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Application of photoactive yellow protein as a photoresponsive module for controlling hemolytic activity of staphylococcal α-hemolysin†

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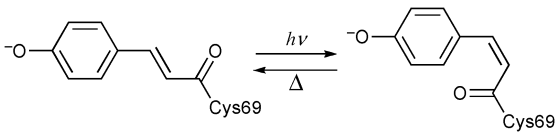
A chimeric protein (*N*-PYP-Hla), consisting of staphylococcal pore-forming toxin α-hemolysin (Hla) and photoactive yellow protein (PYP), exhibited photoresponsive hemolytic activities, where visible light irradiation gave rise to retardation of hemolysis at 25 °C.

Biomacromolecules have been drawing attention as useful substructures for nano-scale molecular devices. Introduction of artificial stimuli-responsive units into these biomacromolecules allows us to control their activities by externally added physical or chemical stimuli; recent examples include chemical modification of the target proteins with photochromic molecules, to reversibly control their functions by UV or visible light irradiation.[1] On the other hand, if the stimuli-responsive units of biological origin could be employed for modification, it would open a way toward *in vivo* use of such semibiological molecular devices. In that sense, photoreceptors could be candidates as stimuli-responsive units.[2] Here we demonstrate that photoactive yellow protein (PYP)[3] could act as a photoresponsive module for controlling the hemolytic activity of a pore-forming protein, staphylococcal α-hemolysin (Hla),[4] where visible light irradiation causes retardation of the hemolysis of sheep red blood cells (SRBC).

PYP known as a photosensor protein has been isolated from *Ectothiorhodospira halophila*.[3] Exposure of PYP to visible light (λ = 446 nm) causes partial unfolding of α-helices at the N-terminal region.[3] PYP contains a *p*-coumaric acid derivative as a chromophore, which binds to Cys69 *via* a thioester bond. This chromophore undergoes *trans*-to-*cis* photoisomerization by irradiation with visible light (λ = 446 nm), followed by thermal *cis*-to-*trans* isomerization to recover the initial *trans*-configuration at room temperature (Scheme 1). It is known that the overall photo-induced configurational and conformational changes of the chromophore and surrounding peptide chains occur within 1 s.[6]



Scheme 1 *trans–cis* isomerization of PYP chromophore.

α-Hemolysin (Hla),[4] isolated from *Staphylococcus aureus*, is a pore-forming toxin that causes lysis of red blood cells. While Hla stays as a monomer in aqueous buffer solutions, it assembles into a cyclic heptamer (Fig. 1) on the surface of the cell membrane. After the formation of the heptameric ring, the stem region forms a β-barrel channel, which penetrates into the membrane to cause lysis of the cell. We expected that PYP could control such a dynamic process of Hla by light, in a recombinant protein fused to Hla.

The chimeric protein *N*-PYP-Hla, where PYP is connected to the N-termini of Hla, has been prepared according to the procedure given in ESI.† We first obtained an apo-protein without a chromophore at the PYP domain, which was then treated with *p*-coumaric anhydride in DMSO at room temperature to afford the desired *N*-PYP-Hla.

The electronic absorption spectrum of *N*-PYP-Hla (17 μM) in Tris-HCl buffer (50 mM, pH 8.0, containing 200 mM NaCl and 6.25 mM sodium deoxycholate) showed an absorption

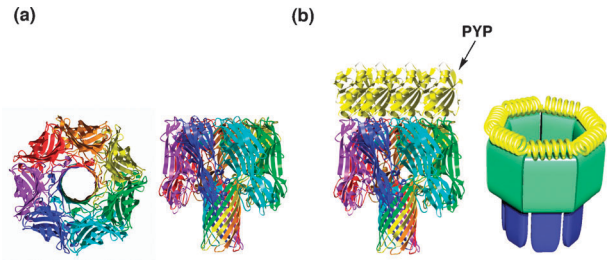


Fig. 1 (a) Crystal structures of a heptameric assembly of α-hemolysin (Hla). Left and right: top and side views, respectively.[5] (b) Schematic images of heptameric assembly of chimeric protein consisting of Hla and photoactive yellow protein (*N*-PYP-Hla).

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† Electronic supplementary information (ESI) available: Preparation of mutants, and hemolytic activities of wild-type Hla and *N*-PYP-Hla-SS. See DOI: 10.1039/c2cc18118e

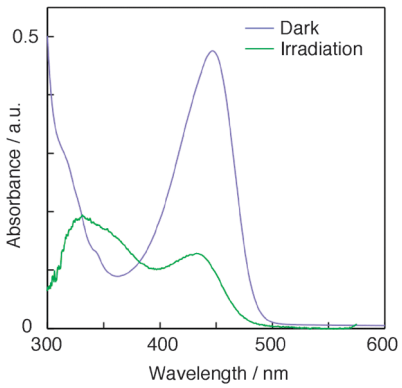

Fig. 2 Electronic absorption spectra of *N*-PYP-Hla (17 μM) in Tris-HCl buffer (50 mM, pH 8.0, containing 200 mM NaCl and 6.25 mM sodium deoxycholate) in the dark (blue) and under irradiation with visible light (green).

band at 445 nm (Fig. 2), similar to that of wild-type PYP in the dark (λ_{max} = 446 nm). Under irradiation with visible light, the spectrum displayed a blue-shifted band at 430 nm with a decrease in its intensity, together with an absorption band at around 350 nm, similarly to the spectrum of wild-type PYP under irradiation with visible light.[7] When irradiation is stopped, the spectrum turns back to the initial one immediately, suggesting that a rapid spontaneous recovery of the *trans* configuration of the chromophore took place in the dark. In relation with this, the intensity of absorption at 445 nm reversibly changed in response to visible light irradiation (Fig. S3, ESI†).

Wild-type Hla exists as a monomer in an aqueous buffer solution, which assembles into a heptameric ring upon binding to cell membranes. This cyclic oligomer formation is also encouraged by sodium deoxycholate micelles even in the absence of lipid membranes.[8] Actually, a transmission electron micrograph of *N*-PYP-Hla in a Tris-HCl buffer (50 mM, pH 8.0, containing 200 mM NaCl and 6.25 mM sodium deoxycholate) clearly displayed doughnut-like assemblies consisting of seven monomer units (Fig. 3). Thus, it is likely that *N*-PYP-Hla is able to form a heptameric cyclic assembly on the membrane, like wild-type Hla.

Hemolytic activity of *N*-PYP-Hla was evaluated by adding a phosphate buffered saline (PBS, pH 7.4) solution of *N*-PYP-Hla (0.18 μM) to a PBS suspension of SRBC, where the progress of hemolysis was monitored by optical density at 700 nm (OD_{700}).[9] Wild-type Hla has strong hemolytic activity and completes hemolysis of SRBC within a few minutes at 25 °C (Fig. S1, ESI†).

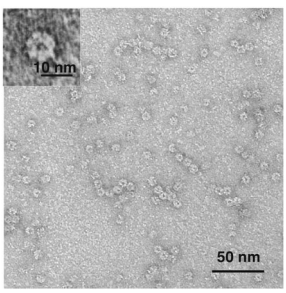

Fig. 3 Transmission electron micrograph (negative staining with uranyl acetate) of *N*-PYP-Hla in Tris-HCl buffer (50 mM, pH 8.0, containing 200 mM NaCl and 6.25 mM sodium deoxycholate).

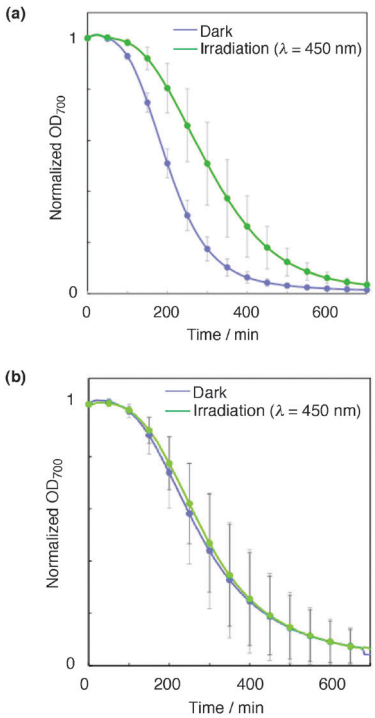

Fig. 4 Time course curves of hemolysis of sheep red blood cells by (a) *N*-PYP-Hla (0.18 μM) and (b) apo-*N*-PYP-Hla (0.25 μM) in PBS in the dark (blue) and under irradiation with visible light (λ = 450 ± 10 nm, green) at 25 °C, monitored by optical density at 700 nm (OD_{700}). OD_{700} was measured every 5 min. Data are means ± standard deviations (every 50 min for clarity) of 3 independent experiments.

The time to lyse 50% of the SRBC (t_{50}) was 2 min. Meanwhile, at 25 °C in the dark, *N*-PYP-Hla showed moderate hemolytic activity (t_{50} = 200 min), and the hemolysis finished in *ca.* 400 min (Fig. 4, blue lines). Of importance, under illumination with visible light (λ = 450 ± 10 nm) at 25 °C, the hemolysis has been significantly retarded (t_{50} = 300 min) (Fig. 4a, green line). Thus, *N*-PYP-Hla clearly exhibited photoresponsive hemolytic activity. It is noteworthy here that while apo-*N*-PYP-Hla, bearing no chromophore, exhibited comparable hemolytic activity with *N*-PYP-Hla, it hardly showed photoresponsiveness upon illumination with visible light (λ = 450 ± 10 nm) (Fig. 4b). t_{50} in the dark and under visible light irradiation were 275 and 285 min, respectively. As expected, irradiation of Hla by visible light (λ = 450 ± 10 nm) does not show any effect on the hemolytic activity (Fig. S1, ESI†). Therefore, photoisomerization of the chromophore, which causes structural changes of the PYP unit, is likely essential for the observed retardation of the hemolysis under the illumination with visible light.

The mechanism of the hemolysis by wild-type Hla has been studied in detail, which is considered to include the following three steps:[10] (1) attachment of monomeric Hla to the cell membrane, (2) assembly of monomeric Hla into a heptameric ring (prepore form)[11] on the cell membrane, and (3) formation of a β-barrel pore penetrating into the membrane to allow for the outflow of the cellular components, thereby resulting in hemolysis (Fig. 5a). In order to investigate the possible process, which is affected by the photoisomerization of the PYP unit, we have prepared a chimeric protein *N*-PYP-Hla-SS, where the Hla unit contains an S–S linkage at the stem region.

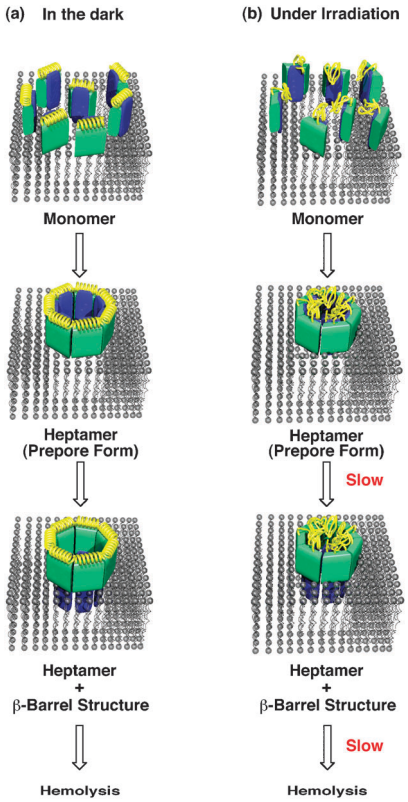

Fig. 5 Schematic images of pore formation on the cell membrane by *N*-PYP-Hla (a) in the dark and (b) under irradiation with visible light.

As for Hla, it is reported that a cysteine mutant D108C/K154C (Hla-SS)[12] forms an S–S linkage in a monomeric unit, which hampers formation of the β-barrel pore on the cell membrane after the formation of a heptameric prepore form. However, under the reductive conditions, for example, in the presence of dithiothreitol (DTT), the β-barrel pore formation takes place immediately to lyse the blood cells. Namely, this mutant is able to keep the prepore form, and step (3) can be initiated separately by addition of reducing agents. We also found a similar effect of DTT on *N*-PYP-Hla-SS. Indeed, in the absence of DTT, *N*-PYP-Hla-SS (0.20 μM) in PBS (pH 7.4) has not shown any hemolytic activity at 25 °C (Fig. S2, ESI†). In contrast, in the presence of DTT (10 mM), lysis of the red blood cells took place moderately at 25 °C (Fig. S2, ESI†), indicating that the prepore form was converted into the β-barrel pore by cleavage of the SS bond triggered by DTT. Of importance, under irradiation with visible light ($\lambda = 450 \pm 10$ nm), hemolysis by *N*-PYP-Hla-SS was retarded significantly, similarly to *N*-PYP-Hla under irradiation (Fig. S2, ESI†). Therefore, it is strongly suggested that the observed retardation of hemolysis by *N*-PYP-Hla under visible-light irradiation is due to the inhibitory effect of the PYP unit on step (3) or on the subsequent processes. In other words, it is likely that the partially unfolded PYP unit generated by irradiation with visible-light disturbs the β-barrel structure formation from the prepore form or blocks permeation of the cellular components through the pore.

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Notes and references

1 H. M. Lee, D. R. Larson and S. Lawrence, *ACS Chem. Biol.*, 2009, **4**, 409; L. Sjulson and G. Miesenböck, *Chem. Rev.*, 2008, **108**, 1588; P. Gorostiza and E. Isacoff, *Mol. BioSyst.*, 2007, **3**, 686; M. R. Banghart, M. Volgraf and D. Trauner, *Biochemistry*, 2006, **45**, 15129; S. Loudwinga and H. Bayley, *J. Am. Chem. Soc.*, 2006, **128**, 12404; S. Muramatsu, K. Kinbara, H. Taguchi, N. i. Ishii and T. Aida, *J. Am. Chem. Soc.*, 2006, **128**, 3764; A. Koçer, M. Walko, W. Meijberg and B. L. Feringa, *Science*, 2005, **309**, 755.
2 M. V. Golynskiy, M. S. Koay, J. L. Vinkenborg and M. Merkx, *ChemBioChem*, 2011, **12**, 353; T. Drepper, U. Krauss, S. Meyer zu Berstenhorst, J. Pietruszka and K. E. Jaeger, *Appl. Microbiol. Biotechnol.*, 2011, **90**, 23; A. Möglich and K. Moffat, *Photochem. Photobiol. Sci.*, 2010, **9**, 1286; S. Morgan, S. Al-Abdul-Wahid and G. A. Woolley, *J. Mol. Biol.*, 2010, **399**, 94.
3 M. A. Cusanovich and T. E. Meyer, *Biochemistry*, 2003, **42**, 4759; T. E. Meyer, *Biochim. Biophys. Acta*, 1985, **806**, 175.
4 M. Montoya and E. Gouaux, *Biochim. Biophys. Acta*, 2003, **1609**, 19; G. Menestrina, M. D. Serra and G. Prévost, *Toxicon*, 2001, **39**, 1661; C.-Y. Chang, B. Niblack, B. Walker and H. Bayley, *Chem. Biol.*, 1995, **2**, 391; B. Walker, O. Braha, S. Cheley and H. Bayley, *Chem. Biol.*, 1995, **2**, 99.
5 L. Song, M. R. Hobaugh, C. Shustak, S. Cheley, H. Bayley and J. E. Gouaux, *Science*, 1996, **274**, 1859.
6 Y. Hoshihara, Y. Imamoto, M. Kataoka, F. Tokunaga and M. Terazawa, *Biophys. J.*, 2008, **94**, 2187; Y. Imamoto and M. Kataoka, *Photochem. Photobiol.*, 2007, **83**, 40; M. A. van der Horst, J. C. Arents, R. Kort and K. J. Hellingwerf, *Photochem. Photobiol. Sci.*, 2007, **6**, 571; Y. Imamoto, H. Kamikubo, M. Harigai, N. Shimizu and M. Kataoka, *Biochemistry*, 2002, **41**, 13595; P. Düx, G. Rubinstenn, G. W. Vuister, R. Boelens, F. A. A. Mulder, K. Hård, W. D. Hoff, A. R. Kroon, W. Crielaard, K. J. Hellingwerf and R. Kaptein, *Biochemistry*, 1998, **37**, 12689; T. E. Meyer, E. Yakali, M. A. Cusanovich and G. Tollin, *Biochemistry*, 1987, **26**, 418.
7 W. D. Hoff, I. H. M. van Stokkum, H. J. van Ramesdonk, M. E. van Brederode, A. M. Brouwer, J. C. Fitch, T. E. Meyer, R. van Grondelle and K. J. Hellingwerf, *Biophys. J.*, 1994, **67**, 1691.
8 S. Bhakdi and J. Tranum-Jensen, *Microbiol. Rev.*, 1991, **55**, 733; S. Bhakdi, R. Füssle and J. Tranum-Jensen, *Proc. Natl. Acad. Sci. U. S. A.*, 1981, **78**, 5475.
9 A. I. Louw and L. Visser, *Biochim. Biophys. Acta*, 1977, **498**, 143.
10 A. Valeva, R. Schnabel, I. Walev, F. Boukhallouk, S. Bhakdi and M. Palmer, *J. Biol. Chem.*, 2001, **276**, 14835; Y. Fang, S. Cheley, H. Bayley and J. Yang, *Biochemistry*, 1997, **36**, 9518.
11 H. Bayley, *Nature*, 2009, **459**, 651.
12 T. Kawate and E. Gouaux, *Protein Sci.*, 2003, **12**, 997.