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Bioconjugation of Silver Nanowires with Photosynthetic Light-Harvesting Complexes

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We demonstrate a way to conjugate a light-harvesting complex, peridinin–chlorophyll–protein, with silver nanowires using biotin-streptavidin linker. In the case of conjugated structure we observe slight increase of the fluorescence intensity of the chlorophyll emission followed by the gradual decrease of the intensity due to photobleaching. For a non-conjugated mixture of peridinin–chlorophyll–protein with silver nanowires only the photobleaching takes place. The results suggest a possible way to fabricate hybrid nanostructures comprising light-harvesting complexes and metallic nanoparticles for achieving the efficient plasmon-induced enhancement of absorption of the light-harvesting complexes.

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1. Introduction

Research aimed at understanding the effects of plasmon excitations upon the optical properties of multichromophoric systems, such as light-harvesting complexes, has recently led to several interesting results [1–5]. It has been shown that coupling light-harvesting systems, which participate in photosynthesis either as sunlight absorbers or charge separators, to metallic nanoparticles lead to pronounced effects: the fluorescence or absorption of chlorophylls and carotenoids can be strongly enhanced. These enhancements could result in increased rate of photoelectron generation [5].

In most cases the hybrid nanostructure involving photosynthetic complexes was assembled in a layer geometry. In other words, the biomolecules were deposited on top of the metallic layer comprising a silver island film or separated metallic nanoparticles. In such an architecture it is relatively straightforward to control the separation between metallic nanoparticles and photosynthetic complexes, through evaporation of dielectric layers [2]. On the other hand, it has been shown for inorganic systems, such as semiconductor nanocrystals or organic dyes, that there are also ways to conjugate them with metallic nanoparticles using biochemical methods based on properly selected biolinkage [6–10]. Depending upon the optical properties of the constituents and the separation between them, fluorescence quenching [10] as well as fluorescence and absorption enhancement [7] can be achieved. In the latter a biolinker based on streptavidin–biotin pair has been used. The ability to conjugate functional proteins, responsible for light-harvesting, with metallic nanoparticles in search for enhanced absorption [11] is highly desirable, in particular from the point of view of keeping the proteins in their native environment, in contrast to the layer geometry.

In this work we show that it is possible to biochemically conjugate peridinin–chlorophyll–protein, a light harvesting complex from algae, with silver nanowires. The reaction is carried out in water-based solution, which protects the protein much better than any polymer matrix used previously. The results of optical spectroscopy demonstrate clearly that conjugation takes indeed place in the system. We find slight increase of the fluorescence intensity of the light-harvesting complex followed by regular photobleaching behavior. We believe these findings suggest that conjugation of light-harvesting complexes with inorganic nanostructures could be a feasible way of building functional hybrid nanostructures.

2. Experimental section

Anhydrous ethylene glycol (EG, 99.5%), dimethyl sulfoxide (DMSO), and isopropyl alcohol (HPLC grade) were obtained from Agros Organics. Peridinin–chlorophyll–protein functionalized with streptavidin was purchased from BD Bioscience Company. Copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$, 99.99+%), silver nitrate ($AgNO_3$, 99+%), poly(vinyl pyrrolidone) (PVP, MW $\approx$ 55 000), biotin disulfide *N*-hydroxysuccinimide ester (BDS) were purchased from Sigma-Aldrich. Pure water was obtained from Fluka. All chemicals were used without further purification.

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Silver nanowires were synthesized using polyol process [12] in the presence of ethylene glycol, copper seeds and PVP polymer, in which the EG served as the reducing and solvent reagent. In a typical synthesis 40 μl of $CuCl_2$ solution (4 mM in EG) was added to 5 mL of ethylene glycol in glass vial, heated beforehand to 150 °C with continuous stirring. After 15 min, 1.5 mL of PVP solution (114 mM in EG) and 1.5 mL $AgNO_3$ solution (94 mM in EG) were then simultaneously added dropwise using a syringe pump over a period of 6 min. The reaction mixture was continuously heated at $\approx$ 160 °C for one hour until all silver nitrate was reduced. The product was next purified by centrifugation processes. The reaction mixture was diluted with isopropyl alcohol and centrifuged at 2000 rpm for 20 min. The supernatant containing silver particles and unreacted substrates was removed using a pipette. This centrifugation procedure was repeated several times until the supernatant became colorless. The final product of the reaction was redispersed in 2 ml of pure water.

For functionalizing silver nanowires with biotin, a 3.5 mM solution of biotin disulfide *N*-hydroxysuccinimide in DMSO was prepared immediately before functionalization process. First we add 100 μl of BDS solution into a 1 mL of silver nanowires solution under vigorous stirring condition. Stirring then continued for 10 s and then the nanowires were left undisturbed overnight at 4 °C. The product was centrifuged twice at 2000 rpm for 20 min and redispersed in phosphate buffer.

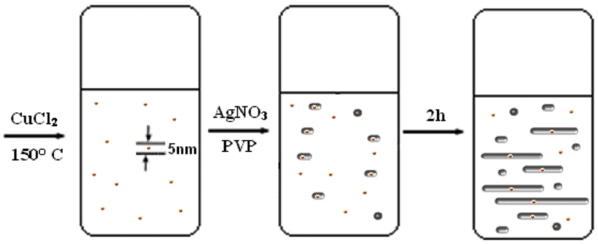
Bioconjugation process was carried out in plastic cuvette in buffer solution PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25 °C) at room temperature. To 100 μl of streptavidin-functionalized PCP in PBS buffer with concentration 2 μg/ml we added 1 μl of silver nanowires without stirring. The reaction rate of bioconjugation progress was measured *in situ* using fluorescence spectroscopy.

Scanning electron microscopy (SEM) studies were carried out with a Zeiss AURIGA microscope, operating at 5 kV, current 89 pA and InLens detector. We devised a method of preparation of metallic nanoparticles for SEM measurement in order to obtain clean and well-separated metallic nanoparticles. In the first step we cleaned a silicon substrate using piranha solution, rinsed with pure water and sonicated in ethanol for 2 min. Next on a silicon surface we spin-coated a solution of (3-aminopropyl)triethoxysilane to form a monolayer. The excess amount of substrate was washed away with pure water. Then we slowly spin-coated (1000 rpm) a small amount (20 μl) of nanowires solution onto silicon substrate. Such a procedure leads to significant improvement of the image quality.

The samples, both light-harvesting complexes and silver nanowires, were characterized using absorption spectroscopy. The absorption spectra of the solutions were taken at room temperature, in a plastic cuvette with a 1 cm optical path, using a Varian model Carry 50 UV-Vis spectrophotometer in the spectral range from 350 to 1100 nm. We have also taken a spectrum of the silver nanowire solution upon functionalization with biotin. The conjugation of peridinin–chlorophyll–protein with silver nanowires was monitored using *in situ* fluorescence spectroscopy. The fluorescence spectra on PCP–SA solution and PCP-AgNW dispersion were registered with a FluoroLog 3 spectrofluorometer (Jobin Yvon/SPEX Horiba) in 30 s intervals for up to 10 min. The excitation wavelength of 485 nm was used, which efficiently excites the PCP complex and at the same time results in plasmon excitation in the silver nanowires. From such datasets we determine the time evolution of the fluorescence intensity. After finishing the sequence, fluorescence excitation spectra were measured with detection at 670 nm, that is the maximum of the peridinin–chlorophyll–protein emission.

3. Results and discussion

We synthesized silver nanowires using polyol procedure [12], which involves the reduction of inorganic salt by polyol at elevated temperature. When ethylene glycol is converted into the glycolaldehyde due by oxidation in ambient condition, it becomes a reducing agent for a silver and copper salt. We can divide this method into two main steps (Fig. 1). In the first one the



$$2HOCH_2{-}CH_2OH \rightarrow 2CH_3CHO + 2H_2O \quad (1)$$

$$2CH_3CHO + CuCl_2 \rightarrow CH_3CO{-}COCH_3 + Cu + 2HCl \quad (2)$$

Fig. 1. Reaction scheme of synthesis of silver nanowires using polyol method.

copper is reduced and forms a seed for further growth process. In the second step, addition of $AgNO_3$ and PVP allowed the nucleation and growth of silver into the wire-shaped nanostructures. Silver nitrate was reduced by ethylene glycol in the presence of PVP, which acts as a stabilizing agent because it contains both nitrogen and oxygen atoms and thus creates a protective coating onto the silver surface, preventing aggregation of silver nanowires [13]. This method of silver nanowire synthesis can be carried out in soft conditions with temperatures < 200 °C and under atmospheric pressure. The morphology of silver nanowires was studied using SEM. A typical image shown in Fig. 2 features nanowires with diameters from 40 nm to 300 nm and lengths reaching up to 50 μm (Fig. 2a). As the size dispersion is relatively broad, it is expected to result in broad range of plasmon resonances in the silver nanowires. The absorption spec-

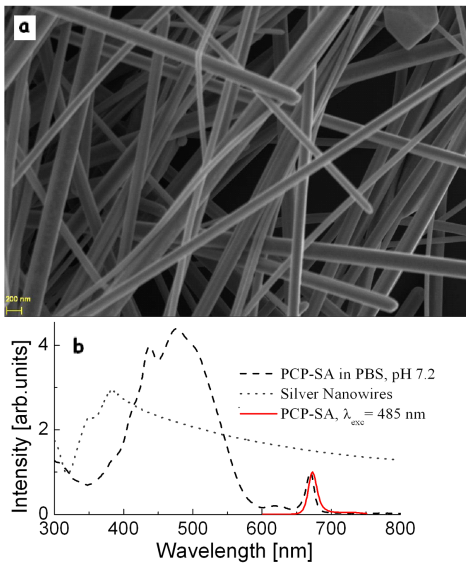


Fig. 2. (a) SEM pictures of silver nanowires; (b) absorption (dashed line) and emission spectra (solid line) of PCP–SA complexes in buffer solution and absorption spectrum of silver nanowires (dotted line).

trum displayed in Fig. 2b confirms that, while the maximum of absorption appears around 390 nm, it features a very broad wing expanding towards longer wavelengths. Therefore we expect that the enhancement can take place over broader spectral range.

Peridinin–chlorophyll–protein is the photosynthetic complex from algae that is responsible for harvesting sunlight energy for photosynthesis. The energy is then transferred to the reaction center in a photosynthetic thylakoid membrane. The monomer of PCP contains two types of pigments, which absorb energy in different spectral regions [14]: the absorption spectrum of PCP shown in Fig. 2b features broad and intense band between 400 nm and 550 nm. They can be attributed to peridinins. On the other hand, chlorophyll *a* absorbs light from 350 to 440 nm and from 600 to 670 nm. The fluorescence of the PCP complex originates from the chlorophyll molecules and appears at 673 nm. In Fig. 2b we compare the absorption of silver nanowires and the PCP complex. It can be seen that there is strong spectral overlap, in particular in the blue-green region of the electromagnetic spectrum. We therefore expect interaction between plasmon excitations in silver nanowires and absorption of the PCP complex. The PCP complex, being water-soluble one, is also very convenient from the point of view of bioconjugation with nanoparticles in aqueous solution.

In order to form a conjugate of silver nanowires and the PCP complexes we use streptavidin–biotin interaction. The streptavidin–biotin forms one of the strongest non-covalently interacting pair; the binding is relatively fast and efficient, it also shows little degradation due to variation of the pH, temperature, solvents, etc. For that purpose it was necessary to functionalize the nanowires with biotin, so that we had to change the chemical properties of the obtained silver nanowires making them connectable to streptavidin.

The procedure is displayed in Fig. 3. We functionalized a surface of silver nanowires using derivates of biotin compound. The biotin disulfide has two sulfur atoms that have high affinity to the metallic surface. The PVP layer can be easily displaced by molecules with disulfide functionality, because sulfur atoms form a strong chemical bond both with gold and silver. The absorption spectrum of the nanowires functionalized with biotin remains essentially unchanged compared to the one shown in Fig. 2. Such behavior is expected. It is in fact somewhat in contrast to functionalization of gold nanoparticles with streptavidin, where clear shifts of the plasmon resonance were observed [8].

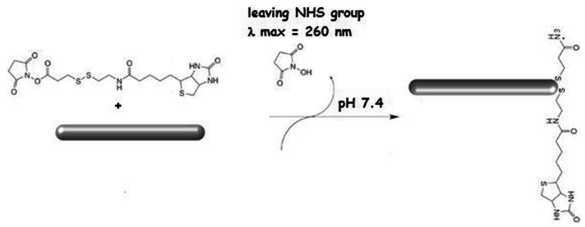


Fig. 3. Bioconjugation of silver nanowires using biotin disulfide derivate.

The process of conjugation between silver nanowires and PCP complexes was monitored using fluorescence spectroscopy. We measured fluorescence spectra of the PCP complexes functionalized with streptavidin upon addition of biotin-functionalized silver nanowires. The metallic nanoparticles were added after 2.5 min. From the sequence of fluorescence spectra the time dependence of the emission intensity was obtained, as shown in Fig. 4a both for the conjugate and for the reference sample containing PCP complexes only (black curve). In the case of the reference sample we observe gradual decrease of the fluorescence intensity, which is typical photobleaching behavior. In contrast, for the conjugate the fluorescence intensity increases just after addition of the silver nanowires into the PCP–SA solution. We attribute these changes as being a result of plasmon induced modifications of the optical properties of the photosynthetic complexes upon coupling with the silver nanostructures. After the initial increase, the emission intensity starts to fall down after couple of minutes in the same manner as observed for the reference solution. In Fig. 4b we show the fluorescence spectrum measured for the conjugate sample. The shape of the emission is identical to pure PCP solution, indicating that interaction with plasmonic nanostructures leads to no structural damage or major modification of the protein. We have also checked that the fluorescence excitation spectrum of the conjugate (Fig. 4b) remains unchanged proving additionally that the protein remains intact, including all the energy transfer pathways. This observation is important for any

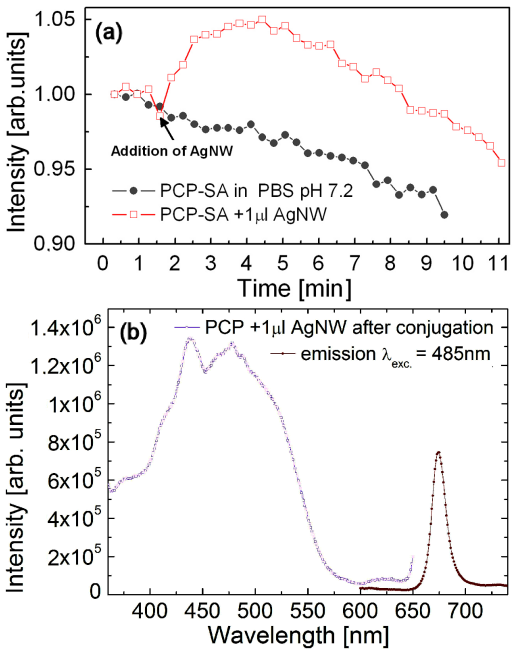


Fig. 4. (a) Changes in maximum of emission of light-harvesting complexes (squares) and light-harvesting complexes conjugated with AgNWr (circles) as a function of reaction time. (b) Fluorescence and fluorescence excitation spectra of the PCP light-harvesting complexes conjugated with Ag NWRs. The excitation wavelength was 485 nm.

future considerations aimed at applying light-harvesting, or more generally, photosynthetic complexes are functional devices.

In conclusion, we demonstrate the possibility to assembly a hybrid nanostructure composed of photosynthetic complexes as silver nanowires using biotin-streptavidin conjugation. The conjugated sample exhibits measurable increase of the fluorescence intensity due to plasmon induced enhancement of the absorption in the light-harvesting complexes. We are convinced that through establishing appropriate conditions, the enhancement could be increased even more, giving thus a possibility to fabricate functional hybrid nanostructures involving photosynthetic complexes.

Acknowledgments

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