J|A|C|S
COMMUNICATIONS

Plasmonic Field Enhancement of the Bacteriorhodopsin Photocurrent during Its Proton Pump Photocycle

Chun-Wan Yen, Li-Kang Chu, and Mostafa A El-Sayed*

Laser Dynamics Laboratory, School of Chemistry and Biochemistry, Georgia Institute of Technology, 901 Atlantic Drive, Atlanta, Georgia 30332

Received February 17, 2010; E-mail: mostafa.el-sayed@chemistry.gatech.edu

Photosynthesis is a process by which nature converts solar energy into the chemical energy that is required for fueling different living processes. There are two main different photosynthetic systems in nature: chlorophyll (using electron pump system) and bacteriorhodopsin (using proton pump system). Bacteriorhodopsin (bR) is found in the purple membrane of Halobacterium salinarum.[1] The absorption of light by its retinal in the ground state (bR_{570}) initiates a photocycle involving a number of intermediates and drives protons across the membrane from the internal cytoplasm to the external medium, resulting in proton gradients that are used for ATP synthesis in the cell.[2] The protons are ejected from the cell at a rate comparable to that of the M_{412} intermediate formation.[3] By CW illumination, bR can transform light energy into electrochemical energy stored in a proton gradient across the membrane and exhibit a stationary photocurrent amplitude. This unique characteristic of bR could make it promising for applications in alternative energy. However, the photocurrent density values reported so far are approximately 0.2−40 pA cm^{-2} $monolayer^{-1}$ in thin film systems.[4] Recently, our group was able to build a solution-based electrochemical cell that did not require bR film preparation and external bias.[5] In this communication, plasmonic field enhancement of the stationary photocurrent, orders of magnitude higher than previous reports, was observed and the mechanism involved was revealed.[4,5]

Surface plasmon resonance (SPR) is an interesting optical phenomenon induced by the coupling of the incident electromagnetic wave of light with the conduction band electrons in the metal. This induces a coherent electronic oscillation of the free electrons of the metal.[6] There is a rich variety of applications utilizing the SPR property of metallic nanoparticles (NPs),[7−9] and our group has previously reported that retinal photoisomerization in the primary step and the proton pump process of the bacteriorhodopsin were both perturbed by plasmonic fields of gold nanoparticles.[10,11] During the photocycle of bR, there are two important forms: the ground state of bacteriorhodopsin, bR_{570}, with a protonated Schiff base ($\lambda_{max} = 570$ nm) and the long-lived intermediate, M_{412}, with a deprotonated Schiff base ($\lambda_{max} = 412$ nm).[12] The M_{412} can be generated following the retinal isomerization (in 70 μs) and revert to the ground bR_{570} either thermally through a number of other intermediates ($M_{412} \rightarrow N \rightarrow O \rightarrow bR_{570}$) in 15 ms or rapidly through the photochemical process upon excitation with blue light in hundreds of nanoseconds (the known blue light effect).[13−15] The results of our experiments suggest that the surface plasmon enhancement of the M_{412} absorption (i.e., of the blue light effect) results in increasing the rate of proton release and forcing the photocycle to follow the short time bypassed path rather than the conventional thermal photocycle path of bR. The stationary photocurrent upon cw excitation of bR could be associated with the steady-state concentration of M_{412} in the $bR_{570} \rightarrow M_{412} \rightarrow bR_{570}$ photocycle.[15−17]

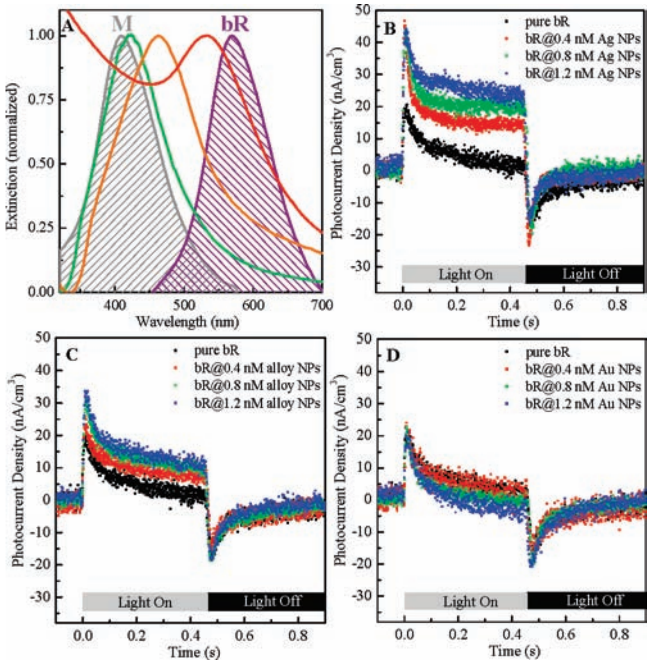

Figure 1. (A) Normalized SPR spectra of Ag NPs, Ag−Au alloy NPs (Ag/Au = 1/1), and Au NPs were at 425 nm (green), 465 nm (orange), and 530 nm (red), respectively. The absorption contours of M state and bR state were represented by gray and violet shadows with corresponding maxima at 412 and 570 nm, respectively. The photocurrent density of bR observed by mixing different nanoparticles (B) Ag NPs, (C) Ag−Au alloy NPs (Ag/Au = 1/1), and (D) Au NPs upon cw white light irradiation.

In this work, we synthesized Ag, Ag−Au alloy (Ag/Au = 1/1), and Au NPs with similar sizes (~30 nm) whose SPR absorption have maxima located at 425, 465, and 530 nm, respectively (Figure 1A). The spectral overlap between the SPR band of NPs and that of the M_{412} absorption band decreased as the gold amounts added to the NPs was increased. The photocurrent measurements were carried out by our recently developed techniques summarized in the Supporting Information.

Figure 1 shows the photocurrent generation from a solution of bR and of bR/NPs mixtures. CW broad band irradiation ($\lambda_{incident} >$ 380 nm) was used to ensure that we can excite bR at 570 nm to produce M_{412} and simultaneously excite Ag NPs at 420 nm to enhance the blue light intensity. During the light-on period, the photocurrent initially showed a sharp rise followed by decay to a near stationary photocurrent. During the light-off period, the observed inverted photocurrent was attributed to the net proton uptake of bR photocycle.

Upon excitation of pure bR, the maximum of the instantaneous rise of photocurrent density was 19.8 nA/cm^3 followed by a stationary offset of 1.6 nA/cm^3 (Figure 1B). When the bR solution

10.1021/ja101301u

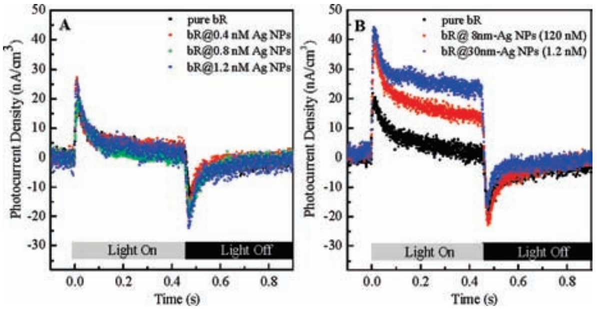

Figure 2. (A) Disappearance of the plasmonic photocurrent enhancement of 30 nm Ag NPs if the blue wavelength of the exciting light is filtered out ($\lambda_{\text{incident}} > 500$ nm is used). (B) Effect of changing the overlap between the SPR extinction band in the blue region with the M_{412} absorption band (see Figure S1). As the overlap decreases (by using 8 nm Ag NPs), the weaker the observed photocurrent becomes.

was mixed with Ag NPs whose SPR band overlapped strongly with the M_{412} absorption band, the initial photocurrent density was increased to 45 nA/cm^3 (Figure 1B). The value of the stationary photocurrent density was 25 nA/cm^3 when bR was mixed with 1.2 nM of Ag NPs, and it was 15 times higher than that of pure bR. The notable increase of photocurrent is due to the enhanced flux of blue photons by the plasmonic field of Ag NPs.

When Ag−Au alloy NPs with similar sizes and concentrations as those of the Ag NPs were used (Figure 1C), the stationary photocurrents were 11.3 nA/cm^3 and increased by 6 times compared with the value of pure bR. This smaller enhancement was probably due to the smaller spectral overlap between the plasmonic absorption of Ag−Au NPs and the M_{412} intermediates compared that of Ag NPs. By shifting the plasmon band more toward bR_{570} absorption by using Au NPs, a negative plasmonic field effect was observed (Figure 1D). There were two strong bands of Au NPs, the SPR band at a long wavelength that overlapped with the bR_{570} absorption and another d−d nonplasmonic band in the blue region of the spectrum (Figure 1A). The bR_{570} absorption of the white light should increase as a result of its overlap with the SPR band from the Au NPs in this region; however the blue light intensity was absorbed by the strong nonplasmonic d−d absorption of the Au NPs, thus decreasing the blue light effect and the photocurrent.

It is found that the plasmonic enhancement effect of Ag NPs on the absorption of the intermediate (M_{412}) is much more pronounced than that of the Au NPs on the ground state (bR_{570}). This might be due to two reasons. One is the filter effect of the blue light by the d−d absorption of Au NPs which eliminates the enhancement of the bR_{570} absorption. The second reason may be the different time scale of the photocycle path; bR follows the full conventional photocycle when exciting the bR_{570}, but the blue light effect causes the bR to follow the much shorter bypass photocycle.[15−17] Both factors might explain the importance of the plasmonic field enhancement of the blue light effect to the bR photocurrent.

The above conclusion can be supported by the results of the following two experiments. The first experiment showed that there was no observed photocurrent enhancement of bR when 30 nm Ag NPs were used under the same intensity of cw illumination but the blue light was filtered out ($\lambda_{\text{incident}} > 500$ nm), resulting in the absence of a plasmonic field (Figure 2A). This suggests that the enhancement of photocurrent density was due to the exciting plasmonic effect of NPs in the blue light region, and not due to the chemical environment or the ionic concentration change by the addition of NPs. The other experiment showed that the larger the overlap between the SPR band of Ag NPs and M_{412} absorption band, the greater the photocurrent enhancement (Figure 2B). Ag NPs of smaller particle sizes (8 nm) were prepared whose SPR band was much narrower than the SPR band of the larger Ag NPs (30 nm) (Figure S1). The difference in photocurrent amplitude and photoelectric profile between the two different sizes of Ag NPs can be explained by the difference in the width of the absorption band of the Ag NPs. The absorption band of 30 nm Ag NPs overlapped better with the absorption contour of M_{412} than that of the 8 nm Ag NPs (Figure S1), and the plasmonic-energy transfer was more efficient to the M_{412} under the same amount of light flux. The results of these above experiments strongly support the conclusion that the plasmonic effect of nanoparticles with an extinction spectrum that overlaps with the M_{412} absorption can excite M_{412} to rapidly form bR_{570} by bypassing the normal photocycle. This increases the duty cycles of the proton release and thus the photocurrent density.

Bacteriorhodopsin's exceptional stability makes it a potential biomaterial for various applications.[18] The reported bR-based electrochemical cell used thin films and required an external bias combination and a laser irradiation source.[19] In our system, cw light is used for the irradiation source and no external bias is needed. Moreover, NPs whose SPR band overlaps well with the M_{412} absorption also overlaps with the maximum intensity region of the solar light at 450 nm.[20] Those factors make a bR-based electrochemical cell using a plasmonically enhanced photocurrent attractive for solar energy conversion.[21]

Acknowledgment. We would like to thank the U.S. Department of Energy, Office of Basic Energy Sciences Grant No. DE-FG02-97 ER 14799. We also thank Mrs. Rachel Near for carefully revising this manuscript.

Supporting Information Available: Synthesis of nanoparticles, UV−vis absorption spectrum, and experimental setup. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Oesterhelt, D.; Stoeckenius, W. *Nature New Biol.* **1971**, *233*, 149.
(2) Mitchell, P. *Nature* **1961**, *191*, 144.
(3) Belliveau, J. W.; Lanyi, J. K. *Arch. Biochem. Biophys.* **1997**, *178*, 308.
(4) Jin, Y.; Honig, T.; Ron, I.; Friedman, N.; Sheves, M.; Cahen, D. *Chem. Soc. Rev.* **2008**, *37*, 2422.
(5) Chu, L.-K.; Yen, C. W.; El-Sayed, M. A. *Biosens. Bioelectron.* Submitted.
(6) Ghosh, S. K.; Pal, T. *Chem. Rev.* **2007**, *107*, 4797.
(7) Neretina, S.; Qian, W.; Dreaden, E. C.; Hughes, R. A.; Preston, J. S.; Mascher, P.; El-Sayed, M. A. *Nano Lett.* **2008**, *8*, 2410.
(8) Gersten, J. I.; Nitzan, A. *J. Chem. Phys.* **1980**, *73*, 3023.
(9) Yen, C. W.; El-Sayed, M. A. *J. Phys. Chem. C* **2009**, *113*, 19585.
(10) Biesso, A.; Qian, W.; El-Sayed, M. A. *J. Am. Chem. Soc.* **2008**, *130*, 3258.
(11) Biesso, A.; Qian, W.; Huang, X.; El-Sayed, M. A. *J. Am. Chem. Soc.* **2009**, *131*, 2442.
(12) Drachev, L. A.; Kaulen, A. D.; Skulachev, V. P. *FEBS Lett.* **1984**, *2168*, 331.
(13) Robertson, B.; Lukashev, E. *Biophys. J.* **1995**, *68*, 1507.
(14) Ormos, P.; Dancshazy, Z.; Karvaly, B. *Biochim. Biophys. Acta* **1978**, *503*, 304.
(15) Hessling, B.; Herbst, J.; Rammelsberg, R.; Gerwert, K. *Biophys. J.* **1997**, *73*, 2071.
(16) Li, R.; Li, C. M.; Bao, H.; Bao, Q.; Lee, V. S. *Appl. Phys. Lett.* **2007**, *91*, 223901.
(17) Uehara, K.; Kawai, K.; Kouyama, T. *Thin Solid Films* **1993**, *232*, 271.
(18) Shen, Y.; Safinya, C. R.; Liang, K. S.; Ruppert, A. F.; Rothschild, K. J. *Nature* **1993**, *366*, 48.
(19) Manoj, A. G.; Narayan, K. S. *Appl. Phys. Lett.* **2003**, *83*, 3614.
(20) Kruse, O.; Rupprecht, J.; Mussgnug, J. H.; Dismukes, G. C.; Hankamer, B. *Photochem. Photobiol. Sci.* **2005**, *4*, 957.
(21) El-Sayed, M. A. *Acc. Chem. Res.* **1992**, *25*, 279.

JA101301U