THE ULTRATHIN POLYMER FILMS BASED ON POLYANILINE AS SPR SENSITIVE DIAGNOSTIC SYSTEMS

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Abstract

In the present work the use of electrochemically synthesized films of polyaniline (PANi/HCl) and its complexes with polyacids (PANi/PSSA and PANi/PAMPSA) is proposed as sensing elements of SPR chips. Adsorption of influenza viruses and antibodies to them at the synthesized thin films of PANi was studied. It is established that these processes occur with higher rate on ultrathin films of PANi with polyacids. It was shown that antibodies to influenza viruses weakly bind to the surface of standard PANi and the reaction "antigen-antibody" leads to desorption of the immunocomplex. The use of PANi/PAMPSA films provides a more efficient binding with antibodies due to their chemical nature, molecular structure and rugged surface.

Keywords: SPR, influenza virus, conducting polymers, polyaniline

1. INTRODUCTION

Diagnostics of viruses and the development of new materials for sensitive elements of sensors is one of the topical problems in modern biotechnology. One of the most promising materials for sensors are conducting polymers, including polyaniline. The chemical structure of polyaniline presents a repeating N-phenyl-p-phenylenediamine and quinondiimine blocks, thus showing greater affinity to proteins. PANi can be easily synthesized on the surface of the electrodes of the various nature, and its physico-chemical properties allow to create devices with different type of registration response to protein binding (electrochemical, optical or impedimetric sensors). Earlier we established the fact that polyaniline is able to interact with influenza viruses [4]. In the present work we have synthesized thin films of new complexes of polyaniline with polysulfonic acids and applied them to detection of influenza viruses by surface plasmon resonance (SPR).

2. EXPERIMENTAL

Polyaniline (PANi) was synthesized by electropolymerization of aniline in aqueous solutions of polysulfonic acids on gilded SPR chips. Polyacids of different structure: poly-2-acrylamido-2-methyl-1-propanesulfonic acid (PAMPSA) (Aldrich, #CAS 27119-07-9, Mw ~ 2 000 000, 15% aqueous solution); poly-4-styrenesulfonic acid (PSSA) (Aldrich, CAS # 28210-41-5, Mw ~ 75 000, 18% aqueous solution) was used for the electrochemical synthesis of PANi. The concentration of the polymeric acid in the solution was 50 mM and concentration of aniline was 25 mM. For comparison, we also synthesized the film of "standard" PANi in 1 M HCl. The procedures of solution preparation for the synthesis of PANi and the film deposition on the substrate were described in [1]. The AFM investigation was carried out with AFM model Enviroscope and controller Nanoscope V (Bruker). SPR studies were carried out using SPR refractometer Biosuplar 6 (Mivitec, Germany) in flat two-chamber thermostatic flow cell attached to a gilded surface of the chip.

Influenza viruses and antibodies to them was provided by NF Gamaleya FRCEM

3. RESULTS AND DISCUSSION

3.1. SYNTHESIS AND PROPERTIES OF THIN FILMS OF PANI

Electrochemical synthesis of PANi films was carried out in the presence of polyacids represented in Figure 1.

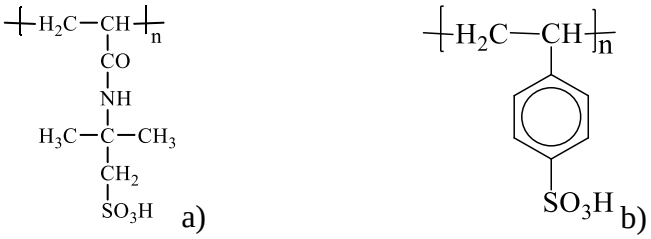

Fig. 1 The structures of the monomer unit PAMPSA (a) and PSSA (b).

The formation of ultra-thin PANi films in the presence of PAMPSA and PSSA, as well as the "standard" PANi films registered by growth of charge coming to the working electrode. It has been found that the synthesis of PANi proceeds faster in the presence of polymeric acids, despite a low concentration of reagents. This is a direct consequence of the association of aniline at polymer chain of these acids [1-3]. It has been found that the synthesis of PANi in the presence of PAMPSA runs a little slower than in the presence of PSSA. This may be due to high molecular weight of PAMPSA and the need to its proper spatial orientation required for co-polymerization with polyaniline. However, almost the equal induction period (1.5-1.7 sec.) was observed for all types of acids. The set of these and other data obtained earlier [1,3], indicates that the film growth occur probably in the same kinetic conditions. It can be assumed that the properties of the films will vary and depend on the type of used acids. Indeed, Figure 2 shows that the morphology of the AFM films PANi-PAMPSA and PANi-PSSA are different. Film PANi-PAMPSA has 1.2 to 1.5 times a rougher surface compared to PANi-PSSA, with larger individual grains (globules).

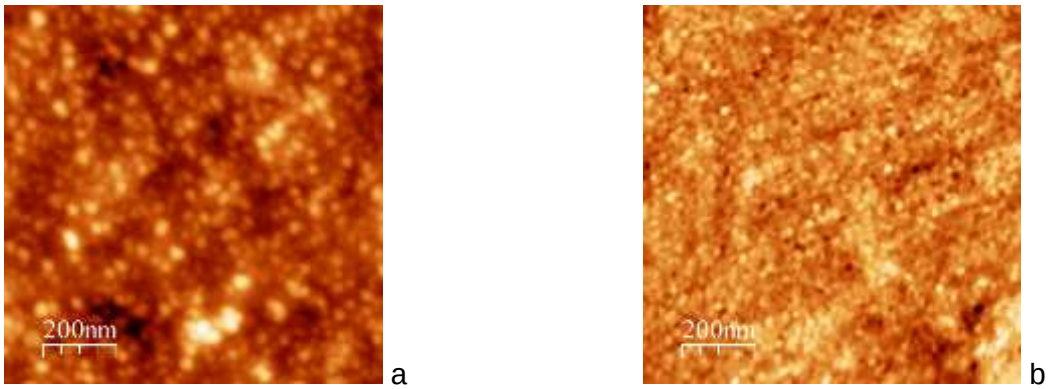

Fig. 2 AFM - investigation of PANi films on gilded SPR chips: a) PANi-PAMPSA (R_q=1,84 nm); b) PANi-PSSA (R_q=1,59 nm)

It was found that the SPR response of the films changes with pH (Fig.3). This dependence has characteristic S-shape during step change of pH. This relationship was more smooth without the characteristic inflection point for the PANi-HCl film. This behavior appears, occurring due to structural changes in the films. Forward and reverse pH dependency was not the same usually. Hysteresis was

amplified with increasing of film thickness, and decreasing with the exposure time at each pH value. However, the position of the inflection point in the curves of SPR responses during increase of the pH hardly changed if the exposure time at each point exceeded 10 minutes. The most dramatic changes in the properties of PANi-PAMPSA and PANi-PSSA films were observed at pH near 6.5.

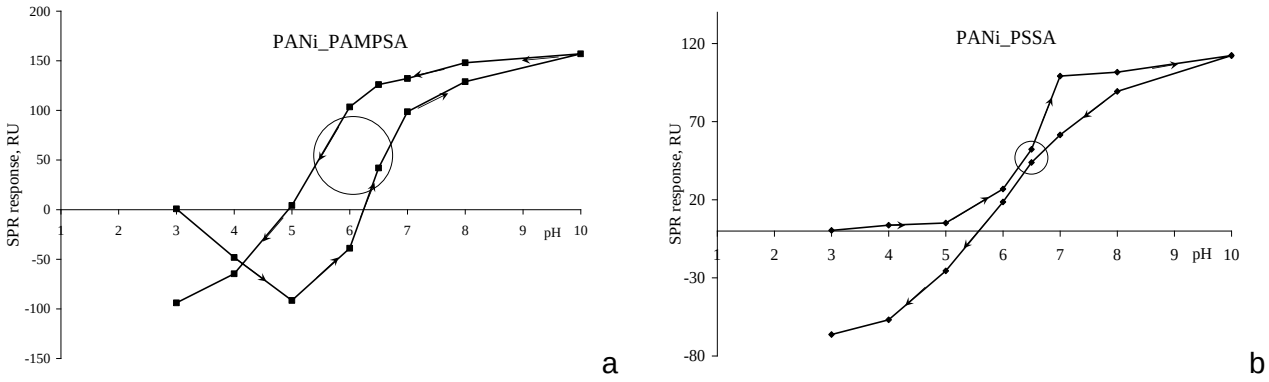

Fig. 3 Changes in SPR signal of PANi films depending on the solution pH. a)PANi-PAMPSA, b) PANi-PSSA.

The obtained films were subsequently used to study the adsorption of biological objects, such as viruses and antibodies to them.

3.2. A study of adsorption of biological objects in the PANi films by surface plasmon resonance

We have previously found that the powders of PANi and its complexes actively interact with influenza viruses [4]. Therefore, we investigated the adsorption of influenza viruses and antibodies to them on thin films of PANi and their complexes by SPR technique. Fig. 4 shows the kinetics of adsorption of the influenza virus A/Texas/50/2012(H3N2) depending on the haemagglutinating titre in solution.

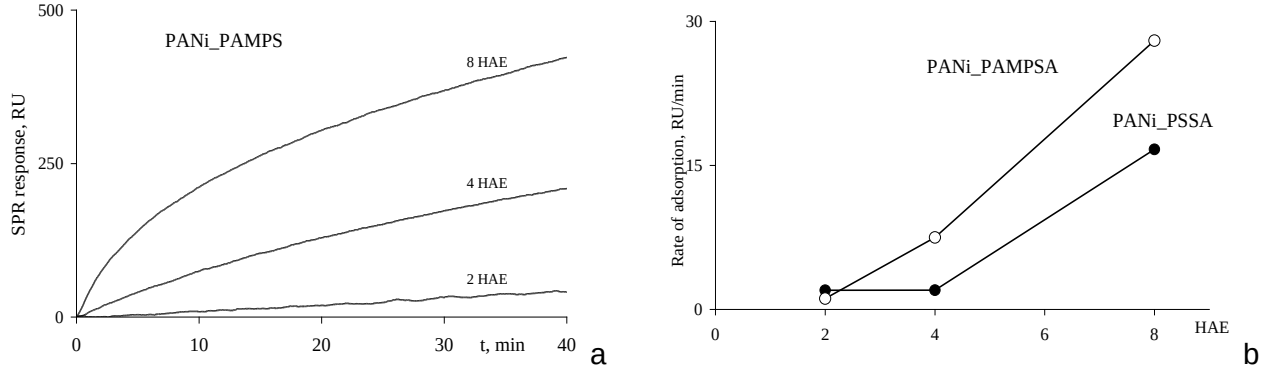

Fig. 4 The kinetics of SPR response growth (a) and the change in the initial rate of adsorption (b) depending on the haemagglutinating titre (HAE) of influenza virus A/Texas/50/2012(H3N2)

For low concentrations of influenza virus (2 HAE) adsorption rate did not depend on the type of PANi film. At higher concentrations of virus adsorption proceeded faster at the films of PANi-PAMPSA. One can offer some explanation for such a high affinity for these films to influenza viruses. As follows from the structure of monomer units (Fig.1) PAMPSA contains carbonyl and amino groups, which are close to proteins in nature, and capable to make hydrogen bonds with them. In addition PANi-PAMPSA has more flexible and mobile structure that contributes to a more close contact with biological objects. Finally, the

PANi-PAMPSA film has a greater surface area, compared with PANi-PSSA (Fig. 2), which also improves ability to adsorb the virus particles.

In spite of significant absorption signals of influenza virus, we could not achieve full coverage of the films surface. As a result, antibodies where adsorbed not only on the viruses, but also on the free surface of the film. Accordingly, it can be registered adsorption of non-complementary antibodies. Attempts to escape surface layer that was not occupied by virus using BSA adsorbtion has not yet yielded positive results. Therefore, we examined another method of registration of antigen-antibody interactions, offering to do a sensitive probe on the basis of a layer of adsorbed antibodies. They showed high affinity for the surface of the polymer films. The prospect of the biosensors based on the films of PANi-PAMPSA with adsorbed antibodies to influenza virus is determined by high affinity of these polymers to proteins that leads to full coverage of the sensor surface by antibodies. As a result after the complete surface covering another proteins and other non-complementary antibodies do not bound to the probe surface and do not distort the results of the determination of antigen-antibody interactions with viral particles.

Figure 5 shows the kinetics of adsorption of antibodies to the virus A/Texas/50/2012(H3N2) on various polymer films. The speed of this process depended on the type of the film. For PANi-PAMPSA films the initial adsorption rate was 3.5 times higher than for PANi-HCl films. The reasons for this is apparently the same as for the adsorption of influenza virus (similarity of chemical nature of the modifying acid, mobility of its structure and surface roughness). For all types of film, adsorption of antibodies was virtually irreversible. Washing the cell with a buffer solution did not lead to appreciable desorption of the antibodies.

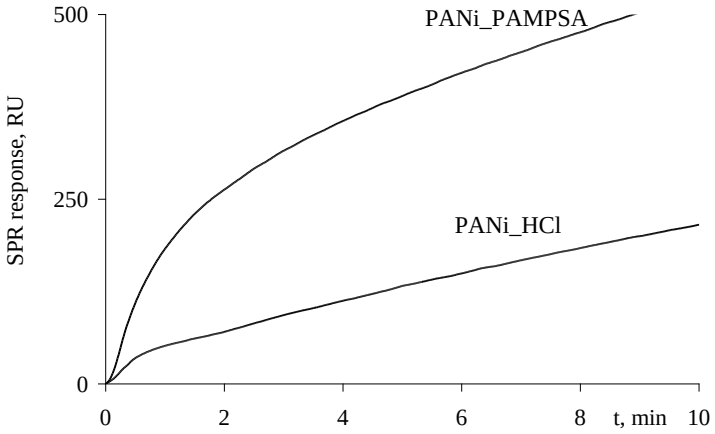

Fig. 5 Kinetics of adsorption of antibodies to the influenza A/Texas/50/2012(H3N2) virus on the films PANi-HCl (curve 1) and PANi-PAMPSA (curve 2). $C_{antibody}$= 0,65 mg/ml

The difference in the binding of different types of films with antibodies to influenza virus was demonstrated in a study of subsequent interaction with the virus. It turned out that after the introduction of the virus solution short adsorption growth a considerable desorption of the pre-adsorbed antibodies from PANi-HCl films was observed by SPR response (Fig. 6). Apparently, a comparatively weak bond with the surface and the strong interaction of viral particles with the antibodies resulted in desorption of immunocomplexes from the surface. Washing by buffer solution enhances this effect.

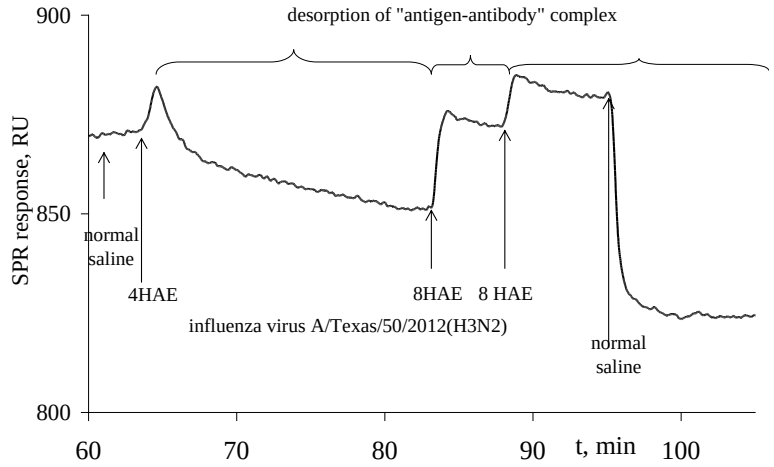

Fig. 6 Sensogram of interaction of influenza virus A/Texas/50/2012(H3N2) on SPR chips with PANi/antibody sensor

The formation of immune complexes on the surface of PANi-PAMPSA has not been accompanied by their desorption during the process of binding with viral particles and subsequent washing by the buffer solution due to a higher affinity of the polymer to the antibodies.

4. CONCLUSION

During the study, we have shown that films based on PANi are promising for creation of SPR sensors for influenza A viruses. The most convenient films are PANi-PAMPSA. Due to chemical nature, molecular structure and rugged surface they have the highest affinity to the antibodies of influenza A. However, a significant effect of pH on the SPR response of these films in the region of pH 6.5 requires controlling the solution acidity and the necessity to use buffer solutions.

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