Surface plasmon resonance biosensor for direct detection of antibodies against human growth hormone

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A direct, label-free detection method of antibodies against the human growth hormone (anti-HGH) using a surface plasmon resonance (SPR) biosensor is reported. The sensing surface of the surface plasmon resonance biosensor chip (SPR-chip) was modified by covalent coupling of the human growth hormone (HGH) to the self-assembled monolayer of 11-mercaptoundecanoic acid (MUA). HGH was immobilized *via* primary amine groups after activation of the MUA carboxyl groups with a mixture of *N*-hydroxysuccinimide (NHS), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC). The specific binding of monoclonal anti-HGH antibody on the HGH-modified surface was examined in the concentration range from 0.25 nM to 10 μM. The experimentally observed detection minimum for anti-HGH was 2.47 nM. A single immunoassay cycle could be done within 30 min including the HGH and anti-HGH association, HGH/anti-HGH complex dissociation and surface regeneration steps. The SPR biosensor response for repeatable detections of anti-HGH was highly reproducible and very stable. On the SPR-chip the formed HGH and anti-HGH complex (HGH/anti-HGH) could be gently dissociated and the sensing surface might be regenerated by 50 mM NaOH and 0.5% sodium dodecylsulfate (SDS) solution. Any changes in the original baseline level were detected during the 40 detection–regeneration cycles. This means that damage of the immobilized HGH-based sensitive layer during regeneration was minimal. It was demonstrated that the developed SPR-chip could be stored for at least 21 days before use without considerable loss of sensitivity towards anti-HGH.

Introduction

Human growth hormone (HGH), also called Somatotropin, is a 22 000 Da polypeptide comprised of 191 amino acids. It is a product of the *GH-1* gene located on chromosome 17 and expressed by acidophilic cells of the anterior lobe of the pituitary gland.[1] HGH is involved in a multitude of biological effects, including somatogenesis, lactation, activation of macrophages, and insulin-like and diabetogenic effects, among others.[2] An excess of the growth hormone causes gigantism,[3] hyperinsulinemia, impaired glucose tolerance, insulin resistance, and finally diabetes.[4] A deficiency of HGH produces significantly different problems at various ages: in newborn infants it may cause hypoglycemia, growth failure in later infancy and childhood, and in adults lean body mass, poor bone density[5] and a number of physical and psychological symptoms, including poor memory, social withdrawal, and even depression.

A variety of clinical applications of HGH are used. Long time ago growth hormone was used as treatment from domestic mammals like cows and pigs, but it did not work in humans. Consequently, for many years the only source of growth hormone for therapy was that extracted from the glands of human cadavers. Due to the fact that several patients died from a rare neurological disease attributed to contaminated glands this therapy was no longer used. Recombinant DNA technology has made it possible to produce recombinant human growth hormone (rHGH), and currently rHGH is used for clinical applications. rHGH is used to treat conditions not related to deficiencies in HGH such as Turner syndrome,[6] chronic renal failure,[7] Prader–Willi syndrome,[8] intrauterine growth retardation,[9] and idiopathic short stature.[10] HGH deficiency treatment involves regular injections of recombinant rHGH.[5] However, in patients treated with rHGH, human growth hormone antibodies (anti-HGH) can form.[11,12] The main concern related to anti-HGH antibodies is their ability to neutralize circulating growth hormone and inhibit its therapeutics effects.[13] This, treatment withholds expected results of treatment by HGH.[1] There are several reports of the adverse effects of growth hormone (GH) therapy. In some cases of GH therapy, local abscesses and lipodystrophy are appearing. Moreover, the slipping of the capital epiphysis of the femur has been reported in some cases of human GH therapy. The major concern has been related to the formation of antibodies against GH. Antibody formation was a common problem with the preparation/application of GH preparations. However, the most important question is whether these antibodies are growth-attenuating or not. This appears to be dependent on the quality and quantity of produced antibodies.[14,15] Growth-attenuating antibodies against pituitary-derived GH appear to be exclusively seen in patients with genetic forms of GH. Changing to a pure form of GH may be helpful to

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restore growth in them. Antibodies are present in significant titres after 6 weeks of treatment with recombinant GH and are maximum after 6 months.[15,16] It is also argued that the antibodies prolong the presence of GH in circulation and this would have potential benefit for the growth of the child.[17] For all mentioned reasons, a rapid selective and sensitive analytical procedure for the determination of the anti-HGH antibodies is critical, during preparation of GH-based medications as well as in the blood of the patient during monitoring and/or optimisation of HGH therapy.

There are many immunoassay methods used in practice such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), fluorescence or other classical immunochemical techniques. Even atomic force microscopy methods could have been applied to detect antigen–antibody complex formation.[18] Although these methods can provide the desired sensitivity, specificity and selectivity, most of these methods are highly disadvantageous because they imply the labelling of antigen or antibody, long analysis times and extensive sample handling.[19] Along with these disadvantages, it is quite difficult to fully automate them. An excellent alternative to the traditional immunoassay methods is immunosensors[20–22] that can be based on electrochemical[23] or optical detection techniques.[24] Surface plasmon resonance (SPR)[25] is a surface-sensitive optical technique very suitable for monitoring of biomolecular interactions occurring in very close vicinity to transducer surfaces.[26,27] It allows real-time and label-free detection of analytes by exploiting the interfacial refractive index changes associated with any affinity binding interaction between a biomolecule immobilized on a transducer surface and its biospecific partner (analyte) in solution.[28] Rapid and real-time analysis without labelling,[29] highly specific detection of analytes with extremely low detection limits[30,31] and possible automation and miniaturization[32] create unique potential for the application of SPR in biosensorics. Furthermore, the ability of SPR to detect and quantify biospecific interactions from complex fluids makes these affinity biosensors amenable to identify specific analytes in complex matrices without sample preparation.[33] Due to these advantages, many applications of SPR biosensors in such areas as biomedical diagnostics,[34] drug discovery,[35] molecular engineering,[36] food analysis[37] and environmental monitoring[38] have been published since SPR's introduction in the early 1990s.

An important class of biomedically interesting analytes is antibodies (in this particular case anti-HGH), because antibodies are used as 'reporters' of many infections and other health disorders. However, currently, the majority SPR-based biosensors and many other bioanalytical protocols are devoted to the detection of antigens. Bioanalytical techniques devoted to the detection of antigens usually exploit immobilized antibodies as affinity agents sensitive to analyte. An SPR immunoassay has been developed to determine human growth hormone directly in human serum samples.[39] However, the detection of antibodies that are also very important biomedical markers is still a challenge.

The aim of this study was to develop an SPR-based biosensor for the detection of anti-HGH antibodies. In the design of this biosensor, the commercially available alkanethiol 11-mercaptoundecanoic acid was applied for the formation of a self-assembled monolayer. The designed biosensor showed highly promising analytical characteristics, such as good sensitivity, stability, repeatability and reusability. This biosensor seems very suitable for rapid and reliable detection of anti-HGH antibodies.

Experimental

Materials and reagents

Purified human growth hormone (HGH), monoclonal mouse antibodies against human growth hormone (anti-HGH) and monoclonal antibodies against the porcine growth hormone (anti-PGH) were purchased from AbD Serotec (Oxford, UK). 11-Mercaptoundecanoic acid (MUA), *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), and sodium dodecylsulfate (SDS) were purchased from Sigma-Aldrich (Steinheim, Germany) and ethanolamine from Merck KGaA (Darmstadt, Germany). All other chemicals were obtained from Sigma-Aldrich (Steinheim, Germany) and were of analytical-reagent grade or better. All aqueous solutions were prepared in HPLC-grade water purified in a Purator-B Glas Keramic (Berlin, Germany). Refractive index matching fluid (refractive index = 1.518) was obtained from Cargille Labs (USA).

SPR measurements

A double-channel SPR analyser 'Autolab ESPRIT' (ECO Chemie, Utrecht, Netherlands) was applied for SPR measurements. One channel was used to perform the assay. The second channel was used to run reference measurements and to exclude systematic errors, to correct the measurements in the first channel. The SPR instrument was equipped with an automatic flow injection system. A glass hemi cylinder prism (BK 7, n = 1.518) was used as a Kretschmann ATR (attenuated total reflection) coupler. A Ge–As diode laser was used as the light source to produce monochromatic light with a wavelength of 670 nm. The intensity of the reflected light was measured over a range of 4000 millidegrees. The SPR angle of buffer solution can be fixed manually by a spindle with an offset SPR angle of 62–78°, which corresponds to a refractive index range of 1.33–1.43 of the sensor surface. The SPR angle scan was performed around the manually fixed SPR position. All samples were monitored at a constant temperature of 20 °C.

Formation of the self-assembled monolayer (SAM)

Before the formation of the SAM, the bare sensor disk (SD AU, XanTec bioanalytics GmbH, Germany) was cleaned using piranha solution (1 : 3 30% H_2O_2 and concentrated H_2SO_4 respectively) at 60 °C for 5 min. Then, it was rinsed with pure ethanol solution and deionised water. Then the SAM consisting of MUA was formed on a cleaned sensor disk. To achieve this goal, the sensor disk was incubated in 1 mM MUA solution in methanol at 4 °C for 24 h. Afterward the MUA-modified sensor disk was rinsed with deionised water and dried. The glass surface of the sensor disk was precisely cleaned with lens cleaning tissue.

Stabilization/rehydratation of the SAM

A MUA-modified sensor disk was deposited on top of a slider with a glass hemi cylinder prism. Then the slider was positioned inside the SPR instrument using a cuvette holder. A repetitive sequence of coupling buffer solution (consisting of 10 mM Na acetate, pH 4.5) and regeneration solution (consisting of 50 mM NaOH and 0.5% SDS), both in two-minute intervals lasting for one hour, was used for stabilization/rehydratation of the monolayer and for obtaining the stabile baseline. Prior to this treatment, the coupling buffer solution pH was adjusted with acetic acid.

Activation of the MUA SAM

A mixture of 0.1 M EDC and NHS in water was used for activation of the SAM consisting of MUA. During this procedure, functionally active NHS esters were obtained due to the reaction of MUA carboxyl groups with a mixture of EDC and NHS (Fig. 1, step 1). The activation step was carried out for 5 min. Then the mixture of EDC and NHS was washed away with the coupling buffer.

Immobilization of the HGH

After activation of the MUA monolayer, immobilization of HGH was performed in both channels of the SPR cuvette. The HGH was coupled covalently by uncharged primary amine functional groups to activated carboxyl functional groups of the MUA SAM (Fig. 1, step 2). Immobilization of 3.0 μM of HGH protein dissolved in a coupling buffer step, which was carried out for 15 min.

Deactivation of excess activated ester groups

After immobilization of the HGH it is essential to neutralize unreacted activated ester groups on the formed MUA SAM. Reactive esters were deactivated with 1 M ethanolamine, pH 8.5 (Fig. 1, step 3). The ethanolamine solution pH was adjusted with hydrochloric acid. The deactivation step was carried out for 15 min.

Surface stabilization/rehydratation prior to interaction of anti-HGH antibody

A repetitive sequence of 10 mM PBS buffer solution, pH 7.4, and regeneration solution, both in two-minute intervals lasting for one hour, was used for stabilization/rehydratation of the surface and for obtaining the stabile baseline.

Interaction of anti-HGH antibody

After the stable baseline was achieved the first aliquot containing the lowest concentration of anti-HGH was added. The interaction between immobilized HGH and anti-HGH present in the aliquot was monitored for 15 min and followed by dissociation in PBS buffer. Then SPR-chip was regenerated with regeneration solution. The regeneration step was carried out for 3 min. After a stable baseline was achieved, a similar interaction cycle was repeated for the other antibody concentrations.

A cross-reactivity study was performed by comparison of SPR signals in the presence of anti-HGH and anti-PGH. This study was performed by injection of 2.47 μM of anti-HGH and anti-PGH in 10 mM PBS buffer, pH 7.4, to different chanels of the same SPR disk.

During the activation, immobilization, deactivation and interaction routine, solutions without any air bubbles were used.

Results and discussion

The experiments presented here were to exploit many advantages offered by SPR-based direct immunoassay: (i) format based on application of a single biological agent, which was immobilized on the sensor disk; (ii) wide measurement range by the same

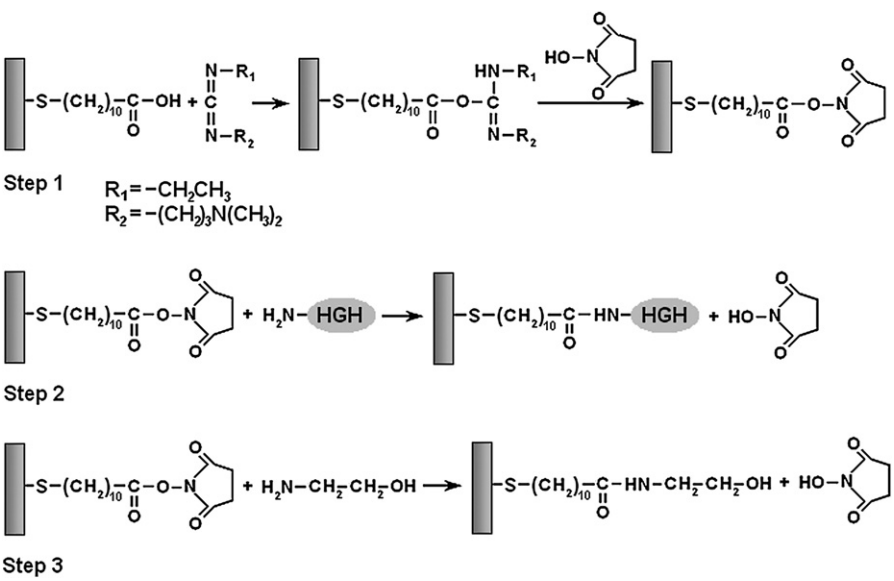

Fig. 1 Principal immobilization scheme of HGH by amine functional groups: step 1, activation of MUA SAM; step 2, immobilization of the HGH; step 3, deactivation of excess activated ester groups.

analytical system;[40] and (iii) direct analyte detection. In the presented experiments, HGH was first immobilized on a MUA self-assembled monolayer-modified sensor disk surface by a covalent coupling method.[41] Immobilization of biomolecules on SAMs is highly advantageous because the SAM provides stable covalent bonding, allows remarkable flexibility with respect to terminal functionality, oriented immobilization of ligands on the transducer surface[42] and helps in minimizing any non-specific adsorption.[43] These are especially important problems in the analysis of real biological or environmental samples. Furthermore, the good stability under extreme conditions of pH and temperature, as well as the reusability and applicability in the flow system, makes the SAM surface a preferred, versatile tool for innovative immunoassays.[44]

Fig. 1 schematically represents the immobilization of HGH over the MUA self-assembled surface. The immobilization of HGH was performed in both channels of the SPR-chip. The first step of immobilization was based on treatment by 10 mM Na acetate buffer, pH 4.5, which was flowed over the MUA-modified SPR-chip surface until a stable resonance angle was maintained in the SPR instrument (Fig. 2, line 1). Then the mixture of EDC and NHS in deionised distilled water was injected and flowed for a period of 5 min to activate the terminal carboxyl groups of MUA and to make an active intermediate of succinimide ester. This activation was essential for the effective immobilization of HGH (Fig. 1, step 1 and Fig. 2, line 2). After the activation step the surface was washed with 10 mM Na acetate buffer, pH 4.5 (Fig. 2, line 3). The resulting changes in the SPR responses were monitored after injection into the cuvette solution of 3.03 μM HGH (Fig. 2, line 4). Sensorgrams showed a gradual increase in SPR angle indicating binding of HGH on the activated MUA surface (Fig. 1, step 2). The SPR angle increased with time and reached saturation in approximately 12 min (Fig. 2, line 4). Saturation was obtained for Na acetate buffer, at pH 4.5, which allowed the removal of any loosely bound HGH from the surface (Fig. 2, line 5). The SPR angle shift for the

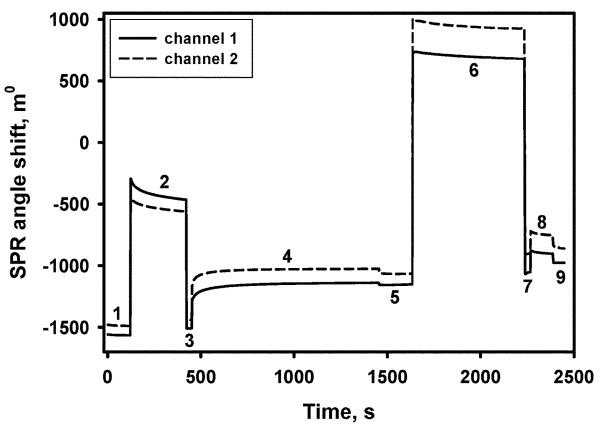

Fig. 2 SPR study of HGH immobilization on a MUA-modified SPR sensor disk: 1, baseline (10 mM Na acetate buffer, pH 4.5); 2, activation of the MUA with a mixture of EDC/NHS; 3, 5, and 7, injection of Na acetate buffer, pH 4.5; 4, immobilization of HGH; 6, deactivation of remaining reactive NHS esters with ethanolamine; 8, injection of regeneration solution (50 mM NaOH and 0.5% SDS); 9, injection of 10 mM PBS buffer, pH 7.4.

binding of the HGH on the SAM was calculated as a difference between the SPR signal prior to the binding of HGH (Fig. 2, line 3) and after washing the HGH-modified sensor disk surface with Na acetate buffer (Fig. 2, line 5). The SPR angle shift obtained for the binding of HGH on the SAM was *ca.* 370 ± 10 millidegrees (m°) and showed quite good reproducibility upon repeated experiments. From the SPR angle shift, the surface concentration of the HGH was calculated as 3.08 ± 0.08 ng/mm^2 whereas the SPR angle shift of 120m° is equivalent to the change in surface concentration of protein by 1 ng/mm^2.[45]

After the HGH's immobilization step some activated ester groups remained unreacted on the MUA SAM. To avoid the binding of other proteins to unreacted ester groups they were deactivated with 1 M ethanolamine solution, pH 8.5[46] (Fig. 1, step 3; Fig. 2, line 6). After the deactivation step, the ethanolamine was washed out by passing 10 mM Na acetate buffer, pH 4.5, solution over the HGH-modified surface (Fig. 2, line 7). The SPR angle registered during the Na acetate buffer, pH 4.5, flow after the deactivation step (Fig. 2, line 7) was higher as in the case of Na acetate buffer, pH 4.5, flow after the immobilization step (Fig. 2, line 5). The obtained results illustrate that the remaining unreacted activated ester groups were successfully blocked by coupling of ethanolamine (Fig. 1, step 3). After washing of the SPR-chip with Na acetate buffer, pH 4.5 (Fig. 2, line 7), the SPR-chip was subsequently treated with the regeneration solution (Fig. 2, line 8) and PBS buffer, pH 7.4 (Fig. 2, line 9). During these procedures the SPR angle goes up (Fig. 2, line 8) and then drops down (Fig. 2, line 9) after switching the sourcing solution from Na acetate buffer, pH 4.5, to the regeneration solution or from the regeneration solution to PBS buffer solution, respectively. This increment and decrement was caused by differences in the refractive indexes of both solutions.

The sensitivity of the fabricated SPR biosensor towards anti-HGH antibodies was studied during the next section of experiments. Interaction of anti-HGH antibody with the immobilized HGH was performed without secondary tracer molecules and anti-HGH binding was registered in real time. To obtain a stable baseline the PBS buffer, pH 7.4, was flowed through the SPR-chip with immobilized HGH for 2 min. From this, a stable baseline was established, and a PBS buffer, pH 7.4, containing the anti-HGH antibodies was introduced into the SPR-chip. The interaction of anti-HGH antibodies with the HGH immobilized on the MUA SAM resulted in an increase of SPR signal (Fig. 3A, 'Association'). After injection of the anti-HGH antibody-containing sample, continuous flow of PBS buffer, pH 7.4, was started, and caused the dissociation of anti-HGH antibodies from the immobilized HGH. This dissociation influenced an inconsiderable decrease in SPR signal (Fig. 3A, 'Dissociation').

Application of biosensors for a number of repeated analyses makes an assay process more cost efficient and in some particular cases it reduces the duration of the analysis. Therefore, the regeneration step is an important procedure in SPR-based immunoassays. In experiments described here the regeneration procedure has been performed after each anti-HGH detection step. The native tertiary structure of anti-HGH antibodies is sensitive to pH.

A variety of regenerating buffers from highly acidic (*e.g.* 0.1 M HCl[29]) to basic (*e.g.* 0.2 M NaOH[47]) or operating in broad pH range (*e.g.* glycine–HCl,[48] pepsin[49]) have been used for

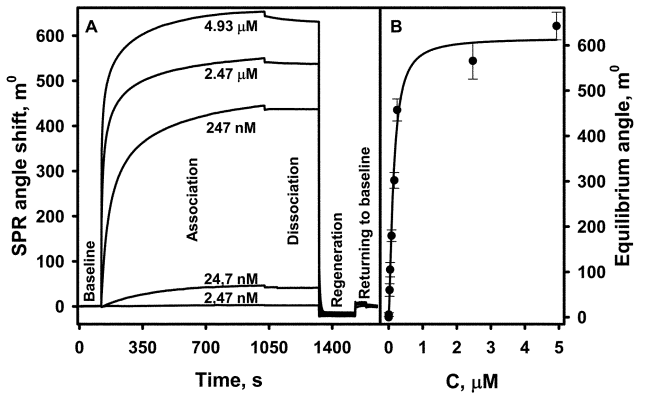

Fig. 3 (A) SPR study of formation of an immune complex between immobilized HGH and anti-HGH antibodies present in the sample at 2.47 nM, 24.7 nM, 247 nM, 2.47 μM and 4.93 μM concentrations. (B) Concentration of anti-HGH *vs.* equilibrium angle.

regeneration of SPR-chips. For repeatable use of the same SPR-chip based on immobilized HGH, the anti-HGH antibodies that were bound to immobilized HGH during the previous analysis cycle should be removed before the next immunoassay cycle. It is important to use a regeneration agent, which efficiently dissociates the antigen–antibody complex without significant damage of analyte-sensitive layer based on immobilized HGH. Several regeneration solutions, such as hydrochloric acid and glycine of different concentrations and of different pH values were tested in terms of their ability to dissociate the formed anti-HGH and HGH complex (anti-HGH/HGH). However, the anti-HGH/HGH complex was not completely dissociated by the exposure to these two regeneration reagents. Another solution consisting of 50 mM NaOH and 0.5% SDS was found to be the most efficient regeneration agent.

When the SPR-chip after detection of anti-HGH was exposed to the flow of 50 mM NaOH and 0.5% SDS solution the SPR angle decreased sharply (Fig. 3A, 'Regeneration'). Yet, in the presence of the PBS buffer the SPR angle attained its original level, which was observed prior to the injection of the anti-HGH. The reported changes is SPR angle indicated the removal of the anti-HGH antibodies from the active SPR-chip surface and efficient SPR-chip regeneration process favoured for multiple analyses. Fig. 3A illustrates that that active SPR-chip surface was completely regenerated with treatment by regeneration buffer for 3 min and then PBS buffer for 2 min.

The detection limit of the sensor is defined as the concentration of analyte which induces the smallest resolvable change in the sensor response; it is usually assumed as three standard deviations of the baseline noise.[50] To estimate the detection limit the anti-HGH biosensor was exposed to the injection of different concentrations of anti-HGH antibodies in the range between 0.25 nM and 10 μM. For the reported SPR biosensor, the minimal detected concentration of anti-HGH was 2.47 nM (0.4 μg/ml). At a flow of this concentration of anti-HGH the SPR angle shift of *ca.* 6m° was obtained. The SPR angle shift for 2.47 nM of anti-HGH is quite significant, whereas the signal-to-baseline noise ratio is 60 (6m°/0.1m°). Fig. 3A shows the SPR sensorgrams for five different concentrations of anti-HGH antibodies ranging from 2.47 nM to 4.93 μM. At the end of each anti-HGH concentration measurement, the SPR angle attained a stable baseline during the flow of PBS buffer. This firmly indicates stable binding of the HGH to the SAM. The shift of the SPR angle was dependent on concentrations of the antibodies (Fig. 3A). The SPR signal at steady-state conditions (equilibrium angle) was plotted against the concentrations of anti-HGH, and the plot is shown in Fig. 3B. The change in SPR angle due to the binding interaction between immobilized HGH and anti-HGH antibody present in the aliquot was proportional to the 3rd concentration of anti-HGH antibodies.[26] In the range of 2.47–250 nM the SPR angle shift was almost linearly dependent on anti-HGH concentration and attained a nearly saturated steady state if concentrations of anti-HGH were higher than 5 μM.

The cross-reactivity study (Fig. 4) illustrates that the SPR signal registered during formation of the immune complex of immobilized HGH and anti-HGH antibodies present in the sample is over 10 times higher if compared with that obtained in the case of non-specific binding of anti-PGH antibodies on the HGH-modified SPR sensor disk surface, if the same concentration of anti-HGH and anti-PGH were added simultaneously to different chanels of the same SPR disk. The study was performed by injection of 2.47 μM of anti-HGH and anti-PGH in 10 mM PBS buffer, pH 7.4.

Repeatability is an important parameter of any analytical system. For this reason it was investigated in current research of the SPR-based biosensor. The PBS buffer was injected into an SPR-chip until a stable baseline was established (Fig. 5 line 1). Then, 2.47 μM solution of anti-HGH flowed over the anti-HGH-modified SPR-chip for 15 min (Fig. 5 line 2). After a short treatment by PBS buffer (Fig. 5 line 3), a regeneration reagent was injected into the SPR-chip and flowed through the chip for 3 min (Fig. 5 line 4). Then the PBS buffer flowed again over the sensor's surface until a stable baseline was reached and then the same concentration of anti-HGH solution was injected. Repeated detection–regeneration cycles were performed three times and consecutive injections of the same amount of the anti-HGH antibodies showed almost similar (±2%) SPR angle shifts.

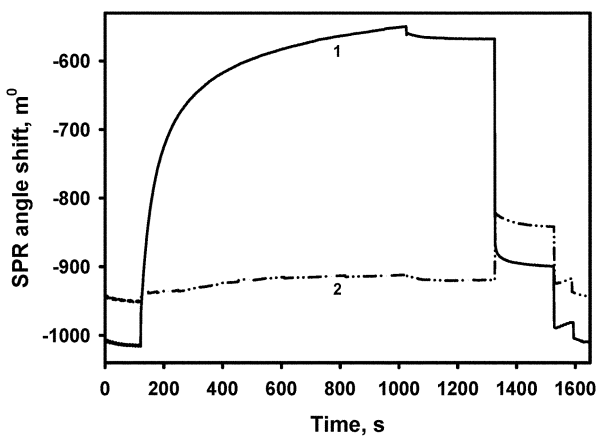

Fig. 4 Cross-reactivity study. The SPR signal: (1) during formation of the immune complex of immobilized HGH and anti-HGH antibodies present in the sample; and (2) non-specific binding of anti-PGH antibodies on the HGH-modified SPR sensor disk surface. Study was performed by injection of 2.47 μM of anti-HGH and anti-PGH in 10 mM PBS buffer, pH 7.4.

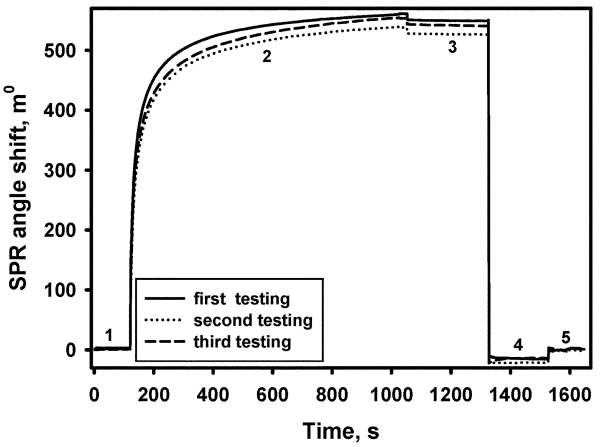

Fig. 5 The repeatability of three consecutively tested SPR signals on the same SPR-chip. Four different stages of immunoreaction between immobilized HGH and anti-HGH antibodies and regeneration of the SPR-chip are represented: 1, baseline (10 mM PBS buffer pH 7.4); 2, immunoreaction between immobilized HGH and anti-HGH antibodies; 3, and 5, injection of 10 mM PBS buffer, pH 7.4; 4, injection of regeneration solution (50 mM NaOH and 0.5% SDS).

These results clearly indicate that the HGH-modified SAM surface retained its reactivity almost completely during the detection–regeneration of the SPR-chip. The standard deviation of the SPR response, which was obtained by the same SPR-chip was found to be in the range of 1.9–2.3%, yielding the measurement repeatability of 99.1–97.7%. The standard deviation of the SPR response calculated for five separate similarly prepared SPR-chips was a little worse and was found to be up to 5.0%, yielding the measurement repeatability of 95%. This insignificant inaccuracy between different SPR-chips may be based on slightly different amounts of HGH immobilized on the MUA that differ for different SPR-chips.

The stability of the proposed SPR-based biosensor was studied during the detection of anti-HGH and SPR-chip regeneration experiments. These experiments were performed using the same procedure as for the determination of biosensor repeatability.

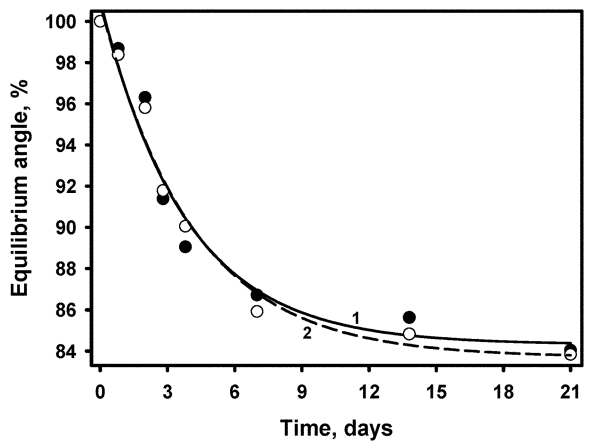

Fig. 6 Operational stability of the anti-HGH biosensor. Study was performed by injection of 2.47 μM (1) or 83.2 nM (2) of anti-HGH in 10 mM PBS buffer, pH 7.4.

Repeated detection–regeneration cycles were performed during the storing of SPR-chips for 21 days in a controlled environment (PBS buffer, pH 7.4; 20 °C). During the first day the differences between the first and tenth detection–regeneration cycles were just 2–3%. However, after the first 4 days a significant 10.9–11.1% decrement in absolute SPR response was observed (Fig. 6, curves 1 and 2). Following 4 days a period of decrement in absolute SPR response became insignificant, and after 7, 14 and 21 days the observed absolute SPR response was 13.3, 14.4 and 15.9%, respectively, if a relatively high concentration antibodies (2.47 μM) was added (Fig. 6, curve 1) and 14.0, 15.1 and 16.2% respectively if the concentration of antibodies was within the linear range of detection (83.2 nM). Generally in biosensorics it is highly desirable to produce a stable and regenerable sensor surface suitable for multiple analyses. In our study, during 21 days lasting operational-stability test of the SPR-chip installed into the analyser the decrease of SPR signal did not exceeded 5% if compared with initially registered signal without. Any appreciable changes in the baseline were registered during the same experiment. The stability of the surface upon regeneration ensures that the surface has remained reactive enough and stable throughout the experiments lasting for 21 days.

Conclusions

An SPR-based biosensor for the detection of anti-HGH utilizing HGH immobilized on the SAM was developed. In the design of this biosensor, the alkanethiol 11-mercaptoundecanoic acid was used for formation of a SAM layer. The designed biosensor showed highly promising sensing characteristics for the rapid and reliable detection of anti-HGH antibodies with good stability, repeatability, and regenerability. For the reported SPR-based biosensor a single immunoassay (detection–regeneration) cycle could be performed within 30 min. This 30 min period includes association of immobilized HGH and anti-HGH that are present in an aliquot, dissociation of the HGH/anti-HGH complex and other SPR-chip regeneration steps, favouring the detection of anti-HGH. The minimal amount of anti-HGH concentration observed experimentally was 2.47 nM. The biosensor response towards anti-HGH exhibited a high degree of repeatability. The optimal procedure for regeneration of the SPR-chip was developed during the experiments presented here. This regeneration procedure was relatively simple and lasted for only 3 min. The rapid and efficient SPR-chip regeneration procedure determined the multiple use of the proposed biosensor without appreciable changes in the sensor response. It was demonstrated that the developed SPR-based biosensor can be stored at room temperature in PBS buffer, pH 7.4, for at least 21 days without considerable loss in its capability to detect anti-HGH. The simple surface preparation steps, good biosensor stability, repeatability and regenerability make this biosensor very attractive for practical biomedical application in the routine monitoring of anti-HGH antibodies.

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