Magnetically tunable control of light reflection in an unusual optical protein of squid

Cite as: AIP Advances **7**, 056722 (2017); https://doi.org/10.1063/1.4976938
Submitted: 21 September 2016 . Accepted: 14 November 2016 . Published Online: 14 February 2017

M. Iwasaka, K. Tagawa, and Y. Kikuchi

Magnetically tunable control of light reflection in an unusual optical protein of squid

M. Iwasaka,[1,a] K. Tagawa,[2] and Y. Kikuchi[2,3]
[1]*Research Institute for Nanodevice and Biosystems, Hiroshima University, Kagamiyama 1-4-2, Higash-Hiroshima, Hiroshima 739-8527, Japan*
[2]*Marine Biological Laboratory, Graduate School of Science, Hiroshima University, Onomichi, Hiroshima 722-0073, Japan*
[3]*Department of Biological Science, Graduate School of Science, Hiroshima University, Kagamiyama 1-3-1, Higashi-Hiroshima, Hiroshima 739-8526, Japan*

(Presented 2 November 2016; received 21 September 2016; accepted 14 November 2016; published online 14 February 2017)

In this study, we focused on the magnetically tunable changes in the reflectivity of the protein reflectin, which is generated by squid and used to control their body surface color for camouflage in seawater. A cellular organelle called an iridosome was separated from the skin of the dorsal part of a squid (cuttlefish; *Sepia esculenta*), and the light reflection dynamics of iridosomes containing reflectin were measured with and without exposure to a magnetic field of 500 mT. The magnetic field induced both steady and transient increases of reflection by the iridosomes, suggesting that a reversible conformational change occurred inside the iridosomes when the magnetic field was switched on and off. The intensity of light scattering perpendicular to the direction of the magnetic field increased when the magnetic field was applied. This kind of behavior (Type I) occurred in the majority (60%) of the measured samples. Another kind of reflection change (Type II) was a transient increase in light reflection. It is speculated that the wave-shaped structure of the lipid membrane connected to reflectin proteins changed to enhance the light reflection of reflectin by altering the diamagnetic orientation of the lipid layer in the bent part of the membrane under the applied magnetic field. Overall, our results suggest that the mesoscale lipid layers changed their alignment diamagnetically and the length between iridescent layers was modified by the magnetic field, even though no obvious change in alignment occurred at the microscale. [http://dx.doi.org/10.1063/1.4976938]

I. INTRODUCTION

Recently, the incorporation of dynamic color control into optical devices has driven development of biomimetic research. Light control involving color changes occurs in many animal species. For example, squid actively control the iridescence of their skin for the purpose of camouflage.[1–5] Squid skin color is controlled by iridosomes, which have a lamellar structure containing a globular protein called reflectin.[6] This protein has a unique hydrophobic region inside its structure that generates unusually intense light scattering. Recent reports have speculated that this protein may have applications in electronic devices.[7–9] However, little is known about the role magnetic fields play in optical control of reflectin. In this study, we evaluate the effect of magnetic fields on the dynamic light reflection of the optical protein reflectin.

[a]E-mail: iwasaka@hiroshima-u.ac.jp

II. METHODS

Iridosomes with a lamellar structure containing reflectin were extracted from a squid (cuttlefish; *Sepia esculenta*), which was supplied by Mukaishima Fishery Coop, Onomichi, Japan. The fresh dead squid were kept at 4 or −20 °C, and a skin section from the edge of the ventral part was separated from the body prior to the experiments. After cutting the section into fragments with a size of less than 10 mm, the fragments were picked by a spatula in seawater to release the iridosomes. The iridosomes floating in seawater were separated in a thin-layer chamber (Frame-seal chamber™, Biorad Laboratories, Inc., California, U.S.A.), which was optically transparent to facilitate the light-scattering measurements. The fraction containing the iridosomes was exposed to ultrasonic irradiation for 5 s and then observed under a high-resolution closed-circuit display (CCD) microscope (VHX-2000, Keyence Co., Ltd., Japan) with and without an applied magnetic field of 500 mT.

The sample chamber was set in the 700-mm gap between the magnetic poles (diameter of 100 mm) of an electromagnet (WS-15-40-5K-MS, Hayama Co., Ltd.), as shown in Fig. 1a and b. The maximum magnetic field generated in the sample position was 500 mT, where an image was obtained by the high-resolution CCD microscope. By changing the directions of the incident light and observation, the effects of a 500-mT magnetic field on the light scattering by the iridosome particles containing reflectin were measured. An example of reflectin iridescence from iridosomes separated from a section of squid dorsal skin is presented in Fig. 1c. Brilliant light scattering was observed in the dark-field image, and the iridosomes displaying this light enhancement were identified in the corresponding bright-field image.

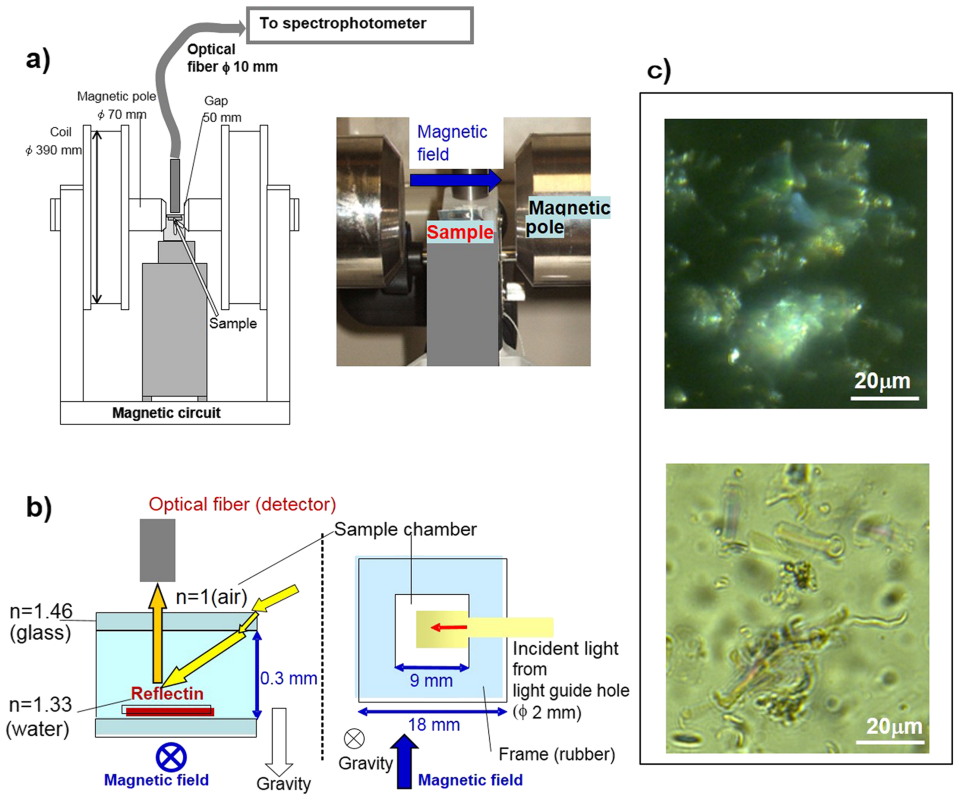

FIG. 1. Experimental setup used to observe the light reflection of iridosomes containing the protein reflectin with and without magnetic field exposure. (a) Electromagnet system and sample set in the magnet pole gap. (b) Schematic diagram of light illumination and detecting sample reflection. (c) Dark-field (top) and bright-field (bottom) images of iridosomes containing reflectin.

III. RESULTS AND DISCUSSION

The applied magnetic field did not cause any obvious change in the orientation of iridosome fractions. It is considered that the diamagnetic anisotropy of the reflectin protein was too small to generate a torque force under a magnetic field of 500 mT. However, the intensity of light scattering perpendicular to the direction of the magnetic field (SS) increased when the magnetic field was applied, as shown in Fig. 2. This kind of behavior (denoted Type I) occurred in the majority (60%) of the measured samples. Comparison of SS with the intensity of light scattering parallel to the magnetic field (SP) revealed that SP and SS increased and decreased, respectively, under the applied magnetic field compared with the intensity of light scattering without an applied magnetic field. Reflection has a globular conformation, so the origin of the change in light scattering was possibly the rotation of iridophore plates or a conformational change induced by diamagnetic torque forces acting on the protein structure inside the globule or linker chains. Considering the diamagnetic energy of the iridophores, the part causing the change of light scattering under an applied magnetic field should have a size of more than 100 nm.[10–13] The prime candidate for this part is the lamellar structure of the iridosome, which consists of a lipid layer membrane containing packed reflectin. This is because a lipid molecule and its layer have minimum diamagnetic energy when they orient perpendicular and parallel to an applied magnetic field, respectively. The flexibility of the lipid membrane allowed magnetically induced changes of the iridosomes, which facilitated light scattering by reflectin. This mechanism can explain the change of reflectivity with the density of reflectin in the lipid membrane, which was confirmed by observing the reflectance of denatured iridosomes (data not shown). Ultrasonic irradiation for more than 30 s dramatically suppressed the iridescence of reflectin because it denatured the structure of the lipid membrane.

Another kind of reflection change (denoted Type II) with more dynamic tuning of light reflection than Type I is illustrated in the time series in Fig. 3a. This group of iridosomes displayed a transient

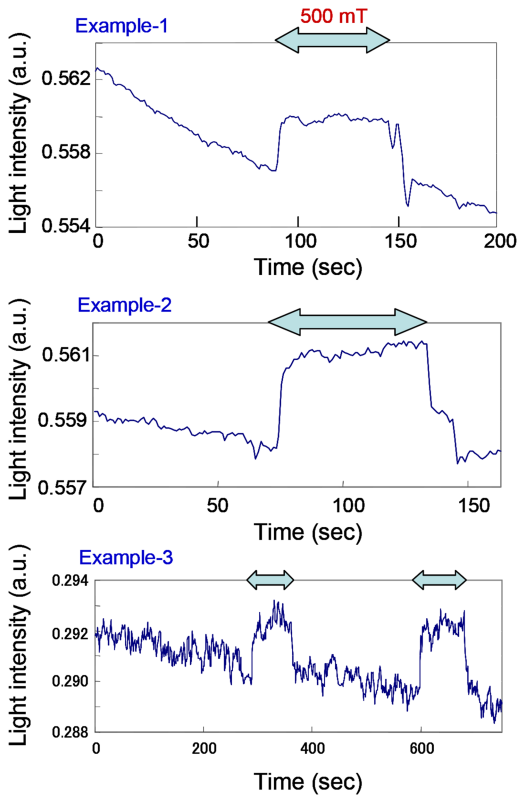

FIG. 2. Effects of 500-mT magnetic field exposure on the time series of the light reflection change of iridosomes containing reflectin. Three typical examples of a Type-I light reflection change are shown.

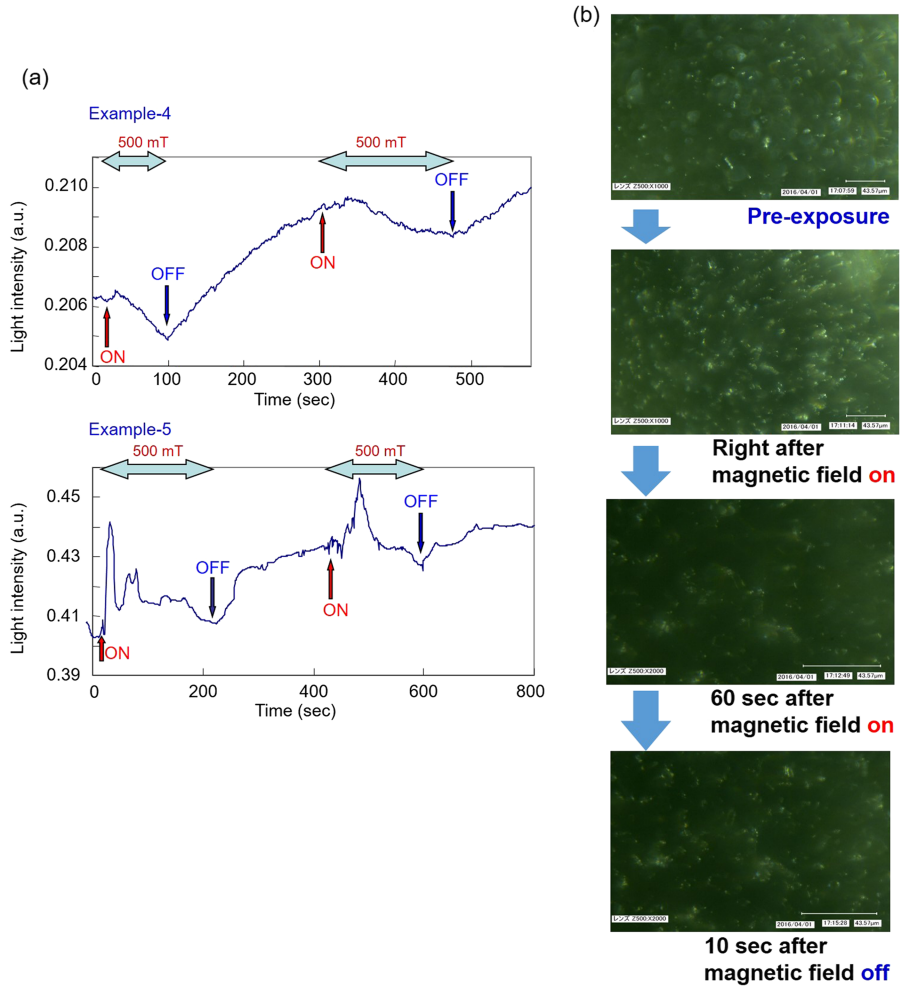

FIG. 3. Effects of a 500-mT magnetic field on the time series of the light reflection change of iridosomes containing reflectin. (a) Two examples of a Type-II light reflection change. (b) Reflection dynamics of reflectin-containing iridosomes observed by a high-resolution optical microscope.

increase in light reflection immediately after the magnetic field was applied, and then the light reflection intensity gradually decreased. The reflection intensity recovered after removing the magnetic field. Similar dynamics of the reflection intensity were detected in real-time microscopic observation (Fig. 3b). The number of reflecting iridosomes increased immediately after magnetic field exposure and then decreased. Similarly, slight recovery of the reflection intensity occurred when the magnetic field was removed (bottom panels of Fig. 3b).

Figure 4 summarizes the effects of a 500-mT magnetic field on the light reflection in the squid iridosomes containing reflectin. The initial change upon applying the magnetic field was analyzed by comparing the reflected light intensity immediately before applying the magnetic field and the maximum intensity during magnetic field exposure. The evaluation of reflection is presented as the relative change during magnetic field exposure. Almost all cases of Type-II reflection showed a relative change of less than 1%. In contrast, the Type-I reflection exhibited a change of 1% to 2% in the initial stage of magnetic field exposure.

The observed simple and complex reflection dynamics of the reflectin-containing iridosomes presented in Fig. 2 and 3, respectively, can be explained by the proposed mechanism in Fig. 5. This mechanism is a type of diamagnetic response that induces magnetic orientation, and has been reported to occur in biological macromolecules such as cell membranes and proteins.[14–16] A model of the native lamellar structure of a reflectin-containing iridosome is illustrated in Fig. 5a. It has

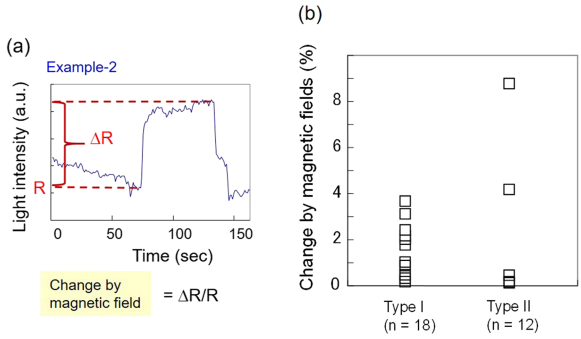

FIG. 4. Type-I and Type-II effects of a 500-mT magnetic field on the light reflection in squid iridosomes. (a) Procedure used to analyze the reflection intensity change. (b) Categorized reflection changes induced by a 500-mT magnetic field.

been reported that the lipid membrane orients parallel to an external magnetic field because the lipid molecules inside the membrane orient perpendicular to the field due to the diamagnetic anisotropy of the lipid molecules and tissues.[14,17–20] Previous studies reported that the lipid membrane caused magnetic orientation under magnetic fields of more than several hundreds of millitesla. It has been speculated that the interaction of lipid membranes with other biological molecules may affect the conformation and function of cellular channels.[14]

Not only lipid membranes consisting of lipid molecules orient perpendicular to external magnetic fields through their diamagnetic anisotropy; protein molecules can also be oriented by magnetic fields when the diamagnetic anisotropy of peptide bonds are integrated by polymerization.[15] In addition, cytoskeletal protein molecules such as tubulin can reorient under magnetic fields during polymerization.[16] As discussed in the previous literature,[14] the most interesting point is the modulation of the function of lipid membrane–protein hybrid structures by the diamagnetic torque force acting on both the lipid molecules and interacting protein molecules. The effect of magnetic fields on channel function realized via membrane deformation is a good example of this modulation.

There is much evidence for the magnetic modulation of biological functions through deformation of lipid membranes and proteins in living tissues. For example, it has been reported that retinal rods from frogs showed magnetic orientation under a 1000-mT magnetic field.[17] In addition, fluorescence measurements suggested that chlorophyll a reoriented under a 1000-mT field.[18] Lecithin bilayers

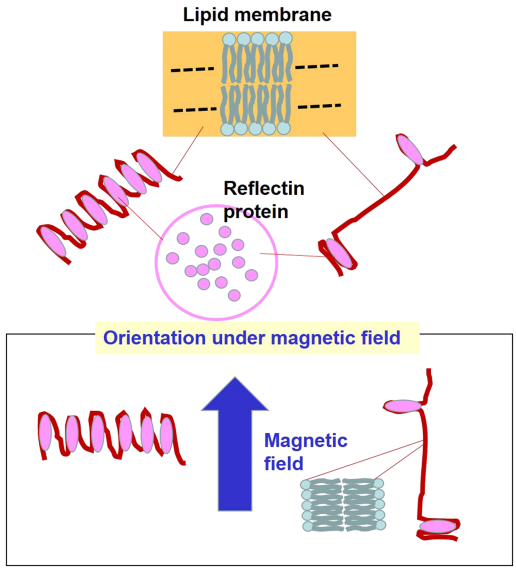

FIG. 5. Possible mechanism explaining the Type-I and Type-II reflection dynamics observed under an applied magnetic field.

needed higher magnetic fields to cause magnetic orientation.[19,20] It has also been reported that the permeability of lipid bilayers becomes sensitive to magnetic fields when they form liposomes.[21]

In the present study, the magnetic field of 500 mT used to change the conformation of iridosomes containing both lipid layers and reflectin protein was moderate. It is considered that the effects of this magnetic field on the conformation of the reflectin protein can be neglected because this protein has a globular structure,[9] which is difficult to magnetically orient.

Probably, the experimental sensitivity to detect the reorientation of lipid bilayers of the iridosomes was enhanced by the light reflected by reflectin proteins contained in the bilayers. The reflectin protein condensed between membranes is expected to exhibit strong light reflection.

The diamagnetic anisotropy of lipid molecules can allow the longitudinal direction of a region containing reflectin protein molecules to align with the direction of the applied magnetic field. In contrast, iridosomes with a denatured structure, as shown in Fig. 5b, can have both regions orienting parallel and perpendicular to the external magnetic field. In addition, the denatured conformation of the iridosomes might allow the change of the periodic length of the lamellar structure, which should result in a change of the density of the reflectin packed between membranes. We speculate that the mixture of both types of regions generated the Type-II reflection dynamics.

IV. CONCLUSION

Iridosomes containing reflectin proteins with a lamellar structure were extracted from squid, and their reflection dynamics were observed with and without an applied magnetic field. Intense light reflections were detected in the iridosomes, and the applied external magnetic field induced a reversible increase in the reflection of the majority of samples, while remaining samples displayed modified dynamics. It is considered that the force balance of diamagnetic torque generated the tunable modification of the reflection intensity of the reflectin-containing iridosomes.

ACKNOWLEDGMENTS

This work was supported by JSPS KAKENHI Grant-in-Aid for Scientific Research (B) Grant Number (16H03169).

[1] L. M. Mäthger, E. J. Denton, N. J. Marshall, and R. T. Hanlon, J. R. Soc. Interface **6**, S149 (2009).
[2] L. M. Mäthger, A. Barbosa, S. Miner, and R. T. Hanlon, Vision Research **46**, 1746 (2006).
[3] L. M. Mäthger and R. T. Hanlon, Biol. Lett. **2**, 494 (2006).
[4] L. M. Mäthger, M. F. Land, U. E. Siebeck, and N. J. Marshall, Journal of Experimental Biology **206**, 3607–3613 (2003).
[5] L. M. Mäthger and R. T. Hanlon, Cell Tissue Res. **329**, 179 (2007).
[6] W. J. Crookes, L. Ding, Q. Huang, J. R. Kimbell, J. Horwitz, and M. J. McFall-Ngai, Science **303**, 235 (2004).
[7] C. Yu, Y. Li, X. Zhang, X. Huang, V. Malyarchuk, S. Wang, Y. Shi, L. Gao, Y. Su, Y. Zhang, H. Xu, R. T. Hanlon, Y. Huang, and J. A. Rogers, PNAS **111**(36), 12998 (2014).
[8] E. Kreit, L. M. Mäthger, R. T. Hanlon, P. B. Dennis, R. R. Naik, E. Forsythe, and J. Heikenfeld, J. R. Soc. Interface **10**, 0601 (2012).
[9] R. M. Kramer, W. J. Crookes-Goodson, and R. R. Naik, Nature Materials **6**, 533 (2007).
[10] M. Iwasaka and Y. Mizukawa, Langmuir **29**(13), 4328 (2013).
[11] M. Iwasaka, Y. Miyashita, Y. Mizukawa, K. Suzuki, T. Toyota, and T. Sugawara, Applied Physics Express **6**(3), 037002 (2013).
[12] Y. Mizukawa, Y. Miyashita, M. Satoh, Y. Shiraiwa, and M. Iwasaka, Scientific reports **5**, 13577 (2015).
[13] M. Iwasaka, Y. Mizukawa, and N. W. Roberts, Langmuir **32**(1), 180 (2016).
[14] A. D. Rosen, Cell Biochem. Biophys. **39**, 163 (2003).
[15] J. Torbet, J. M. Freyssinet, and G. Hudry-Clergeon, Nature **289**, 91 (1981).
[16] W. Bras, G. P. Diakun, J. F. Diaz, G. Maret, H. Kramer, J. Bordas, and F. J. Medrano, Biophys. J. **74**, 1509 (1998).
[17] F. T Hong, D. Mauzerall, and A. Mauro, Proc. Natl. Acad. Sci. USA **68**, 1283 (1971).
[18] N. E. Geacintov, F. Van Norstrand, M. Pope, and J. B. Tinkel, Biophys. Acta **226**, 486 (1971).
[19] E. Boroske and W. Helfrich, Biophys. J. **24**, 863 (1978).
[20] J. B. Speyer, P. K. Sripada, S. K. Das Gupta, and G. G. Shipley, Biophys. J. **51**, 687–691 (1987).
[21] T. S. Tenforde, J. Theor. Biol. **133**, 385 (1988).