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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_2 thin film consisted of TiO_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_2 thin film are described in some previous authors' works [2,8].

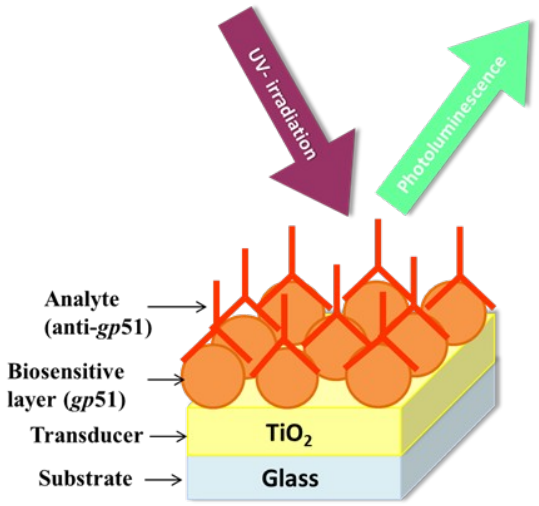

Figure 1. The scheme of photoluminescence immunosensor based on TiO2 nanoparticles.

The PL spectrum of TiO_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 ($V_{[O]}$) [9]. Bovine leucosis antigens *gp*51 were immobilized on the surface of a nanostructured TiO_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_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_2 surface. To prevent a nonspecific interaction (i.e., binding of anti-gp51 antibodies directly to unmodified TiO_2 surface), the surface of TiO_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_2 surface with *gp*51.

It was found that immobilization of *gp*51 leukemia antigens on the surface of TiO_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_2 by adsorbed *gp*51 antigens (Fig. 2). Further interaction of immobilized *gp*51 antigens with *gp*51 antibodies resulted in reversed changes in TiO2 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].

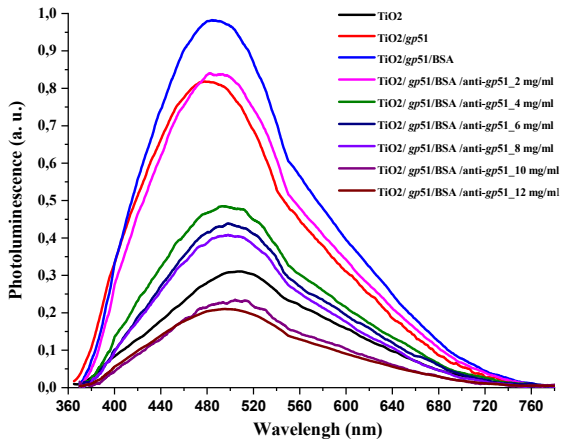

Figure 2. Photoluminescence spectra of TiO_2 nanoparticles before and after the immobilization of *gp*51 antigens on the TiO_2 surface, subsequent BSA deposition and after the interaction of TiO_2/*gp*51 based immunosensor after with analyte (anti-*gp*51 antibodies) of different concentrations.

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 *gp*51 is not a redox protein, i.e. it cannot be involved in reduction-oxidation reactions therefore the charge transfer between *gp*51 antigens and TiO_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 ($-NH_2$) and some other

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 *gp*51 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 *gp*51 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 *gp*51 [10,13].

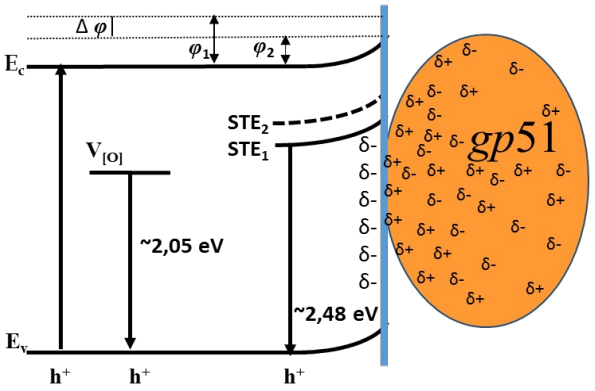

Figure 3. Energetic levels of TiO_2/*gp*51 immunosensing structure.

Further interaction of TiO_2/*gp*51 structure with anti-*gp*51, 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 *gp*51 and anti-*gp*51 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 *gp*51 and anti-*gp*51 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 (δ+ and δ-) 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 *gp*51 and TiO_2 are at least partially compensated by the opposite charge of the anti-*gp*51 protein groups, thereby reducing the direct electrostatic effect from immobilized *gp*51 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/*gp*51 interface due to the charge-charge interaction between TiO_2 and *gp*51. The potential barrier at the interface between TiO_2 and *gp*51 has greater value in TiO_2/*gp*51 structure in comparison with that in TiO_2/*gp*51/anti-*gp*51 due to partial compensation (decrease in value) and/or delocalization of charges, which were initially involved into interaction between TiO_2 and *gp*51 after formation of TiO_2/*gp*51 structure [13,15].

The distribution of charges in TiO_2/*gp*51 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 *gp*51 layer and the TiO_2 surface (Fig. 4). The capacitor is formed as a result of protein *gp*51 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 *gp*51 area that after adsorption appears in close proximity to TiO_2/*gp*51 interface and/or due to the negative electrostatic effect of TiO_2 are induced/attracted closer to negatively charged surface. These charged atoms/groups/domains of *gp*51 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/*gp*51) 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 *gp*51 protein in close proximity to TiO_2/*gp*51 interphase. Hence, the interaction of TiO_2/*gp*51 with anti-*gp*51 antibodies and the formation of *gp*51/anti-*gp*51-based immune complex leads to a 'deformation' and the reduction of charge 'stored' on 'the positive imaginary capacitor plate' (Fig. 4b).

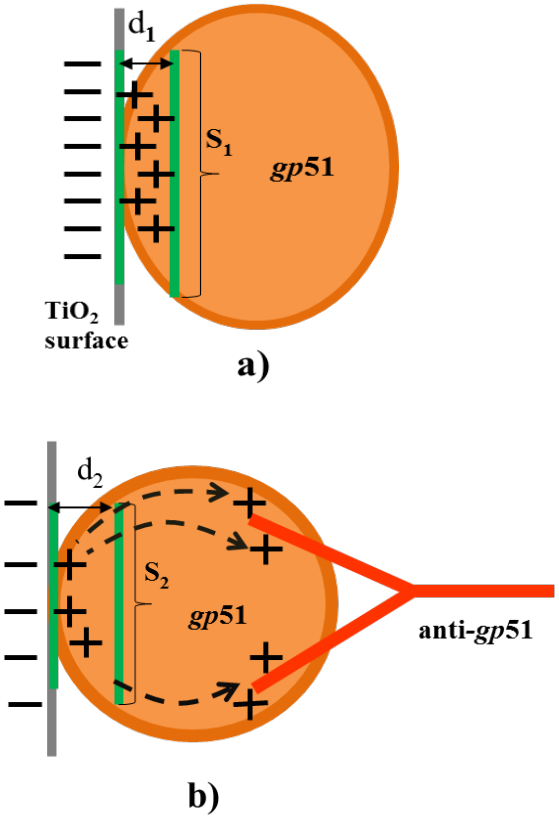

Figure 4. Flat capacitor based model of the charges interaction between TiO_2 surface and *gp*51 proteins: a) electrostatic interaction of partial uncompensated charges of immobilized *gp*51 antigens with negative charges located on the surface of TiO_2; b) model of interaction that takes into account the electrostatic interaction of charges within *gp*51 antigens and anti-*gp*51 antibodies of BLV immune complex.

This is mainly due to the redistribution and partial compensation of charges during the formation of the *gp*51/anti-*gp*51 immune complex, which in turn reduces the charge of 'the imaginary capacitor plate' based on *gp*51 ($q_2 < q_1$). 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 *gp*51 and TiO_2 which leads to

the decrease of capacitance according to equation (1).

$$C = \varepsilon\varepsilon_0 S/d \quad (1)$$

This effect is observed because some of the *gp*51 protein charges move from the TiO_2/*gp*51 interface towards interacting anti-*gp*51 protein and are partially compensated by the charge present in anti-*gp*51, whereby an imaginary positive *gp*51-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 *gp*51 becomes reduced. Therefore, after the interaction of TiO_2/*gp*51 with anti-*gp*51 antibodies and the formation of *gp*51/anti-*gp*51 complex, which is involved into TiO_2/*gp*51/anti-*gp*51 structure, the electrostatic effect of *gp*51 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 *gp*51 protein and negatively charged surface of TiO_2 [10,13,15].

Conclusions

The main aspects of the interaction mechanism between nanostructured TiO_2 layer and BLV proteins *gp*51 have been evaluated during the formation of photoluminescence-based immunosensor. Bovine leucosis protein *gp*51 was adsorbed on the surface of a nanostructured TiO_2 thin film, formed on glass substrates. A photoluminescence (PL) peak shift from 517 nm to 499 nm was observed after modification of the TiO_2 by adsorbed *gp*51 (i.e. formation of the biosensitive layer *gp*51/TiO_2). An incubation of *gp*51/TiO_2 in a solution containing anti-*gp*51 resulted in the formation of a new structure (anti-*gp*51/gp51/TiO_2) and the backward PL peak shift from 499 nm to 516 nm. 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 *gp*51 protein and negatively charged surface of TiO_2. The charge–charge-based interaction in the double charged layers *gp*51/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 *gp*51 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.

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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 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.

Key words: TiO_2 nanoparticles, photoluminescence, biosensor.

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А. Терещенко, В. Сминтина

ВЗАЄМОДІЯ НАНОЧАСТИНОК TIO_2 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 TiO_2 з білком *gp*51 вірусу лейкозу великої рогатої худоби (BLV), який використовується як модельний білок, під час формування імуносенсора на основі фотолюмінесценції. Антигени *gp*51 були іммобілізовані на поверхні наноструктурованої тонкої плівки TiO_2, сформованої на скляних підкладках. В результаті спостерігалося збільшення інтенсивності сигналу фотолюмінесценції (ФЛ) та зсув піку ФЛ з 517 нм до 499 нм. Інкубація структури TiO_2/*gp*51 у розчині, що містить антитіла до *gp*51, 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 *gp*51 на поверхні тонкої плівки TiO_2 є електростатична взаємодія між негативно зарядженою поверхнею TiO_2 та позитивно зарядженими атомами та групами адсорбованого білка *gp*51 через наявність часткових нескомпенсованих зарядів всередині білків.

Ключові слова: TiO_2 наночастинки, фотолюмінесценція, біосенсор.

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А. Терещенко, В. Смынтына

ВЗАИМОДЕЙСТВИЕ НАНОЧАСТИЦ TIO_2 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 TiO_2 с белком *gp*51 вируса лейкоза крупного рогатого скота (BLV), используемым в качестве модельного белка, при формировании иммуносенсора на основе фотолюминесценции. Антигены *gp*51 были иммобилизованы на поверхности наноструктурированной тонкой пленки TiO_2, сформированной на стеклянных подложках. В результате наблюдалось увеличение интенсивности сигнала фотолюминесценции (ФЛ) и сдвиг пика ФЛ с 517 нм до 499 нм. Инкубация структуры TiO_2/*gp*51 в растворе, содержащем антитела против *gp*51, приводила к обратному сдвигу 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 *gp*51 BLV на поверхностной тонкой

пленке TiO_2 является электростатическое взаимодействие между отрицательно заряженной поверхностью TiO_2 и положительно заряженными атомами и группами адсорбированного белка *gp*51 из-за наличия частичных нескомпенсированных зарядов внутри белков.

Ключевые слова: TiO_2 наночастинки, фотолюминесценция, биосенсор.

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