Sequence-Specific Oxidative Cleavage of DNA by a Designed Metalloprotein, Ni(II)·GGH(Hin139–190)†

David P. Mack and Peter B. Dervan*

Arnold and Mabel Beckman Laboratories of Chemical Synthesis, California Institute of Technology, Pasadena, California 91125

Received May 26, 1992

ABSTRACT: A 55-residue protein containing the DNA binding domain of Hin recombinase, residues 139–190, with the tripeptide Gly-Gly-His (GGH) at the NH_2 terminus was synthesized by stepwise solid-phsae methods. GGH(Hin139–190) binds sequence specifically to DNA at four 13 base pair sites (termed *hix*L and *secondary*) and, in the presence of $Ni(OAc)_2$ and monoperoxyphthalic acid, reacts predominantly at a single deoxyribose position on one strand of each binding site [Mack, D. P., & Dervan, P. B. (1990) *J. Am. Chem. Soc. 112,* 4604]. We find that, upon treatment with *n*-butylamine, the DNA termini at the cleavage site are 3′- and 5′-phosphate, consistent with oxidative degradation of the deoxyribose backbone. The nickel-mediated oxidation can be activated with peracid, iodosylbenzene, or hydrogen peroxide. The sequence specificity of the reaction is not dependent on oxidant, but the rates of cleavage differ, decreasing in the order peracid > iodosylbenzene > hydrogen peroxide. Optimal cleavage conditions for a 1 μM concentration of protein are 50 μM peracid, pH 8.0, and 1 equiv of $Ni(OAc)_2$. The preferential cleavage at a single base pair position on one strand of the minor groove indicates a nondiffusible oxidizing species. A change of absolute configuration in the GGH metal binding domain from L-His to D-His [Ni(II)·GG-(-D-)H(Hin139–190)] affords cleavage at similar base pair locations but opposite with regard to strand specificity.

Attachment of ethylenediaminetetraacetic acid (EDTA) to a DNA binding molecule affords a DNA cleaving molecule (Hertzberg & Dervan, 1982). In a formal sense, the nonnatural amino acid EDTA is a metal binding domain capable of oxidizing DNA when bound in a DNA–ligand complex (Hertzberg & Dervan, 1982, 1984). Affinity cleaving has been used to study the sequence-specific recognition of double-helical DNA by small peptides bound in the minor groove (Taylor et al., 1984; Dervan, 1986), oligonucleotides bound in the major groove by triple-strand formation (Moser & Dervan, 1987), and the DNA binding domain of proteins bound in both the major and minor grooves (Sluka et al., 1987, 1990). In an effort to design sequence-specific DNA-cleaving proteins consisting wholly of naturally occurring α-amino acids, the tripeptide H-Gly-Gly-His-OH (GGH) was attached to the amino terminus of the DNA binding domain of Hin recombinase, residues 139–190, affording a new 55-residue protein (Mack et al., 1988; Mack & Dervan, 1990). GGH(Hin139–190) contains two structural domains, each with distinct functions. The GGH segment is a tripeptide ligand for transition metals such as Cu(II) or Ni(II), and Hin(139–190) is a helix–turn–helix domain for specific DNA binding (Figure 1).

DNA Binding Domain, Hin(139–190). Hin recombinase is a 190 amino acid enzyme that inverts a segment of DNA which controls the expression of the flagellin genes of *Salmonella typhimurium* (Zeig et al., 1977). Recombination occurs between two crossover sites, designated *hix*L and *hix*R, on supercoiled DNA (Johnson et al., 1984). Each hix site is 26 base pairs long and has nearly 2-fold symmetry. Hin binds to a hix site as a dimer and protects bases −13 through +13, inclusive, from chemical cleavage reagents (consensus half-site 5′-TTNTCNNAAACCA-3′) (Johnson et al., 1984; Glasgow et al., 1989). A synthetic 52 amino acid protein identical with the COOH terminus of Hin(139–190) has been shown by DNase I and dimethyl sulfate protection experiments to contain the sequence-specific DNA-binding activity of Hin (Bruist et al., 1987).

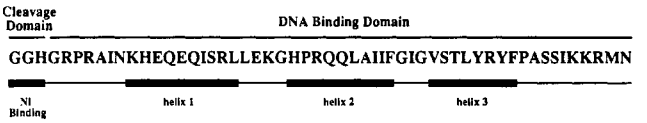

FIGURE 1: Sequence of GGH(Hin139–190) protein combining the DNA binding domain of Hin recombinase (residues 139–190) with the copper binding domain (GGH) from serum albumin.

Hin(139–190) contains extensive sequence similarity to the helix–turn–helix DNA-binding proteins (Pabo & Sauer, 1984; Steiz, 1990; Kissinger et al., 1990) and is thought to recognize DNA through this motif in the major groove (Sluka et al., 1987). Hin(139–190) with EDTA·Fe at the NH_2 terminus reveals that the NH_2 terminus of Hin(139–190) is bound in the minor groove of DNA near the symmetry axis of Hin binding sites (Sluka et al., 1987, 1990a,b). A binding model put forward for Hin(139–190) includes a helix–turn–helix–turn–helix structure in the major groove with residues at the NH_2 terminus extending across the DNA phosphodiester backbone making specific contacts to the adjacent minor groove (Sluka et al., 1987, 1990a,b). Attachment of EDTA·Fe to a lysine side chain (Ser^{183} to Lys^{183}) at the COOH terminus of Hin(139–184) reveals that the recognition helix is oriented toward the symmetry axis of the binding site (Mack et al., 1990).

Metal Binding Domain, GGH. The tripeptide GGH is a consensus sequence for the copper binding domain of serum albumin and binds Cu(II) in a 1:1 complex over the pH range 6.5–11 with a dissociation constant of 1.2×10^{-16} M (Lau et al., 1973; Kruck et al., 1976). A crystal structure of Cu-

† We are grateful to the National Institutes of Health (GM27681) and the National Foundation for Cancer Research for financial support.

* To whom correspondence should be addressed.

0006-2960/92/0431-9399$03.00/0

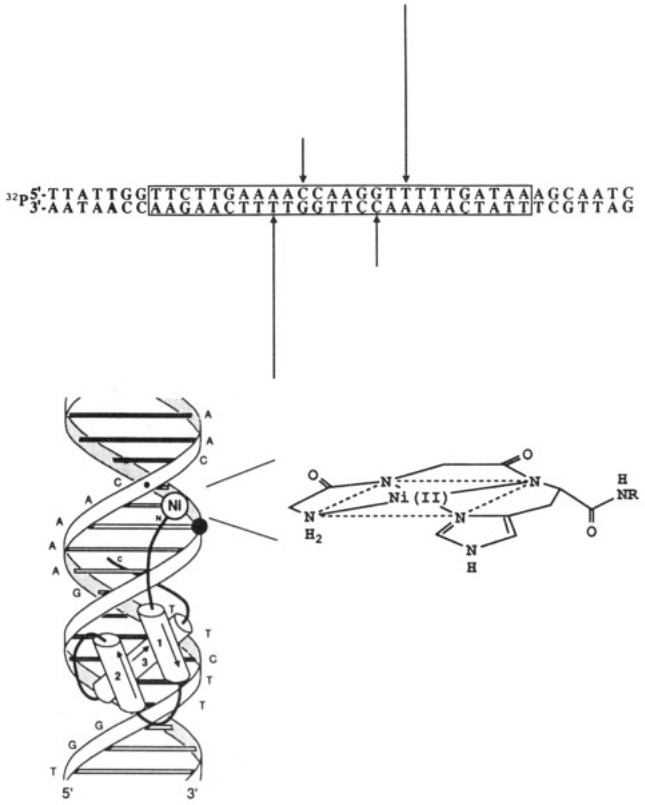

FIGURE 2: (Top) The sequence left to right represents the cleavage data for the dimeric *hix*L binding site. Arrows represent location and extent of cleavage. (Bottom) Schematic representation of a model for the designed metalloprotein Ni·GGH(Hin139–190) binding to one half-site (Mack & Dervan, 1990). Putative α-helices are shown as cylinders with an arrow pointing from NH_2 to COOH terminus. Dots represent the site and extent of cleavage in the presence of monoperoxyphthalic acid.

(II)·GGHa reveals square-planar complexation of the Cu(II) by an imidazole nitrogen, two deprotonated peptide nitrogens, and the terminal amino group (Cameraman et al., 1976). Although a crystal structure of GGH·Ni(II) is not available, the Ni(II) complex of GGH is known and has been studied by other techniques (Bossu & Margerum, 1977; Bannister et al., 1982; Sakuri & Nakahara, 1979). Crystal structures of tetraglycine with Cu(II) or Ni(II) indicate that the metal ions are bound by peptide ligands in a similar fashion (Freeman & Taylor, 1965; Freeman et al., 1968; Bossu et al., 1978). Thus, as in the Cu(II) case, coordination of Ni(II) by GGH is believed to occur via the imidazole nitrogen, two deprotonated peptide nitrogens, and the terminal amino group.

Site-Specific Cleavage of DNA by Ni(II)·GGH(Hin139–190). GGH(Hin139–190) in the presence of $Ni(OAc)_2$ and monoperoxyphthalic acid produces cleavage in minutes (25 °C) at all four DNA binding sites upon sequent treatment with *n*-butylamine (Mack & Dervan, 1990). The cleavage efficiency observed at the *hix*L sites is high and occurs predominantly at a single base pair on one strand of each DNA binding site (Figure 2). Cleavage at the *secondary* sites is less efficient and occurs at two base positions on both DNA strands. Maximal cleavage on opposite strands of the DNA is asymmetric to the 3′ side, consistent with the known location of the NH_2 terminus of Hin(139–190) in the minor groove of DNA (Sluka et al., 1987, 1990a). We report here a study of the nickel-mediated DNA cleavage reaction by the designed metalloprotein including (i) optimization of reaction conditions for DNA cleavage, (ii) analyses of the DNA cleavage products, and (iii) the effect on the cleavage specificity by a change of absolute configuration of a single histidine in the metal binding region.

EXPERIMENTAL PROCEDURES

General. UV–vis spectra were recorded on a Beckman Model 25, Cary Model 219, or Perkin-Elmer Lambda 4C spectrophotometer. Laser densitometry of autoradiograms was performed on an LKB Ultrascan XL densitometer. Gels were exposed to storage phosphor screens and quantitated using a Molecular Dynamics Model 400S PhosphorImager. Doubly distilled or Milli-Q water was used for all aqueous reactions and dilutions. Calf thymus DNA was purchased from Sigma and was sonicated, deproteinized, and dialyzed. Enzymes were purchased from Boehringer Mannheim or New England Biolabs. Monoperoxyphthalic acid was purchased as the magnesium salt hexahydrate from Aldrich.

Syntheses. GGH(Hin139–190) was synthesized by manual protocols on phenylacetamidomethyl (PAM) resin solid support substituted with tBoc-Asn using Boc-protected amino acids with an average yield per step of 99.4% (Mack et al., 1988) as obtained by quantitative ninhydrin analysis (Sarin et al., 1981). In addition, Hin(139–190) could be synthesized by automated methods on an Applied Biosystems 430 protein synthesizer and the last three residues added manually. Peptide sequence analysis by Edman degradation confirmed the sequence shown in Figure 1. Mass spectral analysis of GGH(Hin139–190) was carried out on a Bioion 20 time of flight plasma desorption spectrometer (calcd average mass: 6287.3; found: 6276.8 ± 11.2) (Mack, 1991). Oligonucleotides were synthesized on either a Beckman System 1 Plus or Applied Biosystems 380B DNA synthesizer by standard methods (Matteucci & Caruthers, 1981; Beaucage & Caruthers, 1984; Atkinson & Smith, 1984).

Plasmid Construction. Plasmid pDPM12 was constructed to investigate the end products of DNA cleavage produced by Ni·GGH(Hin139–190). Oligonucleotides 5′-AATTCTATTCGTTCTTGAAAACCAAGGTTTTTGATAAGCAATCA-3′ and 5′-AGCTTGATTGCTTATCAAAAACCTTGGTTTTCAAGAACGAATAG-3′ were synthesized, annealed, and ligated into the *Eco*RI/*Hin*dIII fragment of plasmid pBR322. The resulting ligation mixture was used to transform *Escherichia coli* strain HB101 according to the standard procedures (Maniatis et al., 1982). Recombinant clones were selected for ampicillin resistance and tetracycline sensitivity due to the disruption of the tetracycline gene by insertion between *Eco*RI and *Hin*dIII (Sutcliffe, 1979). Plasmid DNA was isolated from *E. coli* and purified by CsCl centrifugation (Tanaka & Weisblum, 1975; Mendel, 1989).

Labeling of Restriction Fragments. The procedure described is for the *Xba*I/*Eco*RI fragment from pMFB36, but is the same for other plasmids with the appropriate restriction enzymes (Bruist et al., 1987). Plasmid pMFB36 (Bruist et al., 1987) was linearized by cleavage with restriction endonuclease *Xba*I. Labeling at the 3′ end was accomplished with [α-^{32}P]dATP and the Klenow fragment of DNA polymerase I. The 5′ end was labeled with ^{32}P by treatment with calf alkaline phosphatase (CAP) followed by treatment with [γ-^{32}P]ATP and T4 polynucleotide kinase. Cleavage with restriction endonuclease *Eco*RI yielded 3′-(and 5-′)end-labeled fragments, 557 bp in size, which were isolated by polyacrylamide gel electrophoresis. Chemical sequencing reactions for A were carried out as described in Iverson and Dervan (1987).

Ni(II)·GGH(Hin139–190) Cleavage Reactions. Cleavage reactions were done in a total volume of 20 μL. Final concentrations were 20 mM phosphate, pH 7.5, 20 mM NaCl, 100 mM (in base pairs) CT DNA, ≈15 000 cpm of ^{32}P-end-labeled DNA, 5 μM monoperoxyphthalic acid (or appropriate concentrations of other oxidants), and 5 μM Ni(II)·GGH-

(Hin139–190). The protein was allowed to equilibrate with the DNA for 10 min prior to addition of oxidant. The reaction was initiated by the addition of magnesium monoperoxyphthalate and allowed to proceed for 15 min at 25 °C. The reactions were terminated by ethanol precipitation, dried, resuspended in 50 μL of 0.1 N *n*-butylamine, heated to 90 °C for 30 min, frozen and lyophilized, and resuspended in 5 μL of 100 mM Tris–borate–EDTA and 80% formamide solution. The ^{32}P-end-labeled products were analyzed by denaturing polyacrylamide gel electrophoresis followed by autoradiography.

Analysis of DNA Termini by Gel Electrophoresis. Cleavage reactions were run as described above for Ni(II)·GGH-(Hin139–190) without and with *n*-butylamine treatment. The DNA cleavage products were analyzed by 20% polyacrylamide gel electrophoresis. The presence of 5′-phosphate groups was tested using calf alkaline phosphotase to remove the 5′-phosphate groups. After the DNA reaction was complete, the DNA was ethanol precipitated. If base treatment was necessary, the solution was neutralized first with NaOAc. The DNA was then dissolved in 45 μL of 5 mM Tris, pH 7.5, and 10 mM EDTA and heated to 90 °C for 5 min. Calf alkaline phosphatase was added (5 μL of 1 unit/μL) and incubated at 37 °C for 30 min. The reaction was stopped by ethanol precipitation and analyzed on a 20% polyacrylamide gel.

The presence of 3′-phosphate groups was tested using T4 polynucleotide kinase to remove the 3′-phosphate groups (Cameron & Uhlenbeck, 1977). After the DNA reaction was completed, the DNA was ethanol precipitated. If base treatment was necessary, the solution was neutralized first with NaOAc. The DNA was then dissolved in 25 μL of water and heat denatured for 5 min at 90 °C and cooled on ice. Twenty-five microliters of a buffer containing 20 mM Tris-HCl, pH 6.6, 20 mM magnesium chloride, and 10 mM β-mercaptoethanol was added followed by 1 mL of T4 polynucleotide kinase (10 units/mL). The reaction was incubated for 1 h at 37 °C and ethanol precipitated for gel electrophoresis.

RESULTS AND DISCUSSION

Synthesis. The 55-residue protein GGH(Hin139–190) was synthesized by stepwise solid-phase methods with optimized manual protocols (Kent, 1988) on phenylacetamidomethyl (PAM) resin solid support with Boc-protected amino acids. Each coupling cycle was monitored by quantitative ninhydrin analysis, and couplings were repeated until the yield was maximal for each step. After deprotection of the peptide–resin, the crude synthetic protein was purified by reverse-phase preparative HPLC. Peptide sequence analysis by Edman degradation and mass spectrometry data confirmed the identity of the purified protein.

DNA Termini Analyses. The nature of the termini of the DNA cleavage reaction were determined by comparing electrophoretic mobilities of DNA fragments with standards of known chemical composition. A 180 bp DNA restriction fragment labeled at the 3′ end was allowed to react with Ni-(II)·GGH(Hin139–190) in the presence of monoperoxyphthalic acid without and with subsequent *n*-butylamine treatment. The DNA cleavage products were analyzed by denaturing 20% polyacrylamide gel electrophoresis (Figure 3). The resulting fragments comigrate with those produced by MPE·Fe(II), which is known to afford 5′-phosphate termini (Hertzberg & Dervan, 1984). The Ni(II)-mediated DNA cleavage products were treated with calf intestinal alkaline phosphatase (CAP) which removes 5′-phosphate groups from

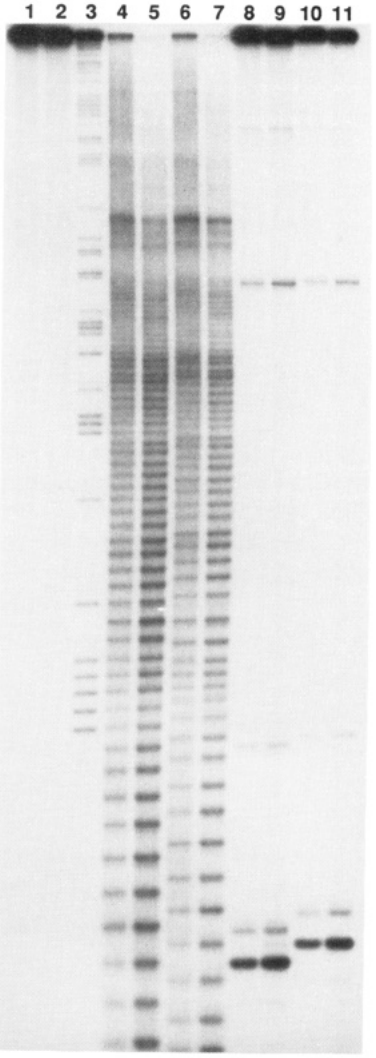

FIGURE 3: Autoradiogram of high-resolution denaturing polyacrylamide gel analyzing the 5′ end products of the Ni(II)·GGH(Hin139–190)–monoperoxyphthalic acid DNA cleavage reaction. Reaction conditions were 20 mM NaCl, 20 μM phosphate, pH 7.5, 100 μM (in base pair) calf thymus DNA, and ≈15 000 cpm of 3′-end-labeled DNA (*Eco*RI/*Rsa*I fragment from pDPM12) in a total volume of 20 mL. Reactions for MPE·Fe(II) (10 μM) were run for 15 min in the presence of 5 mM DTT. Reactions for Ni(II)·GGH(Hin139–190) (5 μM) were run for 15 min in the presence of monoperoxyphthalic acid (5 μM). Nickel-mediated cleavage reactions were treated with 0.1 M *n*-butylamine for 30 min at 90 °C. DNA cleavage products were analyzed on a 20% 1:20 cross-linked 50% urea denaturing polyacrylamide gel. Lanes 1 and 2 are intact DNA without and with base workup, respectively. Lane 2 is a Maxam–Gilbert chemical sequencing G lane. Lanes 4 and 5 are MPE·Fe(II) reactions without and with base workup, respectively. Lanes 6 and 7 are MPE·Fe(II) reactions followed by treatment with calf alkaline phosphatase, without and with base workup, respectively. Lanes 8 and 9 are Ni(II)·GGH(Hin139–190) cleavage reactions without and with base workup, respectively. Lanes 10 and 11 are Ni(II)·GGH-(Hin139–190) reactions followed by treatment with calf alkaline phosphatase, without and with base treatment respectively.

DNA substrates (Chaconas & Van de Sande, 1980). CAP treatment of the DNA cleavage products resulting from treatment of DNA with Ni(II)·GGH(Hin139–190) and MPE·Fe(II) produces DNA fragments with an identical decrease in electrophoretic mobility, confirming that the Ni-(II)·GGH(Hin139–190) cleavage results in the production of 5′-phosphate groups (Figure 3).

A DNA fragment labeled at the 5′ end was treated with Ni(II)·GGH(Hin139–190) in the presence of monoperoxyphthalic acid without and with subsequent *n*-butylamine treatment. The DNA cleavage products were analyzed by

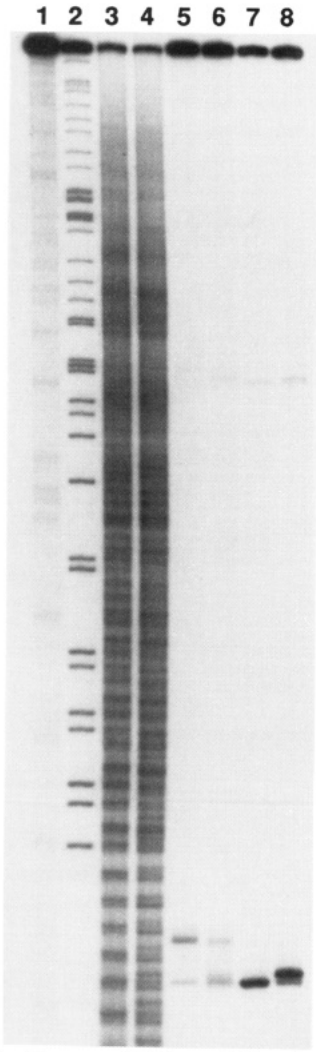

FIGURE 4: Autoradiogram of high-resolution denaturing polyacrylamide gel analyzing the 3′ end products of the Ni(II)·GGH(Hin139–190)–monoperoxyphthalic acid DNA cleavage reaction. Reaction conditions were 20 mM NaCl, 20 μM phosphate, pH 7.5, 100 μM (in base pair) calf thymus DNA, and ≈15 000 cpm of 5′-end-labeled DNA (*Eco*RI/*Rsa*I fragment from pDPM12) in a total volume of 20 μL. Reactions for MPE·Fe(II) (10 μM) were run for 15 min in the presence of 5 mM DTT. Reactions for Ni(II)GGH(Hin139–190) (5 μM) were run for 15 min in the presence of monoperoxyphthalic acid (5 μM). Nickel-mediated cleavage reactions were treated with 0.1 M *n*-butylamine for 30 min at 90 °C. DNA cleavage products were analyzed on a 20% 1:20 cross-linked 50% urea denaturing polyacrylamide gel. Lane 1 is intact DNA. Lane 2 is an A-specific chemical sequencing lane. Lane 3 is an MPE·Fe(II) reaction. Lane 4 is an MPE·Fe(II) reaction followed by treatment with T4 polynucleotide kinase. Lane 5 is a Ni(II)·GGH(Hin139–190) cleavage reaction without base treatment. Lane 6 is a Ni(II)·GGH(Hin139–190) cleavage reaction without base treatment followed by treatment with T4 polynucleotide kinase. Lane 7 is a Ni(II)·GGH(Hin139–190) cleavage reaction with base treatment. Lane 8 is a Ni(II)·GGH(Hin139–190) cleavage reaction with base treatment followed by treatment with T4 polynucleotide kinase.

20% denaturing polyacrylamide gel electrophoresis. The mobilities of the resulting fragments were compared to those of the known products produced by the MPE·Fe(II) reaction, 3′-phosphate and phosphoglycolate (Hertzberg & Dervan, 1984). The cleavage products produced by Ni(II)·GGH-(Hin139–190) upon butylamine treatment have the same electrophoretic mobility as the slower migrating product generated by MPE·Fe(II) cleavage, identified previously as a 3′-phosphate (Hertzberg & Dervan, 1984) (Figure 4). The identity of this DNA cleavage product was confirmed by treatment with T4 polynucleotide kinase to remove the 3′-phosphate groups (Cameron & Uhlenbeck, 1977). Treatment with kinase produces the same shift in the mobility of the cleavage products from reaction with Ni(II)·GGH(Hin139–190) and MPE·Fe(II), indicating that the end product is a 3′-phosphate after butylamine treatment (Figure 4).

The 3′-Termini Composition Depends on Base Treatment. The composition of the DNA termini at the 3′ end of the cleavage sites depends on whether the reaction mixture is treated with base after the Ni(II)·GGH(Hin139–190)/peracid reacts with DNA. With no base treatment, cleavage yields are low. Upon treatment with NaOH, two DNA cleavage products appear; the faster migrating product has similar electrophoretic mobility to a product with the phosphate termini (data not shown). Treatment with *n*-butylamine produces only a single cleavage product that can be identified as 3′-phosphate. These observations are reminiscent of the base treatment required in the cleavage of DNA by bleomycin·Fe(II) [BLM·Fe(II)] under anaerobic conditions (Sugiyama et. al., 1988). BLM·Fe(II) is known to abstract the C-4 hydrogen atom from double-helical DNA, leading to two types of DNA lesions (Hecht, 1984; Stubbe & Kozarich, 1987). In the presence of oxygen, the DNA is cleaved at neutral pH; in the absence of oxygen, strong base is required for strand scission. The nature of the base used in the workup for anaerobic conditions, believed to involve a C-4 hydrolation pathway, affects the composition of the DNA termini. Treatment with NaOH yields two products, a modified sugar fragment and a 3′-phosphate. Treatment with *n*-alkylamine affords only 3′-phosphate end products (Sugiyama et al., 1988).

Optimization of Reaction Conditions. Magnesium monoperoxyphthalate was chosen as oxidant for use in the Ni(II)-mediated DNA cleavage reactions due to its water solubility and stability. Ni(II)·GGH(Hin139–190) will also cleave DNA in the presence of iodosylbenzene or hydrogen peroxide. The rates of cleavage differ, decreasing in the order monoperoxyphthalic acid > iodosylbenzene > hydrogen peroxide. The DNA cleavage patterns obtained with GGH(Hin139–190) (5 μM) and $Ni(OAc)_2$ (5 μM) in the presence of hydrogen peroxide (5 mM), monoperoxyphthalic acid (2 μM), or iodosylbenzene (5 μM) are identical except iodosylbenzene-mediated cleavage at the weak tertiary site is lost (Figure 5). The similarity in cleavage specificity in the presence of different oxidants implies that a common intermediate may be involved. Although a metal-bound oxidant could be a precursor in each case, the different steric bulk of the oxidants appears to rule out three different nickel-bound oxidants as the reactive oxidizing species in the minor groove of DNA.

The DNA cleavage reaction by Ni(II)·GGH(Hin139–190) at 1 μM concentration was studied as a function of peracid and nickel concentration and pH (Figures 6 and 7). The DNA cleavage efficiency increased from a low value at 1 μM peracid concentration to a high of 100% cleavage at 50 μM (Figure 6). Increasing the peracid concentration further resulted in a decrease in overall cleavage yield. The extent of cleavage by Ni(II)·GGH(Hin139–190) (5 μM) with peracid (5 μM) was studied in a pH range of 6–8. The cleavage efficiency increases from pH 6 to 8, consistent with the protonation state of the Ni·GGH ligand ((Bryce et al., 1966). The DNA cleavage reaction of Ni(II)·GGH(Hin139–190) with peracid was also studied as a function of the amount of $Ni(OAc)_2$. The cleavage yield increases up to 1 equiv of Ni-$(OAc)_2$ and then levels off, suggesting a 1:1 Ni(II)·protein complex (Figure 7).

Location of the Ni(II)·GGH Domain. From other work, we believe the GGH segment at the NH_2 terminus is firmly anchored in the minor groove by specific contacts between the adjacent Arg-Pro-Arg sequence and the A,T tract of *hix*L

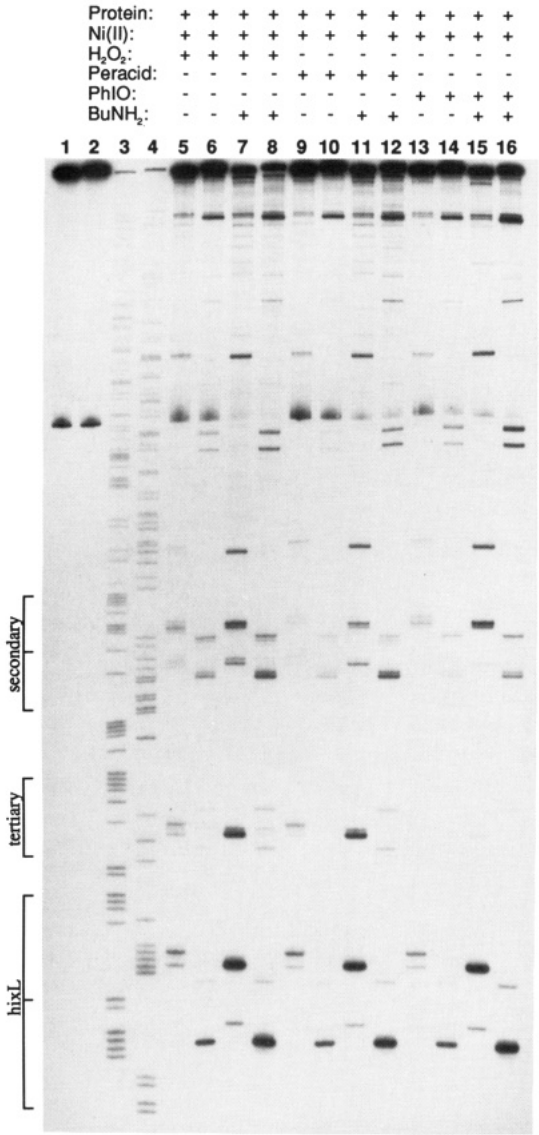

FIGURE 5: Autoradiogram of high-resolution denaturing polyacrylamide gel of Ni(II)·GGH(Hin139–190) cleavage using hydrogen peroxide, monoperoxyphthalic acid, and iodosylbenzene of a ^{32}P-end-labeled DNA fragment (*Xba*I/*Eco*RI) from pMFB36. Reaction conditions were 5 μM $Ni(OAc)_2$, 5 μM GGH(Hin139–190), 20 mM NaCl, 20 mM phosphate, pH 7.5, calf thymus DNA (100 μM in base pair), and ≈15-000 cpm of end-labeled DNA in a total volume of 20 μL. Reactions for Ni·GGH(Hin139–190) were run for 15 min (25 °C). Nickel-mediated reactions were treated with 0.1 M *n*-butylamine for 30 min at 90 °C. Cleavage products were analyzed on an 8%, 1:20 cross-linked, 50% urea polyacrylamide gel. Odd-numbered lanes and even-numbered lanes contain 5′- and 3′-end-labeled DNA, respectively. Lanes 1 and 2 are intact DNA. Lanes 3 and 4 are A-specific sequencing reactions. Lanes 5 and 6, and 7 and 8, contain hydrogen peroxide (5 mM) without and with base workup, respectively. Lanes 9 and 10, and 11 and 12, contain monoperoxyphthalic acid (2 μM) without and with base workup, respectively. Lanes 13 and 14, and 15 and 16, contain iodosylbenzene (5 μM) without and with base workup, respectively.

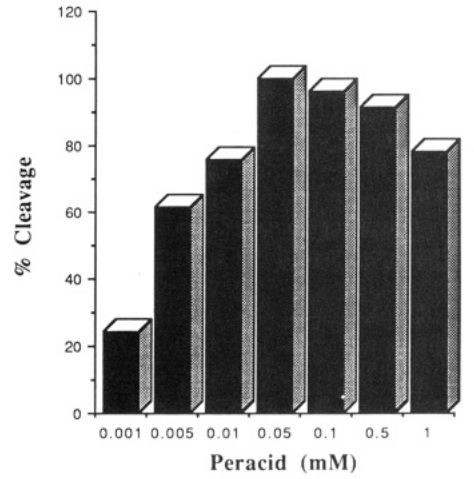

FIGURE 6: Extent of cleavage by Ni·GGH(Hin139–190) in the presence of varying concentrations of monoperoxyphthalic acid.

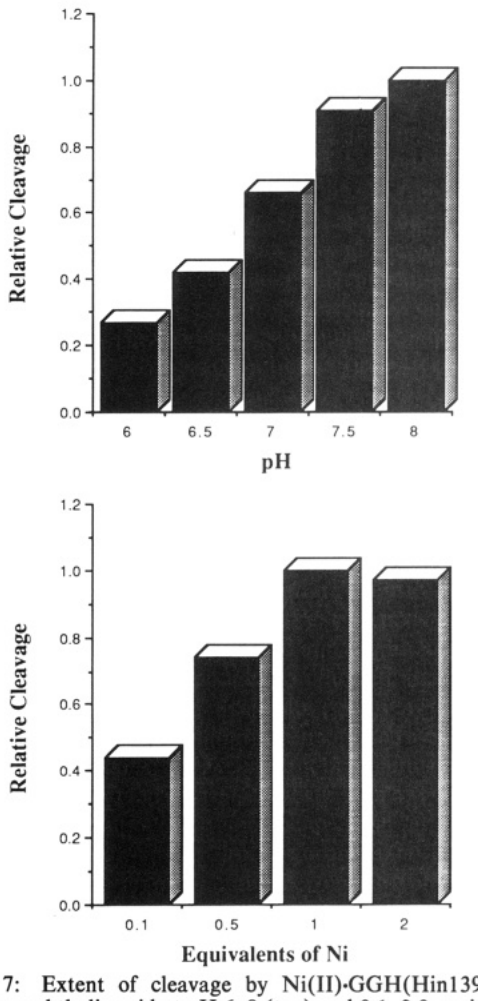

FIGURE 7: Extent of cleavage by Ni(II)·GGH(Hin139–190)–monoperoxyphthalic acid at pH 6–8 (top) and 0.1–2.0 equiv of Ni(II) per GGH(Hin139–190) (bottom).

(Sluka et al., 1987, 1990a,b). It has been shown from footprinting and affinity cleaving data that Arg[140] in the DNA binding domain of Hin is important for recognition of the 5′-AAA-3′ sequence in the minor groove in DNA at the *hix*L site (Sluka et al., 1987, 1990a,b). Although a crystal structure of the Hin·DNA complex does not yet exist, Pabo and co-workers have recently described the crystal structure of the engrailed homeodomain·DNA complex. This helix–turn–helix protein is bound in the major groove, and the N-terminal arm fits into the adjacent minor groove. The side chains of Arg in an Arg-Pro-Arg segment make contact with thymine at two A,T base pairs (Kissinger et al., 1990).

GG(-L-)*H*(*Hin139–190*) *to GG*(-D-)*H*(*Hin139–190*). The preferential cleavage observed on one strand of the DNA at each *hix*L half-site suggests asymmetry for the structure of the nickel·GGH complex bound in the minor groove. The 55-residue protein was synthesized with D-His replacing L-His in the GGH domain. The specificity of cleavage by Ni(II)·GG(-D-)H(Hin139–190) was compared with that of Ni(II)·GG(-L-)H(Hin139–190) by high-resolution gel electrophoresis (Figure 8A). At all sites studied (*hix*L, *secondary*, and

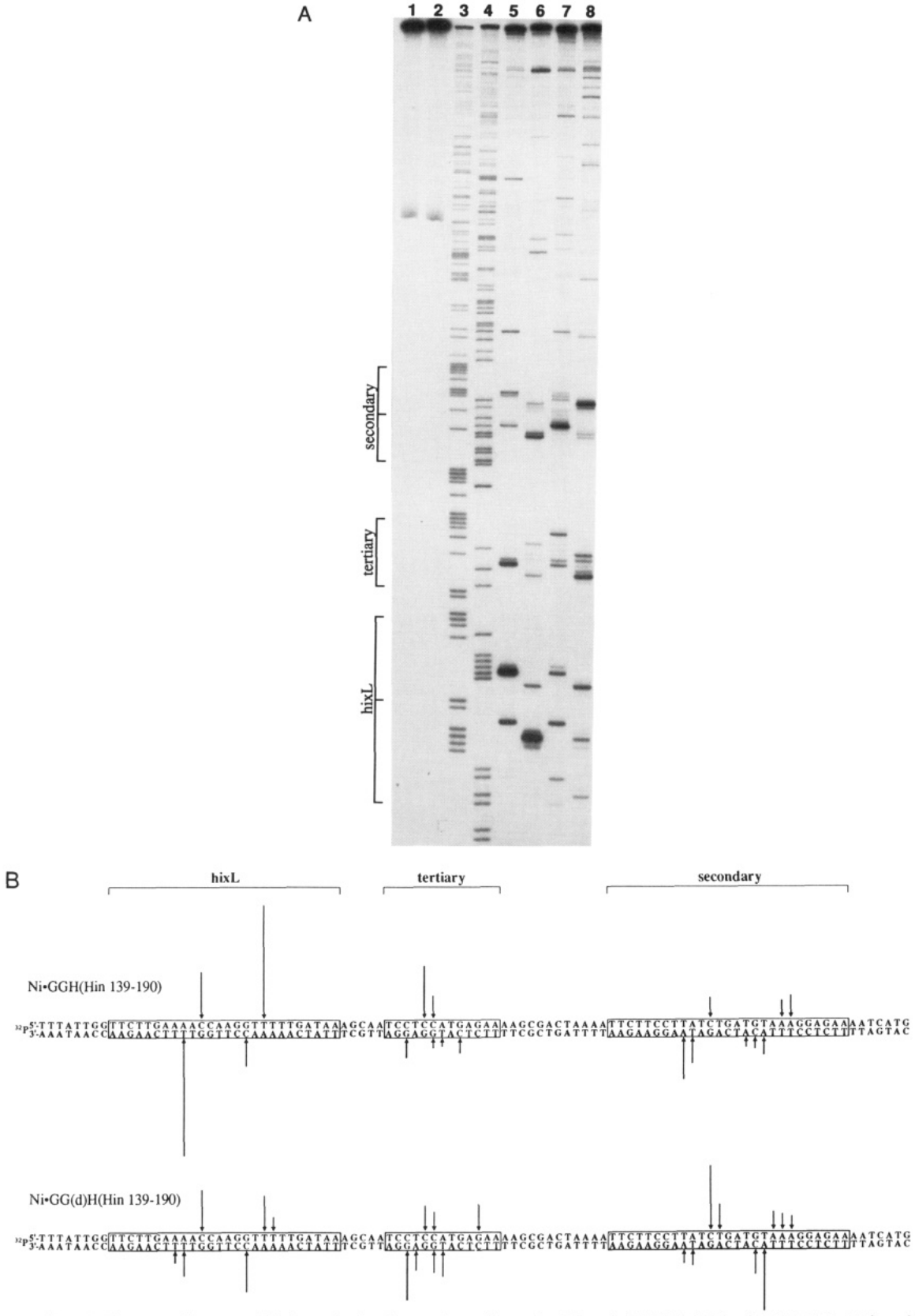

FIGURE 8: (Panel A) Autoradiogram of high-resolution denaturing polyacrylamide gel of Ni(II)·GG(-L-)H(Hin139–190) and Ni(II)·GG(-D-)H(Hin139–190) cleavage of a ^{32}P-end-labeled fragment (*Xba*I/*Eco*RI) from pMFB36. Reaction conditions were 20 mM NaCl, 20 mM phosphate, pH 7.5, calf thymus DNA (100 μM in base pair), and ≈15 000 cpm of end-labeled DNA in a total volume of 20 μL. Reactions for Ni·GGH(Hin139–190) were run for 15 min (25 °C). Nickel-mediated reactions were treated with 0.1 M *n*-butylamine for 30 min at 90 °C. Cleavage products were analyzed on an 8%, 1:20 cross-linked, 50% urea polyacrylamide gel. Odd-numbered lanes and even-numbered lanes contain 5′- and 3′-end-labeled DNA, respectively. Lanes 1 and 2 are intact DNA. Lanes 3 and 4 are A-specific sequencing reactions. Lanes 5 and 6 contain Ni(OAc)$_2$ (5 μM) and GGH(Hin139–190) (5 μM) followed by monoperoxyphthalic acid, magnesium salt (5 μM). Lanes 7 and 8 are identical to lanes 5 and 6 except they contain GG(-D-)H(Hin139–190) (5 μM). (Panel B) Histograms of cleavage sites from panel A. The sequence left to right represents the data from the bottom to the middle of the gel. Boxes indicate the dimeric *hix*L and *secondary* Hin binding sites. (Top) Ni(II)·GG(-L-)H(Hin139–190) in the presence of monoperoxyphthalic acid (panel A, lanes 5 and 6). (Bottom) Ni(II)·GG(-D-)H(Hin139–190) in the presence of monoperoxyphthalic acid (panel A, lanes 7 and 8). Arrows represent extent of cleavage, determined by densitometric analysis of the gel autoradiogram.

tertiary), the same base pair locations are cleaved for the two proteins. However, the positions of major cleavage have changed from one strand to the other. Examination of models of Ni(II)·GGH indicates that if the position of the amide bond between the NH_2-terminal glycine of Hin(139–190) and the histidine residue is fixed in the minor groove, a change of absolute configuration at His changes the direction of the coordination geometry such that the peptide wraps around the metal atom in the opposite direction. This change would be expected to alter the position of a radical species with respect to the walls in the minor groove of DNA.

Implications for Protein Design. The tripeptide GGH is a metal-specific structural domain consisting of naturally occurring amino acids that could be incorporated at the NH_2 terminus of a variety of recombinant proteins (such as other DNA binding proteins, receptors, or antibodies) with the function of precise, efficient substrate-directed oxidation, activated in the presence of Ni(II) and peracid (25 °C, pH 7.5). A full understanding of the mechanism of the nickel-mediated oxidation of double-helical DNA in the minor groove must await further studies, such as detailed characterization of products and reactive intermediates. The highly reactive nature of the oxidizing species and the precision of the cleavage reaction imply that the reactive moiety is a nondiffusible radical species when localized in the minor groove of DNA that abstracts a hydrogen atom(s) from the deoxyribose backbone. It is likely that a higher oxidation state of nickel is involved, such as Ni(III) (Bossu & Margerum, 1977). There is no precedent in the literature for observation of discrete nickel–oxygen intermediates for hydrocarbon activation. Perhaps most relevant are the recent studies of Burrows and co-workers who find that Ni(II), in conjunction with certain macrocyclic polyamine ligands and in the presence of oxidants such as iodosylbenzene, generates catalysts capable of epoxidation of alkenes and even hydrogen atom abstraction from hydrocarbons (Kinneary et al., 1988; Yoon & Burrows, 1988). Undoubtedly, the nature of the ligand modulates the reactivity of Ni(II). The ability of Ni(II) complexes to catalyze oxygen atom transfer is found to be highly ligand dependent (Kinneary et al., 1988; Chen et al., 1991).

REFERENCES

Atkinson, T., & Smith, M. (1984) in *Oligonucleotide Synthesis: A Practical Approach* (Gait, M. J., Ed.) pp 35–81, IRL Press, Oxford and Washington, DC.

Bannister, C. E., Raycheba, J. M. T., & Margerum, D. W. (1982) *Inorg. Chem. 21*, 1106.

Beaucage, S. L., & Caruthers, M. H. (1984) *Tetrahedron Lett. 22*, 1859.

Bossu, F. P., & Margerum, D. W. (1977) *Inorg. Chem. 16*, 1210.

Bossu, F. P., Paniago, E. B., Margerum, D. W., Kirksey, S. T., & Kurtz, J. L. (1978) *Inorg. Chem. 17*, 1034.

Bruist, M. F., Horvath, S. J., Hood, L. E., Steitz, T. A., & Simon, M. I. (1987) *Science 235*, 777.

Bryce, C. F., Roeske, R. W., & Gurd, F. R. N. (1966) *J. Biol. Chem. 241*, 1072.

Camerman, N., Camerman, A., & Sarkar, B. (1976) *Can. J. Chem. 54*, 1309.

Cameron, V., & Uhlenbeck, O. C. (1977) *Biochemistry 16*, 5120.

Chaconas, G., & Van de Sande, J. H. (1980) *Methods Enzymol. 65*, 75.

Chen, X., Rokita, S. E., & Burrows, C. J. (1991) *J. Am. Chem. Soc. 113*, 5884.

Dervan, P. B. (1986) *Science 232*, 464.

Freeman, H. C., & Taylor, M. R. (1965) *Acta Crystallogr. 18*, 939.

Freeman, H. C., Guss, J. M., & Sinclair, R. L. (1968) *Chem. Commun., 485*.

Glasgow, A. C., Bruist, M. F., & Simon, M. I. (1989a) *J. Biol. Chem. 264*, 10072.

Hecht, S. M. (1986) *Acc. Chem. Res. 19*, 83.

Hertzberg, R. P., & Dervan, P. B. (1982) *J. Am. Chem. Soc. 104*, 313.

Hertzberg, R. P., & Dervan, P. B. (1984) *Biochemistry 23*, 3934.

Iverson, B. L., & Dervan, P. B. (1987) *Nucleic Acids Res. 15*, 7823.

Johnson, R. C., Bruist, M. F., Glaccum, B. B., & Simon, M. I. (1984) *Cold Spring Harbor Symp. Quant. Biol. 49*, 751.

Kent, S. B. H. (1988) *Annu. Rev. Biochem. 57*, 957.

Kinneary, J. F., Wagler, T. R., & Burrows, C. J. (1988a) *Tetrahedron Lett. 29*, 877.

Kinneary, J. F., Albert, J. S., & Burrows, C. J. (1988b) *J. Am. Chem. Soc. 110*, 6124.

Kissinger, C. R., Beishan, L., Martin-Blanco, Kornberg, T. B., & Pabo, C. O. (1990) *Cell 63*, 579.

Kruck, T. P. A., Lau, S.-J., & Sarkar, B. (1976) *Can. J. Chem. 54*, 1300.

Lau, S.-J., Kurck, T. P. A., & Sarkar, B. (1973) *J. Biol. Chem. 249*, 5878.

Mack, D. P. (1991) Ph.D. Thesis, California Institute of Technology.

Mack, D. P., & Dervan, P. B. (1990) *J. Am. Chem. Soc. 112*, 4604.

Mack, D. P., Iverson, B. I., & Dervan, P. B. (1988) *J. Am. Chem. Soc. 110*, 7572.

Mack, D. P., Shin, J., Sluka, J. P., Griffin, J. H., Simon, M. I., & Dervan, P. B. (1990) *Biochemistry 29*, 6561.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Matteucci, M. D., & Caruthers, M. H. (1981) *J. Am. Chem. Soc. 103*, 3185.

Mendel, D. (1989) Ph.D. Thesis, California Institute of Technology, Pasadena, CA.

Moser, H., & Dervan, P. B. (1987) *Science 238*, 645.

Pabo, C. O., & Sauer, R. T. (1984) *Annu. Rev. Biochem. 53*, 293.

Sakuri, T., & Nakahara, A. (1979) *Inorg. Chim. Acta 34*, L243.

Sarin, V. K., Kent, S. B. H., Tam, J. P., & Merrifield, R. B. (1981) *Anal. Biochem. 117*, 147.

Sluka, J. P., Horvath, S. J., Bruist, M. F., Simon, M. I., & Dervan, P. B. (1987) *Science 238*, 1129.

Sluka, J. P., Horvath, S. J., Glasgow, A. C., Simon, M. I., & Dervan, P. B. (1990a) *Biochemistry 29*, 6551.

Sluka, J. P., Griffin, J. H., Mack, D. P., & Dervan, P. B. (1990b) *J. Am. Chem. Soc. 112*, 6369.

Steitz, T. A. (1990) *Q. Rev. Biophys. 23*, 205.

Stubbe, J., & Kozarich, J. W. (1987) *Chem. Rev. 87*, 1107.

Sugiyama, H., Xu, C., Murugesan, N., & Hecht, S. M. (1988) *Biochemistry 27*, 58.

Sutcliffe, J. G. (1979) *Cold Spring Harbor Symp. Quant. Biol. 43*, 77.

Tanaka, T., & Weisblum, B. (1975) *J. Bacteriol. 121*, 354.

Taylor, J. S., Schultz, P. G., & Dervan, P. B. (1984) *Tetrahedron 40*, 457.

Yoon, H., & Burrows, C. J. (1988) *J. Am. Chem. Soc. 110*, 4087.

Zieg, J., Silverman, M., Hilmen, M., & Simon, M. (1977) *Science 196*, 170.