Structural Motif of the DNA Binding Domain of γδ-Resolvase Characterized by Affinity Cleaving*

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The DNA binding domain of γδ-resolvase, residues 141–183, is thought to bind DNA by a helix-turn-helix motif based on sequence similarities with other known DNA binding proteins. Incorporation of the DNA cleaving moiety, EDTA-Fe, at the NH2 and COOH termini of γδ(141–183) allows the positions of these residues relative to the DNA bases at three resolvase binding sites, each consisting of inverted copies of an imperfectly conserved 6-base pair sequence, to be mapped by high resolution gel electrophoresis. The cleavage data for EDTA-γδ(141–183) reveals that the NH2 terminus of the DNA binding domain of γδ-resolvase is bound proximal to the minor groove of DNA near the center of the resolvase binding sites. Cleavage by EDTA-Fe attached to a lysine side chain (Asn→Lys) at the COOH terminus of γδ(141–183) reveals that the putative recognition helix is in the adjacent major groove on the same face of the helix, oriented toward the center of the inverted repeats.

The structural class of DNA binding proteins best characterized by crystallographic studies contains the helix-turn-helix motif. Comparison of the three-dimensional structures of α-cro, λ-repressor, and catalytically active gene repressor protein led to the postulate that a conserved α-helix-turn-α-helix motif is involved in recognition of DNA in the major groove and may be a common structural motif for sequence-specific DNA affinity (Anderson et al., 1981; McKay and Steitz, 1981; Pabo and Lewis, 1982; McKay et al., 1982; Ohiendorf and Matthews, 1983; Pabo and Sauer, 1984; Schevitz et al., 1985). The x-ray structure determination of three proteins containing helix-turn-helix motifs bound to their DNA operator sites elucidates the DNA binding domain of the 434 repressor (1-69) (Anderson et al., 1985, 1987; Aggarwal et al., 1988), the DNA binding domain of λ-repressor (1-92) (Jordan and Pabo, 1988), and the trp repressor (Otwinski et al., 1988). These high resolution crystallographic views of repressor-operator complexes reveal the complexity of protein-DNA interactions. The protein-DNA interface includes protein contacts to the sugar-phosphate backbone as well as to base pairs in the major groove. A particular side chain can contact several base pairs, and several side chains can cooperate to recognize a single base. Moreover, sequence-dependent ability of DNA to adopt the required conformation appears important for site-specific recognition. The combination of direct protein-DNA contacts mediated by multiple hydrogen bonds and the sequence-dependent conformational effects in DNA limits our ability to make detailed structural predictions. In the absence of high resolution crystallographic and nuclear magnetic resonance data, affinity cleavage methods can be utilized to correlate sequence similarities with known structural classes (Sluka et al., 1987, 1990; Mack et al., 1990; Oakley and Devan, 1990).

The γδ-resolvase is a 183-residue protein encoded by the transposable element, γδ, a member of the Tn3 family of bacterial transposons (Grindley and Reed, 1985; Heffron, 1983). Resolvase is both a site specific recombinational protein and a repressor. It interacts with a 120-bp site named res which lies within the region between the divergently transcribed tnpA and tnpR genes of the γδ transposon. Resolvase protects three binding sites within res from nucleosome digestion (Grindley et al., 1982; Kitts et al., 1983). Each binding site consists of inverted repeats of a 9-base pair segment consensus sequence, TGCYCNNTA (where Y is a pyrimidine and N means any base), separated by a variable spacer of 7, 10, or 16 bp (Grindley et al., 1982). Site I, which has a 10-bp spacer, contains the recombination of cross-over point; all three sites are required for efficient recombination (Grindley et al., 1992; Kitts et al., 1993; Wells and Grindley, 1984). γδ-Resolvase has been shown to induce a structural change in the DNA at site I that corresponds to a bend (Hatfull et al., 1987; Salvo and Grindley, 1988). The interactions between γδ-resolvase and DNA have been examined by methylation and ethylation interference studies (Favley and Grindley, 1987). Major groove methylations within the 9-bp recognition sequence as well as ethylation of phosphates within and adjacent to this region were found to inhibit resolvase binding. Furthermore, inhibition of resolution by methylation of adenosine at the center of site I suggests that minor groove contacts near the cross-over may be required for resolution activity.

γδ-Resolvase can be cleaved by chymotrypsin into two fragments (Abdel-Meguid et al., 1984). The NH2-terminal fragment, γδ(1-140), which is thought to contain the protein-protein contacts and be responsible for the enzymatic activity, does not bind DNA. The 43-residue COOH-terminal fragment γδ(141–183) binds specifically but independently to both halves of all three DNA sites to which resolvase binds. However, unlike native resolvase, which binds to all three complete

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1 The abbreviations used are: bp, base pair(s); DTT, dithiothreitol; t-Boc, t-butoxycarbonyl; DMF, dimethylformamide; DIAE, diisopropylammonium; IODt, N-hydroxybenzotriazol; DCM, dichloromethane; HPLC, high performance liquid chromatography; Fmoc, 9-fluorenlymethyloxycarbonyl; DNP, dinitrophenol; MPB, methyldimpro- pyr-EDTA; OBzl, benzyl ester; Bzl, benzyl; Cl-Z, 2-chloro-benzoyloxycarbonyl; Tos, 4-toluenesulfonyl.
sites with equal affinities, γδ(141–183) binds to each of the six half-sites with different affinities (Abdel-Meguid et al., 1984). Ethylation interference experiments reveal that phosphate contacts made by the COOH-terminal DNA binding domain are similar to those of the intact resolvase with the exception of a single phosphate at the inside of each contact region (Rimphanitchayakit et al., 1989). Phosphate contact extends across adjacent major and minor grooves on one face of the DNA helix. The minimal binding segment is a 12-bp sequence that includes the 9-bp inverted repeat. γδ(141–183) contains a high degree of sequence similarity with the helix-turn-helix regions of several DNA binding proteins (Pabo and Sauer, 1984). Based on sequence similarities with other DNA

![Diagram](http://www.jbc.org/)

**FIG. 1.** Cleavage patterns produced by a diffusible oxidant generated by EDTA. Fe located in the major (Moser and Dervan, 1987; Griffin and Dervan, 1989) and minor (Taylor et al., 1984; Dervan, 1986) grooves of right-handed DNA. Filled circles represent points of cleavage along the phosphodiester backbone. Sizes of circles represent extent of cleavage.

**A)**

\[
\text{GKRKIDAVLNMWQOG} \\
\text{LGASHKTMIAVRVTYKINESN}
\]

**B)**

\[
\text{H} \quad \text{H} \\
\text{H} \quad \text{H} \\
\text{H} \quad \text{H}
\]

**C)**

\[
\text{H} \quad \text{H} \\
\text{H} \quad \text{H} \\
\text{H} \quad \text{H}
\]

**FIG. 2.** A, the 43-amino-acid DNA binding domain of γδ-resolvase. Underlined regions are possible α-helices assigned according to a secondary structure predicting algorithm (Garnier et al., 1978) (B) EDTA-γδ(141–183), (C) γδ(141–183, Asn134→Lys134)-EDTA.

**FIG. 3.** Synthetic scheme for the attachment of the tricyclohexyl (cHex) ester of EDTA to the ε-amino group of Lys183 on p-methylbenzydrylamine resin (Sluka et al., 1990b). DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; OBzl, benzylxoy.

binding proteins, it has been proposed that resolvase uses the helix-turn-helix motif in the major groove for sequence-specific DNA binding with its NH₂ terminus oriented toward the center of each binding site (Abdel-Meguid et al., 1984; Rimphanitchayakit et al., 1989).

Incorporation of the DNA cleaving moiety, EDTA-Fe, at discrete amino acid residues along a protein allows the positions of those residues in the protein-DNA complex relative to the DNA bases to be mapped to nucleotide resolution (Sluka et al., 1987). Following chemical activation with a reducing agent such as dithiothreitol (DTT), EDTA-Fe localized at a specific DNA binding site cleaves both DNA strands, typically covering 4–6 base pairs via a diffusible species (Schultz et al., 1982; Taylor et al., 1984; Dervan, 1986). Due to the right-handed nature of double-helical DNA, the
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EXPERIMENTAL PROCEDURES

Materials—Manual peptide syntheses were carried out in 20-ml vessels fitted with a coarse glass frit as described by Kent (1988). Automated syntheses were performed on an ABI 430A synthesizer (Kent et al., 1984, 1985), modified by the removal of in-line filters to the top and bottom of the reaction vessel, using a 20-ml Teflon/Kel-F reactor vessel. The synthetic protocols used were developed at the California Institute of Technology (Kent and Clark-Lewis, 1985; Clark-Lewis et al., 1986; Kent et al., 1988, Kent, 1988). Protected amino acid derivatives were purchased from Peninsula Laboratories. Boc-L-His (DNP) was obtained from Fluka and N-α-Lys-L-Fuc-N-F from Chemie-Dynamics Corp. (S. Plainfield, NJ). Phenylacetamidomethyl resin with N-α-T-Boc-L-asparagine was purchased from Applied Biosystems (Foster City, CA) and p-methyl-benzhydrolamine resin was purchased from United States Biological Corp. Dimethylformamide (DMF), diisopropylethylamine (DIEA), diethylamincarbodiimide in dichloromethane, N-hydroxybenzotriazole (HOBT) in DMF, and trifluoroacetic acid (TFA) in dichloromethane from Applied Biosystems. Dichloromethane (DCM) and methanol (HPLC grade) were purchased from Mallinkrodt, 1,4-butanedithiol and anisole from Aldrich, and diethyl ether (low peroxide content) from J. T. Baker Chemical Co. Doubly distilled water was used for all aqueous reactions and dilutions. Calf thymus DNA was purchased from Sigma and sonicated, deproteinized, and dialyzed. Enzymes were purchased from Boehringer Mannheim or New England Biolabs.

Synthesis—N-α-Boc-α-amino acids were used with the following side chain protecting groups, Arg(Tos), Asp(OBzl), Glu(OBzl), His(DNP), Lys(Cl-Z), Ser(Bzl), Trp(formyl), Thr(Bzl), and Tyr(Br-Z). Manual assembly of the protected peptide on the solid support was carried out as described previously (Sluka et al., 1990a; Mack et al., 1990). Automated syntheses were carried out with modified cycles which are similar to the manual procedures (Kent, 1988). Double couplings were performed for every amino acid. Boc protecting groups were removed from the α-amino group of the resin-bound amino acid immediately after the second coupling. The EDTA near the COOH terminus of γδ(141–183) reveals that the recognition helix is oriented toward the center of the inverted repeats, in a manner similar to that seen in the 434 and λ repressor-DNA co-crystals. The location of the cleavage patterns reveal a structural motif very similar to the DNA binding domain of Hin recombinase, Hin(139–184) (Sluka et al., 1987, 1990a; Mack et al., 1990).

We report here chemical syntheses of the 43-residue DNA binding domain of γδ with EDTA at the NH2 terminus, the COOH terminus, and both termini. Affinity cleaving studies using Fe-EDTA-γδ(141–183) reveal that the NH2 terminus of the DNA binding domain of γδ lies proximal to the minor groove near the center of γδ recombination sites. Attachment of EDTA near the COOH terminus of γδ(141–183) reveals that the recognition helix is oriented toward the center of the inverted repeats, in a manner similar to that seen in the 434 and λ repressor-DNA co-crystals. The location of the cleavage patterns reveal a structural motif very similar to the DNA binding domain of Hin recombinase, Hin(139–184) (Sluka et al., 1987, 1990a; Mack et al., 1990).

FIG. 4. Autoradiogram of a high resolution denaturing polyacrylamide gel containing 32P-end-labeled fragments from pRW80. Bars on the left indicate the position of the three binding sites I, II, and III (each consisting of imperfectly conserved inverted repeats) for γδ-resolvase with res. Odd- and even-numbered lanes 1–12 contain 5′- and 3′-labeled DNA, respectively. Lanes 1 and 2, intact DNA; lanes 3 and 4, EDTA-γδ(141–183) (0.5 μM); lanes 5 and 6, EDTA-γδ(141–183) (2.0 μM); and lanes 7 and 8, EDTA-γδ(141–183) (10.0 μM). Lanes 9 and 10 are MPEF-Fe footprinting lanes, γδ(141–183) 2.0 μM. Lanes 11 and 12 are MPEF-Fe control lanes. Lanes 13 and 16 are 5′ and 3′ chemical sequencing G reactions, respectively (Maxam and Gilbert, 1980); lanes 14 and 15 contain 5′ and 3′ chemical sequencing A reactions, respectively (Iverson and Dervan, 1987).

Asparagine, glutamine, and arginine were coupled as the HOBt esters in DCM. For the first coupling the diethylamincarbodiimide in DCM was transferred to the activated peptide, and the HOBt was exchanged for DMF. HOBT and the amino acid in DMF were then added to the concentrator, and the active ester was allowed to form. The ester was transferred to the reaction vessel where the amino acid was allowed to couple. After coupling the resin was neutralized with DIEA in DMF. The second coupling was identical to the first except that the DMF was not removed from the activating solution until after the ester formed. The second coupling for arginine was identical to the first symmetric anhydride coupling. Reaction times for HOBT esters were longer than the symmetric anhydride due to the slower coupling reaction. In a single synthesis, a resin-bound peptide corresponding to the residues 141–183 of γδ-resolvase was produced. Coupling efficiencies ranged from 99.9–99.8% near the beginning of the synthesis to a low of 98.0% at the end. For γδ(141–183) the average yield for 42 couplings was 99.3%. EDTA was attached to the NH2 terminus of the protected peptide as the tribenzylester with a γ-aminobutyric acid linker via an HOBT
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Fig. 5. Histograms of footprinting and affinity cleaving data from Fig. 4. The sequence left to right corresponds to the DNA sequence for Site I to Site III (top to bottom of the gel). Sites I, II, and III are indicated by brackets. Boxes represent the binding sites assigned for γδ-resolvase (Grindley et al., 1982). A, bars represent the extent of protection from MPE.Fe cleavage in the presence of γδ(141-183) (2.0 µM). B, arrows represent the extent of cleavage for Fe.EDTA-γδ(141-183) at 0.5 µM; C, 2.0 µM; D, 10.0 µM.

Ester (Stuka et al., 1987, 1990b). EDTA was positioned at the COOH terminus by covalent attachment to a lysine side chain (Asn183→Lys183) (Mack et al., 1990; Stuka et al., 1990b). Nα-t-Boc-Nα-Fmoc-L-lysine was activated with dicyclohexylcarbodiimide and coupled onto the p-methylbenzhydrylamide resin. Selective removal of the Fmoc protecting group was accomplished with 20% piperidine in DMF for 20 min (Stewart and Young, 1981). The tricyclohexylester of EDTA (Stuka et al., 1990b) was then coupled to this amine as the HOBt ester (Stuka et al., 1990b). γδ(141-183, Asn183→Lys183) equipped with EDTA at both termini was synthesized by the combination of the procedures described above.

Protein Deprotection and Purification—The histidine protecting group, dinitrophenol (DNP), was removed at 25°C using 20% 2-mercaptoethanol and 10% DIEA in DMF; this treatment was repeated twice (two times for 30 min). After removal of the Nα-t-Boc group with trifluoroacetic acid and drying of the resin, all other side chain protecting groups were removed, and the peptide-resin bond was cleaved using anhydrous HF in the presence of anisole and 1,4-butanedithiol as scavengers for 60 min at 0°C. The HF was removed under vacuum. The crude protein was precipitated with diethyl ether,
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FIG. 6. Model for γδ(141–183) binding to Site I of res. The location of the EDTA–Fe moiety at the NH₂ terminus of γδ(141–183) was assigned from the cleavage patterns for Fe·EDTA·γδ(141–183). Filled circles represent the positions of cleavage along the phosphodiester backbone. Sizes of circles represent the extent of cleavage at the indicated base position.

collected on a fritted funnel, dissolved with 5% acetic acid, and washed through, leaving the resin on the frit. A small sample was then removed, filtered, and subjected to analytical HPLC (Brownlee 25 cm × 4.6 mm C₁₈ column, 0–60% acetonitrile, 0.1% trifluoroacetic acid over 60 min). The remaining solution was frozen and lyophilized. Residual DNP groups were removed from the crude peptide by treatment in 4 M guanidine HCl, 50 mM Tris, pH 8.5, and 20% 2-mercaptoethanol for 1 h at 50 °C (Kent, 1988). This solution was injected directly onto a semipreparative C₁₈ HPLC column (25 × 1 cm) and run in H₂O, 0.1% trifluoroacetic acid until the guanidine and 2-mercaptoethanol had eluted. A gradient of 0–60% acetonitrile, 0.1% trifluoroacetic acid was run over 240 min, and fractions were collected. Fractions were analyzed by HPLC. Sequencing by Edman degradation and amino acid analysis of the purified peptide showed its composition to be identical to the sequence for γδ(141–183). Protein concentrations were assayed based on calculated OD₅₀₀ (ε = 6950 based on one tyrosine and one tryptophan). The purified proteins were lyophilized for storage.

DNA Cleavage Reactions—The plasmid PRW80 (Abdel-Meguid et al., 1984) containing two copies of res was digested with restriction endonuclease HindIII to afford two fragments, 4 and 3 kilobase pairs in size. Labeling at the 3′ end was accomplished with [α-³²P]dATP using the Klenow fragment of DNA polymerase I. The 3′ end was labeled with ³²P by dephosphorylation with calf alkaline phosphatase followed by treatment with [γ-³²P]ATP and T₄ polynucleotide kinase. Cleavage with restriction endonuclease Sau3A yielded three different size 3′- and 5′-labeled fragments. The smallest fragment, 240 bp long, containing the intact res site was isolated by nondenaturing polyacrylamide gel electrophoresis.

MPE footprinting reactions were performed in a buffer containing 20 mM NaCl, 100 μM in base pair calf thymus DNA, 20 mM Tris, pH 8.0, and ³²P-end-labeled DNA. The reactions contained MPE·Fe(II) (10 μM), DTT (5 mM), and γδ(141–183) (2.0 μM). For footprinting reactions, γδ(141–183) was added to the buffer and allowed to equilibrate with the DNA for 10 min. MPE·Fe(II) was added and allowed to equilibrate for 5 min. The reaction was initiated by the addition of DTT. After 10 min at 25 °C the reaction was stopped by ethanol precipitation.

DNA cleavage reactions were run in a total volume of 10 μl. Final concentrations were 20 mM Tris, pH 8.0, 20 mM NaCl, 100 μM in base pairs calf thymus DNA, ~15,000 cpm of ³²P-end-labeled restriction fragment, 1 mM dithiothreitol, and 0.5, 2.0, and 10 μM Fe·EDTA-protein. The proteins were allowed to equilibrate with the DNA for 10 min at 25 °C, cleavage was then initiated by the addition of DTT and allowed to proceed for 45 min at 25 °C. The ³²P-labeled DNA products were analyzed by denaturing polyacrylamide gel electrophoresis followed by autoradiography. Densitometric analysis of the gel autoradiogram and comparison of individual lanes with sequence marker lanes (Maxam and Gilbert, 1980; Iverson and Dervan, 1987) allowed assignment of DNA cleavage to nucleotide resolution.
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Site I  site II  site III

r I I I I I

B)

FIG. 8. Histogram of the affinity cleavage data from Fig. 7. The sequence left to right represents sites I, II, and III (top to bottom of the gel). Arrow heights indicate the extent of cleavage at the indicated bases. A, Cleavage by \( \gamma^b(141-183) \)-EDTA-Fe. B, cleavage by Fe-EDTA-\( \gamma^b(141-183) \)-EDTA-Fe.

RESULTS AND DISCUSSION

Synthesis—Four 43-residue proteins, based on the DNA binding domain of \( \gamma^b \)-resolvase (residues 141–183), were synthesized by automated solid-phase techniques using t-Boc-protected amino acids: one with EDTA at the NH\(_2\) terminus, EDTA-\( \gamma^b(141-183) \); one with EDTA at the COOH terminus (Asn\(_{83}^b\)-Lys\(_{83}^b\)), \( \gamma^b(141-183) \)-EDTA; one with EDTA at both the NH\(_2\) and COOH termini, EDTA-\( \gamma^b(141-183) \)-EDTA; and one with no modification at either termini (Fig. 2). Tribenzyl EDTA-Lys\(_{83}^b\) was attached to the NH\(_2\) terminus of the protected peptide-resin as described (Sluka et al., 1987, 1990b) to afford EDTA-\( \gamma^b(141-183) \). Attachment of EDTA near the COOH terminus of the protein was accomplished by a combination of t-Boc and Fmoc protection schemes (Fig. 3) (Mack et al., 1990; Sluka et al., 1990b). N-c Fmoc-N-\( \alpha \)-t-Boc lysine was substituted for Asn\(_{83}^b\) of \( \gamma^b(141-183) \). The Fmoc-protecting group was then removed selectively from the \( \epsilon \)-NH\(_2\) chain using piperidine in DMF. Attachment of the tricyclohexyl ester of EDTA to Lys\(_{83}^b\) and completion of the synthesis afforded \( \gamma^b(141-183) \)-EDTA.

Footprinting—Footprinting studies of the synthetic protein, \( \gamma^b(141-183) \), demonstrated that at 2.0 \( \mu \)M concentrations the 43-mer binds to all six half-sites contained within the \( \gamma^b \)-resolvase binding site (Figs. 4 and 5). At each half-site the synthetic \( \gamma^b(141-183) \) protects a 12-bp region of DNA centered on the consensus binding sequence (Figs. 4 and 5). These observations are consistent with DNase I footprinting studies (Abdel-Meguid et al., 1984) and ethylation interference studies (Rimphanitchayakit et al., 1989) using \( \gamma^b(141-183) \) derived from a chymotrypsin digest of native \( \gamma^b \)-resolvase.

Position of the NH\(_2\) Terminus—Affinity cleaving studies with Fe-EDTA-\( \gamma^b(141-183) \) yields a 3'-shifted cleavage pattern at each half-site located at the center of each binding site (Fig. 5). The 3' shift of the cleavage pattern indicates that the Fe-EDTA group at the NH\(_2\) terminus of \( \gamma^b(141-183) \) is located proximal to the minor groove of DNA near the center of the dimeric binding sites (Fig. 6). This is in agreement with the ethylation interference studies (Rimphanitchayakit et al., 1989). The cleavage of res by Fe-EDTA-\( \gamma^b(141-183) \) at different concentrations (0.5–10 \( \mu \)M) shows that the six half-sites have different affinities for the DNA binding domain. At 0.5 \( \mu \)M concentration, sites II-L and site III-L are cleaved. At 4-fold higher concentrations (2.0 \( \mu \)M), cleavage at sites I-L and I-R, II-R, III-R appear with unequal intensity (Fig. 5). If the amount of cleavage is proportional to the extent of site occupancy, the data suggest that the relative affinities of the DNA binding domain of \( \gamma^b \) is II-L, III-L > III-R, II-R > I-L, I-R. This is somewhat different than that assigned from footprinting studies of the DNA binding domain obtained by chymotrypsin digest (Abdel-Meguid et al., 1984).

Position of the COOH Terminus—The specific cleavage patterns produced by \( \gamma^b(141-183) \)-EDTA-Fe are shifted to the 3' side and indicates that the EDTA-Fe attached near the COOH terminus of the putative recognition helix is positioned within the \( \gamma^b \)-binding site above the minor groove of sequence 5'-TGTGC-3' (Fig. 7, lanes 5 and 6; Fig. 8A). Two possible orientations for the putative recognition helix of \( \gamma^b(141-183) \) can be considered, one oriented toward and one away from the center of the binding site (Fig. 9, A and B). When the position of the EDTA-Fe-Lys\(_{83}^b\) is considered in each case relative to the cleavage data, a better fit is obtained with orientation 9A. This is similar to the orientation of the recognition helix of the \( \lambda^b \) and 434-repressors (Aggarwal et al., 1988; Jordan and Pabo, 1988) and that assigned for the 52-residue DNA binding domain of Hin recombinase (Mack et al., 1990). The fact that the cleavage pattern is seen predominately on one but not both minor grooves adjacent to the
major groove location of the helix-turn-helix motif suggests that the EDTA-Fe moiety (and, hence the putative "recognition helix") is not positioned symmetrically in the major groove. The data would be consistent with the COOH end of the "recognition helix" projecting outward from the floor of the major groove and tilting away from the center of the inverted repeat binding site. Clearly, refinement of these models must await more definitive x-ray crystallographic and nuclear magnetic resonance analyses of the protein-DNA complex.

In controls, γ6(141-183) equipped with EDTA at both the NH₂ and COOH termini, Fe-EDTA-γ6(141-183)-EDTA-Fe affords a pair of cleavage patterns consistent with the combination of patterns from Fe-EDTA-γ6(141-153) and γ6(141-183)-EDTA-Fe (Fig. 7, lanes 7 and 8; Fig. 8B). The fact that the cleavage pattern for EDTA-Fe at Gly141 (NH₂ terminus) appears unchanged when EDTA-Fe is present or absent at the COOH terminus suggests that γ6(141-183) maintains the same structure independent of which termini is modified with EDTA-Fe.

Conclusion—MPE-Fe(II) footprinting studies have shown that the protein protects a 12-bp region of DNA centered on the γ6 recognition sequence. Affinity cleaving studies with EDTA equipped at both the NH₂ and COOH termini extend across the DNA phosphodiester backbone turn-helix motif in the major groove with residues at the NH₂ and COOH termini suggest that γ6(141-183) maintains the same structure independent of which termini is modified with EDTA-Fe.

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