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ORIGINAL PAPER

Biofunctionalized gold nanoparticles for SPR-biosensor-based detection of CEA in blood plasma

Tomáš Špringer · Jiří Homola

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Abstract We report on the use of new biofunctionalized gold nanoparticles (bio-AuNPs) that enable a surface plasmon resonance (SPR) biosensor to detect low levels of carcinoembryonic antigen (CEA) in human blood plasma. Bio-AuNPs consist of gold nanoparticles functionalized both with (1) streptavidin, to provide high affinity for the biotinylated secondary antibody used in the second step of the CEA sandwich assay, and with (2) bovine serum albumin, to minimize the nonspecific interaction of the bio-AuNPs with complex samples (blood plasma). We demonstrate that this approach makes it possible for the SPR biosensor to detect CEA in blood plasma at concentrations as low as 0.1 ng/mL, well below normal physiological levels (approximately nanograms per milliliter). Moreover, the limit of detection achieved using this approach is better by a factor of more than 1,000 than limits of detection reported so far for CEA in blood plasma using SPR biosensors.

Keywords Surface plasmon resonance · Carcinoembryonic antigen · Biosensor · Nanoparticles

Introduction

Surface plasmon resonance (SPR) biosensors are one of the most advanced label-free optical biosensor technologies and hold potential for applications in numerous important areas, such as medical diagnostics, environmental monitoring, food safety, and security [1]. In spite of the substantial advances SPR biosensors have made in terms of both technology and applications over the last two decades, detection of biomolecules present at low levels in complex biological media (e.g., biomarkers present at sub-nanogram per milliliter or nanogram per milliliter levels in bodily fluids [2]) still remains a challenge. As the SPR biosensors measure refractive index changes caused by the binding of target molecules in a liquid sample to biorecognition elements immobilized on the sensor surface, low levels of target molecules can produce a limited sensor response. In addition, when the target molecules are contained in a complex sample, the specific response due to the capture of target molecules at the surface of the sensor can be concealed by the sensor response owing to the adsorption of nontarget molecules (which may be present at much higher concentrations than the target) to the sensor surface.

To improve the sensitivity of SPR biosensors, various methods have been developed to enhance the refractive index change associated with the capture of a target molecule at the sensor surface. These include strategies employing secondary and tertiary antibodies [3–5], antibodies labeled with horseradish peroxidase [6, 7], and dielectric [8, 9] or metallic nanoparticles [7, 10, 11]. Owing to their large size with respect to many biomolecules of interest, nanoparticles have been widely used to improve the limit of detection (LOD) for SPR biosensors. For instance, Huang et al. [10] developed a sandwich assay for prostate-specific antigen in which gold nanoparticles (AuNPs) coated with streptavidin were used for binding to a biotinylated secondary antibody, resulting in an improvement in the LOD from 10 ng/mL (LOD for the direct detection) to sub-nanogram per milliliter levels in buffer. Cao and Sim [7] used a double-enhancement strategy, where both the binding of AuNPs

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T. Špringer · J. Homola (✉)
Institute of Photonics and Electronics AS CR, v.v.i.,
Chaberská 57,
182 51 Prague, Czech Republic
e-mail: homola@ufe.cz

labeled with horseradish peroxidase and the formation of insoluble precipitate were monitored. This approach allowed the detection of prostate-specific antigen in buffer at sub-nanogram per milliliter concentrations. Although these enhancement strategies have been shown to work well in simple buffer solutions, only modest improvements in the LOD have been demonstrated when complex biological samples (e.g., blood plasma) were analyzed. Teramura et al. [9] demonstrated the detection of brain natriuretic peptide using a multistep assay based on repeated binding of streptavidin-coated particles and biotinylated anti-streptavidin antibodies. Although the assay improved the LOD from 10 ng/mL to 25 pg/mL for the detection in buffer, the LOD was limited to 1 ng/mL when the multistep assay was performed in blood plasma.

Carcinoembryonic antigen (CEA) is a 200-kDa cell adhesion glycoprotein [12]. Elevated levels of CEA may indicate gastrointestinal, breast, or lung carcinoma; therefore, CEA is a diagnostically relevant biomolecule of interest and has been targeted by various detection technologies, including SPR biosensors. CEA has previously been detected at nanogram per milliliter levels in buffer [3, 13] and at tens of nanograms per milliliter levels in blood serum [3]. The physiological levels of CEA for healthy individuals are up to 2.5 ng/mL and 5 ng/mL for nonsmokers and smokers, respectively [2, 14, 15].

In this study we report on the use of biofunctionalized AuNPs (bio-AuNPs) for SPR-biosensor-based detection of CEA in human blood plasma. Bio-AuNPs with covalently immobilized streptavidin and bovine serum albumin (BSA) were prepared and optimized in terms of specific and non-specific binding. Subsequently, they were used to enhance the SPR sensor response to CEA in a sandwich detection format and the LOD for CEA in blood plasma was established.

Materials and methods

Reagents

Sodium acetate, KH_2PO4, Na_2HPO_4, KCl, NaCl, ethanolamine, and BSA were purchased from Sigma-Aldrich, USA, in molecular biology grade or higher. *N*-Hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from GE Healthcare, USA. AuNPs of 30 nm functionalized with a mixture of carboxy-terminated and hydroxy-terminated thiols were obtained from Nanopartz, USA, at a concentration of 5 mg/mL (stock solution). Primary antibody (Ab1), biotinylated secondary antibody (Ab2), and CEA were purchased from Fitzgerald, USA. Antibody against *Salmonella* (Ab_S) was obtained from Meridian Life Science. CD166/activated leukocyte cell adhesion molecule (ALCAM) and human chorionic gonadotropin (hCG) were obtained from R&D Systems, USA, and Scripps, USA, respectively. Carboxy-terminated [$HS–C_{11}–(EG)_6–OCH_2–COOH$] and hydroxy-terminated [$HS–C_{11}–(EG)_4–OH$] alkanethiols were purchased from Prochimia, Poland. Ethanol for spectroscopy (purity 99.9 % or greater) was purchased from Merck, USA. The composition of phosphate buffer was 1.4 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.7 mM KCl, pH 7.4 at 25 °C. PBS and PBS_{NaCl} consisted of phosphate buffer with the addition of 137 mM and 750 mM NaCl, respectively. PBS_{BSA} and $PBS_{NaCl/BSA}$ buffers were prepared by adding 500 μg/mL BSA to PBS and PBS_{NaCl}, respectively. SA_1 and SA_{10} consisted of 1 mM and 10 mM sodium acetate, pH 5 at 25 °C, respectively. All buffers were prepared using deionized water (18 MΩ/cm resistance, Direct-Q from Millipore). Human blood plasma was kindly provided by the Institute of Hematology and Blood Transfusion, Prague, Czech Republic (IHBT). The blood samples were obtained at IHBT from informed consent patients in accordance with the rules of the Ethical Committee of IHBT. Plasma was centrifuged from acid–citrate–dextrose whole blood of healthy donors and stored at −80 °C until use. In all the SPR experiments reported herein, human plasma diluted to 50 % was used. The plasma samples were prepared by mixing the undiluted (100 %) human plasma and PBS with 10 mg/mL BSA in a ratio of 1:1.

Preparation and optimization of bio-AuNPs

To obtain the bio-AuNPs with both high affinity for biotin and high resistance to nonspecific binding, streptavidin and BSA were covalently immobilized on the surface of AuNPs. To activate the carboxylic groups on AuNPs, 500 μg of AuNPs in 100 μL of stock solution was incubated for 5 min in 400 μL of a mixture of 0.5 M NHS/0.1 M EDC (dissolved in deionized water). The solution with activated AuNPs was then centrifuged (6 min at 9,500*g*) and washed in four cycles to remove the unreacted NHS and EDC. After the first three cycles, the supernatant was removed and the pellet was dissolved in 500 μL of deionized water. In the last cycle, the pellet was mixed with streptavidin in 300 μL of SA_1 and was incubated for 5 min. The amount of streptavidin was optimized to obtain functional AuNPs with an optimum surface concentration of streptavidin. In the optimization process, weight ratios of 0.008, 0.032, 0.1, and 0.4 μg of streptavidin to 1 μg of AuNPs in stock solution were used. To suppress the nonspecific binding of AuNPs, the solution with streptavidin-coated particles was mixed with 1,700 μL of 500 μg/mL BSA in SA_1. Then, bio-AuNPs were purified by repeated centrifugation (each time for 7 min at 9,500*g*), where the number of centrifugation cycles was also optimized. After each cycle, the supernatant was removed and the pellet was diluted in 2 mL of 500 μg/mL

BSA in SA_1. Prior to the SPR experiments, the pellet was dissolved in PBS_{BSA} to obtain a concentration of 200 or 340 pM. To determine the concentration of bio-AuNPs, absorption at a wavelength of 528 nm was measured using a NanoPhotometer Pearl UV–Vis absorption spectrophotometer (Implen, Germany).

SPR sensor

A laboratory SPR biosensor (Plasmon IV) [16] developed at the Institute of Photonics and Electronics, Prague, Czech Republic, was used in this study. The scheme is shown in Fig. 1. The broadband light generated by a halogen lamp is collimated, polarized, and directed through a glass prism to an SPR chip attached to the base of the prism. The SPR chip is made from a BK7 glass slide that is coated with an adhesion-promoting titanium layer (thickness 1 nm) and a gold layer (thickness 50 nm). Light incident on the gold layer excites surface plasmons on the outer boundary of the gold layer. The light beam reflected on four independent spots is collected by gradient-index lenses and brought to the four-channel spectrometer via optical fibers. Excitation of surface plasmons on the four spots gives rise to the resonance dips in the spectra of reflected light. The spectral position of the dip (sometimes referred to as the SPR wavelength) is monitored in time. The spectral position of the resonance dip is dependent on refractive index changes which are caused by binding of molecules on the SPR chip. For the SPR biosensor used in this study (resonance wavelength about 750 nm), a 1-nm SPR wavelength shift represents a change in the protein surface coverage of 17 ng/cm^2 [17]. The binding of molecules from a liquid sample on the SPR chip occurs in four independent fluidic channels. The fluidic channels (dimensions in the plane of the surface are 2 mm×6 mm) are formed from 60-μm-thick self-adhesive foil which is stuck to a polished acrylic manifold and pressed against the SPR chip. In this work, we used a special version of microfluidics (dispersionless microfluidics) which allows the delivery of the sample to the SPR chip without sample dispersion and intersample mixing. The microfluidic system is composed of independent peristaltic pumps and microelectric valves that allow switching between the buffer and the sample in the proximity of the fluidic channels. This system is described in detail in previous works [18, 19].

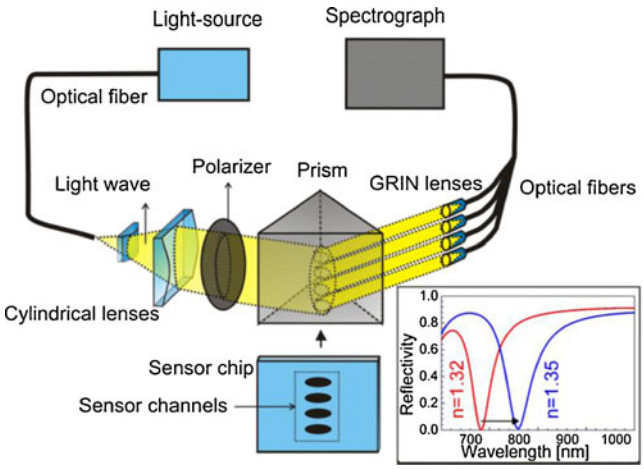

Fig. 1 The scheme of surface plasmon resonance biosensor Plasmon IV. The *inset* illustrates resonance dips in reflected light for two different refractive indexes of solutions

Functionalization of the SPR chip

An SPR chip was functionalized with a self-assembled monolayer of mixed carboxy-terminated and hydroxy-terminated alkanethiols, after which antibodies and BSA were covalently attached to the self-assembled monolayer as described previously [19]. Initially, a 3:7 mixture of HS–C_{11}–$(EG)_6$–OCH_2–COOH and HS–C_{11}–$(EG)_4$–OH thiols was dissolved in ethanol with a total concentration of 0.2 M. A clean SPR chip was immersed in the thiol mixture for 10 min at 40 °C and stored at a room temperature for at least 12 h. Then, the chip was rinsed with ethanol and deionized water and mounted in the SPR sensor. SA_{10} buffer was flowed along the SPR chip surface for 5 min. Subsequently, the chip surface was incubated in 0.5 M NHS/0.1 M EDC (dissolved in SA_{10}) for 10 min to activate the carboxyl groups. After the activation, Ab1 or Ab_S at a concentration of 5 μg/mL in SA_{10} was injected for 15 min. Subsequently, SA_{10} and PBS_{NaCl} were applied to remove the noncovalently bound antibodies. Then, 1 mg/mL BSA in PBS was flowed along the surface for 15 min, followed with SA_{10} buffer and 5-min-long injection of PBS_{NaCl}. Finally, 1 mM aqueous ethanolamine (pH 8 at 25 °C) was injected for 5 min to deactivate any remaining carboxyl groups. The functionalized chip was then washed with SA_{10}. During the functionalization processes described above, the flow rate and the temperature were set to 20 μL/min and 25 °C, respectively.

Optimization of bio-AuNPs and detection of CEA

The sandwich assay shown in Fig. 2 was used for both the optimization of bio-AuNPs and the detection of CEA. In the first step, PBS_{BSA} was flowed along the functionalized SPR chip to obtain a baseline. Then, a solution of CEA was flowed through the channels functionalized with Ab1 for 15 min, where (1) for the characterization of bio-AuNPs we used a solution of CEA in PBS_{BSA} (concentration 25 or 50 ng/mL) and (2) for the detection of CEA we used 50 % plasma either without added CEA or spiked with CEA at a concentration of 0.4, 1, 2, 5, 10, 25, or 100 ng/mL. For referencing, 50 % plasma with and without 5 μg/mL Ab1

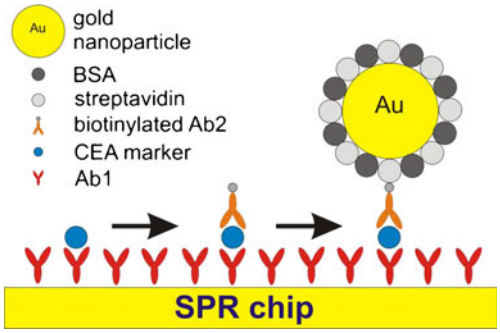

Fig. 2 The sandwich assay for the detection of carcinoembryonic antigen (*CEA*). *Ab1* primary antibody, *Ab2* biotinylated secondary antibody, *BSA* bovine serum albumin, *SPR* surface plasmon resonance

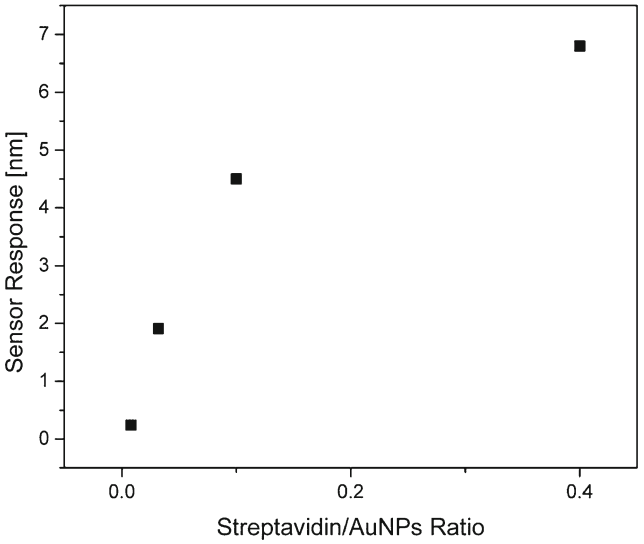

Fig. 3 Sensor response to biofunctionalized gold nanoparticles (bio-AuNPs) prepared with different ratios of streptavidin and gold nanoparticles (*AuNPs*). The concentration of CEA in PBS_{BSA} buffer was 50 ng/mL

was flowed along the chip surface with Ab1 and Ab_S, respectively. To demonstrate the specificity of the enhanced assay for different cancer markers, 50 % plasma was spiked with 100 ng/mL ALCAM or 100 ng/mL hCG. Subsequently, PBS_{BSA} was pumped along the SPR chip for 3 min. For the detection of CEA, $PBS_{NaCl/BSA}$ was injected for 7 min to reduce the nonspecific adsorption from plasma and then PBS_{BSA} was injected again. Then, biotinylated Ab2 at a concentration of 25 μg/mL was introduced for 15 min, after which a 1-min pulse of $PBS_{NaCl/BSA}$ was injected in order to remove any nonspecifically bound Ab2. Finally, bio-AuNPs were flowed for 20 min to enhance the response of the SPR sensor to CEA.

For comparison, direct detection of CEA in PBS was also performed. First, PBS_{BSA} was flowed along the surface of an SPR chip functionalized with Ab1 for 10 min. Then, a solution of CEA in PBS_{BSA} at a concentration of 25, 50, 100, 250, 500, 1,000, 2,500, and 5,000 ng/mL was flowed along the sensor surface for 15 min. For all of the steps described above, the volumetric flow rate and temperature were kept at 20 μL/min and 25 °C, respectively.

Results

Optimization of bio-AuNPs

The bio-AuNPs were first optimized in terms of streptavidin coverage. Figure 3 shows the sensor response to 200 pM bio-AuNPs for different ratios of streptavidin and AuNPs on an SPR chip that was incubated in 50 ng/mL CEA in PBS_{BSA}. As follows from Fig. 3, the sensor response increased with increasing incubation ratios of streptavidin to AuNPs. This dependence was more pronounced at lower streptavidin to bio-AuNP ratios, whereas at higher ratios the binding capacity of bio-AuNPs tended to level off. Following these results, in the subsequent detection experiments we used a ratio of 0.2 μg of streptavidin per 1 μg of AuNPs in stock solution to achieve high affinity of bio-AuNPs while keeping the consumption of streptavidin reasonable.

The preparation of bio-AuNPs was also optimized in terms of the number of centrifugation cycles. The sensor response to 340 pM bio-AuNPs (this concentration was used in all further detection experiments) was monitored on an SPR chip that was incubated with 25 ng/mL CEA in PBS_{BSA} (see Fig. S1). The sensor response increased up to the fifth cycle, after which there was no increase in sensor response with increasing number of centrifugation cycles. The observed behavior can be explained by changes in the purity of the solution, notably the decrease in the presence of unbound streptavidin. It appears that even after the third centrifugation, the solution was not fully purified and both bio-AuNPs and free streptavidin were present in the solution. In this case, free streptavidin interacted with a portion of biotinylated antibodies on the SPR chip, thus preventing the bio-AuNPs from binding to immobilized Ab1. Additional centrifugations further reduced the concentration of unbound streptavidin in the solution, after which only bio-AuNPs bound to the SPR chip. Therefore, in the detection experiments, we used bio-AuNPs that underwent at least six centrifugation cycles.

Detection of CEA in blood plasma

The optimized bio-AuNPs were employed in sandwich assay to detect CEA in 50 % blood plasma. The concentration of CEA ranged from 0 to 100 ng/mL (see Fig. 4). Each concentration was measured in at least four independent experiments. As expected, the sensor response increased

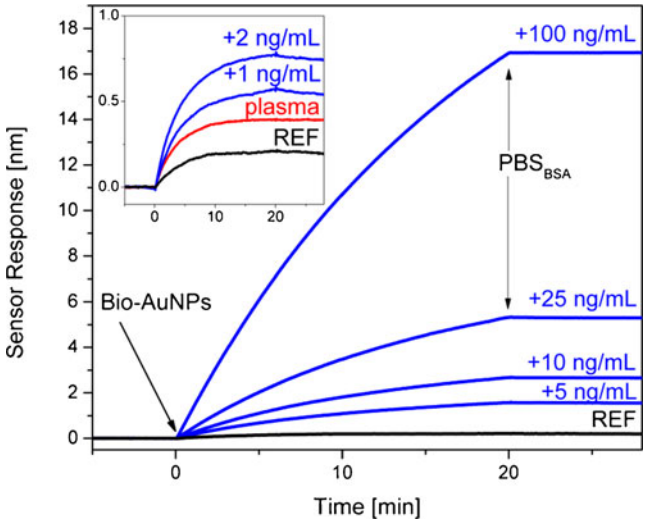

Fig. 4 Bio-AuNP-enhanced sensor response for blood plasma samples spiked with different concentrations of CEA. In reference channels (*REF*) nonspiked blood plasma sample with 5 μg/mL primary antibody Ab1 was used

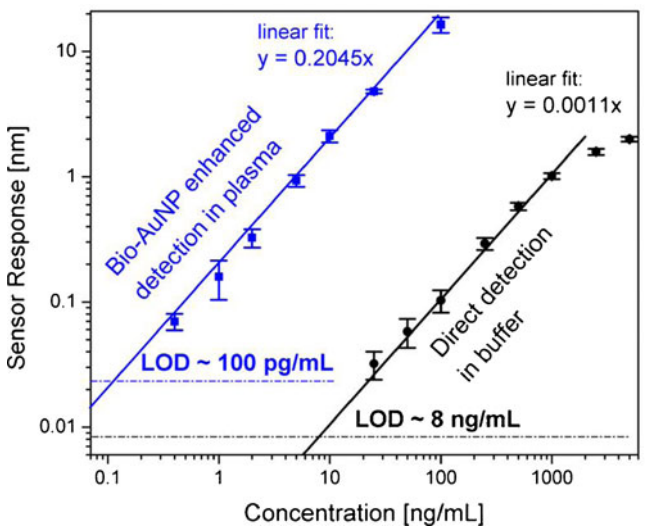

Fig. 5 Calibration curves for the direct and bio-AuNP-enhanced detection of CEA in buffer and plasma, respectively. The *error bars* denote the standard deviation of the sensor response for each concentration. The *solid lines* correspond to linear fits through zero and *dash-dotted lines* represent the sensor response corresponding to three standard deviations of the blank sample. *LOD* is the limit of detection

with increasing concentrations of CEA. Although the sensor response reached equilibrium for low concentrations of CEA, a time of 20 min was not sufficient for the sensor to reach equilibrium for the highest concentration (100 ng/mL). This is likely caused by the increase in the surface density of CEA after incubation with increased CEA incubation concentrations, where it is expected that the time to reach equilibrium is proportional to the number of binding sites [20]. The limited binding of bio-AuNPs was also observed in the reference channel where nonspiked blood plasma with 5 μg/mL Ab1 was used (see the next section for details). This response is caused not by the nonspecific binding of bio-AuNPs, but rather by the limited specificity of Ab2 for CEA (as discussed in the next section).

The sensor response to nonspiked plasma was considerably higher than that observed in the reference channel (see the inset in Fig. 4). We believe that this difference is caused mainly by CEA naturally present in the blood plasma. To explore this aspect of the sensor response, we spiked plasma with 1 ng/mL of CEA and measured the sensor response. From the comparison, it is safe to assume that the concentration of CEA was about 1 ng/mL in nonspiked 50 % plasma. This agrees well with the expected levels of CEA for healthy nonsmokers in undiluted plasma (below 2.5 ng/mL).

The calibration curve for the bio-AuNP-enhanced detection of CEA is presented in Fig. 5. The sensor responses to different concentrations of CEA were reference-compensated by subtracting the response from the channel that was incubated only with nonspiked plasma. A linear fit through zero was applied, and it can be seen from Fig. 5 that the experimental data follow a linear trend in the region from 0.4 to 25 ng/mL. The LOD was calculated as the concentration that corresponds to three standard deviations of the sensor response to a blank sample (0.024 nm), and was determined to be 0.1 ng/mL CEA in 50 % plasma.

The specificity of the enhanced assay for different cancer markers was also tested. Plasma was spiked with ALCAM and hCG cancer markers [2, 21] and the enhanced SPR sensor response was compared with a that of the channel where nonspiked plasma was used. The variation of SPR sensor responses was within the standard deviation of the enhanced SPR sensor response for nonspiked plasma (see Fig. S2). Therefore we conclude that the assay does not interfere with the tested cancer markers in plasma.

The direct detection of CEA in PBS$_{BSA}$ was also performed for comparison purposes. This allowed us to evaluate the binding of CEA to immobilized Ab1 and also the effect of bio-AuNPs. The calibration curve for the direct detection of CEA in PBS$_{BSA}$ is shown in Fig. 5. Each concentration was measured in at least three independent experiments. A linear fit through zero was applied, and the LOD was calculated using the same approach as in the case of the bio-AuNP-enhanced detection in plasma. The three standard deviations of the sensor response to the blank sample were 0.008 nm, which corresponds to an LOD of 8 ng/mL. This result is comparable with the results of previous SPR studies; Su et al. [3] demonstrated the direct detection of CEA in buffer with an LOD of 6.25 ng/mL. We also estimated the LOD for the direct detection of CEA in plasma. The standard deviation of the sensor response for a blank plasma sample was approximately 0.05 nm, and the

LOD for the direct detection of CEA in 50 % blood plasma was thus calculated to be about 100 ng/mL.

Nonspecific effects

The nonspecific effects occurring in the assay were investigated in several experiments (for details, see the table in Fig. 6). It was observed that the nonspecific binding of bio-AuNPs to the SPR chip incubated with blood plasma was negligible (Fig. 6, curve d). The binding of bio-AuNPs was observed (Fig. 6, curve b) when Ab2 was incubated with the SPR chip with covalently immobilized Ab_S that had been previously incubated in plasma. This could be explained by a low specificity of Ab2 for CEA. Some of the biomolecules from plasma were adsorbed on the surface of the SPR chip, which led to binding of low-specificity Ab2 and subsequent capturing of bio-AuNPs by biotin on Ab2. Ab2 remained on the SPR chip even if the surface of the chip was washed with $PBS_{NaCl/BSA}$ after the injection of Ab2. When plasma was mixed with more than 1,000 times excess of Ab1 (Fig. 6, curve c), the response to bio-AuNPs was very similar to that shown by curve b in Fig. 6. This suggests that free Ab1 bound to CEA in plasma and thus prevented CEA from binding to the SPR chip surface (compared with curve a in Fig. 6, where no Ab1 was added to plasma). Therefore, the sensor response shown by curve c in Fig. 6 originated from the low specificity of Ab2. Because it was observed that channels with immobilized Ab_S exhibited lower nonspecific adsorption from blood plasma than the channel with Ab1, we decided to use the channel corresponding to curve c in Fig. 6 as a reference channel for all detections of CEA in blood plasma that were performed in this study.

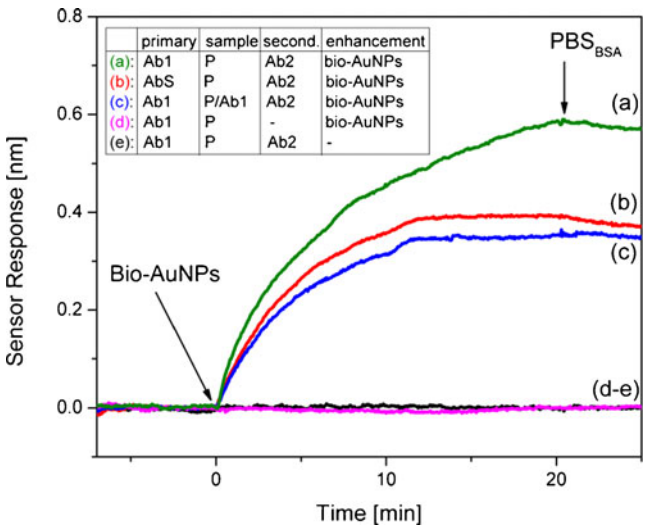

Fig. 6 Nonspecificity in a sandwich assay with bio-AuNPs for the detection of CEA in blood plasma. *Ab1* primary antibody, *Ab2* biotinylated secondary antibody, *AbS* antibody against *Salmonella, P* 50 % plasma, *P/Ab1* 50 % plasma with 5 μg/mL primary antibody

Discussion

The new bio-AuNPs improved the LODs by a factor of 80 and 1,000 compared with direct detection in PBS_{BSA} and plasma, respectively. This result agrees well with the findings of published SPR studies performed in buffer, where nanoparticles were used to enhance detection of cancer and cardiac markers and improvements by a factor of up to 100 (in comparison with the direct detection) were demonstrated [7, 9, 10]. The major difference was obtained for complex samples. Teramura et al. [9] demonstrated nanoparticle-enhanced detection in plasma with an LOD that was reduced to a factor of 10 compared with direct detection in buffer. This relatively modest improvement was caused by high nonspecific adsorption of particles to molecules from plasma that were nonspecifically adsorbed to the SPR chip. Our work shows that bio-AuNPs with optimized composition make it possible to overcome this issue.

The LOD of 0.1 ng/mL is the best LOD achieved for the detection of CEA in plasma using SPR biosensor technology. In [3], CEA was detected in a sandwich assay with two different secondary antibodies at levels down to 1 ng/mL in buffer. In 10 % blood serum, the same approach was used to detect 25 ng/mL CEA. The use of bio-AuNPs reported in this work allowed detection of CEA at 0.1 ng/mL in 50 % blood plasma and thus improved the detection of CEA by a factor of 250 in terms of the detected CEA level and by a factor of more than 1,000 when differences in plasma dilution are taken into account. This improvement is mainly due to (1) the high refractive index change produced by the bio-AuNPs (in comparison with secondary and tertiary antibodies) and therefore the high enhancement effect and (2) the ability of bio-AuNPs to detect CEA in 50 % plasma. Recently, Altintas et al. [13] used two different methods based on sandwich and anti-mouse capture assays to detect CEA and demonstrated an LOD of 3 ng/mL in buffer (no detection in biological fluids was reported).

Conclusions

We have presented the use of new bio-AuNPs with optimized binding and nonfouling properties and further demonstrated their applicability for the sensitive detection of CEA in 50 % human blood plasma. We have shown that bio-AuNPs are about ten times more effective in complex samples than previously reported nanoparticles. Using both the SPR sensor and the sandwich assay enhanced by bio-AuNPs, we achieved an LOD of 0.1 ng/mL in 50 % plasma

(the threshold concentration of CEA in undiluted plasma which may indicate a disease is more than 2.5 ng/mL). The use of bio-AuNPs improved the LOD by a factor of more than 1,000 in comparison with previously published studies when considering CEA in undiluted plasma. The level of performance achieved is caused mainly by two factors-high affinity for the biomolecules captured on the surface of the SPR sensor and negligible nonspecific binding of bio-AuNPs to the SPR chip. This approach presents an avenue enabling label-free optical biosensors to achieve sub-nanogram per milliliter sensitivity in complex biological samples desired in many medical diagnostics applications.

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