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Antibody immobilisation on the metal and silicon surfaces. The use of self-assembled layers and specific receptors

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Abstract

The use of *Staphylococcal* protein A and lectins as intermediate immobilising agents allows operators to orient antibodies (Ab) towards the solution due to the presence of a specific binding sites of immunoglobulin (Ig) molecules. Antibodies of different species of animals have unequal affinities to individual lectins. The effective thickness of immobilised Ab's depends on the type of substrates used and increases in the following sequence: bare gold or silicon surface, the surface treated with self-assembled polyelectrolytes (PESA) or with protein A or some lectins deposited on the preliminary formed polyelectrolyte layer. The glycolysated protein of jp51 may be selectively immobilised from the mixture of retroviral proteins (p24 and jp51), if it is necessary to distinguish infected animals from preliminarily immunised ones by means of a vaccine based on p24 protein. It was shown that the use of *Staphylococcal* protein A, instead of some lectins as intermediate layer for the Ab immobilisation, does not lead to a more sensitive determination of such low-weight toxins as 2,4-dichlorophenoxyacetic acid (2,4-D). The above-mentioned results were obtained with surface plasmon resonance (SPR) technique.

Keywords: *Staphylococcal* protein A; Lectins; Polyelectrolyte self-assembly; Antibodies; Immobilisation; Orientation; Biosensors; Transducers; Surface plasmon resonance (SPR)

1. Introduction

The search for new approaches to increase the sensitivity of biosensors continues, and the main one is to improve methods of immobilisation of biological materials on the transducer surface. It is necessary to provide dense coatings on the metal or silicon surfaces, which contain functioning and properly oriented bio-recognising structures. A number of different procedures were deployed for the integration of biological sensitive molecules with different types of transducer surfaces.

We have shown earlier [1,2] that an intermediate layer of polyelectrolyte adsorbed electrostatically on the sample surface provides higher (up to 10 times) specific signal of SPR and planar polarisation interferometric immune sensors in comparison with either the untreated surface of bare gold, or that modified by dodecanthiol, the surface of silicon nitride treated with glutaraldehyde. In particular, this effect was demonstrated for the immobilisation of both IgG and anti-IgG as well as for their interaction. Moreover, it was found [3] that the use of protein A from *Staphylococcus aureus* as an additional intermediate layer further increases the specific signal of optical immune sensors and, therefore, their sensitivity towards the immobilisation of anti-IgG and the subsequent binding of IgG in solution.

In this article, we present the experimental results on the use of protein A and some lectins together with PESA as intermediate layers for the immobilisation of Ab's or Ag's selected for specific tasks.

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2. Experimental

SPR-4 instrument, which was designed and fabricated in the Institute of Semiconductor Physics of Ukrainian National Academy of Sciences, was deployed in the present study. Transducers in the form of glass slides with thermally evaporated gold layer (about 20 nm in thickness) was connected to the prism with the help of index matching fluid (polyphenyl ether, n=1.6). The transducer surface was preliminarily treated in PESA solution) (Fig. 1), as described in Ref. [2]. In our experiments, four lectins from some vegetables and animals were used: (1) FGÀ-Ð from *Phaseolus vulgaris* (Pharmacia, Sweden); (2) PLA from *P. vulgaris*; (3) STA from *Solanum tuberosum*; (4) HPA from *Helix pomatia*; (5) WGA from *Tuberosum vulgaris*. The last three lectins were obtained from Sigma (USA). They were dissolved in 0.05 M sodium phosphate buffer (PBS; pH 7.5) containing 0.14 M sodium chloride The formation of an intermediate layer of protein A was carried out following the routine described in Ref. [3].

At first, the resonance angle was observed when PBS introduced at 10 μl. Then the cell was filled with the solution of some lectin and left alone for 20 min at room temperature. After removing non-bonded lectin from the surface, the resonance angle was checked again, and the solutions of specific Ab or Ag were introduced in the cell. The time of incubation of these solutions in the cell was 15 min. After that, the cell was washed with PBS. Finally, an appropriate second immune component was pumped into the cell and kept there for 10 min. The measuring cell was filled with PBS each time before the registration of the resonance angle.

The following pairs of Ab–Ag interaction were used in our experiments: (i) human IgG and pig Ab to it; (ii) human mioglobin (Mb) and mouse monoclonal Ab to this protein. All IgG were obtained from Sigma. $F(ab)_2$ fragments of human IgG was from Calbiochem (USA).

Conjugates of 2,4-dichlorophenoxyacetic acid (2,4-D) with bovine serum albumin (BSA) and ovalbumin (Ova) were prepared via benzene ring using Fenton reagent [4]. 2,4-D was dissolved in 2 ml of distilled water with the addition of 1 M NaOH in order to increase the pH up to 9.0. Then, 1 ml of hydrogen peroxide (50%) and 8 mg of $FeSO_4$ were added to the above solution. In parallel, the solution of BSA in 8 ml of distilled water was prepared. Both solutions

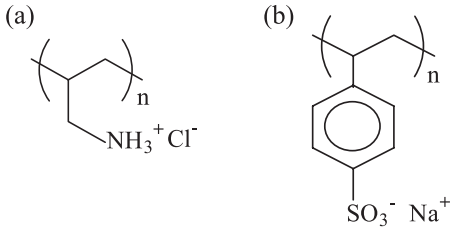

Fig. 1. Polyelectrolites: a—poly(alylamine) hydrochloride (PAA) and poly(sterenesulfonate) sodium salt (PSS).

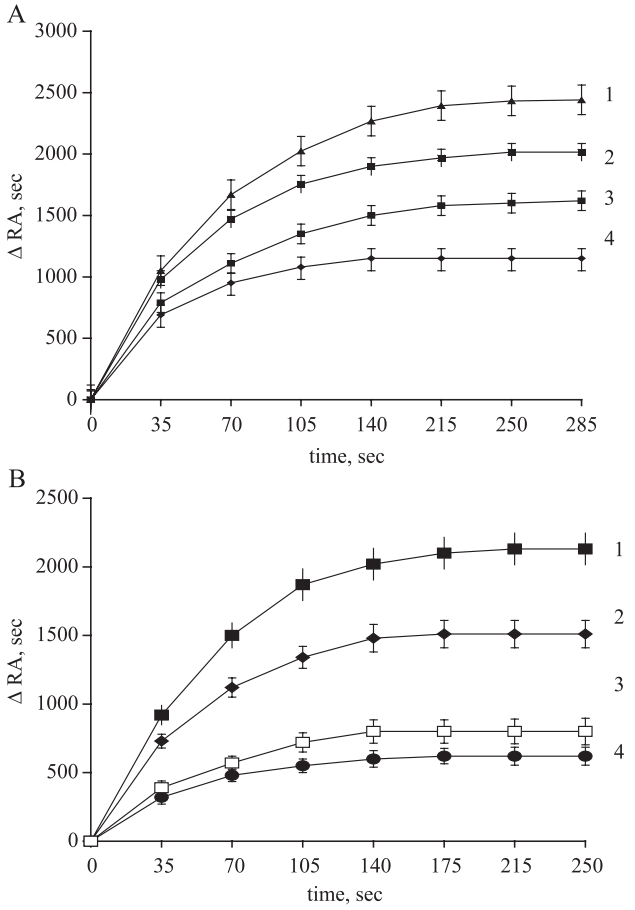

Fig. 2. Changes in the resonance angle (RA) of SPR based immune sensor at the immobilization of (A) human and (B) mouse IgG on the bare gold surface (2) and on the gold surface modified with WGA-, or ÍPA-, or PLA lectins (1, 3 and 4, respectively). Abscissa is the time of the immobilization.

were mixed (in a 2,4-D/BSA ratio of 10:1), and the mixture was stirred for 20 min at 4 °C. Finally, it was dialysed in distilled water, centrifuged and lyophilically dried.

Rabbits were immunised twice by subcutaneous injection of the emulsion of 2,4-D–BSA (or Ova) conjugated with full Freund adjuvant at a ratio of 1:1. After that, rabbits were immunised four times by intramuscular injection of the above solution but without Freund adjuvant. Blood sample was taken 7–8 days after the last step of immunisation, and antiserum was prepared in the usual manner.

Total antigen of bovine leukosis virus was given to us by Prof. L. Nagajeva from joint venture “Leucopol”, Poltava (Ukraine). Serum, blood and milk from cows infected by leukosis virus and immunised by the p24 protein based vaccine, as well as from healthy animals, were obtained from dairies in Poltava region (Ukraine). In order to obtain serum, fresh milk was treated in 5% of acetic acid. Both serums (from blood and milk) were diluted PBS. The analysis of these samples was carried out in the following way. First, the measuring cell was filled with PBS, and the resonance angle was checked. Then the retroviral antigen was introduced into the cell, left there for 20 min and washed away with PBS containing 0.05%

Table 1
Maximal changes in the resonance angle values at the immobilization of pig anti-human IgG on the different surfaces and binding human IgG

No.	Type of the surface	Immobilisating/ binding agent	Changes in the resonance angle (″)
1	bare gold	pig anti-human IgG	2016±60
2	gold with WGA-lectin		2433±120
3	Gold with anti-human IgG	human IgG	2100±100
4	gold with WGA-lectin-anti-human IgG		2816±116

solution of Tween-20. The resonance angle was checked again. Finally, the analysed sample of some particular serum was pumped in the cell, kept there for 10 min, and after washing it out in the PBS mixed with 0.05% of Tween-20, the changes in resonance angle were registered. The gold surface of the transducer was covered by PESA layer with lectin.

3. Results and discussion

Physical sorption of lectins on the transducer surface is very stable and cannot be destroyed after washing in PBS. The formation of a monolayer of lectin causes a shift of the resonance angle in range of 0.27–0.42°. We think that the variation of the resonance angle depends on the molecular mass of individual lectins, varied from 36 to 128 kDa. To check the possibility of additional binding of bio-molecules, the investigated surface latter was brought into the contact with human $F(ab)_2$ fragments preliminary treated by the appropriate lectin. In this case, we did not observe any changes in the resonant angle. It proved that lectin-treated surfaces do not contain free sites capable of direct binding of IgG molecules.

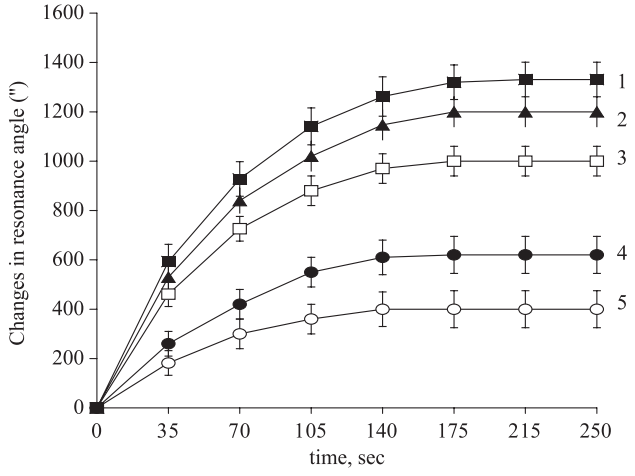

Fig. 3. Changes in the resonance angle (RA) of SPR based immune sensor at the presence of different concentrations of Mb in the solution and the in case of immobilization of specific Ab on the surface of bare gold (3) and the surface treated in HPA-, or PLA-, or WGA-, or STA-lectins (1, 2, 4 and 5, respectively).

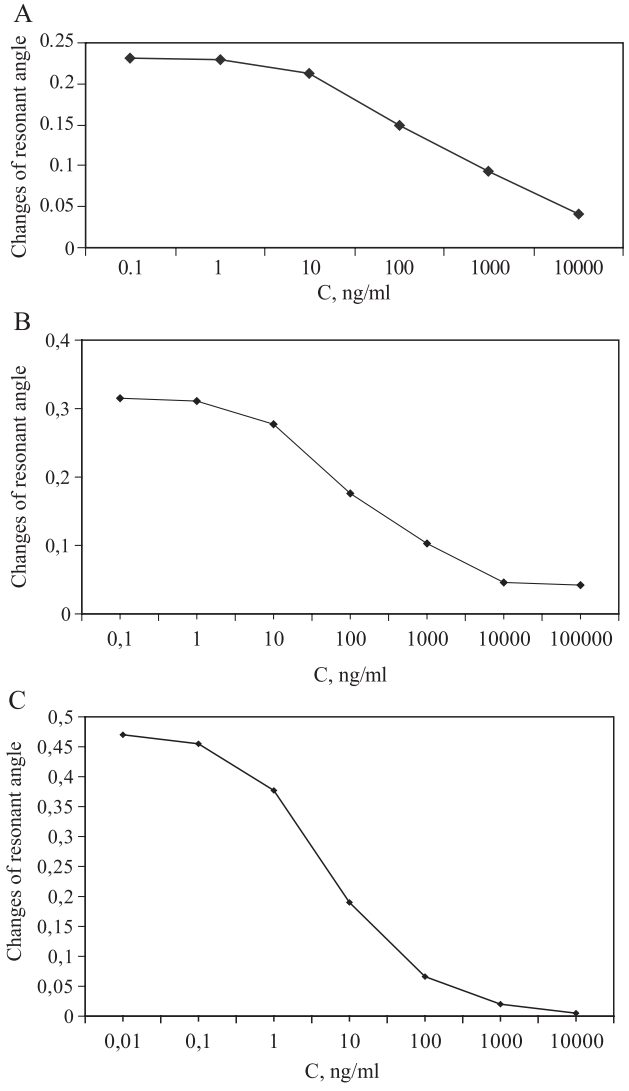

Fig. 4. Changes in the resonance angle of SPR-based immune sensor in the presence of different concentrations of 2,4-D in solution at the immobilisation of antiserum on the gold surface treated by (A) PAA only, (B) by PAA together with HPA-lectin and (C) by PAA together with the *Staphylococcal* protein A.

In the next set of experiments, it was stated that the maximal response of the SPR sensor to the human IgG immobilisation was obtained, when WGA-lectin was used, in comparison with HPA- and PLA-lectins (Fig. 2A). It is necessary to pay attention to the fact that the density of IgG immobilisation on the bare gold surface is higher than that on the surfaces treated with HPA- or PLA-lectins. Its probable that the above lectins modify the surface to such an extent that it becomes excessively hydrophilic, and at the same time the affinity of these lectins to carbohydrates of IgG is very low. To clarify the influence of non-specific binding of IgG to the surface modified by lectins, it was treated with appropriate $F(ab)_2$ fragments and then with full IgG molecules. It was

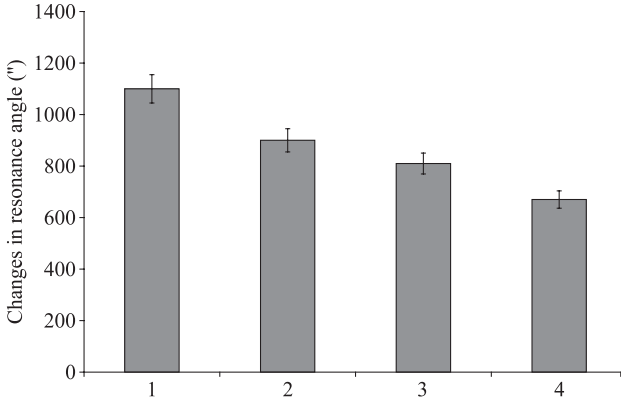

Fig. 5. Changes in the resonance angle of SPR based immune sensor with bare gold surface (1) and the surface modified with FGA-P- or PLA- or HPA- lectins (2–4, respectively). Abscissa—changes resonant angle.

stated that the resonance angle was the same, regardless of the use of $F(ab)_2$ fragments. A similar result was obtained with the application of bovine serum albumin (BSA) as an additional treatment of prepared surfaces.

In the case of immobilisation of pig anti-human IgG, we obtained following results. WGA-lectin, in comparison with HPA- and PLA-lectins, has higher affinity to this IgG. At the same time, it is necessary to note that the changes in resonance angle were more than 20% higher at the immobilisation of pig antibodies to human IgG on the gold surface treated by WGA-lectin than on bare surface (Table 1). It confirms that the density of immobilised pig antibodies is higher in the first case. A specific response of the immune sensor on the presence of human IgG is 34% higher when the surface is treated with WGA-lectin, in comparison to the bare gold substrates. The results presented above show that the sensitivity of the immune sensor is defined by both the density and orientation of IgG molecules on the transducer surface.

Similar experiments were performed with the other Ab–Ag pair, e.g. mouse monoclonal Ab and human Mb (Fig. 2B). It was shown that the affinity of mouse IgG decreases in a row: HPA-, PLA-, WGA- and STA-lectins. The response of the immune sensor to Mb decreases in the same manner, too (Fig. 3).

The affinity of rabbit IgG to the individual lectins decreases in a row: HPA-, WGA-, STA- and PLA-lectins.

Thus the affinity of some lectins to the carbohydrate component of IgG, localised mainly in Fc fragments, may provide the oriented immobilisation of antibodies targeting the exposition of their Fab-binding fragments towards the solution.

We tried to compare the sensitivities of SPR immune sensors, prepared with and without the use of intermediate layers of PESA-lectins or PESA protein A, of the analysis of 2,4-D herbicides. In all cases, the competitive analysis was exploited, when both free 2,4-D and 2,4-D conjugated with some protein were competed for sites of specific antibodies immobilised on the transducer surface. It was shown that in the case of immobilisation of specific antibodies from antiserum on gold surface, covered either with PSA or PSA with HPA-lectin, we obtained the same sensitivity of about 1 ng/ml for 2,4-D determination (Fig. 4A and B). If the procedure of the immobilisation of specific antibodies from antiserum on the gold surface covered by PSA with protein A from *S. aureus* was used, the sensitivity towards 2,4-D increased approximately by 1 order of magnitude (Fig. 4C).

In spite that both declared sites of IgG for binding lectins and protein A localised in the second domain of Fc fragment, the orientation of Fab fragments may be different. It may be related to the presence of non-specific carbohydrate sites in other places of IgG molecule. In this case, the following situation is very likely to occur, when carbohydrates are bonded to Fab fragments, and their interaction with lectins blocks this fragment from the interaction with the specific antigen. Therefore, the use of protein A in this case is preferable than some lectins.

Nevertheless, there exist situations when the immobilisation of biological sensitive element via its carbohydrate

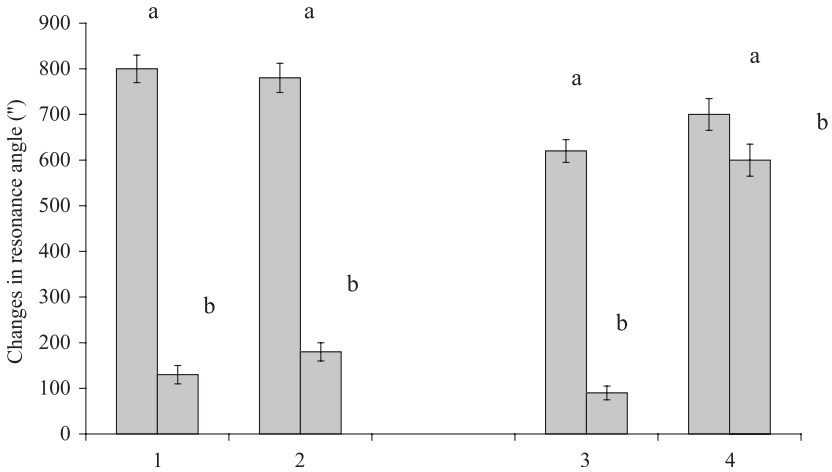

Fig. 6. Changes in the resonance angle of SPR based immune sensor at the analysis of (a) ill and (b) vaccinated cows for non-modified gold surface (4) and the surface modified with FGA-, or PLA-, or HPA-lectin (1–3, respectively).

structure is very much desirable. We have analysed the situation of the discrimination of antiserum from ill animals on the viral leucosis and those preliminarily immunised by specific antigens.

During immunological investigations of retroviral infected animals, there were used medications obtained by the virus cultivation on the embryonal kidney cells of ovine [5]. These medications are mixed with viral membrane glycoproteins and some inner proteins. It is well known [6,7] that antigenic abilities of this retrovirus are mainly caused by membrane glycoproteins. Therefore, the majority of antibodies in ill animals are formed in these antigens. For immunised animals, the situation is different: the vaccines used are mainly based on the p24 protein, which is not glycolised. This information may be utilised for the discrimination of antiserum from ill and immunised animals. It is practically necessary to use gold surfaces, preliminarily treated by appropriate lectin, for the immobilisation of glycoproteins (typically jp51 protein) from the mixture of viral antigens.

In preliminary experiments, the affinity of retroviral jp51 to the following lectins was investigated: FGA-P, PLA, and HPA. The largest sensor response was found in the case of the immobilisation of viral proteins on the surface treated in PSA only. In this case, all (glycolysated and non-glycolysated antigens) were immobilised on the transducer surface. At the same time, in the case of using the transducer surface treated with PSA and some lectins, the largest response was revealed on the application of FGÀ-Ð-lectin (Fig. 5). The experimental data, presented in Fig. 6, demonstrate that the above method of surface preparation is very effective for the discrimination of antiserums from ill and immunised animals.

4. Conclusions

The obtained results confirmed our previous observations [1,2] that the presence of polyelectrolyte layers unifies the surface of transducers and increases the efficiency of the immobilisation of the next intermediate layer of *Staphylococcal* protein A. The same effect was demonstrated on the formation of intermediate layers of some lectins. The affinity of lectins is varied for different iso-types of IgG, so the type of lectins suitable for binding some particular IgG should be chosen in preliminary experiments. Both types of intermediate layers (lectins and *Staphylococcal* protein A) enhance the preferential orientation of Fab fragments of IgG towards the solution; however, *Staphylococcal* protein A is more effective in for this task. At the same time, lectins can provide selective immobilisation of appropriate glycilisated antigens from some complex mixtures. It proved to be very effective to apply this approach for the direct discrimination of antiserum from health and ill animals at the biochemical diagnostics of bovine retroviral leucosis.

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