$FeOCl(ET)_{1/4}$ compared to other FeOCl intercalates, the FTIR data indicating the presence of vibronic bands due to partially oxidized ET molecules, and the broad electronic transitions in the near-IR, it is likely that the guest ET stacks are indeed reponsible for the high conductivity of this material. Herber et al.[20] have found that typically only 10–13% of Fe^{3+} in FeOCl can be reduced to Fe^{2+} upon intercalation, which is consistent with the observation of partially oxidized guest stacks in $FeOCl(ET)_{1/4}$. Thus, in $FeOCl(ET)_{1/4}$ only about half of the ET molecules can be oxidized, suggesting that the increased conductivity relative to other FeOCl intercalates is due to the partially oxidized array of ET molecules.

Acknowledgment. This research was supported in part by the U.S. National Science Foundation, Solid State Chemistry Grant No. DMR-8313252, and the North Atlantic Treaty Organization, Grant No. 0727/87.

Supplementary Material Available: A listing of observed intensities of 00*l* lines and calculated structure factors for $FeOCl(ET)_{1/4}$ (1 page). Ordering information is given on any current masthead page.

(20) Phillips, J. E.; Herber, R. H. *Inorg. Chem.* **1986**, *25*, 3081.

Site-Specific Cleavage of Duplex DNA with a λ Repressor–Staphylococcal Nuclease Hybrid

Dehua Pei and Peter G. Schultz*

Department of Chemistry, University of California Berkeley, California 94720

Received February 6, 1990

The development of reagents capable of selective cleavage of large double-stranded DNA would greatly facilitate the manipulation and mapping of genomic DNA. Several such strategies have been reported including D-loop formation to deliver a semisynthetic nuclease to double-helical DNA,[1] and the use of triple-helix formation[2] or DNA-binding proteins[3] to deliver oxidative cleaving agents to the target sequences. We report here the design and synthesis of a λ repressor–staphylococcal nuclease hybrid protein[4] capable of efficient hydrolysis of both linear and supercoiled duplex DNA. The repressor domain of the hybrid nuclease binds specifically to its recognition sequence, λ operator O_R1 or O_L1, and delivers the nuclease activity to DNA sequences adjacent to the repressor binding site. Upon addition of Ca^{2+}, the nuclease selectively hydrolyzes both DNA strands at the target region.

Staphylococcal nuclease[5] was coupled to a truncated λ repressor[6,7] lacking the C-terminal domain (Figure 1). A unique cysteine (Ser 32 to Cys 32) was introduced into the cysteine-free N-terminal domain (residues 1–102) via site-directed mutagenesis.[8] The mutant cysteine is located in the loop region between α-helices

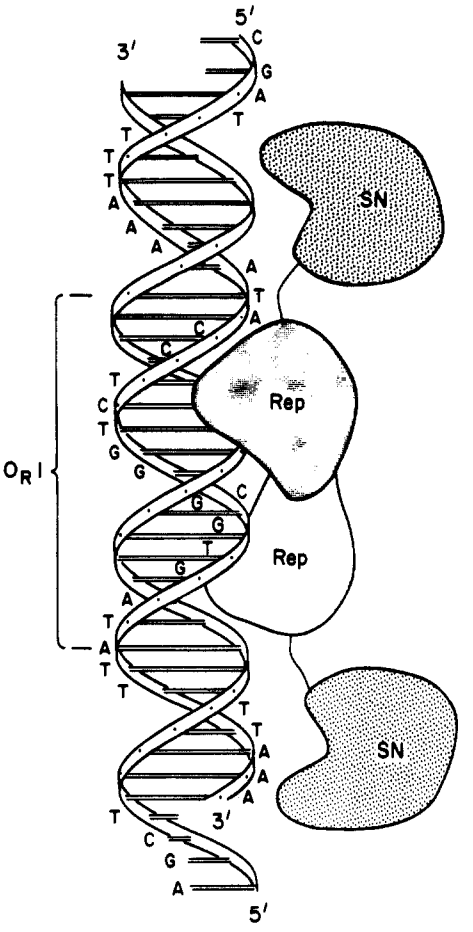

Figure 1. Scheme showing the binding of the hybrid protein to B-form DNA. The two DNA strands, 35 and 37 nucleotides, were chemically synthesized with restriction sites *Cla*I and *Hin*dIII at the termini. Repressor binds to the 17-base-pair O_R1 as a dimer and delivers two nuclease molecules to the two $d(T_4A_4)$ target sites.

1 and 2 and has been previously shown not to appreciably alter the binding properties of the repressor.[10]

A water-soluble tether, methylbis[3-[3-(2-pyridyldithio)-propionamido]propyl]amine (**1**),[11] was used to cross-link staphylococcal nuclease to the repressor. This flexible cross-linker was designed to allow the hybrid protein to bind substrate and hydrolyze both strands of the duplex target. The construction of the hybrid protein involved two consecutive disulfide exchange reactions. Monomeric repressor[13] was reacted with a 50-fold excess of the cross-linking agent (**1**) in water (pH adjusted to 7.0 with HCl). The derivatized repressor was purified by cation-exchange chromatography[14] and subsequently reacted at pH 8.5 with 1 equiv of reduced K84C staphylococcal nuclease which contains a single surface cysteine.[1,15] The reaction was complete within 1 h at room temperature, and the resulting hybrid protein was purified by cation-exchange chromatography.[14]

* Author to whom correspondence should be addressed.

(1) Corey, D. R.; Pei, D.; Schultz, P. G. *J. Am. Chem. Soc.* **1989**, *111*, 8523.

(2) (a) Stroebol, S. A.; Moser, H. E.; Dervan, P. B. *Ibid.* **1988**, *110*, 7927. (b) Francois, J.; Saison-Behmoaras, T.; Chassignol, M.; Thuong, N. T.; Helene, C. J. *J. Biol. Chem.* **1989**, *264*, 5891.

(3) (a) Sluka, J. P.; Horvath, S. J.; Bruist, M. F.; Simon, M. I.; Dervan, P. B. *Science* **1987**, *238*, 1129. (b) Mack, D. P.; Iverson, B. L.; Dervan, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 7572. (c) Chen, B. C.; Sigman, D. S. *Science* **1987**, *237*, 1197.

(4) A fusion protein of *lac* repressor/T7 endonuclease has previously been constructed genetically. Panayotatos, N.; Backman, S. *J. Biol. Chem.* **1989**, *264*, 15070.

(5) Tucker, P.; Cotton, F.; Hazen, E. *Mol. Cell. Biochem.* **1979**, *23*, 67.

(6) Ptashne, M. *A Genetic Switch*; Cell Press & Blackwell Scientific Publications: Cambridge, MA, 1986.

(7) The N-terminal domain alone (wild-type) has previously been shown to bind its cognate sequence O_R1 with K_1 (dimerization) = 1×10^{-4} M and K_2 (dissociation) = 2.5×10^{-9} M.[9]

(8) Mutagenesis was carried out in a fashion similar to that described by Sauer et al.[9]

(9) Sauer, R. T.; Hehir, K.; Stearman, R. S.; Weiss, M. A.; Jeitler-Nilsson, A.; Suchanek, E. G.; Pabo, C. O. *Biochemistry* **1986**, *25*, 5992.

(10) Hecht, M. H.; Sauer, R. T. *J. Mol. Biol.* **1985**, *186*, 53.

(11) Compound **1** was synthesized by reacting 3,3′-diamino-*N*-methyldipropylamine with 2 equiv of *N*-succinimidyl 3-(2-pyridyldithio)propionate[12] in aqueous solution at pH 7.5. The product was purified by chromatography on silica gel (eluent CH_2Cl_2–MeOH–H_2O, 49:49:2) (R_f = 0.20 on silica gel with the same solvent system).

(12) Carlsson, J.; Drevin, H.; Axen, R. *Biochem. J.* **1978**, *173*, 723.

(13) The repressor was isolated in monomeric form in a buffer (10 mM Tris·HCl, pH 8.0, 2 mM $CaCl_2$, 0.1 mM EDTA, 1.4 mM β-mercaptoethanol, 50 mM NaCl, and 5% glycerol). Prior to use, it was treated with 5 mM DTT and purified on a Pharmacia FPLC Fast Desalting column (HR 10/10) eluted with 20 mM Tris·HCl buffer (pH 8.5).

(14) Purification was performed on a Pharmacia FPLC Mono S column (HR5/5), by elution with 50 mM HEPES (pH 6.8), 2 mM EGTA, and a linear gradient of 50–600 mM NaCl. K84C staphylococcal nuclease, repressor derivatized with the tether, and the hybrid protein eluted at 370 mM, 300 mM, and 430 mM NaCl, respectively.

(15) Corey, D. R.; Schultz, P. G. *Science* **1987**, *238*, 1401.

0002-7863/90/1512-4579$02.50/0

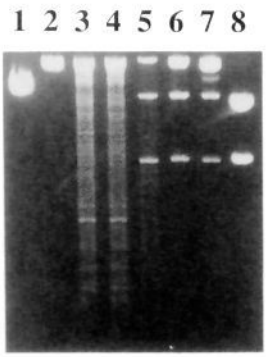

Figure 2. A 1% agarose gel stained by ethidium bromide. Lane 1: supercoiled plasmid pUC19-O_R1 DNA. Lane 2: plasmid DNA linearized by *Sca*I. Lane 3: *Sca*I-linearized plasmid DNA (3 μg, 0.18 μM) treated with free K84C staphylococcal nuclease (0.8 μM). Lane 4: same as in lane 3 but in the presence of monomeric repressor (1.0 μM). Lane 5: *Sca*I-linearized DNA (2 μg, 0.12 μM) treated with the hybrid protein (0.2 μM). Lane 6: same as in lane 5 but in the presence of poly(A) (10 μg). Lane 7: supercoiled plasmid DNA (3 μg, 0.18 μM) treated first with the hybrid protein (0.13 μM) in the presence of poly(A) (10 μg) and then digested with *Sca*I. Lane 8: *Sca*I/*Hin*dIII digest of pUC19-O_R1. Cleavage reactions were performed by mixing the plasmid pUC19-O_R1 (2–3 μg), poly(A) as indicated, and enzyme (0.1–0.8 μM) in 10 mM PIPES, pH 7.0, 0.1 mM EDTA, and 40 mM NaCl, in a total volume of 10 μL. After incubation for 20 min at room temperature, the reaction was initiated by the addition of $CaCl_2$ to a final concentration of 10 mM and terminated after 15 s by the addition of ethylene glycol bis(1-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) to a final concentration of 12 mM.

In order to analyze the ability of the hybrid protein to selectively hydrolyze duplex DNA, a DNA fragment containing the O_R1 repressor binding site (Figure 1) was chemically synthesized and inserted into the *Acc*I/*Hin*dIII site of plasmid pUC19.[16] The resulting plasmid pUC19-O_R1 (2704 bp) contained the 17-base-pair repressor binding site, O_R1, with A,T-rich sites d(T_4A_4) on each side.[17] The supercoiled plasmid DNA (3 μg, 0.18 μM, 500 μM in base pairs) was preincubated with stoichiometric amounts of the hybrid protein, and the cleavage reaction was then initiated by the addition of Ca^{2+} (the nuclease is Ca^{2+}-dependent[18]). The reaction was quenched, and the product was then treated with the restriction enzyme *Sca*I to generate discrete fragments, which were analyzed by agarose gel electrophoresis. Comparison of these fragments with known standards (1730 and 974 bp, generated by *Sca*I/*Hin*dIII[19] digestion of pUC19-O_R1) indicated that the cleavage was highly specific and occurred adjacent to the O_R1 site (Figures 1 and 2).

In a similar experiment, the plasmid DNA was first linearized with *Sca*I and then treated with the hybrid protein. The linear DNA (2 μg, 0.12 μM, 330 μM in base pairs) was also efficiently hydrolyzed (approximately 50% conversion) at the target site (Figure 2). Another plasmid, pLcIIFXβ[20] (approximately 3000 bp), which contains the λ operon but not the eight-base-pair A,T-rich sites, was also selectively cleaved adjacent to the O_L1 sequence. Control experiments showed that neither K84C staphylococcal nuclease alone nor free nuclease in combination with repressor was able to selectively cleave the target sequence. Specific cleavage was accompanied by some nonspecific hydrolysis, presumably by unbound hybrid protein. Addition of poly(A) effectively depressed this nonspecific cleavage. Experiments are currently being carried out to determine the nature of the double strand cleavage reaction.

We have demonstrated that a combination of chemical and genetic modifications can be used to convert a relatively nonspecific enzyme into a sequence-selective DNA-cleaving molecule. This strategy should be applicable to other DNA-binding proteins and may lead to a family of hybrid nucleases capable of selective cleavage of large DNAs.

Acknowledgment. Support for this work was provided by the National Institutes of Health (1R01GM41679-01) and the Alfred P. Sloan Foundation (BR-2766). We also thank Dr. Robert Sauer of MIT for providing the plasmid plac1-102 and D. R. Corey for helpful discussions.

(16) Yanisch-Perron, C.; Viera, J.; Messing, J. *Gene* **1985**, *33*, 103.
(17) Staphylococcal nuclease cleaves DNA preferentially at T,A-rich sites. Corey, D. R.; Pei, D.; Schultz, P. G. *Biochemistry* **1989**, *28*, 8277. Dingwall, C.; Lomonossoff, G. P.; Laskey, R. A. *Nucleic Acids Res.* **1981**, *9*, 2659. Flick, J. T.; Eissenberg, J. C.; Elgin, S. C. R. *J. Mol. Biol.* **1986**, *190*, 619.
(18) Cuatrecasas, P.; Fuchs, S.; Anfinsen, C. B. *J. Biol. Chem.* **1967**, *242*, 1541.
(19) The *Hin*dIII sequence is directly adjacent to the A,T-rich site, which is the hybrid protein cleavage site.
(20) (a) Nagai, K.; Thøgersen, H. C. *Nature* **1984**, *309*, 810. (b) Baldwin, E. P.; Schultz, P. G. *Science* **1989**, *245*, 1104.

cis-Stilbene Fluorescence in Solution. Adiabatic $^1c^* \rightarrow {}^1t^*$ Conversion

Jack Saltiel,* Andrew Waller, Ya-Ping Sun, and Donald F. Sears, Jr.

Department of Chemistry, The Florida State University Tallahassee, Florida 32306-3006

Received November 29, 1989

Though *cis*-stilbene fluorescence has been observed in high-viscosity media,[1-5] starting with the important contribution by Lewis and co-workers,[6] all previous attempts to detect its fluorescence in fluid solution have failed. Recently, however, fluorescence with an extremely long lifetime, 20 ns, was reported from supersonic beams of *cis*-stilbene vapor seeded in inert gas expansions and was attributed to trapping of vibrationally relaxed $^1c^*$ in an inherent minimum on the S_1 potential energy surface.[7] This interpretation sets an upper limit of $k_f \leq 5 \times 10^7$ s^{-1} for the radiative rate constant of $^1c^*$ that is nearly one-third the value based on the 4.7-ns lifetime of $^1c^*$ measured at 77 K in 3-methylpentane glass[8] under conditions for which values of ϕ_f = 0.75–0.79 have been reported.[3,4] An earlier time-resolved investigation of *cis*-stilbene vapor, in which transient decay was monitored by multiphoton ionization,[9] reported single-exponential decay, τ = 0.32 ps, that was associated with motion along the torsional coordinate of $^1c^*$ unimpeded by any appreciable barrier.[9] Somewhat longer lifetimes, τ = 0.9–1.35 ps and 1.0 ps, have been obtained by monitoring $^1c^*$ absorption[10-12] and $^1c^*$ fluorescence,[13] respectively, in *n*-hexane solution at room temperature, suggesting a small medium-imposed torsional barrier.[14]

(1) (a) Stegemeyer, H.; Perkampus, H. H. *Z. Phys. Chem.* (*Munich*) **1963**, *39*, 125. (b) Aurich, F.; Hauser, M.; Lippert, E.; Stegemeyer, H. *Z. Phys. Chem.* (*Munich*) **1964**, *42*, 123. (c) Lippert, E. *Z. Phys. Chem.* (*Munich*) **1964**, *42*, 125.
(2) Lamola, A. A.; Hammond, G. S.; Mallory, F. B. *Photochem. Photobiol.* **1965**, *4*, 259.
(3) Sharafi, S.; Muszkat, K. A. *J. Am. Chem. Soc.* **1971**, *93*, 4119.
(4) Fischer, G.; Seger, G.; Muszkat, K. A.; Fischer, E. *J. Chem. Soc., Perkin Trans. 2* **1975**, 1569.
(5) Hohlneicher, G.; Müller, M.; Demmer, M.; Lex, J.; Pen, J. H.; Gan, L.; Loesel, P. D. *J. Am. Chem. Soc.* **1988**, *110*, 4483.
(6) Lewis, G. N.; Magel, T. T.; Lipkin, D. *J. Am. Chem. Soc.* **1940**, *62*, 2973.
(7) Petek, H.; Fujiwara, Y.; Kim, D.; Yoshihara, K. *J. Am. Chem. Soc.* **1988**, *110*, 6269.
(8) Yoshihara, K.; Namiki, A.; Sumitani, M.; Nakashima, N. *J. Chem. Phys.* **1979**, *71*, 2892.
(9) Greene, B. I.; Farrow, R. C. *J. Chem. Phys.* **1983**, *78*, 3336.
(10) Greene, B. I.; Scott, T. W. *Chem. Phys. Lett.* **1984**, *106*, 399.
(11) (a) Doany, F. E.; Hochstrasser, R. M.; Greene, B. I.; Millard, R. R. *Chem. Phys. Lett.* **1985**, *118*, 1. (b) Hochstrasser, R. M. Private communication.
(12) Courtney, S. H.; Kim, S. K.; Canonica, S.; Fleming, G. R. *J. Chem. Soc., Faraday Trans. 2* **1986**, *82*, 2065.
(13) Todd, D. C.; Jean, J. M.; Rosenthal, S. J.; Ruggiero, A. J.; Yang, D.; Fleming, G. R. Manuscript in preparation.
(14) (a) Saltiel, J.; Sun, Y.-P. *J. Phys. Chem.* **1989**, *93*, 6246. (b) Sun, Y.-P.; Saltiel, J. *J. Phys. Chem.* **1989**, *93*, 8310 and references cited therein.

0002-7863/90/1512-4580$02.50/0