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Obtaining a phototoxic complex based on silver nanoparticles and riboflavin generating reactive oxygen species

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Abstract. As an alternative to antibiotics, antimicrobial photodynamic therapy method leads to the treatment on microorganisms and does not cause the development of resistance. This work demonstrates the use of flavin mononucleotide (FMN), which acts as a photosensitizer capable of generating singlet oxygen and other reactive oxygen species, that have a phototoxic effect to bacteria. The effectiveness of its action increases in the case of the use of silver nanoparticles with antibacterial activity. This study presents the development of approaches for the controlled increase in the effectiveness of antimicrobial photodynamic therapy using FMN-silver nanoparticle complexes. The formation of the complex is accompanied by the generation of radicals upon 365 nm irradiation, that has extreme dependence and also leads to a change in the fluorescence kinetics. The introduction of triethanolamine activator to FMN into the system leads to the generation of radicals. The method of synthesis of silver nanoparticles also significantly affects the optical properties of the formed complex.

Keywords: reactive oxygen species (ROS), DPPH, riboflavin, triethanolamine, AgNPs

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Получение фототоксического комплекса на основе наночастиц серебра и рибофлавина, генерирующего активные формы кислорода

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Аннотация. В качестве альтернативы антибиотикам используется метод антимикробной фотодинамической терапии, приводящий к воздействию на микроорганизмы и не вызывающий развития резистентности. Эта работа демонстрирует использование флавинмононуклеотида (ФМН), который действует как фотосенсибилизатор,

способный генерировать синглетный кислород и другие активные формы кислорода, которые оказывают фототоксическое действие на бактерии. Эффективность его действия повышается в случае использования наночастиц серебра, обладающих антибактериальной активностью. В данном исследовании представлена разработка подходов для контролируемого повышения эффективности антимикробной фотодинамической терапии с использованием комплексов ФМН-наночастиц серебра. Образование комплекса сопровождается имеющей экстремальную зависимость генерацией радикалов при облучении с длиной волны 365 нм, и также приводит к изменению кинетики флуоресценции. Введение в систему активатора триэтаноламина к ФМН приводит к образованию радикалов. Способ синтеза наночастиц серебра также существенно влияет на оптические свойства образующегося комплекса.

Ключевые слова: активные формы кислорода, ДФПГ, рибофлавин, триэтаноламин, наночастицы серебра, генерация радикалов

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Introduction

A promising alternative to antibiotics is antimicrobial photodynamic therapy based on the generation of reactive oxygen species (ROS) upon irradiation of a photosensitizer. ROS destroy biomolecules and lead to the death of microorganisms. The literature demonstrates the successful use of flavin mononucleotide (a derivative of vitamin B2), which is a strong oxidizing agent in the triplet state (after irradiation) and can also act as a photosensitizer capable of generating singlet oxygen. It is known that the efficiency of ROS action increases significantly when used in the form of hybrid organic-inorganic nanoparticles based on metals and metal oxides [1]. Creation of nanoconstructions that combine the antibacterial activity of silver nanoparticles (Ag NPs) and the phototoxicity of flavin mononucleotide (FMN), as well as the study of their phototoxicity, is an urgent task.

Materials and Methods

Chemicals. Silver nitrate “Serva”, Germany; Riboflavin ampoule, JSC Pharmstandard-UfaVITA, 10 mg/ml; Sodium citrate “helicon”, Moscow State University, Russia; 2,2-diphenyl-1-picrylhydrazine, SigmaAldrich; Triethanolamine, SigmaAldrich, Polyvinylpyrrolidone “MERCK” Germany; Sodium borohydride “SigmaAldrich”;

Equipment. “Evolution 200” spectrophotometer, Thermo scientific (Thermo, USA); Raman spectrometer, Renishaw inVia Qontor confocal Raman microscope; LED device “Polyronic”; Dynamic Light Scattering Analyzer, DynaPro NanoStar.

Methods. To prepare a colloidal solution of silver nanoparticles by reducing silver nitrate with sodium citrate, 50 ml of a silver nitrate solution with a concentration of 0.4 mg/ml and 10 ml of sodium citrate with a concentration of 0.5 mg/ml were prepared. A solution of sodium citrate was added dropwise to a solution of silver nitrite with constant stirring on a magnetic stirrer and heating in a water bath to 80 °C for 4 hours.

To prepare a colloidal solution of silver nanoparticles by reducing silver nitrate with sodium borohydride, 10 ml of a silver nitrate solution with a concentration of 1.7 mg/ml and 30 ml of sodium borohydride with a concentration of 0.1 mg/ml were prepared. A solution of sodium borohydride was added dropwise to a solution of silver nitrate with constant stirring on a magnetic stirrer in an ice bath. To stabilize silver NPs obtained by the borohydride method, a solution

of PVP (poly-N-vinylpyrrolidone) at a concentration of 2 mg/mL was added to the prepared colloidal solution of nanoparticles.

The efficiency of radical production was evaluated using stable radicals of 2,2-diphenyl-1-picrylhydrazine (DPPH). DPPH is a stable radical with an intense absorption band at about 525 nm. After interaction with free radicals, a decrease in the absorption of the sample with DPPH at 523–525 nm is observed.

Results and Discussion

Silver NPs were synthesized by using the citrate and borohydride as reducing agents. To explore citrate method of synthesis an experiment was conducted in which a mixture of sodium citrate solution and silver nitrate was irradiated with light at a wavelength of 360 nm for 1, 3, 4, and 5 minutes. As the irradiation time increases, the color of the sample becomes more intensive, which indicates the formation and enlargement in size of silver nanoparticles. This was confirmed by the study of the samples using the dynamic light scattering method. Thus, ultraviolet (UV) irradiation significantly accelerates and simplified the synthesis of nanoparticles, and by varying the irradiation time, one can control the particle size.

The optical absorption of the initial components and their complexes did not change for 1 h. When irradiated with light at a wavelength of 365 nm by laser with power density 100 mW/cm^2, the formation of lumichrome, which is a product of FMN photodegradation, was observed already after 30 s, and the absorption of silver nanoparticles obtained by the citrate method slightly increased with a slight shift of the peak to the blue region, which is probably due to the appearance of a finer fraction of nanoparticles.

The formation of a complex is revealed by a spectrophotometric study of complexes with different concentrations of Ag NPs and a constant concentration of FMN. With an increase in the concentration of silver nanoparticles, the intensity of the peak at 375 nm, which corresponds to the FMN absorption peak, grew, though FMN concentration unchanged. This indicates the change in Ag NPs surroundings associated with the electron transfer between FMN and Ag NPs.

In the case of the FMN–Ag NPs complex, the peaks of FMN photoproducts were not detected upon irradiation (Fig. 1); in this case, the peak was smoothed and shifted to the red region, which indicates an increase in the nanoparticle size. We also compared the mass of the dry residue of nanoparticles before and after irradiation of the complex. The weight increase after irradiation was more than 80% that confirmed the conversion of unreduced silver ion into AgNPs.

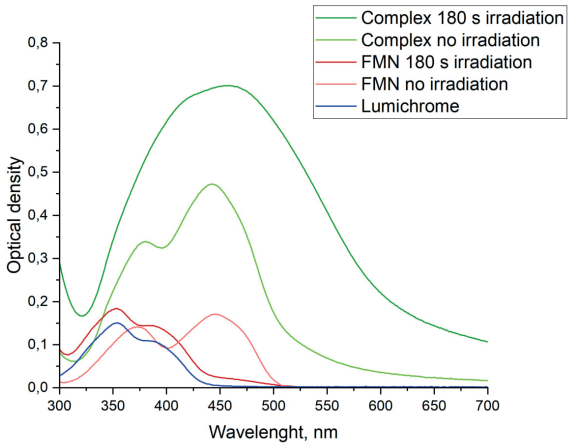

Fig. 1. Absorption spectra of FMN, complex FMN with silver nanoparticles before and after laser irradiation at wavelength of 365 nm and lumichrome, which is a photoproduct of FMN

After addition of AgNPs, FMN fluorescence is quenched by more than 35%. Upon irradiation, fluorescence quenching in the complex was 65%, in contrast to free FMN, whose fluorescence loss was more than 95% (Fig. 2, *a*). We suppose, that AgNPs can prevent FMN quenching due to the formation of a complex based on the electron transfer.

We also studied the fluorescence quenching kinetics of complexes with AgNPs obtained by the citrate and borohydride methods, excited by a 440 nm laser light. To estimate the kinetics

of fluorescence quenching, the characteristic decay time was calculated taking into account the number of incident photons from the laser onto the sample. The results are presented in the diagram (Fig. 2, *b*).

The dependence of the photodegradation time on Ag NPs concentration is clearly observed. With an increase in the concentration of silver NPs obtained by the borohydride method, the time increases, i.e., the addition of these NPs slows down fluorescence quenching. In the case of citrate particles, as their concentration increases, the fluorescence of the complex decays faster.

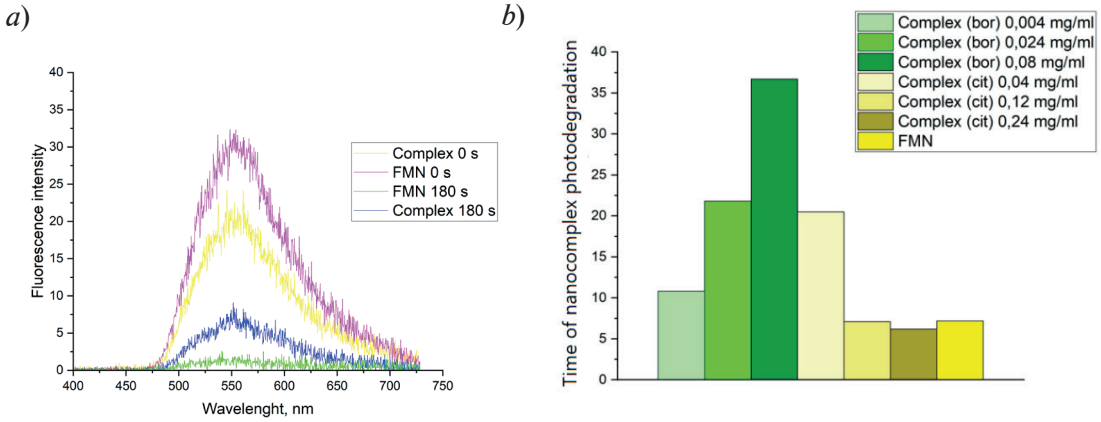

Fig. 2. Fluorescence spectrum of flavimononucleotide, flavimononucleotide complex with silver nanoparticles before and after laser irradiation 365 nm (*a*), Photodegradation of nanocomplex under 440 nm laser light irradiation (*b*)

It is worth noting that the fluorescence of the complex with AgNPs obtained by the borohydride method upon excitation by a laser with a wavelength of 365 nm and a power of 300 mW, photodegradation of FMN to lumichrome was observed. This was confirmed by the appearance of an emission peak at 540 nm, corresponding to FMN, and a peak at 470 nm, which corresponds to lumichrome.

During irradiation, ROS such as superoxide anion ($O_2 \cdot -$), hydroxyl radical ($\cdot OH$), and singlet oxygen ($^1O_2$) are generated [2]. The formation of radicals was controlled by the decrease in the absorption of the stable diphenylpicrylhydrazyl radical at a wavelength of 525 nm, which is responsible for the reaction with the generating radicals [3]. In the case of individual components of the complex, there was practically no change in the peak at a wavelength of 525 nm, but an increase in the shoulder in the spectrum of Ag NPs at a wavelength of 420 nm was noted. In the case of complexes, an extreme dependence was recorded with the maximum production of radicals during irradiation for 150 s. It is known that the efficiency of generating FMN radicals increases when using amines, for example, triethanolamine [4]. We observed a decrease to 30 s in the time required for the appearance of radicals, which can be explained by the electron transfer from the amine to FMN.

Conclusion

Silver NPs and FMN form a complex, and the optical properties of this complex can be controlled by changing the concentration of silver NPs. The formation of the complex is accompanied by the generation of radicals upon 365 nm irradiation. The production of radicals has an extreme dependence. The introduction of triethanolamine activator to FMN into the system leads to the generation of radicals already in the first minutes of irradiation. The formation of the complex leads to a change in the fluorescence kinetics. The method of synthesis of silver NPs significantly affects the optical properties of the formed complex.

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