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Low density lipoprotein sensor based on surface plasmon resonance

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ABSTRACT

Biotinylated heparin has been immobilized onto self-assembled monolayer of 4-aminothiophenol using avidin–biotin specific binding. The modified electrodes have been characterized using surface plasmon resonance technique (SPR), cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), atomic force microscopy (AFM) and contact angle (CA) measurements. The interaction of immobilized biotinylated heparin with low density lipoprotein (LDL) has been studied using surface plasmon resonance technique. The biotinylated heparin modified electrode can be used to detect LDL in the range of 20 to 100 mg/dl with the sensitivity of 513.3 m°/μM.

1. Introduction

Estimation of cholesterol concentration in blood is clinically important for the diagnosis of coronary heart diseases, arteriosclerosis, and cerebral thrombosis [1–3]. Low density lipoprotein (LDL) is the major cholesterol carrier in blood and accumulation of LDL is regarded as the first stage of atherosclerotic lesions. Currently, LDL concentration is estimated in most clinical laboratories by an indirect method that estimates LDL concentration according to the Friedewald equation [4]. Efforts have been made to quantify LDL using ultracentrifugation, nuclear magnetic resonance (NMR) spectroscopy etc. [5,6]. However these methods are expensive and time consuming. There is thus an urgent need for the development of a suitable technique for estimation of LDL. In this context, biosensors have been considered to provide various advantages such as high sensitivity, ease of use and low cost [7–9]. Self-assembled monolayer (SAM) based biosensors are currently attaining much interest due to advantages such as stability, uniform surface structure and relative ease of varying the functionalities. Moreover, the immobilization of biomolecules using SAM requires a small amount of biomolecules resulting in the binding of desired molecule in near vicinity of electrode surface and acts as a molecular wire between biomolecule and the electrode surface [10–12]. Snellings et al. have fabricated a shearing mode sensor for the detection of lipoprotein fractions using dextran sulfate modified alcohol ended SAM on gold (Au) surface [13]. It has been found that the dextran sulfate coating is more selective to LDL fraction as compared to other lipoprotein fractions. These authors could not achieve reproducibility for coating DS onto a similar surface. Also it suffers with the problem of specificity.

Surface plasmon resonance (SPR) has recently been used for estimation of biomolecules of clinical interest. This is because SPR allows both qualitative and quantitative measurements of biomolecular interactions in real-time eliminating the need of labeling reagents. SPR based biosensors are known to provide rich information on specificity, affinity, kinetics of biomolecular interactions and the concentration of analyte of interest in a complex sample. SPR based biosensors are presently geared towards the design of a compact, low cost, and sensitive biosensing device and have implications towards rapid advances in microfabrication technology [12,14–19].

Sugars like heparin (HEP) having SO_3^- groups are known to interact with apolipoprotein B of LDL via highly electrostatic interactions as they are the constituent of glycosaminoglycans responsible for complex formation with LDL and can be used for its detection [20]. Seven distinct HEP-binding sites have been found on the apolipoprotein B-100 by fragmenting protein with cyanogen bromide [20]. Therefore, specific LDL–HEP interaction can be used for detection of LDL. In the present work, biotinylated heparin (B-HEP) has been immobilized onto self-assembled monolayer of 4-aminothiophenol using avidin as a coupling agent. The specific avidin–biotin coupling helps in better immobilization of B-HEP onto the SAM surface. The interaction of immobilized heparin with low density lipoprotein has been studied using surface plasmon resonance technique. The modified electrodes have been characterized using surface plasmon resonance (SPR) technique, cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), atomic force microscopy (AFM) and contact angle measurements.

2. Experimental

2.1. Chemicals and reagents

LDL (3500 kDa), N-hydroxy succinimide (NHS), N-ethyl-N-(3-dimethyl amino propyl carbodiimide) (EDC), bovine serum albumin (BSA), and 4-aminothiophenol have been procured from Sigma-Aldrich (USA).

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Biotinylated heparin and avidin (AVI) have been procured from Merck Biosciences. The 50 nm thick Au-coated BK-7 glass plates (24 mm diameter) have been purchased from Autolab, The Netherlands.

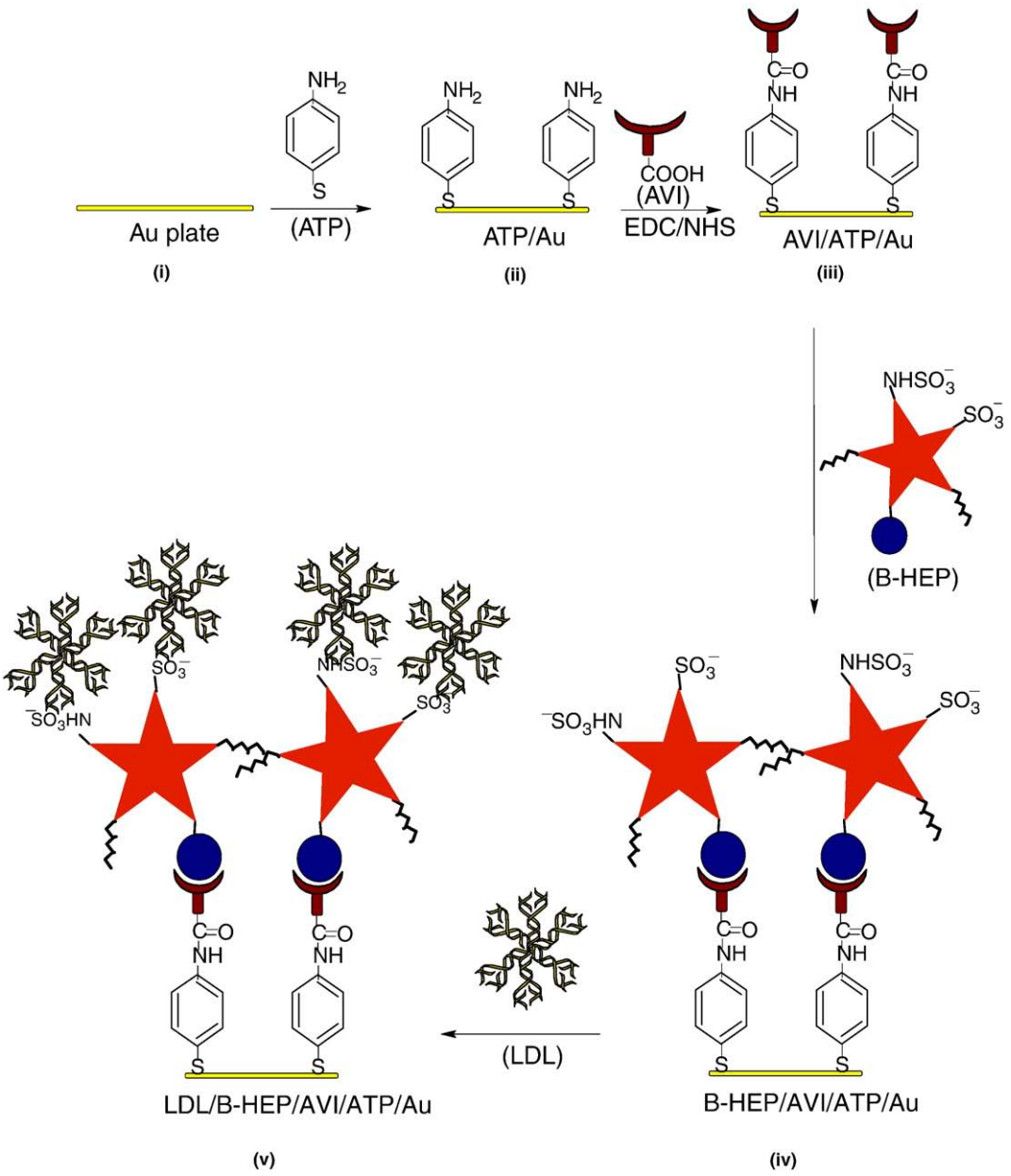
2.2. Solution preparation

Lyophilized powder (5 mg) of LDL is reconstituted with 1 ml of de-ionized water to make a solution containing 150 mM NaCl of pH 7.4 and 0.01% EDTA. Solution of BSA (2 mg/ml) is prepared in 50 mM phosphate buffer saline solution of pH 7.4 containing 150 mM NaCl (PBS). Solutions of B-HEP (100 μM) and AVI (1 mg/ml) are prepared in de-ionized water.

2.3. Formation of 4-aminothiophenol (ATP) self-assembled monolayer (SAM) on gold (Au) and its modification by biotinylated heparin (B-HEP)

Prior to SAM formation, Au plates are cleaned with acetone, ethanol and with a copious amount of de-ionized water. Further, Au plates are treated with piranha solution (H_2SO_4/H_2O_2; 7:3) for about 5 min followed by rinsing with de-ionized water. The pre-cleaned Au plates are then immersed into a 2 mM solution of ATP in ethanol for over-night at room temperature for SAM formation after which the electrodes are sonicated in ethanol for about 10 min and are then rinsed with ethanol and acetone followed by water to remove any unbound ATP molecules. The ATP SAM is modified with avidin (1 mg/ml) by utilizing the amino group functionality of ATP. The –COOH group present in avidin is covalently linked to amino group of ATP using EDC (0.2 M) as the coupling agent and NHS (0.05 M) as the activator.

Specific binding of biotin–avidin has been used for the attachment of B-HEP onto ATP self-assembled monolayer (Scheme 1). 100 μM of B-HEP is immobilized onto avidin modified SAM surface. After B-HEP attachment, the non-specific sites are blocked with BSA (2 mg/ml in PBS solution). The resulting electrode is exposed to different concentrations of LDL. The entire process has been monitored by *in situ* SPR measurements at 25 °C. Scheme 1 shows preparation of LDL/B-HEP/AVI/ATP/Au electrode.



Scheme 1. Schematic for attachment of LDL onto B-HEP/AVI/ATP/Au electrode.