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Study of quantum dots conjugation with antibodies to be used in a lateral flow immunochromatographic assay

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Abstract. Lateral-flow immunochromatographic assays (LFIA) is one of the most common low-cost, simple-to-use and rapid methods for point-of-care determining various types of bacterial, viral and autoimmune diseases. The selectivity and sensitivity of the analysis is determined by the complementarity interactions of protein molecules and attaching an analytical label to the protein. Recently, quantum dots (QDs) have been considered the most promising protein labels. In this study, oriented conjugation of QDs with antibodies (Abs) to glycoprotein B (gB) of the Aujeszky's disease was conducted by the carbodiimide-succinimide method. There has been proposed a polymer coating of QDs suitable for site-specific conjugation based on the vinylpyrrolidone-maleic anhydride-ethylene glycol dimethacrylate (VP-MAN-EGDMA) copolymer. Using a portable fluorescent reader the minimum detection limits of the considered immunochromatographic test-systems with QDs as Abs labels were determined.

Keywords: bioconjugation, immunochromatographic assay, quantum dots, antibodies

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Материалы конференции
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Исследование процесса конъюгации антител с квантовыми точками для создания иммунохроматографических тест-систем на их основе

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Аннотация. Иммунохроматографический анализ (ИХА) является одним из наиболее распространенных экспресс-методов для определения различных видов бактериальных, вирусных и аутоиммунных заболеваний. Селективность анализа определяется комплементарностью взаимодействий белковых молекул, а чувствительность анализа достигается присоединением аналитической метки к белку. В последнее время к наиболее перспективным меткам белков относят квантовые точки (КТ). В данной работе была проведена ориентированная конъюгация КТ с антителами (АТ) к гликопротеину B (gB) вируса болезни Ауески карбодиимид-сукцинимидным методом. Предложено подходящее для ориентированной конъюгации полимерное покрытие КТ на основе сополимера

винилпирролидон – малеиновый ангидрид – этиленгликольдиметакрилат (ВП-МАН-ЭГДМА). С помощью портативного флуоресцентного ридера были определены минимальные пределы обнаружения рассматриваемых иммунохроматографических тест-систем с КТ в качестве меток АТ.

Ключевые слова: биоконъюгация, иммунохроматографический анализ, квантовые точки, антитела

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Introduction

LFIA is an immunochemical method for the qualitative and quantitative identification of viral diseases. The widespread use of LFIA is due to the fact that this method is express (analysis time is 10–30 minutes), reproducible, requires the use of a minimum number of instruments and devices, and is suitable for point-of-care screening of infectious diseases. Currently, one of the most promising fields is the use of colloidal QDs as analytical labels for protein molecules. QDs have a number of unique properties, including a narrow symmetrical fluorescence peak, high fluorescence brightness, a wide excitation band, and high photostability. Due to these properties, it is possible to reduce detection limits and increase the sensitivity of test systems. The surface of the label and protein, as well as the method of conjugation are important parameters affecting the result of conjugation of protein molecules.

To ensure the spatial accessibility of antigen-binding fragments, it is preferable that Fab regions of Ab do not participate in the conjugation process. Some of the protocols used for the functionalization of nanoparticles (NPs) are based on the attachment of Abs to the surface in a random orientation, which leads to a partial loss of sensitivity and increase detection limits of test-systems [1]. Thus, the development of strategies of site-specific conjugation is a key challenge to overcome the reduction of randomly immobilized Abs.

Two-stage approaches to oriented conjugation which consists in a combination of electrostatic adsorption and covalent bonding are promising. Since electrostatic adsorption is a multipoint interaction, the orientation of Abs will depend on the number of positively or negatively charged regions on the surface of the protein molecule. A region with a higher density of charged residues will have a higher adsorption rate and, as a result, Abs will be attached to QDs predominantly through it, providing the optimal orientation on the surface.

Materials and Methods

CdSe/CdS/CdS/ZnS QDs, coated with vinylpyrrolidone-maleic anhydride-ethylene glycol dimethacrylate copolymer (VP-MAN-EGDMA), monoclonal Abs ADV 34/2 gB of the Aujeszky's disease (Institute of Cell Biophysics of the Russian Academy of Sciences, Pushchino, Russia), phosphate-buffered saline (PBS) tablets (for biological research, Sigma Aldrich), 2-(N-morpholino)ethanesulfonic acid (MES) monohydrate (≥ 99.0%, Sigma-Aldrich), N-Hydroxysuccinimide (NHS, ≥ 97%, Sigma-Aldrich), Dimethyl sulfoxide $(CH3)_2SO$ (99.7%, SigmaAldrich), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, ≥98%, Sigma Aldrich), tris(hydroxymethyl)aminomethane $(HOCH_2)_3CNH_2$ (≥ 99.9%, Trizma base, Sigma-Aldrich), bovine serum albumin (Sigma-Aldrich), Superose 6 Prep Grade (Sigma-Aldrich), anti-mouse monoclonal antibodies (Institute of Cell Biophysics of the Russian Academy of Sciences, Pushchino, Russia), deionized water.

Microcentrifuge Eppendorf 5425, magnetic stirrer IKA C-MAG HS 7, analytical balance CAUX-120 (weighing limit 120 g), polyethersulfone (PES) syringe filters with 0.22 μm pores, Amicon ultra-0.5 mL centrifugal filters (Merck, standard deviation - 0.1 mg), spectrophotometer UNICO-2100/2800 (wavelength range: 190–1100 nm), spectrofluorimeter FluoroLog 3 model FL3–21, Zetasizer Nano S Size Analyzer (Malvern Panalytical).

Conjugation of QDs with Abs

The conjugation of QDs with Abs is conducted in a 0.01 M PBS solution with pH = 7.4 with a molar ratio of QDs:Abs 1:10. To activate carboxyl groups on the surface of QDs, a 0.1 M EDC solution (at a molar ratio of QDs:EDC 1:2000) and a 0.1 M solution of NHS (at a molar ratio of QDs:NHS 1:1000) are added to the QDs and left under constant stirring for 30 minutes. After activation of carboxyl groups, the calculated amount of Abs is added to the reaction mixture and left for 1.5 hours in the dark with constant stirring. After completion of the conjugation process, the free NHS-ester groups are blocked with a blocker solution in an amount of 1/10 of the total volume of the conjugate for 30 minutes. The resulting conjugates are purified from free components using gel-filtration chromatography.

Oriented conjugation of QDs with Abs

The conjugation of QDs with Abs is conducted in a 10 mM MES solution with pH = 5 with a molar ratio of QDs:Abs 1:10. To activate carboxyl groups on the surface of QDs, a 0.1 M EDC solution (at a molar ratio of QDs:EDC 1:2000) and a 0.1 M solution of NHS (at a molar ratio of QDs:NHS 1:1000) is added to the QDs and leave under constant stirring for 30 minutes. Abs are incubated in 10 mM MES pH = 5 with constant stirring for 30 minutes at room temperature. After activation of the carboxyl groups, the QDs are purified from excess activating agents by ultrafiltration. Then, the calculate amount of purified Abs is adding to the reaction mixture and leave for 1.5 hours in the dark with constant stirring. After completion of the conjugation process, a blocker solution is added to the reaction mixture in an amount of 1/10 of the total volume of the conjugate and leave for 30 minutes with constant stirring. The resulting conjugates are transferred into a 0.01 M PBS solution, then purified from the components of the reaction mixture using gel-filtration chromatography.

Results and Discussion

To use QDs as analytical labels in LFIA, it is necessary to conjugate them with analyte-specific protein molecules. Immobilization of Abs by antigen-binding fragments on the QDs surface leads to a decrease in sensitivity and an increase in the detection limits of immunochromatographic test-systems. Currently, have been developed various approaches for the site-specific conjugation of NPs with biomolecules. They include the selective interaction of a crystallizing antibody fragment with proteins A, G, and A/G [2, 3], binding through sulfhydryl groups formed during the reduction of antibodies [4], aldehyde groups [5], and histidine labels [6] located in the constant region of Abs.

The binding of Abs to the A/G protein does not require chemical modification of Abs, but it is due to physical adsorption, which leads to low stability of the NP-Abs complex. This approach is also costly. The other methods described above are labor intensive as they require the chemical modification of the Abs for the conjugation.

The most promising are site-specific covalent binding approaches that do not require chemical modification of Abs. These methods include the two-stage immobilization of biomolecules through electrostatic interactions and the subsequent formation of a covalent bond [7, 8].

In this study, oriented conjugation of CdTe/CdSe/CdS/ZnS-VP-MAN-EGDMA QDs with Abs to gB of the Aujeszky's disease was conducted by using the carbodiimide-succinimide method.

The absorption and luminescence spectra of CdTe/CdSe/CdS/ZnS-VP-MAN-EGDMA QDs and their conjugates with antibodies to gB of the Aujeszky's disease shows in Fig. 1. The peak in the absorption spectrum of QDs and conjugates in the region of 470 nm match to the formation energy of an electron-hole pair (exciton). After immobilization of Abs on the QD surface, the intensity of the exciton peak decreases, which indicates the formation of conjugates. The maximum luminescence intensity is at a wavelength of 680 nm. Abs immobilized on the QD surface screen the secondary radiation, which leads to a decrease in the luminescence intensity. After performing oriented

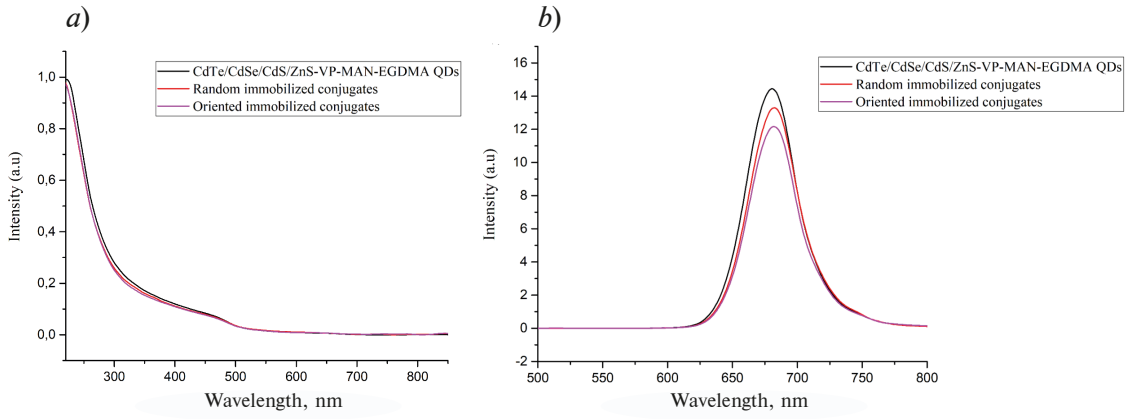

Fig. 1. Absorption (*a*) and luminescence (*b*) spectra of QDs and their conjugates

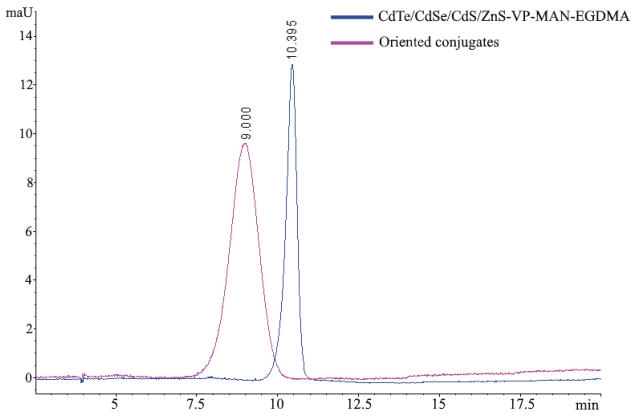

Fig. 2. Overlay of electrophoregrams of QDs and their conjugates. Analysis conditions: detection wavelength 220 nm, voltage +30 kV, supporting electrolyte 2 mM PBS pH = 7

conjugation based on fast electrostatic interactions, Abs are immobilized in a flat orientation, which leads to a larger area of QD coverage, and hence a larger reduction in quantum yield. However, with this conjugation approach, the number of antigen-binding fragments available for binding increases, which leads to a decrease in the detection limits of immunochromatographic test systems.

After conjugation of Abs with QDs, the resulting conjugates are purified from unbound components by gel chromatography. The purity of conjugates was determined by capillary zone electrophoresis. Electrophoregrams of CdTe/CdSe/CdSe/ZnS-VP-MAN-EGDMA QDs and their conjugates are shown in Fig. 2.

The migration time of QDs is 10.4 minutes, and that of their conjugates is 9 minutes, which evidence an increase in the hydrodynamic size and surface charge of QDs after conjugation due to the immobilization of protein molecules. The electrophoretic zone is evidence for the homogeneity and purity of the conjugates.

Dynamic light scattering (DLS) was used to determine the sizes of QDs and their conjugates. The QDs size was 67.8 nm, random immobilized, oriented immobilized conjugates 71 nm and 70.6 nm, respectively.

The orientation of immobilized protein molecules can be estimated by the minimum detection limit of test-systems created on their basis. Conjugates with optimal orientation are capable of interacting with a large number of antigens, which leads to an increase in the number of QD-Ab-antigen complexes in the test zone of the test-strip. It is possible to quantify antigens in the analyzed sample using a fluorescent reader. When studying the intensity of the analytical fluorescence signal on the test zone of the test strip, it was found that the minimum limit of antigen detection in the sample was 0.8 μg/ml.

Conclusion

There were study the optical properties of QDs of composition CdTe/CdSe/CdS/ZnS-VP-MAN-EGDMA and their conjugates with Abs to gB of the of the Aujeszky's disease. As a result of site-specific conjugation of protein molecules, the fluorescence intensity of the conjugate decreases compared to QDs, however, the spatial availability of sites for antigen binding leads to a decrease in the detection limits of immunochromatographic test systems.

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