J. Microbiol. Biotechnol. (2017), 27(12), 2173–2179
https://doi.org/10.4014/jmb.1708.08068

jmb

Characterization of ATPase Activity of Free and Immobilized Chromatophore Membrane Vesicles of *Rhodobacter sphaeroides*

Hyeonjun Kim[1†], Xiaomeng Tong[1†], Sungyoung Choi[2], and Jeong K. Lee[1*]

[1]*Department of Life Science, Sogang University, Seoul 04107, Republic of Korea*
[2]*Department of Biomedical Engineering, Kyung Hee University, Gyeonggi-do 17104, Republic of Korea*

Received: September 1, 2017
Revised: October 7, 2017
Accepted: October 10, 2017

First published online
October 14, 2017

**Corresponding author*
Phone: +82-2-705-8459;
Fax: +82-2-704-3601;
E-mail: jgklee@sogang.ac.kr

†These authors contributed equally to this work.

pISSN 1017-7825, eISSN 1738-8872

The intracytoplasmic membrane of *Rhodobacter sphaeroides* readily vesiculates when cells are lysed. The resulting chromatophore membrane vesicle (CMV) contains the photosynthetic machineries to synthesize ATP by ATPase. The light-dependent ATPase activity of CMV was lowered in the presence of O_2, but the activity increased to the level observed under anaerobic condition when the reaction mixture was supplemented with ascorbic acid (≥0.5 mM). Cell lysis in the presence of biotinyl cap phospholipid (bcp) resulted in the incorporation of bcp into the membrane to form biotinylated CMV (bCMV), which binds to streptavidin resin at a ratio of approximately 24 μg bacteriochlorophyll *a*/ml resin. The ATPase activity of CMV was not affected by biotinylation, but approximately 30% of the activity was lost by immobilization to resin. Interestingly, the remaining 70% of ATPase activity stayed constant during 7-day storage at 4°C. On the contrary, the ATPase activity of bCMV without immobilization gradually decreased to approximately 40% of the initial level in the same comparison. Thus, the ATPase activity of CMV is sustainable after immobilization, and the immobilized bCMV can be used repeatedly as an ATP generator.

Keywords: *Rhodobacter sphaeroides*, chromatophore membrane vesicle, immobilization, biotinyl cap phospholipid, light-dependent ATPase

Introduction

Rhodobacter sphaeroides is a purple non-sulfur photosynthetic bacterium performing anoxygenic photosynthesis [1]. The bacterium has a specialized intracellular structure for photosynthesis, which is an invaginated cytoplasmic membrane (also called an intracytoplasmic membrane (ICM) or chromatophore membrane). The ICM houses the light-harvesting complexes (LH1 and LH2), photosynthetic reaction center (RC), F_OF_1-ATPase (ATP synthase), and proteins for the electron transport chain [2, 3]. Once light is absorbed by LH1 and LH2 complexes, the energy is eventually transferred to a special pair of bacteriochlorophyll *a* (Bchl *a*) of the RC to trigger charge separation, followed by electron transfer in a cyclic manner [4]. Then, proton-motive force is generated inside the ICM and utilized for ATP synthesis by ATPase [5].

When *R. sphaeroides* grown photoheterotrophically is ruptured, its ICM readily forms a chromatophore membrane vesicle (CMV) of approximately 70 nm diameter [6], which contains the photosynthetic apparatus [7]. The periplasmic content, typically cytochrome c_2, is localized inside the vesicle, whereas ATPase is exposed outside [8]. Thus, CMV is able to perform the light-dependent synthesis of ATP in vitro.

Enzymes and living cells can be attached or confined to inert and insoluble material in order to sustain the bio-catalytic activities of interest. There have been several methods for the immobilization of biocatalysts, which include adsorption, covalent linking, ionic binding, entrapment, and affinity binding [9, 10]. The materials for immobilization may be chosen among natural polymers, synthetic polymers, or inorganic materials, depending on the property of the biocatalyst and the nature of the reaction [10]. The advantage of immobilization is to facilitate the availability of the biocatalyst. Accordingly, the turnover

of biocatalyst is greatly increased, and product separation and bioreactor operation get easier [11].

In this work, we prepared biotinylated CMVs (bCMVs) by integrating biotinyl cap phospholipid (bcp) into the membrane of CMV. The bCMV was attached to streptavidin-linked agarose bead resin, and the immobilized bCMV showed a higher sustainability of ATPase activity, compared with that of free bCMV. This is the first demonstration to immobilize CMV and characterize the sustainability of the ATPase activity of the immobilized CMV.

Materials and Methods

Bacterial Strain and Growth Conditions

R. sphaeroides 2.4.1 [12] was grown photoheterotrophically in Sistrom's succinate-based minimal medium [13] at 28°C as described by Sistrom [14].

Purification of CMV and Preparation of bCMV

CMV was purified as previously described [15] with some modifications. All procedures were carried out in an anaerobic chamber (Model 10; Coy Laboratory Products, USA) filled with 90% N_2, 5% H_2, and 5% CO_2. The photoheterotrophic culture of *R. sphaeroides* was harvested by centrifugation at 4,000 ×*g* for 10 min. The pellet was resuspended in buffer A (10 mM Tris-Cl, pH 8.0, containing 10% (w/v) sucrose, 5 mM sodium ascorbate, and EDTA-free protease inhibitor cocktail (cOmplete; Roche, Swizerland)). Cells were lysed by sonication, followed by centrifugation at 6,000 ×*g* for 10 min to obtain the supernatant. The cell-free supernatant was subjected to ultracentrifugation at 150,000 ×*g*, 4°C for 2 h using a Beckman SW41Ti rotor, and the resulting membrane pellet was resuspended in buffer A. The membrane suspension was layered on a discontinuous sucrose gradient composed of 20% (w/v), 40%, and 60% sucrose in buffer A. After ultracentrifugation at 110,000 ×*g*, 4°C for 4 h using a Beckman SW41Ti rotor, the CMV at the interface between the 20% and 40% sucrose layer was collected and diluted by the same volume of buffer A. For the preparation of bCMV, all the procedures were exactly the same except that 2.0 mg of bcp (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-cap biotinyl; Avanti Polar Lipids, USA) was added per 10^{10} cells immediately before cell lysis. The CMV concentration was quantified by determining the Bchl *a* content and the CMV-incorporated bcp was analyzed through determination of the biotin level.

Quantification of Bcp of bCMV through Determination of Biotin

The bcp in the outer leaflet of the membrane was considered only in this work. The outward-facing biotin was released by digestion of bCMV with 10 U phospholipase C (P7633; Sigma, USA) in 50 mM Tris-Cl (pH 7.3) containing 5 mM $CaCl_2$ at 37°C for 12 h. The liberated biotin was determined with a FluoReporter Biotin Quantitation Assay Kit (Thermo Fisher Scientific, USA). Varying amounts of bcp were treated in the same way to plot a standard curve of bcp vs. biotin.

ATPase Assay

The ATPase reactions of CMV and bCMV were performed in a reaction buffer (50 mM Tris-Cl (pH 8.0), 10 mM Na_2HPO_4, 10 mM $MgCl_2$, 5 mM sodium ascorbate (added only under aerobic condition), and 1 mM ADP) under the illuminated condition of 15 W/m^2. When the ATPase activity of the immobilized bCMV was determined, 100 μM of DAPP (diadenosine pentaphosphate) was added to the reaction mixture to block adenylate kinase activity. The ATP level was determined with an ATP Bioluminescent Assay Kit (Sigma, USA).

Determination of Bchl *a*

Bchl *a* from CMV and bCMV was extracted in acetone-methanol (7:2 (v/v)), and the Bchl *a* level in the extract was determined with an extinction coefficient (ε) of 83.9 mM^{-1} cm^{-1} at 771 nm [16].

Determination of Light-Harvesting Complexes

CMV in phosphate-buffered saline (pH 7.4) was spectrally analyzed by a UV-Vis spectrophotometer (UV 2550; Shimadzu, Japan). The level of B875 complex was calculated with $A_{878-820}$ and an extinction coefficient of 73 mM^{-1} cm^{-1}, and that of B800-850 complex was calculated with $A_{849-900}$ and an extinction coefficient of 96 mM^{-1} cm^{-1} [17].

Immobilization of bCMV to Streptavidin Resin

Varying amounts of bCMV were mixed with streptavidin [18]-linked agarose bead resin (Strep-Tactin Sepharose 50% suspension; IBA Lifesciences, Germany) pre-equilibrated with a binding buffer (10 mM Tris-Cl (pH 8.0) containing 150 mM NaCl). The mixture was incubated at 25°C for 5 min and transferred onto an empty column. Non-bound bCMV was removed by washing the column with binding buffer. The bCMV-bound streptavidin resin was taken from the column and stored at 4°C in the binding buffer supplemented with 5 mM sodium ascorbate. To determine the amount of bCMV bound to resin, the resin was soaked in acetone-methanol (7:2 (v/v)). After 5-min incubation at 25°C, the extract was separated from the resin and Bchl *a* was determined as described above.

Results and Discussion

Characterization of CMV

Given the photosynthetic machineries of CMV sufficient for light-driven ATP formation, ATP has to be synthesized from ADP and inorganic phosphate in light. As expected, ATP was formed by the purified CMV in a light-dependent manner (Fig. 1). The kinetic parameters of CMV for ATP synthesis were determined at 30°C under anaerobic condition. Approximately 2,481 Bchl *a* molecules are assumed to be

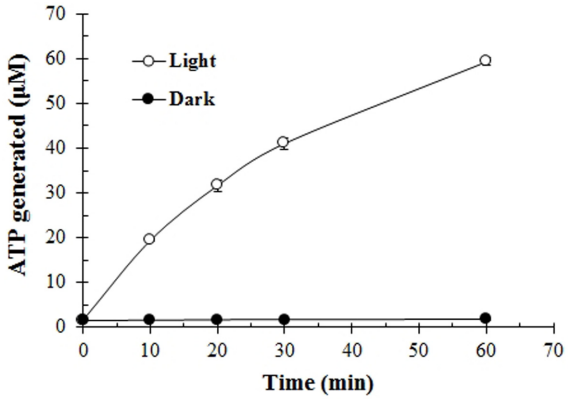

Fig. 1. Light-dependent ATP generation of chromatophore membrane vesicles (CMVs).

The ATPase reaction of CMV (1 μg Bchl *a*/ml) was performed in light (open circle) and in the dark (closed circle) under anaerobic condition. The experiments were independently repeated three times; data shown represent the means with standard deviation (SD) from triplicate experiments.

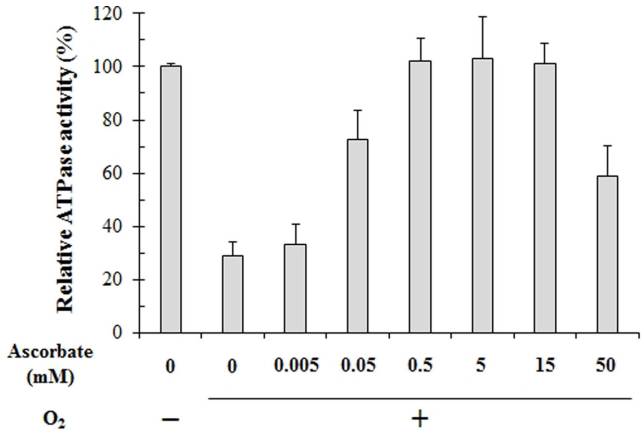

Fig. 2. Reduction in the ATPase activity of chromatophore membrane vesicles (CMVs) by O_2 and its restoration by ascorbate.

ATPase activities of CMVs were measured under anaerobic and aerobic conditions. The detrimental O_2 effect on the ATPase activity of the CMV was relieved by ascorbate in a dose-dependent manner except for concentration at 50 mM. The experiments were independently repeated three times; the average values are shown with the SD on each bar.

present per CMV, which is calculated from the number of its light-harvesting complexes and RC complex [19]. The apparent K_m of CMV ATPase for ADP was 11.0 ± 4.7 μM, which is similar to the average K_m 9.3 ± 6 μM of *E. coli* ATPase on putative inside-out membrane vesicles [20]. The apparent k_{cat} for ATP synthesis by CMV ATPase was approximately 27.7 ± 9.7 s^{-1}. Since one CMV is supposed to have two ATPase complexes [19], the k_{cat} for ATP synthesis by a CMV would be 55.4 s^{-1}. Recently, Cartron *et al*. [19] theoretically proposed that k_{cat} for ATP synthesis by a CMV was approximately 101 under the illumination intensities between 10 and 30 W/m^2. The k_{cat} shown in this work was about half that of the theoretical value, the reason for which is not yet clear but may reside in the low purity of CMV. The ICM has to be sealed with the inside-out orientation to form a functional CMV. If ICM is sealed with the right-side-out orientation, the vesicle may look similar to a functional CMV, but it would no longer have active ATPase. The way to estimate the purity of CMVs remains to be determined.

Since *R. sphaeroides* performs anoxygenic photosynthesis [21], the ATPase activity of CMVs could be affected by O_2. As expected, the activity decreased by 70% under ambient atmospheric condition compared with the control measured under anaerobic condition (Fig. 2). Excitation of Bchl *a* in light and subsequent electron flow in the chromatophore membrane under aerobic condition may generate reactive oxygen species [21]. Interestingly, addition of ascorbate, which is known as a superoxide scavenger [22], resulted in alleviation of the oxygen effect in a dose-dependent manner (Fig. 2). However, ascorbate at 50 mM instead decreased the ATPase activity. It has been known that high-dose ascorbate may act as a prooxidant in the presence of a catalytic metal [23]. In the following experiments, the ATPase activity of the CMV was measured under ambient atmospheric conditions in the presence of 5 mM ascorbate, which fully alleviated the oxygen effect. Moreover, it was 10-fold higher than the minimal ascorbate (0.5 mM) showing no oxygen effect (Fig. 2).

Construction of Biotinylated CMV

The specific interaction between biotin and streptavidin [18] was exploited for the immobilization of CMVs. In order to label CMVs with biotin, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-cap biotinyl was added to the cell suspension right before sonication for cell lysis. Sonic wave disturbs the membrane and the bcp can be integrated into the membrane of the CMV to generate bCMV. Most CMVs in this treatment were thought to have bcp integrated, and the resulting bCMV was isolated by ultracentrifugation.

The CMV-incorporated bcp level increased in proportion to bcp added to the cell suspension before lysis (Fig. 3A), but the percentage incorporation of bcp (CMV-incorporated bcp/bcp added) was in a range of approximately 7% to 10%. In order to know the relative bcp incorporation per

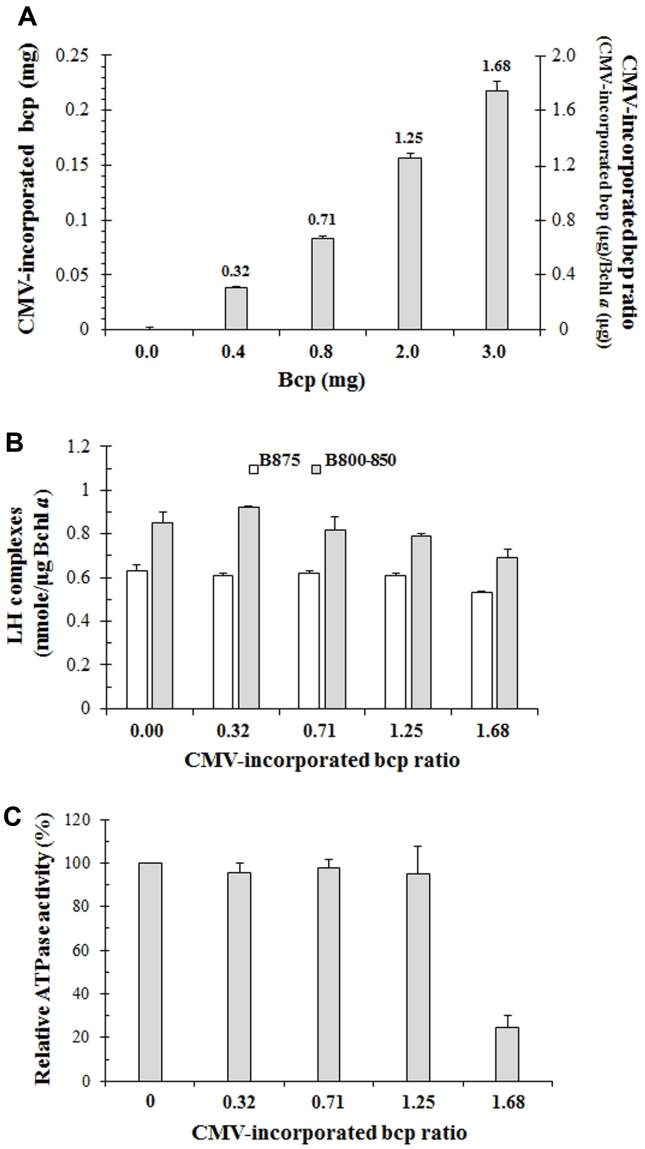

Fig. 3. Optimization of the biotinyl cap phospholipid (bcp) level to construct biotinylated chromatophore membrane vesicles (bCMVs).

(**A**) Varying amounts of bcp were added to the cell suspension immediately before cell lysis, and bCMVs were purified to determine the CMV-incorporated bcp. The same data were also expressed as the ratio of CMV-incorporated bcp (μg) per bacteriochlorophyll *a* (Bchl *a*) (μg), whose values are specified on top of each bar. CMV without bcp was included as a control. (**B**) The light-harvesting (LH) complex and (**C**) the relative ATPase activity were determined with bCMVs having a CMV-incorporated bcp ratio from 0 to 1.68. The experiments were independently repeated three times; the average values are shown with the SD on each bar.

CMV, the data were further expressed as the ratio of CMV-incorporated bcp (μg) per Bchl *a* (μg) (Fig. 3A), which also

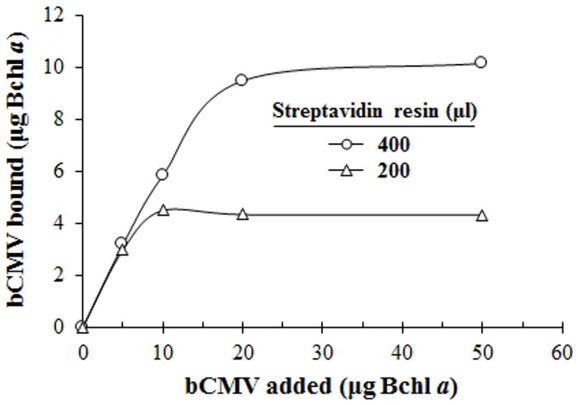

Fig. 4. Binding of biotinylated chromatophore membrane vesicles (bCMVs) to streptavidin resin.

Varying amounts of bCMV were mixed with streptavidin-linked agarose bead resin (400 μl, open circle; 200 μl, open triangle), followed by removal of non-bound bCMV by washing. The amounts of immobilized bCMV were determined by measuring bacteriochlorophyll *a* (Bchl *a*) after extraction of the bCMV-bound resin with acetone-methanol (7:2 (v/v)). The experiments were independently repeated three times; the data shown represent the means with standard deviation from triplicate experiments.

increased in proportion to bcp added. The levels of the B875 complex and B800-850 complex of CMV were not much affected by bcp, but 10% reduction in LH complexes was observed at 1.68 CMV-incorporated bcp ratio (Fig. 3B). The ATPase activity was significantly reduced at the same CMV-incorporated bcp ratio (Fig. 3C). The phospholipids surrounding the ATPase of the CMV may be occupied significantly by bcp at the high CMV-incorporated bcp ratio, which might affect the ATPase structure and activity. Accordingly, bCMV at 1.25 CMV-incorporated bcp ratio, whose ATPase activity was not affected by bcp, was used in the following experiments.

Immobilization of bCMV to Streptavidin Resin

In order to characterize the binding of bCMVs to streptavidin resin, varying amounts of bCMV were mixed with a fixed amount of streptavidin resin. Then, the resin was washed and bCMV bound to streptavidin resin was quantified with Bchl *a*. It was found that the amount of bCMV bound to resin was proportionally increased with the level of bCMV in binding buffer until the resin was saturated (Fig. 4). The capacity of streptavidin resin to bind bCMV was calculated as approximately 24 μg Bchl *a*/ml resin from the binding curve. It was also found from the linear range of the binding curve that approximately 60% of bCMV was immobilized to streptavidin resin. The binding

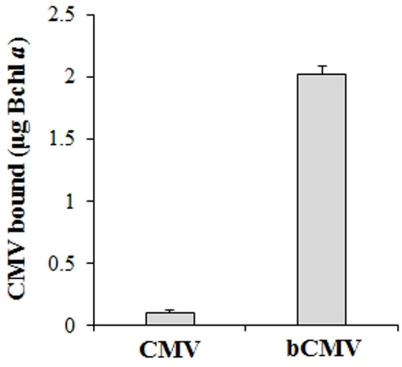

Fig. 5. Non-specific binding of biotin-free chromatophore membrane vesicles (CMVs) to streptavidin resin.

Biotinylated CMV (bCMV) was prepared at the CMV-incorporated bcp ratio of 1.25. CMV prepared without bcp was used as a control. Both CMV and bCMV (each at 10 μg Bchl *a*) were mixed with 200 μl of streptavidin resin, followed by determination of bound Bchl *a*. The experiments were independently repeated three times; the average values are shown with the SD on each bar.

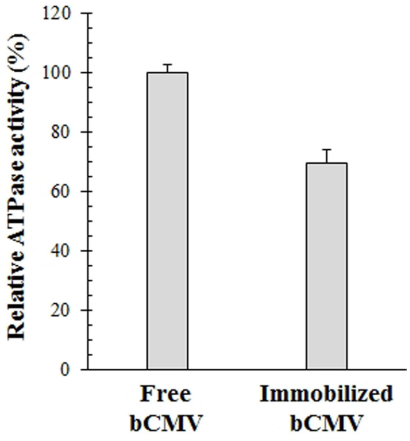

Fig. 7. ATPase activity of free and immobilized biotinylated chromatophore membrane vesicles (bCMVs).

The ATPase activity of the bCMV immobilized to streptavidin resin was measured and compared with that of free bCMV after normalization with the bacteriochlorophyll *a* level. The experiments were independently repeated three times; the average values are shown with the SD on each bar.

of biotin-free CMV to streptavidin resin was negligible (Fig. 5), so nonspecific binding between the CMV and streptavidin resin was ignored.

We found that the streptavidin resin used in this study has adenylate kinase activity, which catalyzes the transfer of phosphate between two ADP molecules, resulting in formation of ATP and AMP [24]. Thus, the ATPase activity of the immobilized bCMV is overestimated owing to the resin-associated adenylate kinase activity. The inhibitor DAPP was used to block the effect by adenylate kinase [25]. As DAPP increased above 100 μM, the adenylate kinase activity decreased by approximately 90% (Fig. 6A). However, the ATPase activity of CMV was not affected by 100 μM DAPP (Fig. 6B). Therefore, the ATPase activity of the immobilized bCMV was measured in the presence of

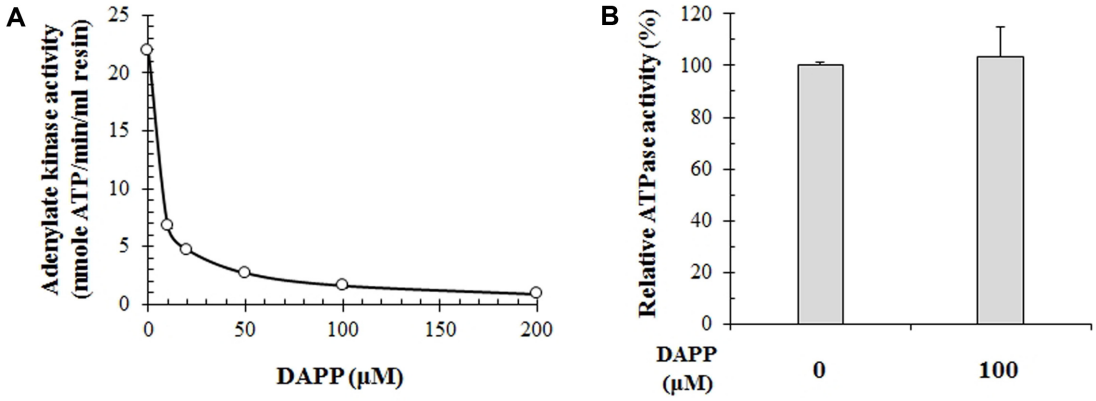

Fig. 6. Inhibition of adenylate kinase associated with streptavidin resin by diadenosine pentaphosphate (DAPP) and its effect on ATPase activity of chromatophore membrane vesicles (CMVs).

(**A**) The activity of streptavidin-resin-associated adenylate kinase was determined with 500 μl of streptavidin resin in the presence of varying concentrations of DAPP, an adenylate kinase inhibitor. The reaction buffer consisted of 50 mM Tris-Cl (pH 8.0), 10 mM Na_2HPO_4, 10 mM $MgCl_2$, and 1 mM ADP. (**B**) Since adenylate kinase activity was severely inhibited at 100 μM DAPP, the ATPase activity of the CMV was investigated in the presence or absence of 100 μM DAPP. The experiments were independently repeated three times; the data shown are one of three representative experiments.

100 μM DAPP.

The ATPase activity of the immobilized bCMV was measured and compared with that of free bCMV after normalization with the Bchl *a* level (Fig. 7). Approximately 30% of the ATPase activity of bCMV was lost by immobilization. The reason for the decrease of ATPase activity is not yet clear, but one plausible explanation is the masking of the CMV ATPase by immobilization. The binding of bCMV to streptavidin resin may be through multiple biotins, and the surface region of bCMV, which is enclosed by biotin-streptavidin interactions, might be in direct contact with the resin, affecting ATPase. If ATPase is located in that region, it may not work properly.

Sustainability of the Immobilized bCMV

The ATPase activity of the immobilized bCMV was examined for a week and compared with that of free bCMV. Both bCMVs were kept at 4°C during the period. Remarkably, the ATPase activity of the immobilized bCMV was not affected during 7 days, whereas that of the free bCMV gradually decreased, reaching 40% of its initial value in 4 days, and then remained constant (Fig. 8). The mechanisms of the stabilization of bCMV by immobilization is not yet clear. However, immobilization of soluble enzymes has been found to exhibit better functional properties, including enzyme stability [26–28]. Although the enhancement of enzyme stability appears to be dependent on the immobilization method, it has been proposed that the increase in enzyme rigidity, reduction in conformational change leading to enzyme inactivation, and protection of the enzyme from contact with any hydrophobic interface or material could result from immobilization [26, 29]. Immobilization might also provide the bCMV with structural consistency, leading to its enhanced sustainability.

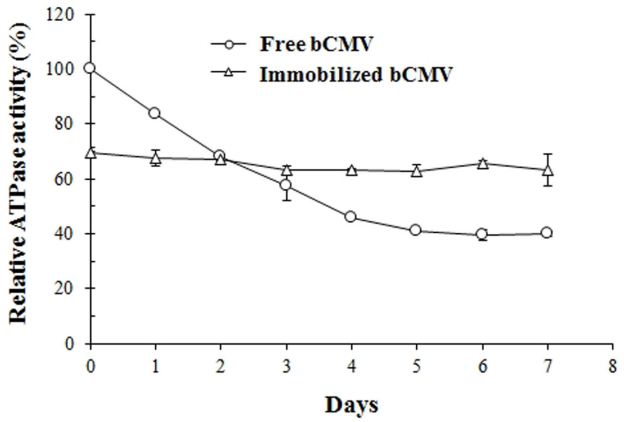

Fig. 8. Sustainability of free and immobilized biotinylated chromatophore membrane vesicles (bCMVs).
The ATPase activity of the immobilized bCMV was examined every day for a week and compared with that of free bCMV. Both bCMVs were kept at 4°C during the period. The experiments were independently repeated three times; the data shown represent the means with standard deviation from triplicate experiments.

It has been shown that cells were permeated by the surfactant Triton X-100 and it was used as the ATP-generating source in the presence of glucose [30–32]. The immobilized bCMV shown in this work may be used as a sustainable supplier of ATP without using energy-rich chemicals. Once ATP is used in an ATP-consuming reaction, the released ADP and inorganic phosphate are condensed to form ATP again by immobilized bCMV in light, constituting ATP-ADP recycling. Moreover, the immobilized bCMV can be easily separated from the reaction mixture and reused in other cycles. One intrinsic limitation of this method is the low ratio of bCMV binding to streptavidin resin (24 μg Bchl *a*/ml resin), which is attributed to the limited surface area of the resin and the relatively large size of CMV. However, the binding ratio may be increased significantly by orienting the CMVs in multilayers on the resin, which can be achieved by direct cross-linking of bCMVs through free streptavidin exogenously added to the solution.

In summary, bCMV was prepared by integrating bcp into the membrane of CMV, and bCMV was bound to streptavidin resin. The immobilized bCMV showed a higher sustainability of ATPase activity compared with that of free bCMV.

Acknowledgments

This work was supported by the Pioneer Research Center Program through the NRF (No. 2013M3C1A3064325). This work was also supported by the Basic Science Research Program through the NRF (No. 2009-0093822) funded by the Ministry of Science, ICT & Future Planning.

References

1. Bryant DA, Frigaard N-U. 2006. Prokaryotic photosynthesis and phototrophy illuminated. *Trends Microbiol.* **14:** 488-496.
2. Jackson PJ, Lewis HJ, Tucker JD, Hunter CN, Dickman MJ. 2012. Quantitative proteomic analysis of intracytoplasmic membrane development in *Rhodobacter sphaeroides*. *Mol. Microbiol.* **84:** 1062-1078.
3. Zeng X, Roh JH, Callister SJ, Tavano CL, Donohue TJ, Lipton MS, *et al*. 2007. Proteomic characterization of the *Rhodobacter sphaeroides* 2.4.1 photosynthetic membrane:

identification of new proteins. *J. Bacteriol.* **189:** 7464-7474.
4. Drews G, Oelze J. 1981. Organization and differentiation of membranes of phototrophic bacteria. *Adv. Microb. Physiol.* **22:** 1-92.
5. Okuno D, Iino R, Noji H. 2011. Rotation and structure of F_oF_1-ATP synthase. *J. Biochem.* **149:** 655-664.
6. Gubellini F, Francia F, Turina P, Levy D, Venturoli G, Melandri BA. 2007. Heterogeneity of photosynthetic membranes from *Rhodobacter capsulatus*: size dispersion and ATP synthase distribution. *Biochim. Biophys. Acta* **1767:** 1340-1352.
7. Michels PA, Konings WN. 1978. The electrochemical proton gradient generated by light in membrane vesicles and chromatophores from *Rhodopseudomonas sphaeroides*. *Eur. J. Biochem.* **85:** 147-155.
8. Hellingwerf KJ, Michels PA, Dorpema JW, Konings WN. 1975. Transport of amino acids in membrane vesicles of *Rhodopseudomonas spheroides* energized by respiratory and cyclic electron flow. *Eur. J. Biochem.* **55:** 397-406.
9. Nisha S, Arun KS, Gobi N. 2012. A review on methods, application and properties of immobilized enzyme. *Chem. Sci. Rev. Lett.* **1:** 148-155.
10. Datta S, Christena LR, Rajaram YRS. 2013. Enzyme immobilization: an overview on techniques and support materials. *3 Biotech* **3:** 1-9.
11. Guisan JM. 2006. *Immobilization of Enzymes and Cells*, pp. 15-30. 2nd Ed. Humana Press, Totowa, NJ.
12. Cohen-Bazire G, Sistrom WR, Stanier RY. 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. *J. Cell. Comp. Physiol.* **49:** 25-68.
13. Sistrom WR. 1960. A requirement for sodium in the growth of *Rhodopseudomonas spheroides*. *J. Gen. Microbiol.* **22:** 778-785.
14. Sistrom WR. 1962. Observations on the relationship between the formation of photopigments and the synthesis of protein in *Rhodopseudomonas spheroides*. *J. Gen. Microbiol.* **28:** 599-605.
15. Fraley RT, Lueking DR, Kaplan S. 1978. Intracytoplasmic membrane synthesis in synchronous cell populations of *Rhodopseudomonas sphaeroides*. Polypeptide insertion into growing membrane. *J. Biol. Chem.* **253:** 458-464.
16. Tanaka K, Kakuno T, Yamashita J, Horio T. 1982. Purification and properties of chlorophyllase from greened rye seedlings. *J. Biochem.* **92:** 1763-1773.
17. Meinhardt SW, Kiley PJ, Kaplan S, Crofts AR, Harayama S. 1985. Characterization of light-harvesting mutants of *Rhodopseudomonas sphaeroides*. I. Measurement of the efficiency of energy transfer from light-harvesting complexes to the reaction center. *Arch. Biochem. Biophys.* **236:** 130-139.
18. Weber PC, Ohlendorf DH, Wendoloski JJ, Salemme FR. 1989. Structural origins of high-affinity biotin binding to streptavidin. *Science* **243:** 85-88.
19. Cartron ML, Olsen JD, Sener M, Jackson PJ, Brindley AA, Qian P, *et al*. 2014. Integration of energy and electron transfer processes in the photosynthetic membrane of *Rhodobacter sphaeroides*. *Biochim. Biophys. Acta* **1837:** 1769-1780.
20. Etzold C, Deckers-Hebestreit G, Altendorf K. 1997. Turnover number of *Escherichia coli* F_oF_1 ATP synthase for ATP synthesis in membrane vesicles. *Eur. J. Biochem.* **243:** 336-343.
21. Berghoff BA, Glaeser J, Nuss AM, Zobawa M, Lottspeich F, Klug G. 2011. Anoxygenic photosynthesis and photooxidative stress: a particular challenge for *Roseobacter*. *Environ. Microbiol.* **13:** 775-791.
22. Nandi A, Chatterjee IB. 1987. Scavenging of superoxide radical by ascorbic acid. *J. Biosci.* **11:** 435-441.
23. Castro ML, Carson GM, McConnell MJ, Herst PM. 2017. High dose ascorbate causes both genotoxic and metabolic stress in glioma cells. *Antioxidants* **6:** 58.
24. Dzeja PP, Zeleznikar RJ, Goldberg ND. 1998. Adenylate kinase: kinetic behavior in intact cells indicates it is integral to multiple cellular processes. *Mol. Cell. Biochem.* **184:** 169-182.
25. Valentine WN, Paglia DE, Nakatani M, Brockway RA. 1989. Inhibition of adenylate kinase by P1,P5-di(adenosine 5') pentaphosphate in assays of erythrocyte enzyme activities requiring adenine nucleotides. *Am. J. Hematol.* **32:** 143-145.
26. Mateo C, Palomo JM, Fernandez-Lorente G, Guisan JM, Fernandez-Lafuente R. 2007. Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme Microb. Technol.* **40:** 1451-1463.
27. Martinek K, Klibanov AM, Goldmacher VS, Berezin IV. 1977. The principles of enzyme stabilization. I. Increase in thermostability of enzymes covalently bound to a complementary surface of a polymer support in a multipoint fashion. *Biochim. Biophys. Acta* **485:** 1-12.
28. Klibanov AM. 1979. Enzyme stabilization by immobilization. *Anal. Biochem.* **93:** 1-25.
29. Klibanov AM, Kaplan NO, Kamen MD. 1978. A rationale for stabilization of oxygen-labile enzymes: application to a clostridial hydrogenase. *Proc. Natl. Acad. Sci. USA* **75:** 3640-3643.
30. Yoshida H, Hara KY, Kiriyama K, Nakayama H, Okazaki F, Matsuda F, *et al*. 2011. Enzymatic glutathione production using metabolically engineered *Saccharomyces cerevisiae* as a whole-cell biocatalyst. *Appl. Microbiol. Biotechnol.* **91:** 1001-1006.
31. Hara KY, Shimodate N, Hirokawa Y, Ito M, Baba T, Mori H, *et al*. 2009. Glutathione production by efficient ATP-regenerating *Escherichia coli* mutants. *FEMS Microbiol. Lett.* **297:** 217-224.
32. Hara KY, Mori H. 2006. An efficient method for quantitative determination of cellular ATP synthetic activity. *J. Biomol. Screen.* **11:** 310-317.