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Effect of Nucleic Acids on Oxidation and Photoluminescence of Porous Silicon

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In this work, porous silicon surface was modified by aqueous solutions of nucleic acids and the effect of such modification on the porous silicon photoluminescence was studied. The treatment of porous silicon with the nucleic acid solutions was found to cause an increase in the photoluminescence intensity, the change being greater with DNA rather than RNA (homopolymer poly(A)). By means of infrared spectroscopy, it was found that the number of Si-O bonds at the silicon surface after treatment by nucleic acid solutions is much higher than that after treatment by distilled water. It is found that the porous silicon photoluminescence weakly depends on the concentration of the molecular oxygen in the DNA solution. At the same time, illumination of the DNA-treated porous silicon samples by the visible light enhances the porous silicon photoluminescence intensity. Nucleic acid stimulated increase in the porous silicon photoluminescence is attributed to thinning of the silicon skeleton, which, according to the quantum-size model of photoluminescence, leads to a radiative transition probability increase. The thinning could be related to enhancement of dissolution and, to a greater extent, to oxidation of porous silicon in aqueous solution by the nucleic acids. The effect of nucleic acids in aqueous solutions on the porous silicon modification was assumed to be twofold. Firstly, nucleic acids, being polyanions in aqueous solutions, can enhance the corrosion of porous silicon by water. Secondly, an increased concentration of reactive oxygen species is generated in aqueous solutions of nucleic acids under visible light illumination. The latter is supposed to be the main reason of porous silicon oxidation. The results of the work can be useful for the development of PS-based biosensors.

Keywords: Porous silicon, Photoluminescence, Oxidation, Nucleic acid, Biosensors, Reactive oxygen species.

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1. INTRODUCTION

Nowadays, porous silicon (PS) in its various modifications, including silicon nanoparticles, nanowires and thin films, is a promising material for biology and medicine due to its unique physical and chemical properties. Special attention is paid to the development of techniques allowing application of PS in therapy, *in vitro* and *in vivo* imaging and sensing [1-3].

Instability of PS properties, hindering its application in optoelectronics, turns into an advantage in living organisms, where the main role is played by metastable structures. Too stable structures, like gold nanoparticles, fullerenes and other nanomaterials, could be toxic and, therefore, potentially dangerous. Nanostructured silicon is characterized by a rather high and easily controlled rate of degradation in biological media, producing non-toxic products, which are easily removed from the body [4].

Currently developed techniques of PS formation allow to change its structure in a wide range. The features of geometry and architecture, as well as surface chemistry of PS, determine the way of its interaction with biological objects. In addition, this material is suitable for immobilization of the biologic objects due to the large specific surface area (up to 1000 m^2/cm^3) and possibility to form the pores with various configurations, while its optical and electrical properties, sensitive to these objects, make it possible to use PS as a material for biosensors [1, 3, 5, 6]. As a rule, PS based optical biosensors detect changes in either its refractive index due to the formation of different molecular complexes or its photoluminescence (PL). The effect of PS PL change was proposed to be used in enzyme and immune sensors [3]. The variation of PL intensity in such sensors is related mainly to a change in the number of silicon dangling bonds being the centers of non-radiative recombination in this material.

As for the PS-based DNA sensors, more attention is currently paid to the development of devices, in which the change in refractive index is monitored. However, it was found that the DNA-DNA hybridization, which is detected in such an interference biosensor, can both increase and decrease the refractive index. The increase is predicted and takes place due to rising refractive index of the material filling the porous matrix as a result of the reaction of the target DNA with the immobilized nucleic acid (NA) probe [1, 5]. The decrease was found to be caused by modification of the porous matrix itself because of the corrosion of silicon surface in the aquatic environment, which was greatly enhanced by NAs [6]. However, the suggested in [6] mechanism of the effect cannot explain some experimental facts, *e.g.*, formation of a significant amount of silicon oxide in the presence of NAs in comparison with the plain water [2].

It is important to note that PL of PS is also a sensitive indicator of silicon interaction with the aquatic environment and, in particular, aqueous solutions of NAs [2, 7]. Unlike the changes in refractive index, those in PL always occur in a predictable way.

In this work, we suggest to use PS PL to monitor the processes occurring in PS-NAs systems. We show that the oxidation in the PS treated by aqueous solutions is accelerated in the presence of DNA, weakly

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depending on the concentration of molecular oxygen in the solutions. The obtained results can be used not only for the improvement of the existing sensors, but also for the development of the new ones.

2. EXPERIMENTAL

The PS samples were fabricated on (100) KDB-10 (10 Ω·cm) silicon wafers by etching for 5 min in 1:1 solution of 48 % HF and ethanol at 5 mA/cm^2. Thereafter, the samples for more than a month were kept in air under natural light for their partial oxidation and stabilization of luminescent properties. It is worth noting that such samples, unlike as-prepared ones, reveal only a monotonous increase in the PL intensity with further interaction with ambient air and aquatic environment [7, 8].

As the nucleic acids, a calf thymus DNA ("Fluka", Switzerland) and polyadenine acid (poly(A)) ("Serva", Germany) were used. Distilled water was used as the solvent. The PS surface was treated by the solutions of the nucleic acid salts with a concentration of 1 g/l. In one of the experiments, a DNA solution additionally saturated with molecular oxygen by means of bubbling for 20 min was used as well. Before all the measurements, the samples treated by the solutions were dried for 6 h under room conditions. For comparison, some initial PS samples were studied after immersion in distilled water for 6 h or more and further drying for 30 min.

The PL of the samples was excited by 337 nm light, which is not absorbed by NAs. The spectra were measured at room temperature. IR spectroscopy was used for estimation of changes in the chemical composition of PS. IR spectra were measured by means of a Specord IR-80 spectrophotometer at room temperature in air in the spectral range of 400-4000 cm^{-1}.

3. RESULTS AND DISCUSSION

At the first stage, we compared the changes in the PL spectra of the PS, whose surface was treated with aqueous solutions of such NAs as DNA and RNA (poly(A)), as well as distilled water for comparison (Fig. 1). The increase in PL intensity of the PS after DNA solution application was more essential than that after the treatment with poly(A), whereas the changes after the water treatment were negligible.

An increase in PS PL intensity after NA solution application was reported in [2] to be caused by modification of the PS structure. Namely, as the silicon skeleton is dissolved and oxidized, the sizes of the nanocrystallites decrease. According to the quantum-size model, this increases the probability of radiative transitions [9]. At the same time, the number of non-radiative recombination centers remains almost unchanged [2]. So, the correlation between PS dissolution/oxidation and PL intensity allows one to use the latter as an indicator of PS interaction with the aquatic environment.

Analyzing the interaction of NA solutions with PS, which changes its properties and chemical composition, one should take into account the conformation features of the polynucleotide molecules and the presence of a hydration shell [10, 11]. It is worth noting that NA molecule strongly bonds water in its hydration shell and the contact between the PS and the DNA occurs predominantly through the water.

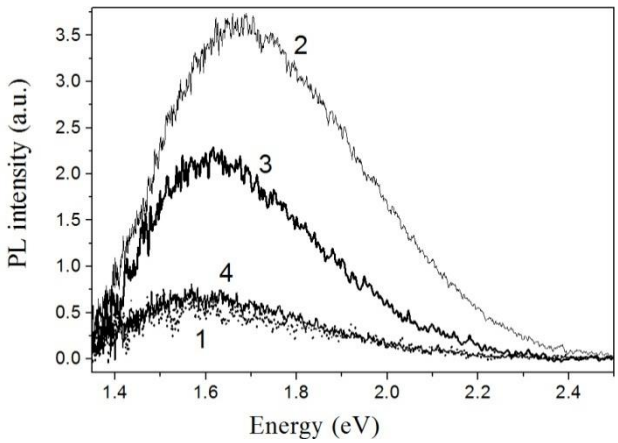

Fig. 1 – PL spectra of the initial PS (1) and the PS after the treatment in aqueous solutions of DNA (2), poly(A) (3) and distilled water (4)

We believe that the effect of NA on the process of PS structure modification can be twofold. Firstly, NAs, being polyanions in aqueous solutions, can enhance the corrosion of PS by water [2, 6]. As known, PS reacts with water only under the action of the electric field, which can appear in the presence of holes in PS and electrons outside, near the PS surface. Different kinds of polynucleotides, like single-stranded poly(A) and double-stranded DNA, have different densities of negative charges on the molecule surfaces [11]. Therefore, the silicon corrodes more intensely in the presence of double-stranded DNA molecules with a higher density of the negative charges. Secondly, such solutions can contain reactive oxygen species (ROS) which easily oxidize the PS surface.

The latter assumption is supported by the experiments on determining the chemical composition of the PS before and after treating it with DNA aqueous solution and distilled water. Because the change of silicon oxide amount on the PS surface is of interest, in Fig. 2

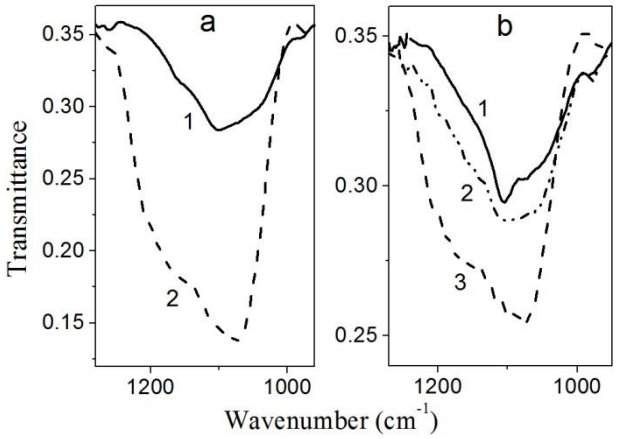

Fig. 2 – Si-O-Si- and SiO_x-related band in the IR spectra of: a) initial PS (1) and PS after the DNA application (2), and b) initial PS (1) and PS after the treatment with distilled water for 6 (2) and 24 (3) hours

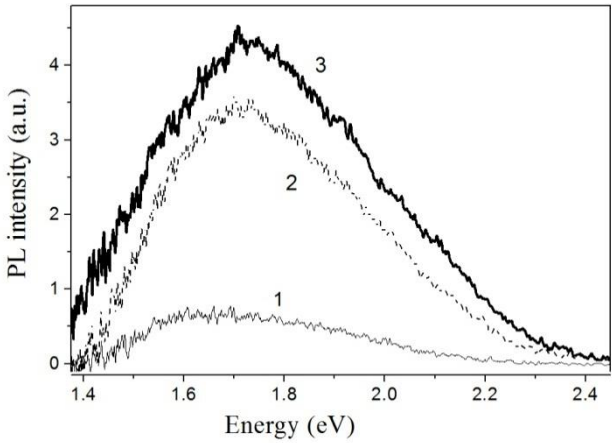

Fig. 3 – PL spectra of the initial PS (1) and PS after the treatment with DNA solution without (2) and with (3) the bubbling-enhanced amount of molecular oxygen

we illustrate the IR transmission spectra in the range of 1000-1300 cm^{-1}. One can see a wide absorption band related to a Si-O-Si mode and SiO_x groups [12].

The band intensity significantly (about threefold) increases 6 h after the DNA solution application (see Fig. 2a). At the same time, only a slight change in the Si-O bond amount is observed after the treatment of PS by water for 6 h (Fig. 2b). Even after 24 h of that kind of treatment the Si-O-Si-mode-related band intensity increases no more than twice. Further treatment of the PS sample in water causes no essential changes in the sample composition. It should be noted that such evolution of the PS chemical composition and structure is expectable for aqueous solutions with pH from 4 to 8.5, the difference being in the rate only [7]. The solutions in our experiments had *pH* about 7.

Thus, our studies show that a greater amount of Si-O bonds can be formed on the PS surface in the presence of NAs than in distilled water. This can be evidence of additional oxidation of the PS.

The modification of PS PL in DNA solutions with different concentrations of molecular oxygen is shown in Fig. 3. In this experiment, a conventional DNA solution was used as well as the one with the oxygen amount increased by bubbling. An increase in PS PL efficiency was observed in both cases. It was higher for the sample treated with the bubbled solution, but not so high as expected, taking into account the estimated twofold difference in the oxygen concentrations [13] (although the concentration could considerably decrease during the interaction with PS).

So, the concentration of dissolved O_2 weakly affected the interaction between PS and DNA solution, judging by the PL behavior. Indeed, O_2 is known to slightly react with the PS surface, however the silicon oxide is formed rather effectively in the presence of such ROS as singlet oxygen or hydrogen peroxide H_2O_2 [14, 15].

A lot of ROS are known nowadays. Moreover, some species of reactive oxygen can be transformed into other ones [13]. The singlet oxygen and hydrogen peroxide can be easily detected by special methods. Furthermore, H_2O_2 has a longer lifetime compared to other ROS. The effect of these forms of ROS is therefore the most studied.

The solutions of NAs may be the source of ROS in our experiments. Indeed, an increased concentration of H_2O_2 was found in the aqueous solutions of DNA compared to distilled water [16]. Moreover, it was shown by the four-photon scattering technique that the H_2O_2 amount in the hydration shell of NA molecules depended on their conformation and could differ several times. Such an effect can be caused by the fact that NAs promote the formation of ROS, in particular, H_2O_2 or by an increase in their lifetimes in the aquatic environment ROS [17]. The latter may be related to the formation of NA complexes with some kinds of ROS [18].

It is known that the ROS generation in media with NAs, as well as their oxidative modification, occurs under various factors including UV, visible, IR and radio frequency irradiation [13, 18, 19].

In this work, we also revealed the effect of visible light on ROS generation in the studied objects. Fig. 4 shows that natural daylight considerably enhances the PS reaction with NA solutions. Indeed, after the DNA solution treatment, the PS PL intensities increased 6 and only 2 times, respectively, for the samples kept under the daylight and in the dark.

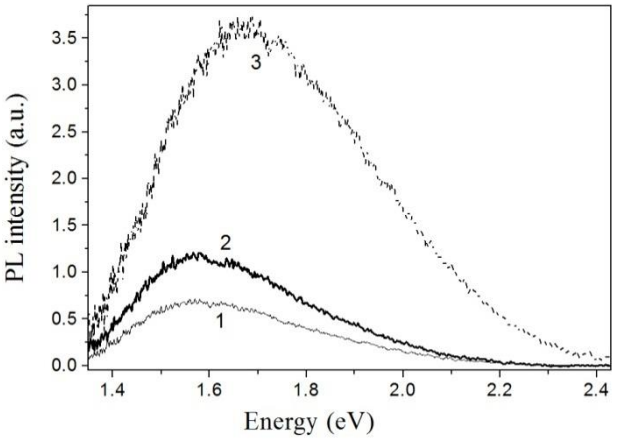

Fig. 4 – PL spectra of the initial PS (1) and PS stored for 6 h in the dark (2) and under daylight (3) after the DNA solution application

It should be noted that the ROS formed in the aquatic environment in the PS-NA system can oxidize both PS and NAs. Several workgroups (for example, [20]) have shown that the RNA is more susceptible to ROS-induced oxidative damage than the DNA. This may be a cause of the weaker oxidative effect of ROS on PS in the PS-RNA system compared to the PS-DNA one, resulting in a weaker modification of PS PL revealed in Fig. 1.

So, we found that the PL intensity of PS increases after its treatment with aqueous solutions of NAs. This effect is more pronounced for double-stranded DNA than for single-stranded poly(A). These results as well as the information on the effect of dissolved molecular oxygen and visible light on the interactions in PS-NA system can be taken into account in the development of the DNA-sensors. There is reason to believe that part of highly reactive ROS is spent on silicon oxidation, resulting in a possible antioxidative effect of PS on NAs. The obtained results can be useful in terms of application of PS PL to ROS detection in biological media and, particularly, NA solutions.

4. CONCLUSIONS

It is established that treatment of PS by aqueous solutions of polynucleotides enhances the PS PL, as well as the PL intensity is sensitive to the applied molecule conformation. It is found that a greater number of silicon-oxygen bonds at the PS surface is created in the NA solutions than in distilled water. These changes in the PS properties can be due to the enhancement of dissolution and, to a greater extent, due to oxidation of PS in the aqueous solution by nucleic acids. It is found that the concentration of molecular oxygen in the DNA solution weakly affects the PS PL. At the same time, modification of PS surface by NA solutions is significantly enhanced by illumination with the visible light. The obtained results allow to make the following suggestions concerning the mechanisms of NA effect on PS: enhancement of PS corrosion by water in the presence of NAs, which are polyanions, and stimulation of the PS oxidation due to an increase in the concentration of reactive oxygen species in NA aqueous solutions compared to distilled water under illumination by the visible light. DNAs exert a stronger influence on PS modification than poly(A). This can be related to a higher density of the negative charge on DNA molecule surface and, in turn, corrosion rate as well as a less susceptibility to the oxidation by ROS, resulting in enhanced oxidative effect of the active oxygen on PS.

The dissolution and oxidation cause thinning of the silicon skeleton and, therefore, size reduction of the nanocrystallites. As a result, an increase in the PS PL intensity after NA application is observed due to increased probability of the radiative transitions according to the quantum-size model of PS PL. The reported results could be useful for the development of PS-based sensors of DNA and application of the PS luminescent properties in the sensors designed for ROS identification.

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Вплив нуклеїнових кислот на окислення та фотолюмінесценцію поруватого кремнію

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В роботі здійснювалася модифікація поверхні поруватого кремнію водними розчинами нуклеїнових кислот та вивчався вплив такої модифікації на інтенсивність його фотолюмінесценції. Показано, що обробка поруватого кремнію водними розчинами нуклеїнових кислот призводить до зростання інтенсивності його фотолюмінесценції, причому для ДНК зміни є більшими ніж для РНК (гомополімер полі(А)). За допомогою інфрачервоної спектроскопії було виявлено, що в присутності нуклеїнових кислот на поверхні кремнію формується значно більша кількість зв'язків Si-O ніж в дистильованій воді. Встановлено, що концентрація молекулярного кисню в розчині ДНК слабо впливає на фотолюмінесценцію поруватого кремнію, в той час як опромінення оброблених розчинами ДНК зразків поруватого кремнію видимим світлом сприяє зростанню інтенсивності його фотолюмінесценції. Стимульований нуклеїновими кислотами ефект зростання фотолюмінесценції поруватого кремнію пояснюється потоншенням кремнієвого скелету, в результаті чого згідно з квантово-розмірною моделлю його фотолюмінесценції, ймовірність випромінювальних переходів зростає. Причиною цього може бути підсилення процесів розчинення і, особливо, окиснення кремнію в водному розчині нуклеїновими кислотами. Були запропоновані два шляхи впливу нуклеїнових кислот на зазначені процеси. По-перше, підсилення корозії поруватого кремнію поліаніонами, якими є нуклеїнові кислоти у водному розчині. По-друге, підвищена концентрація активних форм кисню в водних розчинах нуклеїнових кислот, генерація

яких відбувається під впливом видимого світла. Останнє вважається основною причиною окислення поруватого кремнію. Представлені результати роботи можуть бути корисними для створення біосенсорів на основі поруватого кремнію.

Ключові слова: Поруватий кремній, Фотолюмінесценція, Окислення, Нуклеїнова кислота, Біосенсор, Активні форми кисню.