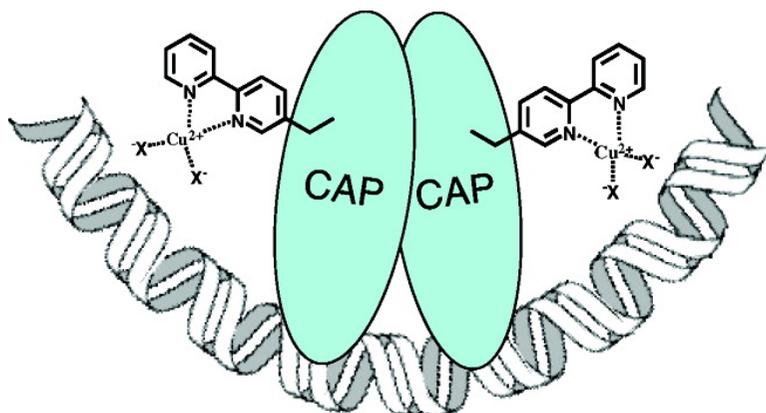


Biosynthesis of a Site-Specific DNA Cleaving Protein

Hyun Soo Lee, and Peter G. Schultz

J. Am. Chem. Soc., **2008**, 130 (40), 13194-13195 • DOI: 10.1021/ja804653f • Publication Date (Web): 13 September 2008

Downloaded from <http://pubs.acs.org> on February 8, 2009



More About This Article

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

- Supporting Information
- 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](#)



ACS Publications
High quality. High impact.

Biosynthesis of a Site-Specific DNA Cleaving Protein

Hyun Soo Lee and Peter G. Schultz*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received June 18, 2008; E-mail: schultz@scripps.edu

A number of chemical tools have been developed to map biomolecular interactions, including chemical and photocrosslinking reagents,¹ DNA footprinting methods,² and fluorescent resonance energy transfer pairs.³ These reagents have helped to elucidate the molecular details of protein–protein and protein–nucleic acid interactions, as well as the higher level architecture of cellular regulatory and signaling networks. A major challenge with all of these methods involves placement of the chemical or spectroscopic probe at a defined site in the protein. This is generally accomplished by exploiting the unique reactivity of cysteine or lysine side chains but can be complicated by the presence of multiple such residues in a protein and cannot easily be carried out within cells. Recently, we developed methods that allow one to genetically encode additional amino acids, beyond the common 20, directly in prokaryotic and eukaryotic organisms. This approach has been used to site-specifically introduce chemically reactive and photocrosslinking amino acids⁴ as well as spectroscopic probes⁵ into proteins. Here we demonstrate that redox active metal ions can be selectively introduced at defined sites in a DNA binding protein to directly map its binding site on duplex DNA.

It has been shown that attachment of EDTA-Fe(II) or 1,10-phenanthroline-Cu(II) derivatives to a DNA binding agent near the DNA interface leads to oxidative cleavage of the phosphosugar moiety of the nucleic acid backbone.⁶ Subsequent high resolution gel electrophoresis allows precise determination of the binding site size, sequence, orientation, and groove location of the ligand on DNA. Recently, the metal-chelating amino acid, (2,2'-bipyridin-5-yl)alanine (Bpy-Ala, Figure 1a), was genetically encoded in *E. coli* with an orthogonal tRNA/aminoacyl-tRNA synthetase (aaRS) pair from *Methanococcus jannaschii*.⁷ The evolved tRNA/aaRS pair is able to incorporate Bpy-Ala into proteins with high efficiency and specificity in response to the amber nonsense codon. Since 2,2'-bipyridyl strongly chelates many transition-metal ions such as Cu²⁺ and Fe²⁺, the genetic incorporation of Bpy-Ala into DNA-binding proteins should similarly make possible oxidative cleavage of the DNA backbone adjacent to the Bpy-Ala residue.

To demonstrate the feasibility of this approach, Bpy-Ala was introduced into the *E. coli* catabolite activator protein (CAP). CAP is a structurally characterized transcriptional activator which regulates a number of catabolite-sensitive operons in *E. coli*.⁸ CAP functions by binding a 2-fold symmetric 22-bp consensus binding site upstream of CAP-dependent promoters in the presence of the allosteric effector cAMP.^{8,9} Upon binding to DNA as a homodimer, CAP induces an approximate 90° bend in the double helix.¹⁰ Based on the crystal structure of the CAP-DNA complex,^{10,11} Lys26 was substituted by Bpy-Ala. Lys26 lies at the protein–DNA interface near bp 1 and 22 of the DNA recognition site (Figure 1b). Indeed, earlier studies showed that chemical derivatization of a Lys26Cys CAP mutant with an electrophilic phenanthroline derivative afforded a selective affinity cleaving agent.^{12b}

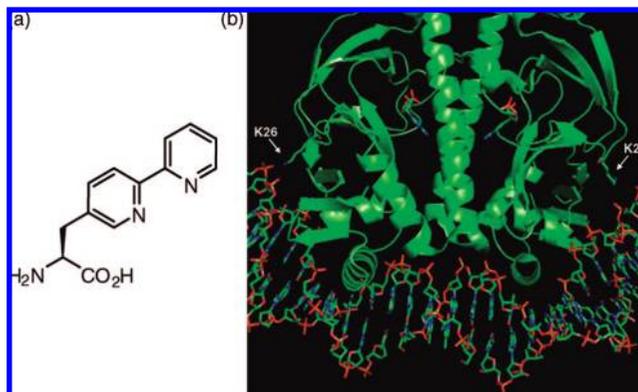


Figure 1. (a) Structure of Bpy-Ala. (b) Crystal structure of the CAP-DNA complex with K26 highlighted.^{10,11}

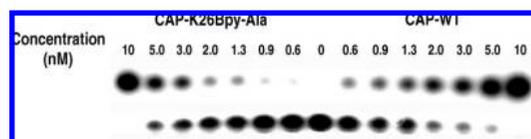


Figure 2. Gel-retardation assay of CAP-WT and CAP-K26Bpy with 50-bp 5'-³²P-end-labeled DNA fragment. Conditions: 2 nM DNA, CAP, 50 μg/mL BSA, 0.2 mM cAMP, 10 mM MOPS (pH 7.3), 200 mM NaCl, 10 μL total volume, 37 °C, 10 min.

To incorporate Bpy-Ala, an amber codon was substituted for Lys26 in a CAP derivative containing a C-terminal His₆ tag. Bpy-Ala was prepared according to a literature procedure with minor modifications.⁷ Expression of CAP was carried out in the presence of the evolved tRNA/aaRS pair and 1 mM of Bpy-Ala in *E. coli* strain DH10B grown in glycerol minimal medium.¹³ Mutant and wildtype (WT) proteins were purified by Ni-NTA, and the yield of the mutant CAP was 4–8 mg/L (CAP-WT yield was 10–15 mg/L). SDS-PAGE analysis showed that a small amount of full-length protein was produced in the absence of Bpy-Ala. However, ESI-mass spectrometric analysis indicated that the full-length mutant protein contained Bpy-Ala with no detectable common amino acid incorporation (Figures S1 and S2).

The binding affinity of the CAP-K26Bpy-Ala to a 50-bp double-stranded DNA fragment containing the 22-bp DNA recognition site for CAP was determined by a gel-retardation assay (Figure 2). The dissociation constants for CAP-WT ($K_d = 0.85$ nM) and CAP-K26Bpy-Ala ($K_d = 1.9$ nM) determined from the gel shift data show that CAP-K26Bpy-Ala has comparable binding affinity to that for CAP-WT and the Bpy-Ala group does not significantly perturb the interaction of CAP with its operator sequence. In contrast, previous chemical labeling experiments of the endogenous Cys178 of CAP^{12a} with an iodoacetamide phenanthroline derivative led to a 400-fold reduction in affinity to the operator sequence, possibly due to nonspecific modification of the protein or a

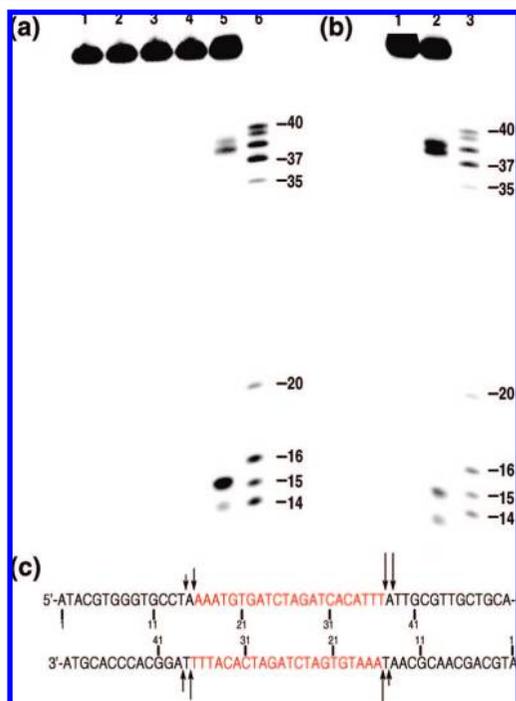


Figure 3. Autoradiogram of a 15% TBE-Urea gel of CAP-K26Bpy-Ala cleavage of a 5'-³²P-end-labeled 50-bp DNA fragment. Conditions: 2 nM DNA (50 bp), 100 nM CAP, 200 nM CuSO₄, 2.5 mM 3-mercaptopropionic acid, 50 μg/mL BSA, 0.2 mM cAMP, 2.2% EtOH, 10 mM MOPS (pH 7.3), 200 mM NaCl, 30 μL total volume, 37 °C, 12 h. (a) Data for the bottom strand. Lane 1, DNA only; lane 2, CAP-WT with all components; lane 3, all components without CAP; lane 4, CAP-K26Bpy-Ala without reducing agent; lane 5, CAP-K26Bpy-Ala with all components; lane 6, DNA marker. (b) Data for the top strand. Lane 1, DNA only; lane 2, CAP-K26Bpy-Ala with all components; lane 3, DNA marker. (c) Arrows indicate the cleavage sites and the lengths of arrows indicate the relative extents of DNA cleavage.

necessarily longer linker between the phenanthroline side chain and protein backbone.

The site-specific cleavage of DNA by CAP-K26Bpy-Ala was investigated using the same 50-bp DNA fragment that was used in the gel-retardation assay. The 5'-³²P-end-labeled DNA fragment was incubated with CAP-K26Bpy, Cu(II) or Fe(II), reducing agent, and cAMP. Both Cu(II) and Fe(II) produced cleavage products when 3-mercaptopropionic acid or ascorbic acid was used as a reducing agent. The cleavage reactions for each strand of DNA were analyzed by denaturing gel electrophoresis. The analysis revealed that CAP-K26Bpy-Ala cleaves the DNA fragment at two different sites corresponding to nucleotides 14 and 15 at the 5'-side of the binding site and 37 and 38 at the 3'-side (Figure 3a,b). Cleavage at two nucleotides on each side likely results from a diffusible oxidizing agent, since the Bpy-Ala side chain has limited flexibility. No cleavage occurred with CAP-WT under the same conditions, or in the absence of CAP-K26Bpy-Ala or reducing agent. These results indicate that the Bpy-Ala-Cu(I) complex is responsible for the cleavage reaction (Figure 3a). The crystal structure of the CAP-DNA complex shows that Lys26 is located close to both boundaries of the homodimeric CAP-binding site, ~5 Å from the DNA backbone. Denaturing gel analysis showed that there is a very close correlation between the nucleotides at which DNA cleavage was expected to occur and the sites at which DNA cleavage was observed (Figure 3c). This result is comparable to that of Ebright and co-workers in which 1,10-phenanthroline was chemically attached to CAP.¹² Moreover, cleavage of a larger

restriction fragment (3.3kb) was also selective (Figure S3), indicating that the genetic incorporation of the Cu²⁺-bipyridyl side chain into a DNA binding protein effectively localizes its oxidative chemistry at a defined site on DNA.

In conclusion, we have demonstrated that CAP can be converted into a site-specific DNA cleaving protein by genetically introducing Bpy-Ala into the protein. The mutant DNA binding protein, CAP-K26Bpy-Ala, was able to cleave double-stranded DNA at its consensus sequence with high specificity. Theoretically, any DNA binding protein can be converted into a DNA cleaving protein by this method. Because Bpy-Ala can be placed at almost any surface site on a protein, this method should provide a high resolution picture of interactions between specific regions of the protein and nearby sites on DNA. Moreover, because introduction of Bpy-Ala only requires simple mutagenesis, it should be possible to rapidly generate a series of Bpy-Ala mutants to define an optimal site of incorporation even in the absence of structural information. Finally, it may also be possible to use a similar approach to map protein-RNA¹⁴ or protein-protein interfaces, as it has been previously shown that selective delivery of Cu²⁺-phenanthroline to these biopolymers also results in selective backbone cleavage.¹⁵

Acknowledgment. We thank Dr. Mary Sever, Dan Groff, and Dr. Youngha Ryu for experimental assistance and helpful discussions. We are grateful to the U.S. Department of Energy, Division of Materials Sciences, under Award No. DE-FG03-00ER46051, and the Skaggs Institute for Chemical Biology for support of this work.

Supporting Information Available: Materials and Methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Singh, A.; Thornton, E. R.; Westheimer, F. H. *J. Biol. Chem.* **1962**, *237*, PC3006-PC8. (b) Johnson, A. E.; Miller, D. L.; Cantor, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3075-3079. (c) Wollenzien, P. L.; Youvan, D. C.; Hearst, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1642-1646. (d) Chowdhry, V.; Westheimer, F. H. *Annu. Rev. Biochem.* **1979**, *48*, 293-325.
- (2) (a) Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5470-5474. (b) Tullius, T. D.; Dombroski, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 5469-5473. (c) Tullius, T. D. *Annu. Rev. Biophys. Chem.* **1989**, *18*, 213-237.
- (3) (a) Miyawaki, A.; Llopis, J.; Heim, R.; McCaffery, J. M.; Adams, J. A.; Ikura, M.; Tsien, R. Y. *Nature* **1997**, *388*, 882-887. (b) Stryer, L. *Annu. Rev. Biochem.* **1978**, *47*, 819-846.
- (4) (a) Chin, J. W.; Martin, A. B.; King, D. S.; Wang, L.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11020-11024. (b) Chin, J. W.; Santoro, S. W.; Martin, A. B.; King, D. S.; Wang, L.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 9026-9027. (c) Tippmann, E. M.; Liu, W.; Summerer, D.; Mack, A. V.; Schultz, P. G. *ChemBioChem* **2007**, *8*, 2210-2214. (d) Wang, L.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 34-66.
- (5) Summerer, D.; Chen, S.; Wu, N.; Deiters, A.; Chin, J. W.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 9785-9789. (b) Wang, J.; Xie, J.; Schultz, P. G. *J. Am. Chem. Soc.* **2006**, *128*, 8738-8739.
- (6) (a) Schultz, P. G.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6834-6837. (b) Sluka, J. P.; Horvath, S. J.; Bruist, M. F.; Simon, M. I.; Dervan, P. B. *Science* **1987**, *238*, 1129-1132. (c) Chen, C. B.; Sigman, D. S. *Science* **1987**, *237*, 1197-1201.
- (7) Xie, J.; Liu, W.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2007**, *46*, 9239-9242.
- (8) De Crombrughe, B.; Busby, S.; Buc, H. *Science* **1984**, *224*, 831-838.
- (9) (a) Berg, O.; von Hippel, P. J. *Mol. Biol.* **1988**, *200*, 709-723. (b) Stormo, G.; Hartzell, G. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1183-1187.
- (10) Schultz, S. C.; Shields, G. C.; Steitz, T. A. *Science* **1991**, *253*, 1001-1007.
- (11) Parkinson, G.; Wilson, C.; Gunaseker, A.; Ebright, Y. W.; Ebright, R. H. *J. Mol. Biol.* **1996**, *260*, 395-408.
- (12) (a) Ebright, R. H.; Ebright, Y. W.; Pendergrast, P. S.; Gunasekera, A. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2882-2886. (b) Pendergrast, P. S.; Ebright, Y. W.; Ebright, R. H. *Science* **1994**, *265*, 959-962.
- (13) Studier, F. W. *Protein Expr. Purif.* **2005**, *41*, 207-234.
- (14) (a) Chen, C. B.; Sigman, D. S. *J. Am. Chem. Soc.* **1988**, *110*, 6570-6572. (b) Muth, G. W.; Hill, W. E. *Methods* **2001**, *23*, 281-232.
- (15) (a) Hoyer, D.; Cho, H.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 3249-3250. (b) Ermacora, M. R.; Delfino, J. M.; Cuenoud, B.; Schepartz, A.; Fox, R. O. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6383-6387.

JA804653F