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Colloidal gold based electrochemical immunoassays for the diagnosis of acute myocardial infarction

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Abstract

The performed immunoassays are directed to the determination of protein markers for acute myocardial infarction. The determination assays for human myoglobin, cardiac troponin complex and the MB isoform of the enzyme creatine kinase are presented. Cyclic voltammetry measurements are adopted for the determination of colloidal gold used as a label in the immunuassays. The measurements are performed with a screen-printed graphite electrode in a sample volume of 50 μl. The influence of different diameter colloidal gold particles on the assay sensitivity is also investigated.

Keywords: Electrochemical immunoassay; Colloidal gold; Cardiac markers

1. Introduction

The electrochemical detection of immunoassays is not very widely used but it is gaining growing interest because it has the potential of being applied to low cost miniaturizable electrodes. The transducer material is very cheap and by the screen-printing technology a large-scale production of graphite and other conductive ink electrodes is possible. Different designs can be adopted in order to perform the measurements in low volumes.

There are different electrochemical techniques adoptable for the revelation of immunoassays, depending on the labelling principle chosen. Colloidal gold is used as a label especially for histological samples in optical as well as in electron microscopy but it has also very interesting electrochemical properties [1,2]. It can in fact be detected quantitatively with various voltammetric as well as potentiometric methods. One of the simplest methods which can be used is the cyclic voltammetry whose operating principle consists in the application of a forward and reversed potential scan (triangular wave) to an electrochemical cell.

The assays performed are directed to the determination of protein markers for acute myocardial infarction (AMI). The choice of this application has been done since cardiovascular diseases are the most lethal diseases in western world and the interest in developing fast and sensitive tests based on specific markers is very high [3,4].

Cardiac isoforms of Troponin I and T are specific components of the cardiac muscle and even a small increase in their concentration in blood indicates that a cardiac damage has occurred. Troponin is present in binary and ternary complexes of its isoforms (cTnI–TnC and cTnI–TnC–cTnT). They can be detected 16–30 h after onset and last for 5–8 days (useful for late diagnosis).

Several pharmaceutical firms have developed assays for troponin detection, some of which are fast (15 min.) and sensitive (0.5 ng/ml) but a number of other cardiac markers have been identified, the role of which in prediction and/or diagnosis and/or prognosis of AMI is valuable.

Myoglobin is not a specific cardiac marker since the molecule is present in skeletal muscle too, but its blood level rises very quickly in patients after AMI and so it is useful for the early diagnosis: it appears in patients blood 1–3 h after the onset of symptoms (peak within 8–12 h) so it is the earliest marker and its predictive value in case of negative result is 100%, so a negative result can reliably

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exclude an AMI episode. Creatine kinase is an enzyme present in three isoforms MM, BB, and MB, the last one more heart specific. The presence of this enzymatic activity in blood is indicative of muscle damage but the immunoassay permits to discriminate the tissue specific isoforms [5,6].

2. Experimental

Monoclonal antibodies against myoglobin and troponin ternary complex have been purchased from HyTest, Finland, while anti-CK MB are from Medix Biochemica, Finland. All other reagents are from Sigma–Aldrich. The screen-printed graphite electrodes are from Gwent Electronics, UK. The electrochemical measurements were performed with an Autolab PGstat 10, Ecochemie, NL.

The immunoassays have been performed in microtiter plates like an ELISA and then the bound colloidal gold has been oxidized and solubilized in a small volume (50 μl) of Br_2 0.25 mM, 1 M HCl, 1 M NaBr. A few minutes after 300 μl of 1 M HCl, 1 M NaBr are added before the measurement (to dilute the residual bromine). Then a 50 μl drop of this solution is applied on the surface of a screen-printed graphite electrode. The measuring configuration (shown in Fig. 1) consists also of a platinum wire counter electrode and a standard Ag/AgCl reference electrode. The cyclic voltammetries have been recorded between 0.5 and 0.7 V and backwards (three cycles) at a scan rate of 10 mV/s.

The conjugation of the antibody molecules with the gold particles has been performed in borate buffer 40 mM pH 9.0. The optimal antibody to particle ratio has been assessed with a preliminary titration step in which different ratios aliquots have been prepared and tested for ionic strength stabilization by adding NaCl to a final concentration of about 160 mM.

Fig. 1. Experimental set up for the electrochemical measurements in a 50 μl drop: the working electrode is a screen-printed graphite one, the counter electrode is a platinum wire and a standard reference electrode Ag/AgCl just touches the 50 μl drop.

3. Results

The principle of the electrochemical revelation method is based on the reversible oxidation of the colloidal gold at the working electrode. In the cyclic voltammetry measurements current/potential curves are obtained in which the oxidation peak amplitude can be correlated quantitatively to the Au concentration (data not shown).

The measurements are performed in a sample volume of 50 μl with the set up shown in the photo (Fig. 1).

The immunoassays performed in this work follow the two schemes shown in the drawing below (Fig. 2).

While the gold-labelled streptavidin is commercially available, the conjugation with colloidal gold particles has been performed for the antibodies specific for the three cardiac markers analyzed. The conjugation of the antibody molecules with the gold particles is a simple process, since the interaction of the protein molecules with the gold surface is a spontaneous reaction which is favoured by electrostatic interactions, hydrophobic interactions and dative bonds between sulfur atoms of SH-groups and gold. The latter interaction assures a quite stable binding. An important aspect of the conjugation is the protein to gold ratio, in fact the particle surface should be saturated, but using an excess of antibody should be avoided because the remaining unconjugated molecules would compete with the conjugated ones lowering the signal. To this aim a titration step is usefully performed taking advantage from the fact that the saturation of the particle surface stabilizes them against high ionic strength disruption, which can be visualized as a change in colour from cherry pink to violet and finally to greyish.

The electrochemical immunoassay is performed in microtiter plates and the last step is the oxidative solubilization of gold with a solution containing HBr and Br_2 [1]. Then a 50 μl drop is withdrawn and placed on the electrode surface for the measurement.

For each well of the microtiter plate a cyclic voltammogram is obtained whose oxidation current peak is plotted against the analyte concentration, giving a calibration curve as the one shown in Fig. 3 on the right.

Mouse monoclonal anti-myoglobin, anti-troponin and anti-creatine kinase MB antibodies have been conjugated to gold.

The electrochemical immunoassays for human myoglobin, cardiac troponin complex and creatine kinase MB have been performed.

In order to assess the performance of the gold conjugated antibodies, the electrochemical immunoassays have been performed following both assay models illustrated below in Fig. 2 and the result are compared in Fig. 4. The streptavidin-colloidal gold conjugated used was purchased from Sigma (cat. number S-9059), two different lots have been tested and one preparation was also done in the lab, but in all the experiments the signal obtained with the streptavidin assay was sensibly lower than that obtained with the conjugated antibody.