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APPLICATIONS OF NANODIAMONDS

Design of a Luminescent Biochip with Nanodiamonds and Bacterial Luciferase

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Abstract—An "aluminum oxide film–adhesive layer–nanodiamond–luciferase" supramolecular structure is prepared on a flat plate. It is demonstrated that, in this structure, the enzyme retains the catalytic activity. The structure prepared can be treated as a luminescent biochip prototype for use in bioluminescent analysis. *© 2004 MAIK "Nauka/Interperiodica".*

1. INTRODUCTION

In recent years, considerable attention has been focused on the development of new techniques for solving applied problems in biology, protein chemistry, biophysics, molecular biology, ecology, etc. In particular, the design of various indicator recording systems based on the use of protein molecules with marker properties holds much promise. Among marker proteins are light-emitting proteins, which are capable of generating photons in the visible spectral region. The attractiveness of these testing methods is related to their high sensitivity, rapidity of analysis, simplicity of recording a light signal, broad potential application, etc. [1–4].

Earlier [5], we developed a luminescent biochip prototype based on detonation nanodiamond particles and light-emitting protein obelin. When interacting with Ca^{2+} ions, this protein generates visible-light photons and, hence, the biochip can be used for their recording in different-type liquids, including biological liquids [5]. At the same time, it is of interest to create similar indicator test systems on the basis of other light-emitting proteins. This will permit one to develop the concept regarding the mechanisms of interaction of protein molecules with the surface of nanodiamond particles, on the one hand, and to expand the field of possible applications of such bioluminescent sensor, on the other. In particular, for this purpose, it is possible to use luciferases of marine bacteria, which have found wide application in bioluminescent analysis as monoenzymes in bienzyme [luciferase–nicotinamide adenine dinucleotide (NADH) : flavin mononucleotide (FMN) oxidoreductase] and related systems [6]. Proteins of this group have a molecular mass of approximately 80 kDa, are heterodimers (consist of α and β subunits), and involve flavin as a cofactor in the structure.

In the present work, we designed a luminescent biochip prototype based on detonation nanodiamond particles and bacterial luciferase.

2. SAMPLE PREPARATION AND EXPERIMENTAL TECHNIQUE

The nanodiamonds used in the experiments were synthesized at the Department of Physics of Finely Dispersed Materials (Krasnoyarsk Research Center) and were purified by the gas-phase method in the presence of boron oxide [7]. Hydrosols from nanodiamond particles were prepared according to the procedure proposed in our earlier work [5]. Luciferase and NADH : FMN oxidoreductase samples were produced using the procedures described in [8, 9]. The luciferase activity was measured with a bienzyme reaction and photoreduced FMN [9]. The measurements were performed on a BLM 8801 bioluminometer (Special Design Bureau "Nauka," Krasnoyarsk) calibrated against the Hastings–Weber radioactive reference sample [10]. One luminescence unit corresponded to 10^7 photons/s. When determining the luciferase activity according to the bienzyme reaction, the reaction mixture in the measuring cell contained 4 mM Tris-HCl buffer (450 μl, pH 7.0), 4.7×10^{-6} M C_{14} aldehyde (10 μl), 7.8×10^{-5} M FMN (10 μl), NADH : FMN oxidoreductase (1–3 μl; enzyme activity, 1 E/ml), and the sample to be studied (5–50 μl). The reaction was initiated by the addition of 10^{-2} M NADH (5 μl). In the case when the luciferase activity was determined with photoreduced FMN, the reaction mixture contained the aforementioned buffer (450 μl), 4.7×10^{-6} M C_{14} aldehyde (50 μl), and the sample to be studied (5–50 μl). The reaction was initiated by the introduction of reduced FMN (7.8×10^{-5} M, 500 μl).

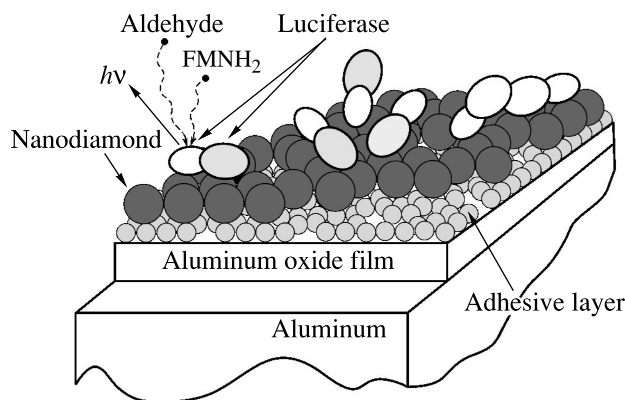

Fig. 1. A hypothetical "aluminum oxide film–adhesive layer–nanodiamond–luciferase" supramolecular structure of the biochip for the components arranged in monolayers.

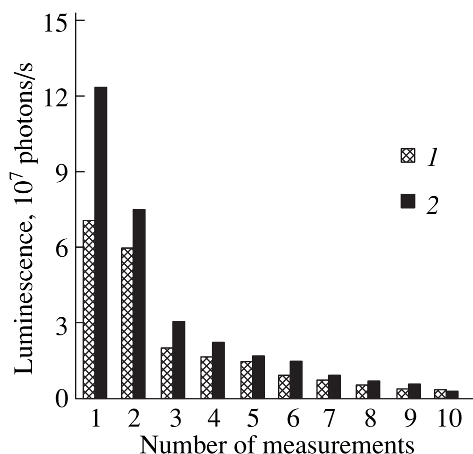

Fig. 2. Variations in the luminescence signal intensity upon multiple measurements with the use of a flat plate involving the supramolecular structure in which a nanodiamond–luciferase complex acts as an indicator element: (*1*) the plate in the reaction mixture in a measuring cell and (*2*) the reaction mixture after removing the plate.

3. RESULTS AND DISCUSSION

At the first stage of our investigations, we demonstrated that the addition of an aqueous solution of luciferase to a suspension of nanodiamond particles and subsequent stirring of the mixture leads to fast and complete adsorption of the enzyme on the particle surface. After sedimenting nanodiamonds by centrifugation at 16000 *g* for 1–3 min, no luciferase activity is observed in a supernatant. The protein-to-nanodiamond ratio necessary for complete adsorption of the enzyme from the solution can be calculated from the adsorption capacity of nanoparticles. With the use of marker proteins (bovine serum albumin, cytochrome C), we found that 1 mg of nanodiamond particles can adsorb 0.3–0.5 mg of the protein on the surface. After adsorption, luciferase molecules are firmly retained on the nanodiamond surface and are not washed off upon multiple washing of the particle sediment with water and the 20 mM Tris-HCl buffer (pH 7.0). The enzyme adsorbed on nanodiamond particles can exhibit catalytic activity both in the bienzyme reaction and with photoreduced FMN. However, in both cases, luciferase molecules are partially desorbed from the surface of nanodiamond particles. After the reaction is performed and particles are removed from the reaction mixture, this mixture is characterized by a luciferase activity. At the same time, the particles hold a considerable number of enzyme molecules on their surface. When nanodiamond particles are washed free of the reaction mixture components and reaction products, they can be used again in luminescence measurements. It was experimentally found that one sample of nanodiamond particles can be used to carry out ten or more measurements. Note that part of the enzyme molecules are desorbed in each experiment. Most likely, this is associated with the conformational transformations of enzyme molecules, which occur at the instant they are involved in the catalytic reaction. This assumption is confirmed by the fact that the individual components of the bioluminescent reaction (C_{14} aldehyde, FMN, NADH), their combinations, and the addition of oxidoreductase do not result in the desorption of luciferase from the nanodiamond surface.

At the next stage, we analyzed the possibility of fabricating a luminescent biochip prototype in which a nanodiamond–luciferase complex acts as an indicator element. An aluminum plate was used in the biochip design. It was revealed that aluminum oxide films do not adsorb luciferase molecules from protein solutions and nanodiamond particles with adsorbed enzyme molecules from suspensions. Therefore, as in the biochip based on nanodiamond particles and obelin [5], the nanodiamond–luciferase complex was attached to the plate by an adhesive layer, which was preliminary applied to the aluminum oxide film. A hypothetical "aluminum oxide film–adhesive layer–nanodiamond–luciferase" supramolecular structure of the biochip is shown in Fig. 1. This structure with the nanodiamond–luciferase complex as an indicator element is not washed out from the plate by water and the 20 mM Tris-HCl buffer (pH 7.0). This suggests a sufficiently high stability of the structure and a strong adhesion to the plate surface. The luciferase activity on the plate prepared was also examined by two techniques (in the bienzyme reaction and with photoreduced FMN). For this purpose, the plate was placed in the measuring cell with the buffer and the reaction was initiated by adding the required components. In both cases, the luminescence signal was observed in the system. Thereafter, the plate was removed from the measuring cell and the luminescence signal was recorded once again. Despite the absence of the plate, the luciferase activity was observed in the reaction mixture. This indicates that, as in the experiments with suspensions, part of the enzyme

molecules are desorbed from the chip surface in the course of the reaction. The plate was washed with a stream of distilled water in order to remove the reaction products and the remaining desorbed protein. Then, the plate was placed in a pure measuring cell with the buffer and the luminescence was measured in the system. It was found that the intensity of the luminescence signal from the plate decreases with each subsequent measurement (Fig. 2). However, this decrease is not a serious obstacle to the use of the proposed test systems in the bioluminescent analysis. Actually, the intensity of the luminescence signals from the plate is sufficiently high and can be recorded despite the decrease in the amount of enzyme on the chip surface.

4. CONCLUSIONS

Thus, the results obtained in this work demonstrated the possibility of fabricating a luminescence biochip whose main element consists of detonation nanodiamond particles with a light-emitting luciferase protein on the surface. The test system proposed can be used in bioluminescent analysis. Stronger fixation of enzyme molecules to the surface can be achieved using chemical (covalent bonding with chemical reactants) and biological (protein–protein interactions with additional molecules) methods. Verification of the aforementioned potentialities of these methods is a subject for further investigation.

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