Metal enhanced fluorescence of flavin mononucleotide using new plasmonic platform

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Abstract

New plasmonic platform was successfully obtained to investigate the increase of fluorescence intensity of a fluorophore in the presence of silver nanoparticles. A flavin mononucleotide, was selected by us as a fluorophore for this study as a very important biological compound playing a key role in many biochemical process. Plasmonic platforms were characterized by means of luminescence spectroscopy. Flavin mononucleotide deposited on plasmonic platform exhibits dramatic emission enhancements in presence of silver nanoparticles deposited on gold mirror.

Keywords: plasmonic platform; FMN; emission enhancement;

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1. Introduction

Over the last decades silver nanoparticles have been applied in many different fields from catalysis through optics and electronics and many other areas due to their unique size-dependent optical, electrical and magnetic properties [1-3]. Currently, most of the applications of silver nanoparticles are related to light harvesting and enhanced optical spectroscopy including metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS) [4-6]. It is known that silver nanoparticles can influence the fluorescence properties of an organic fluorophore on account of the localized surface plasmon resonance. Moreover, fluorophores close to noble metallic films or nanostructures show exciting and desired properties like higher brightness and increased photostability.

This work shows the results of enhanced fluorescence intensity of a flavin mononucleotide (FMN) in the presence of silver nanoparticles on especially designed and prepared plasmonic platform. The plasmonic platform consists of silver nanoparticles deposited and arranged on semitransparent gold mirrors. Light-induced localized plasmons in assemblies of nanoparticles interact with traveling plasmons in a conductive surface and create extremely strong local electric fields. Fluorescent molecules deposited on the platform surface are exposed to the enhanced local fields and are excited with higher rate constant. In addition, excited molecules interact with surface plasmons in metallic nanostructures resulting in the enhanced radiation of the excitation energy. Obviously, the brightness of fluorophores is then also greatly enhanced and their lifetime is significantly shortened. A shorter lifetime of fluorophore prevents photobleaching due to reduced excited state residence time as photodegradation occurs predominantly in the excited state [7-10].

A flavin mononucleotide, which was selected by us as a fluorophore for this study, is a very important biological compound as it plays a key role in many biochemical processes [11,12]. FMN and other flavins can for example participate in the process of destroying

pathogens and inactivation of many microorganisms [13]. Some studies indicate also that flavins exhibit anti-cancer activity and therapeutic properties in the treatment of several other diseases [14]. Furthermore they participate in many signaling events occurring in the cell membranes, mitochondria, or cytoplasm. They also act as photoreceptors of blue light in plants (for example phototropism) [15-17]. Spectroscopic properties of FMN and its biological actions depend strongly on its local environment, concentration and temperature [18,19]. Therefore, the detection of FMN enhanced fluorescence and the introductory recognition of FMN spectroscopic properties in the presence of metal nanostructures is an important task which herein is undertaken.

2. Experimental part

All materials used to prepare the plasmonic platform were purchased from commercial sources (analytical grade) and used without further treatment. Silver nitrate, AgNO_3 (99,99%); trisodium citrate dihydrate, $(\text{OH})\text{C}_3\text{H}_4(\text{COONa})_3 \cdot 2\text{H}_2\text{O}$ (99%) were purchased from Aldrich Company. Flavin mononucleotide (riboflavin-5'-monophosphate sodium salt $\text{C}_{17}\text{H}_{20}\text{N}_4\text{NaO}_9\text{P} \cdot \text{H}_2\text{O}$) (FMN) analytically pure was purchased from Lancaster. For the preparation of all the samples deionized water was used which was obtained from Hydrolab system installed in our laboratory.

2.1. Preparing of silver nanoparticles

The silver nanoparticles used in this work were obtained by the reduction of silver nitrate in aqueous solution in the presence of trisodium citrate according to the developed procedure presented earlier [20]. First, the sodium citrate (2 ml at 34 mM) was added dropwise to a stirred solution of AgNO_3 (100 ml at 1 mM). During the process, all solutions

were mixed vigorously and heated to 90-95°C for 15 min or until change of color was evident (yellow). Then the mixture was incubated in an ice bath for 15-20 min. Finally, the silver nanoparticles were purified by centrifugation at 3500 rpm for 8 min and the precipitate was then suspended in 1 ml of 1mM trisodium citrate.

2.2. Preparation of plasmonic platform

The plasmonic platforms were obtained as follows. First, gold mirrors (Thin Metals Films Ltd. (UK)) were cleaned and drop coated with silver nanoparticles. Then, the mirrors were dried in air. When a liquid containing silver nanoparticles was evaporated, different structures were formed. In the final stage, FMN in water ($C = 10^{-3}$ M) was applied on gold mirror with Ag nanoparticles by spin-coating method. The spin coating was done at 300 rpm for 60 s to disperse the solution. After that, the thin films were allowed to dry in a dark room and in air atmosphere for 24 hours.

For comparison and control experiment, FMN was deposited on the gold mirror and a microscopic glass without silver nanoparticles. Microscopic glasses used were kept in a mixture of 33% H_2O_2 and H_2SO_4 in molar ratio 2:1 over 24 h and rinsed with deionized water. The spin coating was done at 300 rpm for 60 s. After that, the thin films were allowed to dry in a dark room and in air atmosphere for 24 hours. The graphical idea of the platform is shown in Fig. 1.

Fig. 1.

2.3. Apparatus

Steady-state fluorescence intensity measurements of all the samples were carried out using a Carry Eclipse spectrofluorometer (Varian Inc., Australia) with the front face

geometrical format described previously in detail [21]. The emission was scanned from 480 nm to 750 nm following a 470 nm excitation and using 495 nm long pass filter on the emission side.

Fluorescence lifetimes of all the samples were measured using FluoTime 200 (PicoQuant, GmbH, Berlin, Germany) Fluorescence Lifetime Spectrometer. It contains a microchannel plate detector (Hamamatsu, Japan) and a 470 nm laser diode was used as the excitation source. The front face setup was used for these measurements as well. To minimize polarization effects the fluorescence intensity decays were collected from the front side upon so called magic angle (54.7° between the excitation beam and the normal to the sample surface) excitation. Data was analyzed with FluoFit 4.0 version software using multiexponential intensity decay model as follows:

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i} \quad (1)$$

where, α_i is the amplitude of the fluorescence intensity decay of the i -th component at time t and τ_i is the lifetime of the i -th component. The intensity weighted average lifetime (τ_{Avg}) was calculated using following equation:

$$\tau_{Avg} = \sum_i f_i \tau_i \quad \text{where} \quad f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \quad (2)$$

The morphology of the samples was investigated by Schottky field Emission Scanning Electron Microscopy (FEI Quanta FEG 250) with an ET secondary electron detector. The beam accelerating voltage was kept at 10 kV.

A confocal MicroTime 200 (Picoquant GmbH, Germany) system coupled with an Olympus IX71 microscope was used to obtain time resolved images. Fluorescence photons were gathered from different places on the sample using 60x water immersed objective (N.A 1.2, Olympus). A 500-nm long-pass filter with additional two 488 R long-pass filters (Shemrock) were applied to remove scattered light. A pulsed laser (470nm-LDH-P-C470B)

with repetition rate 20 MHz was used as a light source. Fluorescence photons were collected with a photon counting module (SPCM=AQR-14, Perkin Elmer) with processing accomplished by the PicoHarp300 time correlated single photon counting (TCSPC) module. Data analysis was performed using a SymPhoTime (5.2.4) software package.

3. Results and discussion

Electron microscopy was done in order to study the morphology and size distribution of silver nanoparticles. Representative SEM image of the prepared Ag nanoparticles and size distribution of grains are presented in Fig. 2A and 2B, respectively. In the SEM photograph round-shaped nanoparticles can be seen with an average size of 67 ± 5 nm. The observed particle size varies from 44 to 97 nm and as seen From Fig. 2B it can be approximated with a typical Gaussian distribution of FWHM = 25 nm.

Fig.2A.

Fig.2B.

Fig.3A.**Fig.3B**

Figure 3 A shows the con-focal FLIM (Fluorescence-lifetime imaging microscopy) image of FMN deposited on the plasmonic platform. Uniform green color (related to lifetime) shows quenching of FMN fluorescence due to the contact with nanoparticles. The average lifetime in the green areas is about 0.5 ns. Figure 3B shows the intensity (bottom) and lifetime (top) profile of the red line drawn in Figure 3A. The intensity along the line varies depending on the presence/absence of silver nanoparticles in contact with FMN. Black areas in the image acts as control areas where there is no silver nanoparticles and hence no enhancements. The intensity counts in the black areas are *ca.* 12 counts and can be used to calculate the enhancements. It appears that fluorescence intensity enhancements vary from 15 to 50 times if one examines the line profile. The fluorescence lifetime in the areas of enhancements is severely shortened (about 0.5 ns or less) while the areas void of Ag nanoparticles show significantly longer lifetimes.

Fig.4.

Fluorescence spectra of FMN deposited on different surfaces were measured in front face setup configuration and are shown in Fig. 4. All spectra present broad fluorescence bands with maximum located at about 530 nm. The fluorescence peak location of FMN in our platform is slightly shifted to the red compared to that observed in polyvinyl alcohol films and in water at low FMN concentration [18]. The shape of the bands visible in Fig. 4 is quite similar; however the intensity of the bands is drastically different. It is clear from the figure

that the FMN fluorescence is strongly enhanced on the plasmonic platform compared to other two cases: FMN fluorescence on gold nanolayer (about 7 times enhancement) and FMN fluorescence on glass substrate (about 14 times enhancement). It should be mentioned that the measurements were performed at the same conditions for all three samples, including FMN concentration and optical density of the samples. These results corroborate our findings using FLIM and intensity measurements.

It is known that, the enhancement efficiency of nanoparticles depends on the distance between the fluorophore and nanoparticles and also the nature of the metal surface. The short fluorophore-metal distances (less than 4 nm) may quench the fluorescence as a result of excitation energy transfer to metal [22]. In the case of silver nanoparticles, enhanced fluorescence is however observed when fluorophores are localized at longer distances of 6-10 nm from silver nanoparticles where the quenching effects become insignificant [23]. There are several interactions between fluorophore and metallic nanoparticles. Strong enhancement of fluorescence emission in the case of the plasmonic platforms may be due to two mechanisms responsible for metal-enhanced fluorescence. First, it is possible that a local enhanced field increases the rate of excitation. The second mechanism is connected with the interaction between fluorophore and silver nanoparticles. The effect of this interaction is a rapid release of the excitation energy and its radiation into free space. This emitter antenna-like effect results in an increase of a quantum yield of the molecule and in decrease of its lifetime. A simultaneous increase of the quantum yield and decrease of the lifetime is possible only when radiative rate constant increases. This effect is also known as radiative decay engineering (RDE) [24,25].

Fig.5.

Table 1

Furthermore, the same samples were used to measure the fluorescence intensity decays and these decays were presented in Figure 5. Red curve is ascribed to fluorescence intensity decay for FMN deposited on glass, blue curve corresponds to the fluorescence intensity decay of FMN on gold mirror and green curve corresponds to the fluorescence intensity decay of FMN deposited on the plasmonic platform. Moreover, the measured fluorescence lifetimes were determined to be: 3.7 ns, 1.9 ns and 0.3 ns for FMN deposited on the plain glass, on the plain gold mirror and on the plasmonic platform, respectively.

It appears from the fitting procedure that fluorescence intensity decays of FMN are of complex character: they are biexponential on glass surface while 3 exponentials were needed to fit the data on gold mirror and plasmonic platform (Table 1). This result is different from that previously reported for FMN in water [15] where at low concentration FMN fluorescence decay was purely single exponential. These deviations from single exponential character of fluorescence decay are of more complex nature. The multiexponential character of fluorescence decay on plasmonic platform has been also observed in the case of other dyes [26] and it can be caused by many different reasons. Firstly, it is possible that FMN forms dimer at its locally increased concentration [18,19]. Also the interaction between fluorophore and silver nanoparticles may lead to the deviation from single exponential decay. Moreover, a decrease of lifetime is found when the reflected field is in-phase with the fluorophore's oscillating dipole. As expected in this case the lifetime was quenched more than 10 times. These results are in agreement with those obtained from microscopic measurements. All these effects may contribute to more complex nature of the fluorescence decay compared to aqueous solution.

4. Conclusion

Plasmonic platform exploiting FMN as a fluorophore was successfully constructed. Moreover, the effect of silver nanoparticles deposited on the gold mirror on the fluorescence enhancement of FMN was investigated in detail. The results show a dramatically increased fluorescence emission which corresponds to the reduced lifetime. This is important as it leads to increased photostability of fluorescent molecules. The increased stability effect occurs because, molecules spend less time in the excited state and thus it decreases the possibility for photochemical reactions. We hope that the use of such platforms will enable further studies with other important photosensitive biologically active substances.

Acknowledgments

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Captions

Table 1. Intensity Decay Parameters of FMN on different surfaces. Tail-fitting protocol was used to analyze the decays.

Fig. 1. Scheme of plasmonic platform.

Fig. 2A. SEM image of the silver nanoparticles.

Fig.2B. Grains of silver nanoparticles size distribution. Results obtained from the SEM photographs.

Fig.3.A. FLIM image (80x80um) of FMN deposited on plasmonic platform.

Fig.3B. Average lifetime and corresponding intensity profile along the red line in a FLIM image of FMN deposited on plasmonic platform.

Fig.4. Fluorescence spectra of FMN on different surfaces: plain glass without silver nanoparticles (red), plain gold without silver nanoparticles (blue) and plasmonic platform (green).

Fig.5. Fluorescence intensity decays of FMN on different surfaces: plain glass without silver nanoparticles (red), plain gold without silver nanoparticles (blue) and plasmonic platform (green).

HV	det	mode	mag	WD
10.00 kV	ETD	SE	100 000 x	5.3 mm

500 nm

Sample	A ₁	A ₂	A ₃	1	2	3	Avg. Int. (ns)	Avg. Amp. (ns)	χ^2
On Glass	0.48	0.52	-	1.60	5.50	-	4.70	3.70	0.96
On Gold	0.33	0.53	0.14	0.68	1.92	4.7	2.70	1.90	0.95
On Plasmonic Platform	0.84	0.14	0.02	0.19	0.70	1.95	0.58	0.30	0.95