepared $2x10^{-5}$ mol dm^{-3} hydroquinone solution in PBS. The same procedure as described above for the tests of the Ig-G - HRP tests was done and the corresponding current time response recorded. The values obtained are summarized on the following table:

	A B C D		E F G H		I J K L		M N O P
[LPS]	5 µg/ml		10µg/ml		----------		----------
Serum	30 µl 1:5000	30 µl 1:1000	30 µl 1:5000	30 µl 1:1000	30 µl 1:5000	30 µl 1:1000	----------
IgG-HRP	30 µl IgG-HRP 1:500						
ΔI/nA	0,10	0,12	0,18	0,22	0,32	0,39	0,52

RESULTS AND DISCUSSION

An amperometric immunosensor for LPS detection was developed.

Immobilization of LPS on Immobilon AV membranes was successfully obtained after ≅ 18h of incubation.

The capping procedure used was found to be effective, as shown by values obtained for controls where no LPS was added to the membranes and serum was directly coupled to them.

The method is sensitive to LPS concentration and to serum dilution and, so, is both qualitatively and quantitatively appropriate for use as an immunosensor.

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