Bioorganic & Medicinal Chemistry Letters 19 (2009) 5368–5371

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Photoregulation of thrombin aptamer activity using Bhc caging strategy

YiMing Li [a], Jing Shi [a,*], ZhaoFeng Luo [b], Hao Jiang [b], XiaoYun Chen [a], FengLiang Wang [a], Xu Wu [b], QingXiang Guo [a,*]

[a] Department of Chemistry, University of Science and Technology of China, Hefei 230026, China
[b] Hefei National Laboratory for Physical Sciences at Microscale and School of Life Science, University of Science and Technology of China, Hefei 230026, China

ARTICLE INFO

Article history:
Received 7 May 2009
Revised 26 July 2009
Accepted 28 July 2009
Available online 6 August 2009

Keywords:
Aptamer
Caged compound
Light-activated
Bhc
Activity regulation

ABSTRACT

Thrombin aptamer was attempted to cage with Bhc (6-bromo-7-hydroxycoumarin-4-ylmethyl) group for controlling its specific affinity to target molecular through photolysis. By multiple-caging strategy, aptamer could be rendered biologically inert and partially restored with subsequently illumination. This provides a convenient method for photoregulating aptamer activity with exact spatiotemporal resolution.

Chemical approaches for regulating nucleic acid activity within living cells offer exciting possibilities for elucidating complex biological processes.[1,2] The term 'caging' refers to installation of a photoremovable group on a biologically active molecule and encapsulates molecule in an inactive form. Irradiation with light removes the caging group and restores biological activity.[3] Monroe and coworkers firstly demonstrated the photoregulation of GFP expression in HeLa cells by labeling DNA plasmid with multiple 1-(4,5-dimethoxy-2-nitrophenyl)ethyl (DMNPE) groups.[4] In other pioneering works, light-activated (caged) oligonucleotides including DNA hybridization,[5–7] polymerase,[8–10] RNase H activity,[6,11] RNA interference,[12,13] as well as gene expression in cells and embryos,[3,4,12,14–16] provide unique possibilities for controlling oligonucleotides function with high spatial and temporal resolution.[15,17]

Aptamers are small single-stranded nucleic acids that fold into a well defined three-dimensional structure.[18,19] It shows a high affinity and specificity to target molecules and inhibits their biological functions. These properties make aptamer very interesting tool for molecular biology as well as diagnostic and therapeutic applications.[20,21] Thus, the regulation of their specific function using light is a challenging and significant issue.[18] Previous effort was carried out by post-selective and site-specific caging method. One or two of the six thymidine residues of thrombin aptamer was modified with photolabile protecting groups, such as *o*-nitrophenylpropyl (NPP) or *o*-nitrophe-nylethyl (NPE), and further synthesized through solid-phase approach.[7,18] This strategy gains control over the spatially and temporally high resolution availability of the aptamer's function. However, due to complicated and time-consuming processes of site-specific caging nucleotide and solid-phase synthesis, the use of this technology was limited. Likewise, since different caging position results in varying de-caging efficiency, the optimal position might be determined primarily by comparing the efficiency of each caged nucleotide. Based on this principle, the more nucleotides that an aptamer includes, the less suitable for the method applied. Moreover, we assumed that NPP and NPE group own weak two photon properties and unsuitable for further used in living cell or tissue. Considering the aforementioned drawbacks, it is necessary to trace some simple methods to fulfill the task.

It has been demonstrated that the Bhc (6-bromo-7-hydroxycoumarin-4-ylmethyl) is an outstanding two photon caged compound.[16,24,25] It owns several advantages such as high photolysis efficiency and biologically useful cross-sections upon two-photon excitation.[22] Comparing with nitrobenzyl- and DMNPE-caged molecules, Bhc-caged molecules are several-fold more sensitive to illumination with ultraviolet light and ~30-fold more sensitive to photolysis by pairs of coincident infrared photons. These positive properties not only contribute to minimize cellular-induced responses and reduce possibility of photodamage to neighboring cells, but also permit better three-dimensional spatial localization and greater penetration into scattering or absorbing tissues.[23] In general, Bhc-diazo (6-bromo-4-diazomethyl-7-hydroxycoumarin) was used as the caging reagent, which forms a covalent bond with the phosphate moiety of the sugar-phosphate backbone of nucleic acid. The whole caging process was completed within 1–2 h at room temperature just through co-incubation of the caging reagent

* Corresponding authors.
E-mail address: shijing@ustc.edu.cn (J. Shi).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.128

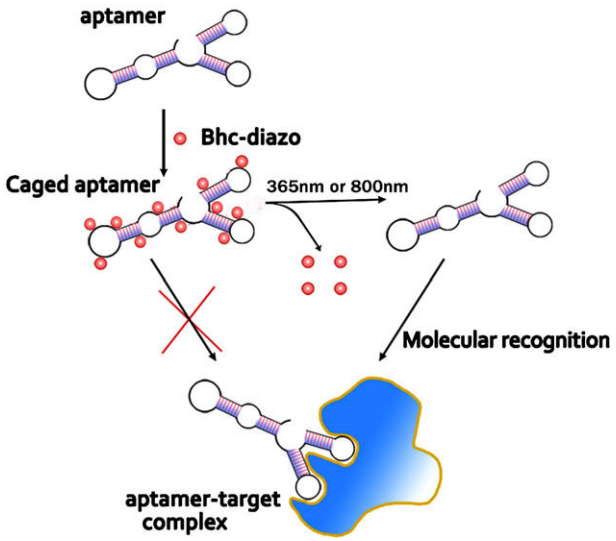

Figure 1. Bhc bind aptamer and resist affinity to target molecule until released with light.

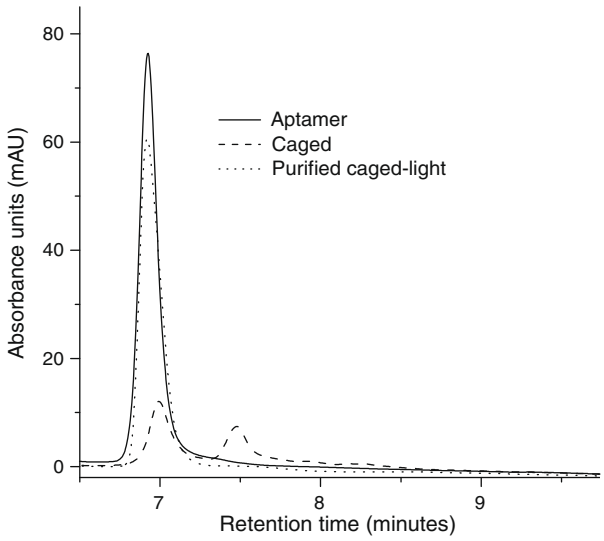

Figure 3. RP-HPLC elution profiles for caged and light-activated aptamer (determined by 260 nm UV–vis). Two peaks of the caged sample indicate the aptamer in various caging degrees.

with biologically active molecule.[16] This approach has been used to cage mRNA by statistically (multiple) labeling method, thereby perturbing mRNA structure and controlling induction of gene expression in zebrafish embryos spatially and temporally.[24,25]

Thrombin aptamer is a 15mer ssDNA molecule (5′-GGTTGGTGTG GTTGG-3′) that shows a high affinity and specificity to R-thrombin, which has already been well studied.[18] Herein, we would like to try this multiple-caging strategy by using Bhc-diazo for controlling its activity in exact time and space with illumination (Fig. 1).

Bhc-diazo was synthesized in five steps as previously described and stored at −20 °C under dark condition.[22] The method of caging reaction was carried out by co-incubating aptamer and Bhc-diazo within 2 h and then purified using Microcon YM-3 (3000 MW cutoff) centrifugal filters.[5,16] Firstly, we estimated the caging efficiency of Bhc-diazo binding with aptamer. The spectrophotometric analysis exhibited two characteristic absorbance peaks at 333 nm and 383 nm in addition to main peak at 260 nm. The appearance of the additional peaks was attributed to the attachment of Bhc, as the Bhc group shows absorbance peaks at 333 nm and 383 nm in protonated and ionized states, respectively.[16] Following illumination with

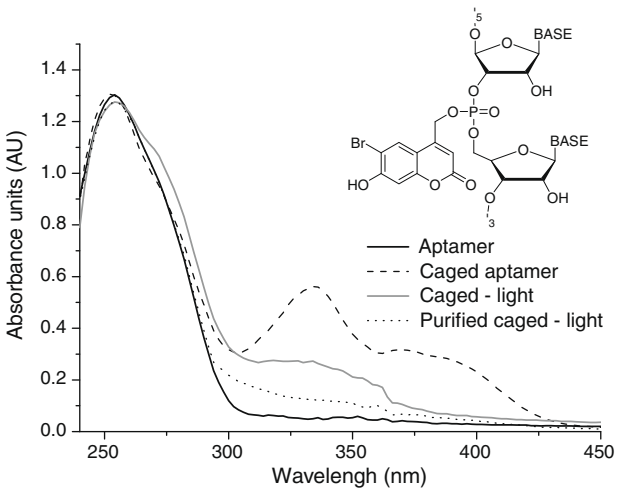

Figure 2. Spectrophotometric analysis of caged and light-activated aptamer. All samples were processed in parallel. Inset shows the attachment of the Bhc to the phosphate backbone of aptamer, considered to be the predominant site of caging.

365 ± 5 nm ultraviolet light and purification, the photocleaved product resembles the control (non-caged) aptamer (Fig. 2). Measurement of the intensity of these additional peaks and the main peak for aptamer (260 nm) gives an estimated binding of 6.8 Bhc cage groups per 15mer aptamer strand on average.[16]

We subsequently characterized the caged and light-activated aptamer using reversed phase high performance liquid chromatography (RP-HPLC).[26] The elution profile of aptamer shown in the representative chromatogram was determined by standard UV–vis at 260 nm (the characteristic absorbance of aptamer) (Fig. 3). Aptamer control samples eluted quickly with a sharp peak elution profile. One part of caged apatmer gave the peak at 7.5 min, which retained longer than their control counterparts. Upon exposure to light, the elution profile of the light-activated species resembled the elution profile of control aptamer, suggesting the removal of the caging groups. Because of incomplete caging efficiency of this method, caged-aptamer showed another elution profile similar with control sample which indicated some remained unreactive aptamer. Other two chromatograms determined by 333 nm and 383 nm UV–vis represent the characteristic elution profile of caging compound (Fig. S1). Likewise, the result of denaturing gel electrophoresis further confirmed characteristic changes in mobility and intensity of band corresponding to the addition and removal of the Bhc caging groups (Fig. S2).

Next, we prefer to investigate the effect of the caged and light-activated aptamers on the specific binding to target molecule. Therefore, the fluctuation of affinity between aptamer and thrombin was measured using surface plasmon resonance (SPR) strategy.[27,28] As shown in Figure 4A, the responds unit (RU) of caged aptamer decreased to 14% of control sample and restored to more than 60% with subsequent photoirradiation within 30 s. Likewise, the dissociation constants (K_D) of aptamer, caged and light-activated aptamer were performed under the different concentrations with a steady state affinity method, and the result is 3.2×10^{-7} M, 6.1×10^{-6} M, 3.9×10^{-7} M, respectively. Furthermore, the nonspecific binding between other oligonucleotides with thrombin can be eliminated. Above results clearly demonstrated that, comparing with nonspecific oligonucleotides, illumination ensured the caged aptamer which was encapsulated into an inactive form and lost affinity to target molecule most recovery to natural state and might act well to complete an aptamer's function. Actually, caged aptamer still had about 14% affinity toward thrombin, while re-

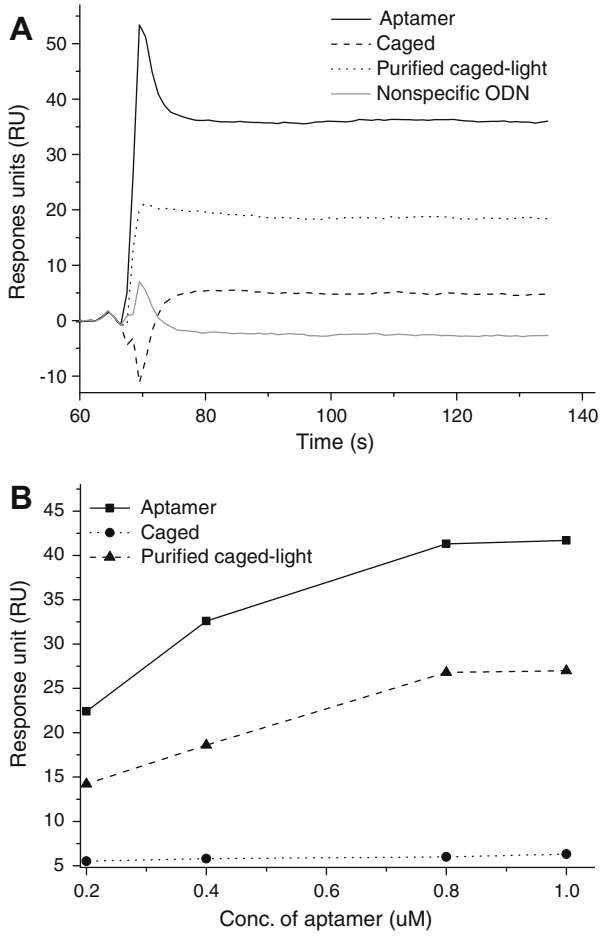

Figure 4. SPR analyses of the affinity fluctuating in the presence and absence of caged and light-activated aptamers.

stored sample showed just 60% RU compared with control aptamer after the irradiation. We consider that the remaining affinity was due to incomplete caging, which was matched with the remained unreactive aptamer shown in Figure 3. It is supposed that, if the caging compound doesn't fully bind with the specific position of aptamer, which plays key roles for specific affinity to target molecules, the partly remaining affinity is possible. Likewise, we analyze that nearly 40% loss of restored efficiency might result from the partial removal of Bhc groups.

It was necessary to further examined whether caging and restoration efficiency should be potential influenced by the change of concentration. For this experiment, we used each sample from 0.2 to 1 μM for SPR assay. The results indicated the similar caging and restoration efficiency of 0.8 and 1 μM sample. In maximum, the affinity decreased to 14.5–14%, and restored to 63–64.7% of control aptamer. (Fig. 4B). Simultaneously, higher concentration samples (more than 5 μM) were used but led to invariable results (data not show). These findings confirmed that the range of concentration we chose was suit for distinguishing the fluctuation of affinity between caged and light-activated samples. To further determined the proper time of irradiation required for rescuing the activity of caged aptamer, time course experiments from 15 s to 5 min were carried out. As shown in Figure 5, the affinity of caged aptamer restored from 14% to 63% under first 1 min illumination. In theory, increasing the time of irradiation should lead to a complete restoration of active aptamer.[5] We have found, however, that prolonged irradiation beyond 1 min barely contributes to farther affinity recovery but a little decrease.[26] Therefore, we conclude that less than 1 min photoreleased period is available for

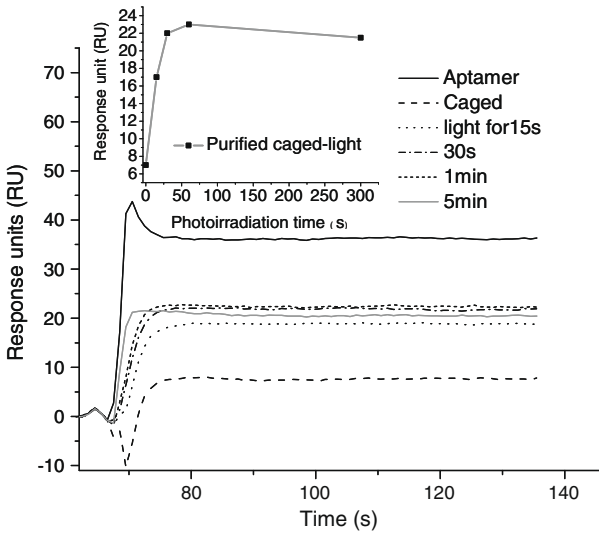

Figure 5. Effect of cumulative photoactive time on affinity restoration of caged aptamer.

activity recovery. Comparing with 30 min irradiation for DMNPE-caged oligonucleotides, Bhc is more light-sensitivity and potentially contributes to lower cellular-induced responses. The results of gel electrophoresis and spectrophotometric analysis indicate the similar trends (Fig. S3).

In summary, our results demonstrated that the spatial and temporal regulation of bioactive aptamer by multiple attachments of the photolabile compound Bhc is possible. This non-site specific caging species resisted the specific affinity of aptamer to target molecule and the effect was partially reversible with the application of illumination. We have further indicated that this effect can be modulated by varying the time of photodeprotection. Indeed, comparing with site-specific caging, this style of inactive reaction leads to incomplete blockade and restoration of aptamer's activity.[26] However, the concise caging process and moderate reaction condition would contribute to expand the application of photoregulating aptamer activity. Furthermore, transitory light-active period may lead to less phototoxicity of biomolecules. Additional potential advantage is more available of Bhc for two-photon uncaging than other site-specific caging group. This approach could minimizes cellular damage and increases targeting precision for liberating caged molecule.[22,29] In order to maximize caging efficacy, further modifications might be obtainable with alternative caged compounds such as BHQ, NHQ, CHQ, and CyHQ that own higher quantum yields and biologically useful two-photon cross sections.[30,31] It should anticipate that better caging group would result in improvements of blockade and subsequent restoration efficiency.

All the results presented here provide a possible and convenient strategy for regulating aptamer's function under illumination with own advantages. This light-controllable method has potential applications in precisely probing biological systems and eventually enabling targeted therapeutics in the cellular and organic environments.[32]

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant Nos. 90713009, 30727001).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.128.

References and notes

1. Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900.
2. Tang, X. J.; Dmochowski, I. J. *Mol. Biosyst.* **2007**, *3*, 100.
3. Tang, X. J.; Maegawa, S.; Weinberg, E. S.; Dmochowski, I. J. *J. Am. Chem. Soc.* **2007**, *129*, 11000.
4. Monroe, W. T.; McQuain, M. M.; Chang, M. S.; Alexander, J. S.; Haselton, F. R. *J. Biol. Chem.* **1999**, *274*, 20895.
5. Ghosn, B.; Haselton, F. R.; Gee, K. R.; Monroe, W. T. *Photochem. Photobiol.* **2005**, *81*, 953.
6. Tang, X. J.; Dmochowski, I. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 3523.
7. Krock, L.; Heckel, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 471.
8. Young, D. D.; Lusic, H.; Lively, M. O.; Yoder, J. A.; Deiters, A. *Chembiochem.* **2008**, *9*, 2937.
9. Asanuma, H.; Tamaru, D.; Yamazawa, A.; Liu, M. Z.; Komiyama, M. *Chembiochem.* **2002**, *3*, 786.
10. Tang, X.; Richards, J. L.; Peritz, A. E.; Dmochowski, I. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5303.
11. Matsunaga, D.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2004**, *126*, 11452.
12. Shah, S.; Rangarajan, S.; Friedman, S. H. *Angew. Chem., Int. Ed.* **2005**, *44*, 1328.
13. Mikat, V.; Heckel, A. *RNA* **2007**, *13*, 2341.
14. Shestopalov, I. A.; Sinha, S.; Chen, J. K. *Nat. Chem. Biol.* **2007**, *3*, 650.
15. Tang, X.; Swaminathan, J.; Gewirtz, A. M.; Dmochowski, I. J. *Nucleic Acids Res.* **2008**, *36*, 559.
16. Ando, H.; Furuta, T.; Tsien, R. Y.; Okamoto, H. *Nat. Genet.* **2001**, *28*, 317.
17. Richards, J. L.; Tang, X. J.; Turetsky, A.; Dmochowski, I. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6255.
18. Heckel, A.; Mayer, G. *J. Am. Chem. Soc.* **2005**, *127*, 822.
19. Mayer, G. *Angew. Chem., Int. Ed.* **2009**, *48*, 2672.
20. Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505.
21. Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818.
22. Furuta, T.; Takeuchi, H.; Isozaki, M.; Takahashi, Y.; Kanehara, M.; Sugimoto, M.; Watanabe, T.; Noguchi, K.; Dore, T. M.; Kurahashi, T.; Iwamura, M.; Tsien, R. Y. *ChemBioChem.* **2004**, *5*, 1119.
23. Furuta, T.; Wang, S. S. H.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1193.
24. Ando, H.; Okamoto, H. *Methods Cell Sci.* **2003**, *25*, 25.
25. Ando, H.; Kobayashi, M.; Tsubokawa, T.; Uyemura, K.; Furuta, T.; Okamoto, H. *Dev. Biol.* **2005**, *287*, 456.
26. Blidner, R. A.; Svoboda, K. R.; Hammer, R. P.; Monroe, W. T. *Mol. Biosyst.* **2008**, *4*, 431.
27. Homola, J. *Anal. Bioanal. Chem.* **2003**, *377*, 528.
28. Boozer, C.; Kim, G.; Cong, S. X.; Guan, H. W.; Londergan, T. *Curr. Opin. Biotech.* **2006**, *17*, 400.
29. Piston, D. W. *Trends Cell Biol.* **1999**, *9*, 66.
30. Pelliccioli, A. P.; Wirz, J. *Photochem. Photobiol. Sci.* **2002**, *1*, 441.
31. Davis, M. J.; Kragor, C. H.; Reddie, K. G.; Wilson, H. C.; Zhu, Y.; Dore, T. M. *J. Org. Chem.* **2009**, *74*, 1721.
32. Casey, J. P.; Blidner, R. A.; Monroe, W. T. *Mol. Pharm.* **2009**, *6*, 669.