INTERACTION OF TIO$_2$ NANOPARTICLES AND PROTEINS OF AN IMMUNE COMPLEX IN PHOTOLUMINESCENCE BASED BIOSENSOR

Interaction between nanostructured semiconductor thin layers and immune complex based proteins is a fundamental issue of the formation of nanobiointerface in the various biosensors, in particular, optical biosensors. In this work, the main aspects of the interaction between photoluminescent TiO$_2$ nanoparticles and Bovine leucosis virus (BLV) protein gp51, used as a model protein, during the formation of photoluminescence based immunosensor have been discussed. The antigens of gp51 were immobilized on the surface of a nanostructured TiO$_2$ thin film formed on the glass substrates. As a result, an increase of the photoluminescence (PL) signal intensity and PL peak shift from 517 nm to 499 nm were observed. An incubation of TiO$_2$/gp51 structure in a solution containing anti-gp51 antibodies resulted in the backward PL peak shift from 499 nm to 516 nm and decrease of the PL intensity. The main reason of the changes in the PL spectra (i.e. PL maxima shifts and PL intensity variations) as a result of BLV protein gp51 adsorption on the surface TiO$_2$ thin film is an electrostatic interaction between negatively charged surface of TiO$_2$ and positively charged atoms and groups provided by the adsorbed gp51 protein due to the presence of partial uncompensated charges within the proteins.

Introduction

Nanostructured Titanium dioxide (TiO$_2$) is a well-known material for biosensors application due to its good bio-compatibility and high chemical stability [1-4]. As a wide band gap semiconductor that has an intense photoluminescence (PL) at room temperature, TiO$_2$ is broadly applied in optical biosensors and immunosensors due to its affinity towards proteins [5,6]. Optical transduction is an attractive technique for biosensing due to its high sensitivity and specificity, applicability in real-time monitoring, capability for high throughput and simple sample pre-treatment. Both label-free and labelled determination of the target analyte are possible by TiO$_2$ based optical transducers [7]. Recently, immunosensors based on optical transducers e.g. photo-luminescence, absorbance, reflectance etc, are of great interest since they demonstrate simple, fast and accurate determination of the target analytes [6,7]. Among various optical immunosensors, the photo-luminescence-based sensors seem to be the most promising for the improvement in the diagnosis of virus induced diseases, such as Bovine leucosis that is a lethal cancerous disease caused by Bovine leukemia virus (BLV) [5,8]. Immunosensors are the class of biosensors that based on the reaction between antibody and antigen by formation of an immune complex (Fig. 1) [7]. Interaction between antigen-antibody couple is highly specific and selective one. The main advantage of the optical systems is that optical signal can detect the bio-molecular interaction contactless, i.e. without contamination or significant damage of the bio-samples. Besides, no additional labels (such as quantum dots or dyes) for the target analytes and no electrical contacts for measurements are required. However, despite of many reports on photoluminescence-based immunosensors, the interaction of biomolecules with semiconducting materials, which are used as analytical signal transducers in the most promising PL-based immunosensors, is poorly discussed [4-8]. In this research an optical immunosensor based on TiO$_2$ thin film which consisted of TiO$_2$ nanoparticles for the determination of Bovine Leucosis antibodies has been developed and the origin of the changes in the photoluminescent properties of TiO$_2$ nanoparticles as a result of the adsorption of Bovine leucosis proteins have been determined.
Experimental

The TiO\textsubscript{2} thin film consisted of TiO\textsubscript{2} nanoparticles (anatase, d~32 nm, purchased from Sigma Aldrich) was formed by sol-gel synthesis on the glass substrates. The details of the deposition procedure and structural characterization of TiO\textsubscript{2} thin film are described in some previous authors’ works [2,8].

It was found that immobilization of gp51 leukemia antigens on the surface of TiO\textsubscript{2} is accompanied by an increase of photoluminescence signal of the sample as well as the shift of the photoluminescence peak from 517 nm to 499 nm was observed after modification of the TiO\textsubscript{2} by adsorbed gp51 antigens (Fig. 2). Further interaction of immobilized gp51 antigens with gp51 antibodies resulted in reversed changes in TiO\textsubscript{2} photoluminescence spectra, i.e. a decrease in PL intensity and the backward PL peak shift from 499 nm to 516 nm. The sensitivity of the obtained immunosensor was in the range of 2-8 mg/ml (Fig. 2) [5,8].

The PL spectrum of TiO\textsubscript{2} nanoparticles, shown in figure 1a, is characterized by broad non-symmetric maximum around 500 nm, which can be splinted (using Origin 8.0 Pro) in two peaks, related to self-trapped exciton (STE) emission and luminescence caused by oxygen vacancies (V\textsubscript{O}) [9]. Bovine leucosis antigens gp51 were immobilized on the surface of a nanostructured TiO\textsubscript{2} thin film by direct adsorption similarly using the process described in [5,6,8,10]. In brief: a solution of PBS containing gp51 antigens at a high concentration was directly immobilized on the TiO\textsubscript{2} surface (Fig.1). Then the sample was placed into a Petri cup for the incubation in a medium saturated with water vapor at 25°C. After 10 minutes of incubation, the surface of the sample was washed with PBS solution in order to remove non-immobilized antigens on the TiO\textsubscript{2} surface. To prevent a nonspecific interaction (i.e., binding of anti-gp51 antibodies directly to unmodified TiO\textsubscript{2} surface), the surface of TiO\textsubscript{2} was further treated with a solution of bovine serum albumin (BSA) that filled possible adsorption sites that remained free after the modification of TiO\textsubscript{2} surface with gp51.

Results and Discussion

Interaction of proteins and semiconductor nanostructures can take place in a few possible mechanisms of interaction: charge transfer, electrostatic interaction, resonance energy transfer and some others [1]. Since Bovine Leucosis protein gp51 is not a redox protein, i.e. it cannot be involved in reduction-oxidation reactions therefore the charge transfer between gp51 antigens and TiO\textsubscript{2} nanoparticles is not possible [11]. Meanwhile, the proteins consist of amino acids that might contain positively and/or negatively charged radicals that are determining the charge of the different protein domains [12]. A large quantity of negatively charged groups such as aldehyde (-CHO), hydroxyl (-OH), carboxyl (-COOH) and primary amine (-NH\textsubscript{2}) and some other

![Figure 1. The scheme of photoluminescence immunosensor based on TiO2 nanoparticles.](image)

![Figure 2. Photoluminescence spectra of TiO2 nanoparticles before and after the immobilization of gp51 antigens on the TiO2 surface, subsequent BSA deposition and after the interaction of TiO2/gp51 based immunosensor after with analyte (anti-gp51 antibodies) of different concentrations.](image)
groups, involved into the structure of amino acids, are responsible for the partial (δ+ and δ-) charges of particular protein domains. Therefore the proteins are characterized by electrostatic properties, and sometimes even significant electrostatic ‘asymmetry of protein molecule’ because the atoms and functional groups forming the protein molecules are charged differently both in their sign and in absolute charge value. Naturally, the charges at least partly are compensating each other, but since the ternary structure of proteins is relatively rigid and the charged groups have only limited degree of freedom to move within the protein globule, therefore in some parts of the protein some uncompensated charge on the surface and inside of the protein still remains. It should be taken into account that even if the structure of the most proteins is at some extent ‘rigid’ there is some degree of flexibility because both secondary and tertiary structures of the protein are supported by a large number of hydrogen bonds but many of them are not very strong. The electrostatic bonds, which are based on Coulomb forces, between the opposite charges, van der Waals forces and disulfide bonds also play an important role in the formation of both secondary and tertiary structures of protein [13,15].

TiO$_2$ (anatase) is known as a semiconductor of n-type conductivity, usually with an ‘upward’ band bending of the energy levels when closing the surface of TiO$_2$, which indicates the accumulation of a negative charge (bound at surface levels) on its surface [12]. The adsorption of the most of molecules is known to introduce an additional charge on the solid state surface and it can change the existing surface energy levels or form the additional ones that are involved in the exchange of charges with the volume of a solid material [14]. The presence of the mentioned above partial charges "δ-" and "δ +" suggests that the electrostatic influence on the surface charge of TiO$_2$ from the side of partially uncompensated charges in those parts of the gp51 protein that located on the surface of TiO$_2$ is responsible for the adsorption of this protein on the TiO$_2$ surface. The Coulomb interaction takes place between charged groups in the gp51 protein and the negatively charged surface of the TiO$_2$ because such electrostatic interactions are very strong at small distances ranging from several Angstroms to few nanometers. Therefore, among the other interactions such as hydrogen bonds, disulfide bonds, Van der Waals interaction, etc, which also have significant role during the adsorption of proteins, the electrostatic interaction plays one of the most important role during the adsorption of proteins to electrically charged surfaces, such as TiO$_2$. In addition, the local electric fields of charged domains of adsorbed proteins are affecting the PL-centers of TiO$_2$ and it causes the shift in the photoluminescence spectra of TiO$_2$ nanoparticles (Fig. 3). Therefore, the photoluminescence maximum caused by STE shifts from 517 to 499 nm (i.e., to 18 nm), which corresponds to ~ 0.086 eV that is less than 0.1 eV, and it is one of the proofs of electrostatic interaction based physical adsorption of gp51 [10,13].

Further interaction of TiO$_2$/gp51 structure with anti-gp51, which is also a protein, led to the inverse changes in the photoluminescence spectra, i.e., to UV-shift of the spectrum and decrease the photoluminescence intensity to the value that corresponds to the pure TiO$_2$ (Fig.2). The latter effect is based on the formation an immune complex between immobilized antigens gp51 and anti-gp51 antibodies, which were present in aliquot. Formation of this immune complex, besides of the van der Waals interaction and other interactions, at a very high extent is based on the interaction between oppositely charged domains, functional groups and atoms in gp51 and anti-gp51 antibody molecules (including the formation of number of hydrogen bounds, which can be estimated as specific kind of electrostatic interaction). It
can be assumed that uncompensated charges ($\delta^+$ and $\delta^-$) of both proteins are involved in electrostatic interactions during the formation of immune complex (Fig. 3). As a result, some of the charged groups that were originally involved in the interaction between gp51 and TiO$_2$ are at least partially compensated by the opposite charge of the anti-gp51 protein groups, thereby reducing the direct electrostatic effect from immobilized gp51 proteins to the charged surface of TiO$_2$ and to light emitting centers. The effects described above have an effect on the shift of PL-maximum and on the decrease in the potential barrier on TiO$_2$/gp51 interface due to the charge-charge interaction between TiO$_2$ and gp51. The potential barrier at the interface between TiO$_2$ and gp51 has greater value in TiO$_2$/gp51 structure in comparison with that in TiO$_2$/gp51/anti-gp51 due to partial compensation (decrease in value) and/or delocalization of charges, which were initially involved into interaction between TiO$_2$ and gp51 after formation of TiO$_2$/gp51 structure [13,15].

The distribution of charges in TiO$_2$/gp51 structure can also be interpreted as a model based on an ‘imaginary flat capacitor’, formed as a result of the electrostatic interaction between oppositely charged protein gp51 layer and the TiO$_2$ surface (Fig. 4). The capacitor is formed as a result of protein gp51 adsorption on TiO$_2$ surface, after which the charges are distributed in energetically most favorable way, partially compensating each other. Consequently, the positive ‘imaginary capacitor plate’ is based on the positive charges, which are predominant in the protein gp51 area that after adsorption appears in close proximity to TiO$_2$/gp51 interface and/or due to the negative electrostatic effect of TiO$_2$ are induced/tracted closer to negatively charged surface. These charged atoms/groups/domains of gp51 that are localized in the close proximity to the TiO$_2$ surface and they electrostatically affect the TiO$_2$ emission centers and the energy value of the surface potential barrier. Hence, the position of the energy levels of the TiO$_2$ emission maximum depends on TiO$_2$ surface modification stage (TiO$_2$ or TiO$_2$/gp51) shifts from/ backwards the initial position of the demarcation level. Figure 4a demonstrates an imaginary flat capacitor consisting of a negatively charged plate on the surface of TiO$_2$ and an ‘imaginary positively charged plate’ formed in gp51 protein in close proximity to TiO$_2$/gp51 interphase. Hence, the interaction of TiO$_2$/gp51 with anti-gp51 antibodies and the formation of gp51/anti-gp51-based immune complex leads to a ‘deformation’ and the reduction of charge stored on ‘the positive imaginary capacitor plate’ (Fig. 4b).

This is mainly due to the redistribution and partial compensation of charges during the formation of the gp51/anti-gp51 immune complex, which in turn reduces the charge of ‘the imaginary capacitor plate’ based on gp51 ($q_1 < q_2$). Due to this reduced charge it can be interpreted as the reduction of the area of the same plate ($S_2$) and/or the increase of the distance ($d_2$) between the two imaginary capacitor plates based on gp51 and TiO$_2$ which leads to
the decrease of capacitance according to equation (1).

\[ C = \varepsilon \varepsilon_0 S/d \]  

This effect is observed because some of the gp51 protein charges move from the TiO\(_2\)/gp51 interface towards interacting anti-gp51 protein and are partially compensated by the charge present in anti-gp51, whereby an imaginary positive gp51-based capacitor plate of the capacitor is reduced in imaginary surface area and/or correspondingly moving apart from the negative TiO\(_2\) plate. This effect leads to a decrease in the capacitance of this imaginary capacitor and the electric field induced by gp51 becomes reduced. Therefore, after the interaction of TiO\(_2\)/gp51 with anti-gp51 antibodies and the formation of gp51/anti-gp51 complex, which is involved into TiO\(_2\)/gp51/anti-gp51 structure, the electrostatic effect of gp51 initially adsorbed on TiO\(_2\) towards the TiO\(_2\) surface significantly decreases. The PL shifts are attributed to the variations in the self-trapped exciton energy level, which were induced by the changes of electrostatic interaction between positively charged atoms and groups, provided by the adsorbed gp51 protein and negatively charged surface of TiO\(_2\). The charge–charge-based interaction in the double charged layers gp51/TiO\(_2\) can also be interpreted as a model based on ‘imaginary capacitor’, formed as a result of the electrostatic interaction between oppositely charged protein gp51 layer and the TiO\(_2\) surface. Development of advanced bioanalytical systems based on photoluminescence immunosensors seems to be very promising direction in the development of new biosensors.

**References:**

7. A. Tereshchenko, V. Fedorenko, V. Smyntyna, I. Konup, A. Konup, M. Eriksson, R.
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Summary.

It has been investigated an interaction between nanostructured semiconductor thin layers and immune complex based proteins that is a fundamental issue of the formation of nanobiointerface in the various biosensors, in particular, optical biosensors. In this work, the main aspects of the interaction between photoluminescent TiO$_2$ nanoparticles and Bovine Leucosis virus (BLV) protein gp51, used as a model protein, during the formation of photoluminescence based immunosensor have been discussed. The antigens of gp51 were immobilized on the surface of TiO$_2$ thin film formed on the glass substrates. As a result, an increase of the photoluminescence (PL) signal intensity and PL peak shift from 517 nm to 499 nm were observed. An incubation of TiO$_2$/gp51 structure in a solution containing anti-gp51 antibodies resulted in the backward PL peak shift from 499 nm to 516 nm and decrease of the PL intensity. The main reason of the changes in the PL spectra (i.e. PL maxima shifts and PL intensity variations) as a result of BLV protein gp51 adsorption on the surface TiO$_2$ thin film is an electrostatic interaction between negatively charged surface of TiO$_2$ and positively charged...
атоми та групи надані адсорбованим 
gp51 
протеїном завдяки присутності 
парцialsьких невкомпенсованих зарядів в середині протеїнів.

**Ключові слова:** TiO2 наночастинки, фотолюмінесценція, біосенсор.

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**ВЗАЄМОДІЯ НАНОЧАСТИНКОК ТіО₂ ТА БІЛКІВ ІМУННОГО КОМПЛЕКСУ У 
БІОСЕНСОРІ НА ОСНОВІ ФОТОЛЮМІНЕСЦЕНЦІЇ**

**Резюме.**

Досліджена взаємодія між тонкими наноструктурованими напівпровідниковими шарами та білками на основі імунних комплексів, яка є фундаментальним питанням формування нанобіоінтерфейсу в різних біосенсорах, зокрема, в оптичних біосенсорах. В роботі обговорено основні аспекти взаємодії фотолюмінесцентних наночастинок TiO2 з білком gp51 вірусу великого рогатої худоби (BLV), який використовується як модельний білок, під час формування імуносенсора на основі фотолюмінесценції. Антитіла gp51 були іммобілізовані на поверхні наноструктурованої тонкої плявки TiO2, сформованої на скляних підкладках. В результаті спостерігалося збільшення інтенсивності сигналу фотолюмінесценції (ФЛ) та спека піку ФЛ з 517 нм до 499 нм. Інкубація структури TiO2/gp51 у розчині, що містить антитіла до gp51, призвела до зсуву піку ФЛ у зворотному напрямку від 499 нм до 516 нм та зниження інтенсивності ФЛ. Основною причиною змін у спектрах ФЛ (тобто зсувів максимумів ФЛ та зміни інтенсивності ФЛ) в результаті адсорбції білка BLV gp51 на поверхні тонкої плявки TiO2 є електростатична взаємодія між негативно зарядженою поверхнею TiO2 та позитивно зарядженими атомами та групами адсорбованого білка gp51 через наявність часткових нескомпенсованих зарядів всередині білків.

**Ключові слова:** TiO2 наночастинки, фотолюмінесценція, біосенсор.

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**ВЗАЙМОДЕЙСТВИЕ НАНОЧАСТИЦ ТІО₂ И БЕЛКОВ ИММУННОГО 
КОМПЛЕКСА В БИОСЕНСОРЕ НА ОСНОВЕ ФОТОЛЮМИНЕСЦЕНЦИИ**

**Резюме.**

Исследовано взаимодействие между тонкими слоями наноструктурированных полупроводников и белками на основе иммунных комплексов, которое является фундаментальной проблемой формирования нанобиоинтерфейса в различных биосенсорах, в частности, оптических биосенсорах. В работе обсуждаются основные аспекты взаимодействия фотолюминесцентных наночастиц TiO2 с белком gp51 вируса лейкоза крупного рогатого скота (BLV), используемым в качестве модельного белка, при формировании иммуносенсора на основе фотолюминесценции. Антигены gp51 были иммобилизованы на поверхности наноструктурированной тонкой пленки TiO2, сформированной на стеклянных подложках. В результате наблюдалось увеличение интенсивности сигнала фотолюминесценции (ФЛ) и сдвиг пика ФЛ с 517 нм до 499 нм. Инкубация структуры TiO2/gp51 в растворе, содержащем антитела против gp51, приводила к обратному свижу пика ФЛ с 499 нм до 516 нм и снижению интенсивности ФЛ. Основной причиной изменений спектров ФЛ (т.е. сдвигов максимумов и вариаций интенсивности ФЛ) в результате адсорбции белка gp51 BLV на поверхности тонкой
пленке TiO$_2$ является электростатическое взаимодействие между отрицательно заряженной поверхностью TiO$_2$ и положительно заряженными атомами и группами адсорбированного белка gp51 из-за наличия частичных нескомпенсированных зарядов внутри белков.

Ключевые слова: TiO$_2$ наночастицы, фотолюминесценция, биосенсор.

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