COMPARATIVE STUDY OF ANTI-HGH ANTIBODY IMMOBILIZATION STRATEGIES FOR HGH IMMUNOASSAY

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Human Growth Hormone (HGH) is a peptide hormone involved in many physiological processes, so both of its deficiency and excess can cause various disorders for people of different ages. In adults, an excess of the HGH produces a disorder known as acromegaly, whose initial symptom is typical enlargement of the hands and feet. Complications of the disorder may include type II diabetes, sleep apnea and high blood pressure. In children, increased HGH levels can lead to excessive growth of long bones, resulting in the child being abnormally tall. This disorder is commonly known as gigantism. The available data suggest that an excess of the HGH in more than 95 % is due to a benign tumor, known as a pituitary adenoma [1]. The HGH can also be secreted by endometrial, breast, liver, prostate or colon cancer cells. A deficiency of the HGH may cause hypoglycemia in newborn infants, growth failure in later infancy and childhood. In adults, the HGH deficiency causes loss of body weight and bone density and leads to a number of physical and psychological symptoms, including poor memory, social withdrawal, and even depression [2]. Hence, determination of the HGH concentration is undoubtedly important in diagnosis both its deficiency and its excess. There are many HGH immunoassays used in practice [3]. Although they can provide the desired sensitivity, specificity and selectivity, most of them are highly disadvantageous, because they are time consuming, require specialized labels and in many cases they require complex sample preparation. Therefore, new methods for the determination of HGH are still required. An excellent alternative to them is surface plasmon resonance (SPR) immunosensors, which offer several significant advantages including real-time and label-free detection of analytes. This is achieved by measuring changes in the refractive index caused by the antigen-antibody interaction taking place at a surface of SPR sensor chip. SPR immunosensors allow detection of very low analyte concentrations in a relatively small volume of the sample. Moreover, an ability to detect and quantify biospecific interactions in the complex solutions makes SPR immunosensors capable of identifying specific analytes without time-consuming sample preparations. An analysis with SPR immunosensor can be performed within a few minutes and very often it is possible multiple analysis, if proper regeneration of the SPR sensor surface is performed [4]. Due to these advantages, SPR immunosensors are powerful tool for bioanalytical and biomedical investigations.

Efficient immobilization of antibodies on a surface of the SPR sensor chip is an essential step in the preparation of the SPR immunosensor [5], since the choice of the immobilization technique greatly affects antibody-antigen interactions efficiency. This is due to asymmetric structure of antibody molecule, which determines that it can be immobilized in a random or oriented manner. Meanwhile, sensitivity of the SPR immunosensor depends on orientation and surface concentration of immobilized antibodies [6]. A great variety of methods suitable for immobilization of antibodies have been developed and described in the literature, but this area of research is still very relevant. Therefore, the objective of this study was to compare the effect of different immobilization techniques on the surface concentration of monoclonal mouse antibodies against human growth hormone (anti-HGH) on SPR sensor chip and estimate binding capacity of the immobilized anti-HGH with HGH. In the present study, anti-HGH antibodies were immobilized using five different methods. First immobilization method was random immobilization of intact anti-HGH (intact-anti-HGH) via 11-mercaptoundecanoic acid (MUA) self-assembled monolayer (SAM). For this purpose, SPR sensor chips for some time were incubated in 11-mercaptoundecanoic acid (MUA) in methanol. Then carboxyl functional groups of MUA were activated with a mixture of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). After activation anti-HGH antibodies were immobilized covalently through their primary amine functional groups. Second, random immobilization of intact anti-HGH within carboxymethyl dextran hydrogel by direct covalent amide coupling with a mixture of EDC and NHS technique. Third immobilization method was oriented coupling of intact-anti-HGH to Protein-G layer assembled on MUA SAM via Fc-fragment of antibodies. Fourth immobilization method was oriented immobilization of fragmented anti-HGH antibodies (frag-anti-HGH) via their native thiol-groups directly coupled to the gold. To liberate these thiol groups, the intact-anti-HGH was chemically "divided" into two frag-anti-HGH fragments by chemical reduction with 2-mercaptoethylamine. And finally fifth immobilization method was oriented coupling of intact-anti-HGH to 3-aminophenylboronic acid (BA), which was assembled on MUA or MUA and 3,3'-dithiodipropionic acid mixed SAM, via cis-diols in the oligosaccharide chains of antibodies.

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