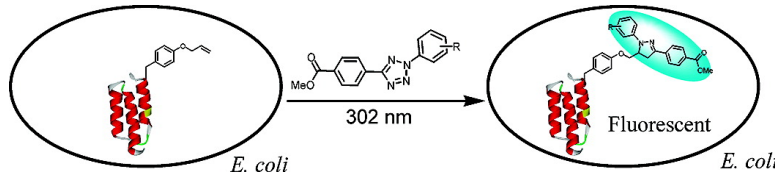
Communication

Selective Functionalization of a Genetically Encoded Alkene-Containing Protein via "Photoclick Chemistry" in Bacterial Cells

Wenjiao Song, Yizhong Wang, Jun Qu, and Qing Lin

J. Am. Chem. Soc., **2008**, 130 (30), 9654-9655 • DOI: 10.1021/ja803598e • Publication Date (Web): 02 July 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 07/02/2008

Selective Functionalization of a Genetically Encoded Alkene-Containing Protein via "Photoclick Chemistry" in Bacterial Cells

Wenjiao Song,[†] Yizhong Wang,[†] Jun Qu,[‡] and Qing Lin*,[†]

Departments of Chemistry and Pharmaceutical Sciences, State University of New York at Buffalo, Buffalo, New York 14260

Received May 14, 2008; E-mail: qinglin@buffalo.edu

Selective functionalization of proteins via bioorthogonal chemistry provides a powerful strategy to study protein function in living systems.[1] A major bottleneck in this approach has been the dearth of unique, bioorthogonal reactions that can be employed to functionalize chemical reporters incorporated site-selectively into proteins. As a result, only a few chemical reporters such as aldehyde,[2] ketone,[3] azide,[4] and acetylene[5] have been used successfully in living systems. The functionalization of these reporters relies essentially on three bioorthogonal reactions: (1) nucleophilic addition to carbonyl by hydrazine and hydroxylamine; (2) azide-acetylene cycloaddition either catalyzed by Cu^{I} ("click chemistry")[6] or accelerated by ring strain;[7] and (3) Staudinger ligation.[4] To expand structural diversity of chemical reporters for multiplexed applications in living systems,[8] there is a strong demand for new enabling bioorthogonal reactions.

Alkene is a versatile functional group in organic transformation for which several selective, water-compatible chemistries have been developed, including olefin metathesis,[9] Diels−Alder reaction,[10] and 1,3-dipolar cycloaddition reactions.[11] A number of alkene-containing amino acids have been incorporated site-specifically into proteins, including homoallylglycine,[12] *O*-allyl-tyrosine,[13] and dehydroalanine,[14] because of their unique reactivity. To our knowledge, however, alkene functionalization using cell-permeable reagents in living cells has not been reported. Herein, we report the first selective functionalization of *O*-allyl-tyrosine genetically encoded in a Z-domain protein using a photoactivated, nitrile imine mediated 1,3-dipolar cycloaddition reaction ("photoclick chemistry") in *Escherichia coli* (Figure 1). While we have recently reported the selective labeling of tetrazole-containing proteins in biological media using this chemistry,[15] the work described here represents the first time that this photoclick chemistry is applied to living cells and in a reverse manner involving an unactivated alkene.

As a first step to establish the utility of photoclick chemistry in selectively functionalizing a genetically encoded alkene, we incubated a panel of diaryltetrazole compounds with a mutant Z-domain genetically encoding *O*-allyl-tyrosine at residue 7 position (alkene-Z).[13] In parallel, we also performed the same incubations with the wild-type Z-domain protein (wt-Z) as a control. The mixtures were photoirradiated with a 302-nm hand-held UV lamp for 10 min, and then the products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Since the photoclick chemistry produces fluorescent pyrazoline cycloadducts,[15] we performed in-gel fluorescence analyses to follow the product formation (Figure 2). Among 12 diaryltetrazoles analyzed, 3 showed selective product formation only with alkene-Z, but not with wt-Z (**1**, **2**, **7** in Figure 2), suggesting that both neutral (e.g., H in tetrazole **1**) and electron-withdrawing substituents (e.g., F in tetrazoles **2** and **7**) on the *N*-aryl ring can give rise to selective reactivity toward *O*-allyl-tyrosine. The cycloadduct formation involving tetrazole **1** was further confirmed by unambiguous identification of the pyrazoline mass (+292.13 Da) in both trypsin- and V8-digested samples in the nanoLC-tandem mass spectrometry analyses.[16]

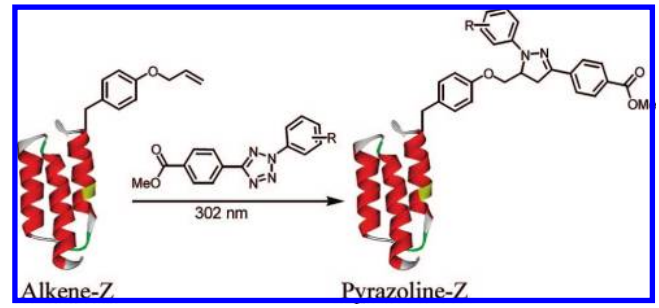


Figure 1. Scheme for selective functionalization of Z-domain protein encoding *O*-allyl-tyrosine via a photoclick chemistry.

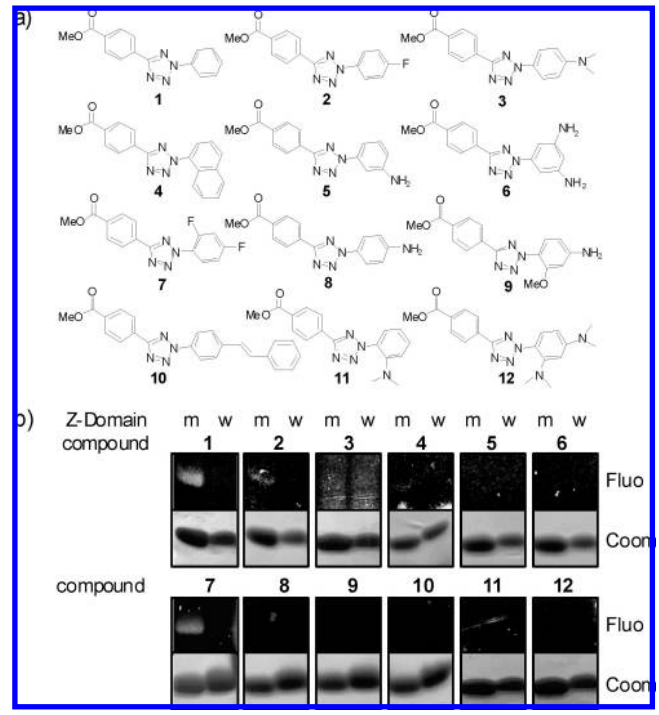


Figure 2. Screening for diaryltetrazoles that selectively react with *O*-allyl-tyrosine-containing Z-domain (m) over the wild-type Z-domain (w) by in-gel fluorescence analysis: (a) structures of diaryltetrazoles; (b) SDS-PAGE gel fluorescence images of the pyrazoline cycloadducts under 365-nm UV illumination (top panels) and by Coomassie blue staining (bottom panels). Reaction conditions: 250 μM tetrazole, 15 μM Z-domain in PBS buffer, pH 7.5, 302-nm photoirradiation for 10 min.

To determine the rate of cycloaddition, we performed a kinetic study of a model reaction between tetrazole **1** and allyl phenyl ether.[17] The photolysis of tetrazole **1** to generate the nitrile imine was found to be very rapid, and essentially complete within 2 min. The subsequent cycloaddition reaction, however, was much slower with an apparent

[†] Department of Chemistry.
[‡] Department of Pharmaceutical Sciences.

10.1021/ja803598e CCC: $40.75

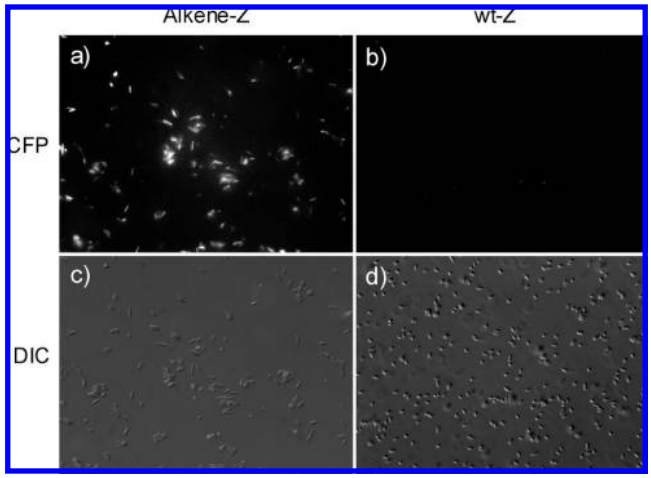


Figure 3. Selective functionalization of alkene-Z by tetrazole **1** in *E. coli* cells: CFP channel (top row) and DIC channel (bottom row) images of bacterial cells expressing either alkene-Z (a,c) or wt-Z (b,d) proteins after treatment with 100 μM tetrazole **1**. An 100× oil immersion lens was used.

second-order rate constant of 0.00202 ± 0.00007 M^{-1} s^{-1}, based on the HPLC analysis.[16] By comparison, the cycloaddition reaction between tetrazole **1** and acrylamide showed an apparent second-order rate constant of 0.15 M^{-1} s^{-1}, 75-fold faster than that of allyl phenyl ether,[16] presumably due to the lower LUMO energy of acrylamide, and thus better dipole HOMO-dipolarophile LUMO overlap.[18]

To examine whether this photoclick chemistry can be used to functionalize alkene-containing proteins in *E. coli*, BL21(DE3) cells expressing either wt-Z or *O*-allyl-tyrosine containing Z-domain proteins were suspended in the PBS buffer containing 5% glycerol and 100 μM tetrazole **1**. After incubation at 37 °C for 30 min, the cell suspensions were irradiated with a hand-held 302-nm UV lamp for 4 min. The bacterial cells were then incubated at 4 °C overnight to allow the cycloaddition reaction to proceed to completion. Because spectroscopic properties[16] of the pyrazoline match closely to that of cyan fluorescent protein (CFP), we were able to use a fluorescent microscope equipped with the CFP filter set (ex 438/24 nm, em 483/32 nm) to monitor the cycloaddition reaction in vivo. In the CFP channel, only *E. coli* cells expressing alkene-Z showed strong fluorescence while cells expressing wt-Z did not (Figure 3a,b), indicating that the cycloaddition reaction is selective toward the alkene functional group.[19] In the DIC channel, the cell density for the alkene-Z expressing cells was lower than that of the wt-Z expressing cells (Figure 3c,d), which can be attributed to significantly slower bacterial growth rate in the GMML medium (the culture medium used for the genetic incorporation of *O*-allyl-tyrosine). There was no apparent treatment-induced toxicity observed, including potential photo damages, presumably due to short irradiation time (4 min).

To verify that the fluorescence observed in *E. coli* arose from the photoclick chemistry, the treated bacterial cells were lysed and the lysates were subjected to in-gel fluorescence analysis. Only one fluorescent band with the size matching that of Z-domain was observed in the alkene-Z lysate, but not in the wt-Z lysate (Figure 4), indicating that indeed the fluorescence was due to selective formation of the fluorescent pyrazoline-Z adduct.

In summary, we have demonstrated that a tetrazole-based, photoclick chemistry can be employed to selectively functionalize an alkene genetically encoded in a protein inside *E. coli* cells. The reaction procedure was simple, straightforward, and nontoxic to *E. coli* cells with the only required external reagents to be tetrazoles and photons. Additionally, fluorescent cycloadducts were formed, which enabled a facile monitoring of the reaction in vivo. The efforts to further optimize

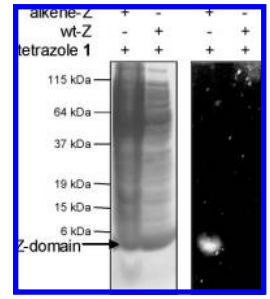


Figure 4. SDS-PAGE analysis of *E. coli* cell lysates expressing either alkene-Z or wt-Z after treatment with tetrazole **1**: left panel, Coomassie blue staining; right panel, fluorescence in-gel imaging (λ_{ex} = 365 nm). The experiment was repeated three times independently and similar results were obtained.

the tetrazole reactivity and apply this chemistry to alkene functionalization in mammalian cells are currently underway.

Acknowledgment. We gratefully acknowledge the Petroleum Research Fund (PRF No. 45503-G4), SUNY Buffalo, and New York State Center of Excellence in Bioinformatics and Life Sciences for financial support. We thank Prof. Peter Schultz at Scripps Research Institute for providing the pLEIZ, pLEIZ-7TAG, pBK-ALRS plasmids used in this study.

Supporting Information Available: Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol* **2005**, *1*, 13–21. (b) van Swieten, P. F.; Leeuwenburgh, M. A.; Kessler, B. M.; Overkleeft, H. S. *Org. Biomol. Chem.* **2005**, *3*, 20–27. (c) Barglow, K. T.; Cravatt, B. F. *Nat. Methods* **2007**, *4*, 822–827.
(2) For a recent example, see: Carrico, I. S.; Carlson, B. L.; Bertozzi, C. R. *Nat. Chem. Biol.* **2007**, *3*, 321−322.
(3) (a) Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125–1128. (b) Zhang, Z.; Smith, B. A. C.; Wang, L.; Brock, A.; Cho, C.; Schultz, P. G. *Biochemistry* **2003**, *42*, 6735–6746. (c) Chen, I.; Howarth, M.; Lin, W.; Ting, A. Y. *Nat. Methods* **2005**, *2*, 99–104.
(4) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007–2010.
(5) Deiters, A.; Cropp, T. A.; Mukherji, M.; Chin, J. W.; Anderson, J. C.; Schultz, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 11782–11783.
(6) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
(7) (a) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046–15047. (b) Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16793–16797.
(8) (a) van Kasteren, S. I.; Kramer, H. B.; Jensen, H. H.; Campbell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; Davis, B. G. *Nature* **2007**, *446*, 1105–1109. (b) Chang, P. V.; Prescher, J. A.; Hangauer, M. J.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2007**, *129*, 8400–8401.
(9) (a) Mortell, K. H.; Gingras, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **1994**, *116*, 12053–12054. (b) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606–9614. (c) Nicolaou, K. C.; Hughes, R.; Cho, S. Y.; Winssinger, N.; Labischinski, H.; Endermann, R. *Chem.—Eur. J.* **2001**, *7*, 3824–3843.
(10) (a) Otto, S.; Engberts, J. B. F. N. *Pure App. Chem.* **2000**, *72*, 1365–1372. (b) de Araújo, A. D.; Palomo, J. M.; Cramer, J.; Köhn, M.; Schröder, H.; Wacker, R.; Niemeyer, C.; Alexandrov, K.; Waldmann, H. *Angew. Chem., Int. Ed.* **2005**, *45*, 296–301.
(11) Gothelf, K. V.; Jorgensen, K. A. *Chem. Rev.* **1998**, *98*, 863–909.
(12) van Hest, J. C. M.; Kiick, K. L.; Tirrell, D. A. *J. Am. Chem. Soc.* **2000**, *122*, 1282–1288.
(13) Zhang, Z.; Wang, L.; Brock, A.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2002**, *41*, 2840–2842.
(14) (a) Wang, J.; Schiller, S. M.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2007**, *46*, 6849–6851. (b) Bernardes, G. J.; Chalker, J. M.; Errey, J. C.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 5052–5053.
(15) Song, W.; Wang, Y.; Qu, J.; Madden, M. M.; Lin, Q. *Angew. Chem., Int. Ed.* **2008**, *47*, 2832–2835.
(16) See Supporting Information for further details.
(17) Tetrazoles **1**, **2**, and **7** showed similar reaction kinetics in the initial study.
(18) Houk, K. N.; Sims, J.; Watts, C. R.; Luskus, L. J. *J. Am. Chem. Soc.* **1973**, *95*, 7301–7315.
(19) In the absence of suitable dipolarophiles, the in situ generated nitrile imines undergo slow water quenching reactions; see ref 15.

JA803598E