Modification of immune SPR biosensor surface at nonylphenol analysis

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The influence of the state of transducer surface of immune SPR biosensor on the sensitivity and stability of its "direct" detection of nonylphenol in solutions has been investigated. The biosensor surface was modified with polyelectrolytes, dodecanethiol, and dextran sulfate and glutaraldehyde. It was shown that the highest sensitivity (up to 10 ng/ml) and stability of the biosensor response were achieved at the surface modification with dodecanethiol and simultaneous creation of intermediate layer with protein A Staphylococcus aureus for the orientation of active sites of specific antibodies toward solution.

Keywords: *nonylphenol, immune biosensor, polyelectrolytes, dodecanethiol, dextran sulfate, glutaraldehyde*

Introduction. An extensive number of chemical compounds are represented by environment pollutants, which have negative effect on the human health via disordering of normal functioning of endocrine system. These compounds are called endocrine disrupters and include some alkylphenols and nonylphenol (NPh). Nuclear receptors of steroid hormones, via which the regulation of the embryonic growth and physiological processes in adult rats takes place, are known to be the targets for these substances [1]. The treatment of nerve stem cells with NPh for 24 h results in sharp inhibition of the cell growth. 4-NPh revealed powerful cytotoxic activity due to caspase cascade apoptosis and cell cycle blocking in G2/M phase [2]. NPh is regarded as one of the most widespread environment pollutants, which can be detected in near-bottom and surface waters. Recent investigations showed NPh to be of high toxicity and being capable of accumulating in water organisms [3].

The majority of tests for NPh detection are expensive, time-consuming, and do not allow making the database of programs on ecological situation observance in analysing a great number of samples [4-7]. There is a need in fast, inexpensive, reliable, and sensitive approaches for detection of microquantities of pollutants, in particular, NPh, in water, in order to determine the toxicity level of waste products. All aforementioned drawbacks of NPh detection could be avoided by the use of modern instrumental analytical devices based on biosensor technology. Some of them have already been described.

Thus, we have developed an optical immune biosensor based on the effect of the surface plasmon resonance (SPR) for high sensitive and specific determination of nonylphenol (NPh) in solutions. As it was shown previously [8], in case of "competitive" way (free NPh competed with NPh, immobilized on transducer surface, for specific binding sites on antibodies, and consequently the shift of resonance

angle has been observed) the level of sensitivity was 7-10 ng/ml and the operational range was up to 1000 ng/ml. The developed algorithm of the "up to saturation" method (after the immune reaction between NPh and specific antibodies, NPh, which had not undergone the reaction, was artificially saturated by injection of antiserum; the value of a resonance angle shift was proportional to the concentration of free NPh in the solution) allows detecting NPh with the sensitivity of about 2-5 ng/ml and working controlled concentrations of 5-1000 ng/ml. The time of analysis by immune SPR biosensor is about 10 min (at the previously prepared transducer surface, including immobilization of sensitive structures) [8].

The development of SPR biosensor on the basis of standard *Biocore* equipment, which allows the determination of NPh using the competition method at the concentration range of 2–5 ng/ml and SPR-sensitive biosensor which is based on NPh interaction with estrogenic receptor [10], has been reported [9]. The data on the development of amperometric immunosensor which provides determination of NPh with sensitivity of 10 ng/ml have been presented, but it requires marked antibodies and special highly effective dyes. Therefore, it is noteworthy that the mentioned above methods are considered to be relatively complex as they require additional reagents and expensive stages of determination.

For instance, *Biacore* equipment costs more than € 500 000,00. Therefore, there is a need in the development of simple, inexpensive, and sensitive methods for the determination of NPh, applicable in environment field. Successful development in this area does not depend on electronics merely, but on selection of adequate methods of biochemical treatment of transducers surface for the optimization of highly-sensitive registration of the optical signal generated at the antigen-antibody interaction. *Pharmacia Co.*, (Sweden) used the branched dextran, which was the subject of a special patent [9]. At the same time it is noteworthy that this preparation method is neither simple nor inexpensive, and, therefore, it is necessary to search for new original and practical variants, satisfying practical needs in portative and semi-portative biosensor devices in field conditions.

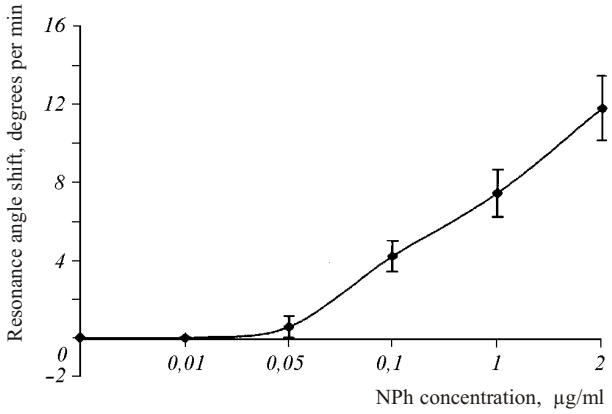

Fig.1 Response of the immune sensor to the introduction of different concentrations of NPh into the measuring cell with non-modified transducer surface

The aim of the current work was the investigation of the immune SPR biosensor continued dependence on the type of surface modification by the way of direct detection of NPh in solutions. Polyelectrolytes, dodecanethiol, and dextran sulphate, with glutaraldehyde (GA) were used for transducer surface modification.

Materials and Methods. SPR-4 equipment, manufactured at the Institute of Semiconductor Physics of National Academy of Sciences of Ukraine, was deployed in the present study. It is based on the SPR phenomena. All experiments were performed in 0.01 M tris-HCl buffer, pH 7.4.

NPh-BSA antiserum in the concentration of 10 mg/ml (determined in the previous research [8]) was used for direct determination of NPh. Initially, the measuring cell was filled with 0.01 M of tris-HCl buffer, pH 7.4 to obtain a base line. Then antiserum was introduced into measuring cell for 20 min, following which the antibodies, which had not been immobilized by physical absorption, were washed with tris-HCl buffer. Then 1% gelatine was rendered on the surface to fill empty seats of linkage, after that NPh solutions with various concentrations were pumped into the measuring cell and the calibration curve was built (Fig.1).

In accordance to the aim of the work, the next stage was to investigate the effect of the modification of the transducer surface by polyelectrolytes self-assembled films and orientation of antibodies using protein A on

the sensitivity of the determination of NPh in solutions. Thereto, polyalylaminhydrochloride (PAA) (*Aldrich*, USA) was immobilized on the transducer surface from the solution of 1 mg/ml concentration, and then protein A in the concentration of 1 mg/ml was introduced into the measuring cell. After that the antibodies were immobilized from antiserum solution (10 mg/ml) and then NPh solutions in the concentration range of 10 ng/ml – 2 μg/ml were introduced.

For the modification of SPRE biosensor surface with dodecanethiol its 98% solution was sustained on the surface for 18 hours. Then the transducer surface was washed with 40% ethylene alcohol. Immobilisation of protein A (1 mg/ml) was performed on this surface. Direct determination of NPh was conducted in accordance to the earlier described method.

The last experiment was dedicated to modifications of the biosensor surface with dextran sulphate and GA. The measuring cell was introduced with 0.01 M of phosphate-citrate buffer, pH 4.0, for base line obtaining, and then antibodies were immobilised on the surface (from the antiserum solution in phosphate-citrate buffer, 10 mg/ml). Then, dextran sulphate solution was applied to the surface (phosphate citrate buffer, 1 mg/ml) and non-immobilised molecules were washed off using the same buffer. This procedure was repeated once more, and then antibodies were immobilised for the third time. The obtained layers of antibodies were cross-linked by the introduction of 0.5% GA solution. Dextran sulphate was washed with the buffer physical solution (BPS), pH 7.4 and "empty" seats of linkage were filled with 0.5% gelatine solution in BPS. After that NPh solutions with various concentrations were pumped into the measuring cell.

Results and Discussion. Thus, it was shown that the direct way of analysis allows detecting NPh with sensitivity of 80-100 ng/ml and the region of working controlled concentrations is up to 2000 ng/ml (Fig.1). The main drawback of this way of analysis was its low sensitivity. But it can be overcome by previous modification of the sensor surface. For this purpose we have investigated the effect of the modification of the sensor surface (with polyelectrolyte self-assembled films, dodecanethiol and dextran sulphate with

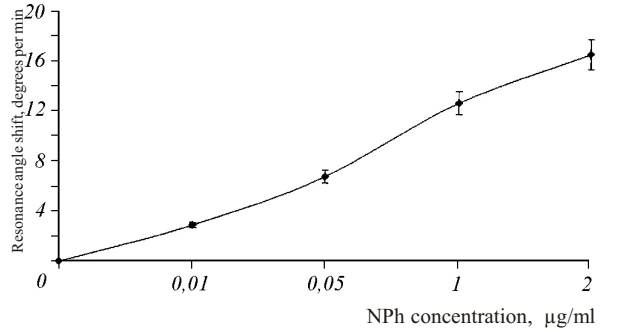

Fig.2 Response of the immune sensor to the introduction of different concentrations of NPh into the measuring cell with preliminary modification of the sensor surface with PAA/protein A/antiserum

glutaraldehyde) on the sensitivity of the biosensors response.

Polyelectrolytes are widely used for the immobilisation of biological material or separation of output components from the products of immune reaction [13]. Obtaining of films using small charged organic molecules is commonly used, as these molecules are absorbed on charged surface and establish an insoluble polymer, capable of electrostatic absorption of oppositely charged molecules [14]. Besides, its immobilisation on SPRE sensor surface provides the positive charge.

Increase in the biosensor response may be achieved by presenting a maximally tight layer of specific immunoglobulins, orientated by antigen-recognising structures towards the solution. As polyelectrolyte intermediate layers provide necessary density of immunoglobulin immobilisation on the transducer surface, their orientation, therefore, may be defined by the formation of the additional intermediate layer (between polyelectrolytes and immunoglobulins) with protein A, obtained from *Staphylococcus aureus* [13]. The latter assists the exposition of $F(ab)_2$-fragments of immunoglobulin towards the solution via binding its site in Fc-fragment region. We have demonstrated that the application of PAA and protein A for surface modification allows increasing biosensor sensitivity up to 50 ng/ml (Fig.2).

In other cases, dodecanethiol has been used for obtaining of stable analysis results and increasing biosensor sensitivity. Dodecanethiol SH-groups interact with metal ions and form mercaptans. Consequently, the surface is evenly (with some

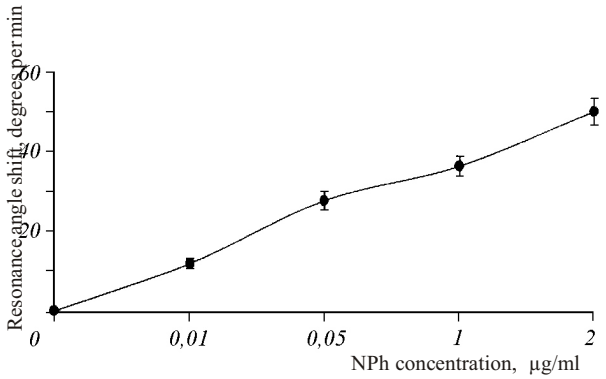

Fig.3 Response of the immune sensor to the introduction of different concentrations of NPh into the measuring cell with preliminary modification of the sensor surface with dodecanethiol

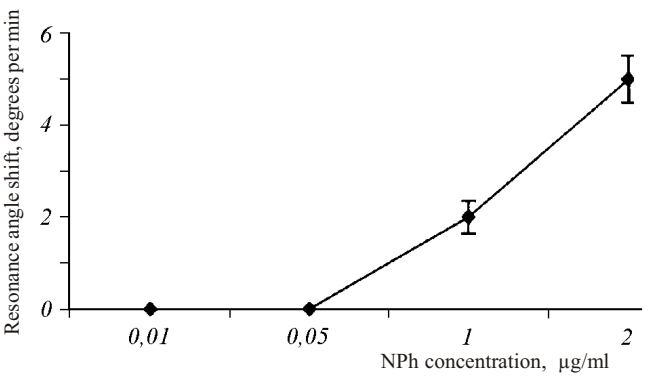

Fig.4 Response of the immune sensor to the introduction of different concentrations of NPh into the measuring cell with preliminary modification of the sensor surface with dextran sulphate and glutaraldehyde

Table

Response of the immune sensor to the introduction of different concentrations of NPh at different types of surface at direct determination of NPh in the solutions

Method of transducer surface modification	NPh concentration, µg/ml			
	0.01	0.05	1	2
Non-modified surface	0	0.6±0.28	7.6±0.71	11.8±1.13
Modification with				
PAA and protein A	3±0.63	7.1±0.89	13±1.32	16.8±1
dodecanethiol and protein A	12.1±0.41	28.3±1.02	37±1.33	51.2±1.51
glutaraldehyde and dextran sulphate	0	0.21±0.12	2.1±0.35	4.6±0.62

probability) covered with the hydrophobic layer which is capable of interacting with molecules (in this case with protein A molecules, immobilised on the surface for antibodies orientation). This modification allows determining NPh with the sensitivity level of 10 ng/ml (Fig.3).

The method of antibodies immobilisation on the SPR immune biosensor surface using dextran sulphate is capable of forming special 3D complex [13]. In this case, sorption of antibodies and dextran sodium sulphate (SD) and covalent binding of antibodies using GA and washing out of dextran from already formed surface were alternated. Antibodies are positively charged at pH level lower than isoelectric point and SD is presented by polyanion. Biological membrane formed has got a selective structure which is more derived, comparing to simple physical sorption of antibodies. However, we have demonstrated that such modification of the surface is inefficient, as sensor response to antigen introduction is lower than in the case of non-modified surface; and the sensitivity was at the level of 1.5 µg/ml (Fig.4). Evidently it is connected with disorders in the tertiary structure of antibodies at their treatment with GA and consequently with inactivation of their active centres.

Later on, the stability of SPR biosensor response in dependence on the type surface at direct determination of NPh in solutions was studied, comparing sensor responses to the introduction of equal NPh concentrations (Table). It has been revealed that the most stable results were achieved at the surface modification with dodecanethiol.

Conclusions. The approach for the high sensitive and specific determination of NPh using immune biosensor based on SPR has been developed. The sensitivity and stability of SPR based immune

biosensors in dependence on the type of surface at the "direct" detection of NPh in solutions have been investigated. It has been shown that the effective layer thickness of immobilised immune components, as well as formed specific immune complex on the gold-SPR surface is increased significantly in the following sequence: their direct physical absorption on the bare gold surface > with intermediary layer of polyelectrolytes and binding with protein A, immobilised on the same layer > immobilisation on the surface, modified with dodecanethiol and binding with protein A. The most stable results were obtained in the experiment with dodecanethiol modification.

Therefore, this method of modification is highly recommended. At the same time it is noteworthy-that the highest sensitivity (up to 10 ng/ml) and stability of the biosensors response were achieved at the surface modification with dodecanethiol and simultaneous creation of the intermediary layer with protein A from *Staphylococcus aureus* for the orientation of active sites of specific antibodies toward solution.

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Модификация поверхности иммунного биосенсора на основе поверхностного плазмонного резонанса при анализе нонилфенола

Резюме

Исследовано влияние состояния поверхности преобразователя иммунного биосенсора, основанного на эффекте поверхностного плазмонного резонанса, на чувствительность и стабильность его отклика при прямом определении нонилфенола в растворах. Поверхность биосенсора модифицировали при помощи полиэлектролитов, додекантиола, а также глутарового альдегида и сульфата декстрана. Показано, что наибольшая чувствительность (10 нг/мл) и стабильность отклика биосенсора достигается при модификации поверхности додекантиолом и одновременном создании промежуточного слоя из белка А Staphylococcus aureus для ориентации активных центров специфических антител в сторону раствора.

Ключевые слова: нонилфенол, иммунный биосенсор, полиэлектролиты, додекантиол, глутаровый альдегид (ГА), сульфат декстрана (СД).

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