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C-terminal deletion mutants of the *Fok*I restriction endonuclease

(Catalytic domain; *Flavobacterium okeanokoites*; hydroxyl radical footprinting; protein-DNA interaction; recognition domain)

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SUMMARY

We have constructed two C-terminal deletion mutants of the *Fok*I restriction endonuclease by using the polymerase-chain-reaction technique and expressed them in *Escherichia coli*. The two mutant proteins (MP) of 41 and 30 kDa, were purified to homogeneity and their DNA-binding properties were characterized. The 41-kDa MP specifically binds the DNA sequence, $\begin{matrix}5'\text{-GGATG}\\3'\text{-CCTAC}\end{matrix}$, like the wild-type (wt) *Fok*I, but does not cleave DNA. The 30-kDa MP does not bind DNA. The affinity of the 41-kDa MP for the DNA substrate is comparable to that of wt *Fok*I. The 41-kDa MP interacts with its substrate like the wt *Fok*I, as revealed by hydroxyl radical footprinting experiments. In the presence of a DNA substrate, the 41-kDa MP is cleaved by trypsin into a 30-kDa N-terminal fragment and an 11-kDa C-terminal fragment. Addition of the HPLC-purified 11-kDa C-terminal fragment to the 30-kDa MP restores its sequence-specific DNA-binding property. These results confirm that the N-terminal 41-kDa fragment of the *Fok*I ENase constitutes the DNA recognition domain of the ENase.

INTRODUCTION

*Fok*I is a type-IIS class restriction endonuclease. It recognizes the nonpalindromic pentadeoxyribonucleotide, $\begin{matrix}5'\text{-GGATG}\\3'\text{-CCTAC}\end{matrix}$, and cleaves 9/13 nt away from its recognition site (Sugisaki and Kanazawa, 1981). Since 10 bp are needed to form one turn of the DNA helix, *Fok*I probably interacts with one face of the DNA by binding at one point and cleaving at the next turn of the helix. This implies the presence of two separate protein domains within this enzyme: one for sequence-specific recognition (the DNA-binding domain) and the other for endonuclease activity (the catalytic domain). Indirect support for this hypothesis comes from the work of Szybalski (1985) and Szybalski et al. (1991). By combining *Fok*I with properly designed oligo adapters, they have cleverly devised a method that utilizes the separation between the recogni-

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Abbreviations: *A*, absorbance (1 cm); aa, amino acid(s); Ap, ampicillin; bp, base pair(s); buffer A, 10 mM Tris·phosphate pH 8.0/7 mM β-mercaptoethanol/1 mM EDTA/10% glycerol; *E.*, *Escherichia*; ENase, restriction endonuclease; HPLC, high-performance liquid chromatography; IPTG, isopropyl-β-D-thiogalactopyranoside; kb, kilobase(s) or 1000 bp; MP, mutant protein(s); N, A or C or T or G; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; PAGE, polyacrylamide-gel electrophoresis; PCR, polymerase chain reaction; RBS, ribosome-binding site(s); SDS, sodium dodecyl sulfate; Tc, tetracycline; TE buffer, 10 mM Tris·HCl/1 mM EDTA pH 8.0; wt, wild type; 2XYT, 1.6% tryptone/1% yeast extract/0.5% NaCl pH 7.2; Δ*fokIR*, truncated gene coding for *Fok*I MP with C-terminal deletion; [ ], denotes plasmid-carrier state.

tion site and the cut site to confer additional cleavage specificities (Szybalski, 1985; Podhajska and Szybalski, 1985; Kim et al., 1989).

Three laboratories including ours have independently cloned the complete *Fok*I restriction-modification system from *Flavobacterium okeanokoites* (Looney et al., 1989; Kita et al., 1989a; L.P.W. and S.C., unpublished work). The purification of *Fok*I endonuclease has been reported by several groups (Kaczorowski et al., 1989; Kita et al., 1989b). Analysis of the domain structure of *Fok*I by trypsin digestion has shown that it has two distinct and separable functional domains, namely a recognition domain and a cleavage domain (Li et al., 1992). More recently, we have shown that insertion of aa between the two domains of *Fok*I can shift the cleavage distance further away from the recognition site within the DNA substrate (Li and Chandrasegaran, 1993).

The aim of present study was to construct two C-terminal deletion mutants of *Fok*I (41-kDa and 30-kDa N-terminal fragments of *Fok*I) to purify them to homogeneity and to characterize their DNA-binding properties.

EXPERIMENTAL AND DISCUSSION

(a) Construction of *Fok*I C-terminal deletion mutants and purification of MP

PCR was used to construct overproducer clones that express the 41-kDa and the 30-kDa C-terminal deletion mutants of *Fok*I. The use of PCR to alter transcriptional and translational signals surrounding a gene so as to achieve high expression of the desired protein in *E. coli* is described elsewhere (Skoglund et al., 1990). Precise deletions in the *fokIR* gene from the C terminus were done using oligo primers shown in Fig. 1A. DNA corresponding to the C-terminal 196 aa of the *fokIR* gene was deleted to construct the Δ*fokIR* mutant for the expression of the 41-kDa N-terminal fragment. DNA corresponding to the C-terminal 296 aa of the *fokIR* gene were deleted to construct the Δ*fokIR* mutant for the expression of the 30-kDa N-terminal fragment. Two stop codons are incorporated in the 3′ primers used in the PCR reactions. The PCR-generated DNA fragments (Δ*fokIR*) were cleaved with *Bam*HI and gel-purified. These were then ligated into *Bam*HI cleaved and dephosphorylated high expression vector pRRS (Skoglund et al., 1990). The recombinant plasmids pRRSΔ*fokIR* were transformed into competent *E. coli* RR1[pACYC*fokIM*] cells which express *Fok*I methylase constitutively. After tetracycline (Tc) and ampicillin (Ap) antibiotic selection, several transformants were picked, and their plasmid DNA were examined by restriction analysis for the presence of Δ*fokIR* in the correct orientation to the vector promoter. RR1[pACYC*fokIM*, pRRSΔ*fokIR*] cells were grown in 6 liters of 2XYT (1.6% tryptone/1% yeast extract/0.5% NaCl, pH 7.2) containing 20 μg Tc/ml and 50 μg Ap/ml at 37°C to $A_{600}=0.8$ and induced overnight with 1 mM

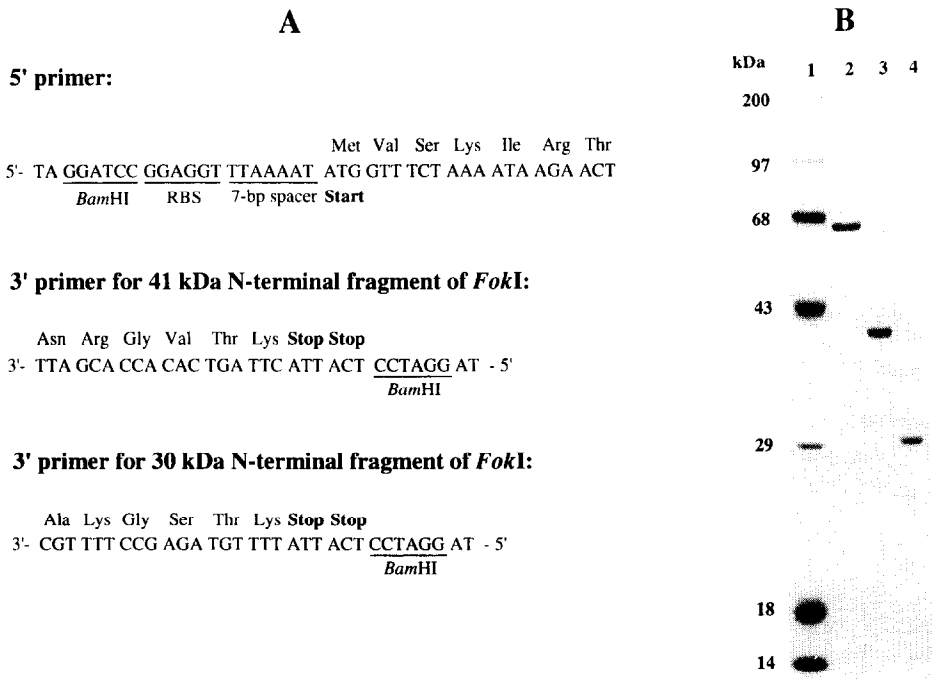

Fig. 1. Construction and analysis of MP. (**A**) Sequences of the 5′ and 3′ primers used to construct the C-terminal deletion mutants of *Fok*I. All primers are flanked by *Bam*HI sites. New translational signals were introduced to achieve high expression of the mutant proteins. A 7-bp spacer separates the RBS for *E. coli* from the ATG start codon. (**B**) 0.1% SDS-12% PAGE profile of the purified 41-kDa and 30-kDa MP. Lanes: 1, protein standards (from BRL, Gaithersburg, MD, USA); 2, wt *Fok*I ENase; 3, the 41-kDa MP; 4, the 30-kDa MP. The procedures for cell growth and purification of the MP are similar to the one described elsewhere (Li and Chandrasegaran, 1993).

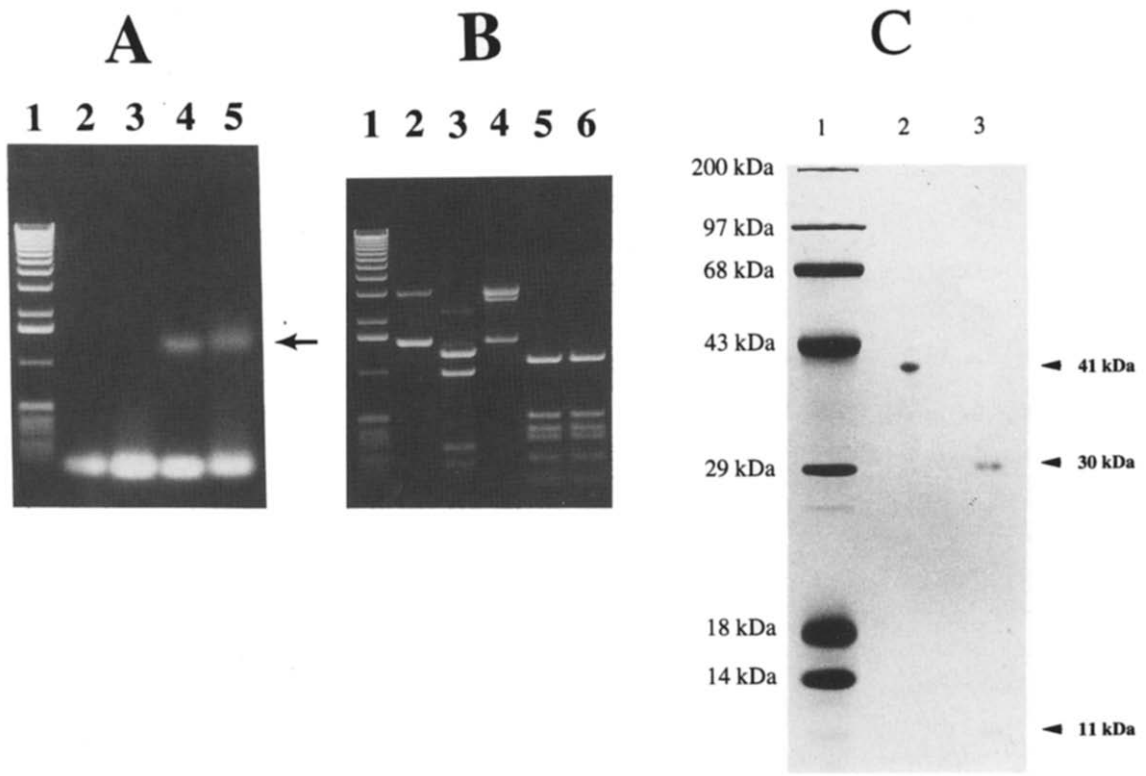

Fig. 2. Sequence-specific DNA-binding by the mixture of the 30-kDa MP and the 11-kDa fragment. (**A**) The 30-kDa MP and the 11-kDa fragment can associate to bind the DNA substrate. The 15-mer oligo duplex (50 pmoles) containing a single *Fok*I site (underlined): 5′-CCTCTGGATGCTCTC / 3′-GGAGACCTACGAGAG was used as the substrate. The mixtures were incubated in 10 mM Tris·HCl, pH 8.0/50 mM NaCl at 25°C for 1 h. Lanes: 1, DNA size markers; 2, oligo substrate; 3, the substrate incubated with 70 pmoles of purified 30-kDa MP; 4, the substrate incubated with 20 pmoles of purified 41-kDa MP; 5, the substrate incubated with 70 pmoles of purified 30-kDa MP and 30 pmoles of HPLC-purified 11-kDa fragment. The observed gel-retarded bands (indicated by the arrow) represent the oligo-protein complexes. The samples were resolved using 1% agarose gel electrophoresis in Tris·acetate, pH 8.0/EDTA buffer at 10 V per cm at 25°C for 1 h. The 11-kDa fragment alone did not bind the oligo substrate (data not shown). (**B**) The effect of the reconstituted 30-kDa MP and the 11-kDa fragment on the *Fok*I or *Hin*fI digestion of pTZ19R. In each reaction, 1 μg pTZ19R (0.5 pmole, 2 pmoles of *Fok*I recognition sites) was incubated with 30-kDa MP and 11-kDa HPLC-purified fragment in 10 mM Tris·HCl, pH 8.0/50 mM NaCl/10 mM $MgCl_2$ at 25°C for 1 h. Then the incubation mixtures were digested by addition of either *Fok*I or *Hin*fI endonuclease at 37°C for 1 h and the digestion profiles were analyzed by 1% agarose gel electrophoresis. Lanes: 1, DNA size markers; 2, pTZ19R (0.5 pmole); 3, pTZ19R digested by *Fok*I; 4, *Fok*I digestion of pTZ19R preincubated with the 30-kDa MP (35 pmoles) and the 11-kDa fragment (15 pmoles); 5, pTZ19R digested by *Hin*fI; 6, *Hin*fI digestion of pTZ19R preincubated with the 30-kDa MP (35 pmoles) and the 11-kDa fragment (15 pmoles). (**C**) 0.1% SDS-12% PAGE profiles of gel-retarded bands from **A**. Lanes: 1, protein standards (see Fig. 1B); 2, 41-kDa MP-DNA complex (lane 4 in **A**); 3, 30-kDa and 11-kDa protein-DNA complex (lane 5 in **A**).

IPTG. The induction was for 6 h in the case of the 30-kDa mutant protein. The 41-kDa and 30-kDa MP were purified as described elsewhere (Li and Chandrasegaran, 1993). The SDS-PAGE profiles of the purified mutant proteins are shown in Fig. 1B.

(b) DNA-binding properties of the 41-kDa and 30-kDa MP

We have studied the DNA-binding properties of MP by restriction enzyme-inhibition assay. DNA substrate (pTZ19R) was incubated with different amounts of the 41-kDa or 30-kDa *Fok*I MP. *Fok*I or *Hin*fI was then added to the DNA-mutant protein mixture. Addition of increasing amounts of 41-kDa mutant protein inhibits *Fok*I endonuclease cleavage of pTZ19R, whereas it does not have any effect on *Hin*fI digestion (data not shown). These results suggest that the 41-kDa MP has the same DNA sequence specificity as the wt *Fok*I. Addition of increasing amounts of the 30-kDa MP does not inhibit the digestion of pTZ19R by *Fok*I (data not shown), indicating that this MP does not bind DNA specifically.

In the presence of a DNA substrate, the 41-kDa MP is cleaved by trypsin into a 30-kDa N-terminal fragment and a 11-kDa C-terminal fragment (see next section **c**). Addition of the HPLC-purified 11-kDa fragment to the 30-kDa MP restores its sequence specific DNA-binding property (Fig. 2). The 30-kDa MP and the purified 11-kDa fragment readily form the protein-DNA complex when they are mixed with the DNA substrate (Fig. 2A). The complex appears as a shifted band similar to that of the 41-kDa protein-DNA complex. To confirm this observation, the gel-shifted protein-DNA bands were excised

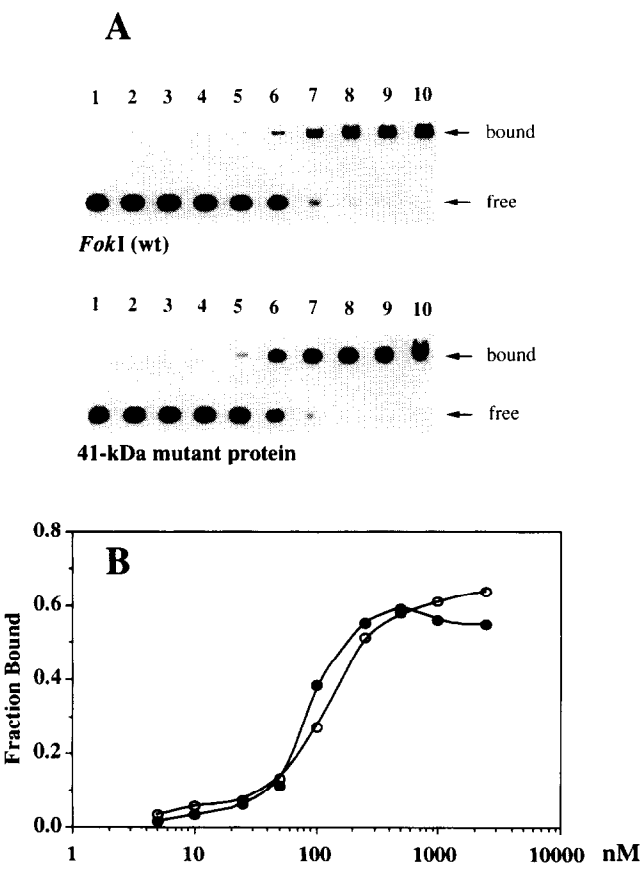

Fig. 3. Determination of equilibrium binding coefficients of the wt *Fok*I and the 41-kDa MP. 1 pmole of ^{32}P-labeled oligo (15-mer duplex; see Fig. 2 legend) was incubated with increasing amounts of the wt *Fok*I or the 41-kDa mutant protein in buffer A with 50 mM NaCl at 25°C for 1 h. The protein-bound oligo was separated from the free oligo by agarose gel electrophoresis. Lanes: 1, oligo duplex; 2–10, oligo incubated with 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 pmoles of *Fok*I or 41-kDa MP respectively. **(A)** The autoradiograph of the dried gel. **(B)** Quantitation of the autoradiograph using a Molecular Dynamics PhosphorImager. Rectangles of same dimension were drawn around each band on computer monitor screen. The counts of each rectangle were integrated. The percentage of oligo bound was plotted against the concentration of protein. The open circles (○) represent the binding curve for the wt *Fok*I, the closed circles (●) represent the binding curve for the 41-kDa MP.

from the agarose gel and run on SDS-PAGE. Their protein profiles are shown in Fig. 2C. Both the 30-kDa MP and the 11-kDa fragments are observed in the protein profile indicating that both these fragments are part of the complex. The mixture of the 30-kDa MP and the 11-kDa fragment also inhibit the *Fok*I cleavage of the DNA substrate (pTZ19R), but does not have any effect on the *Hin*fI digestion (Fig. 2B). The 11-kDa fragment by itself does not bind DNA (data not shown). This suggests that the 30-kDa MP and the 11-kDa fragment can associate in solution to reconstitute the *Fok*I recognition domain.

The affinities of the wt *Fok*I and the 41-kDa MP for the DNA substrate were estimated by measuring their equilibrium binding coefficients. The ^{32}P-labeled oligo DNA was titrated with increasing amounts of *Fok*I or 41-kDa MP. The protein-bound oligo and free oligo were separated by agarose gel electrophoresis (Fig. 3A). The data were quantitated by using a Molecular Dynamics PhosphorImager and plotted (Fig. 3B). The equilibrium binding coefficients were estimated to be approximately 110 nM for the wt enzyme and 80 nM for the 41-kDa MP, respectively. These results suggest that the deletion of the C-terminal 25-kDa portion of *Fok*I has little if any effect on its affinity for DNA substrates. Our values are higher than the one determined for *Fok*I (1.3–1.7 nM) by Skowron et al. (1993). This difference may be attributed to the small size of the oligo substrate used in our study.

(c) Analysis of the 41-kDa MP by trypsin cleavage

Trypsin digestion of the purified 41-kDa MP was carried out in the absence of a DNA substrate (Fig. 4A) and in the presence of a DNA substrate (Fig. 4B). The 41-kDa MP cleaved into a 8-kDa N-terminal fragment and a 33-kDa C-terminal fragment in the absence of a DNA substrate. In the presence of a DNA substrate, it is cleaved into a 30-kDa N-terminal fragment and a 11-kDa C-terminal fragment. All these fragments were purified by using reversed phase HPLC and their N-terminal aa sequences were determined (Table I). These results are consistent with the data obtained from the trypsin cleavage of the *Fok*I endonuclease (Fig. 4C). The 41-kDa MP appears to maintain a conformation similar to the wt *Fok*I in solution as well as in the DNA-protein complex.

(d) The mode of interaction of *Fok*I and 41-kDa MP with the DNA substrate

The hydroxyl radical footprinting method was used to study how *Fok*I and the 41-kDa MP bind DNA. A 100-bp pTZ19R DNA fragment containing a single *Fok*I-recognition sequence was amplified by using PCR and this served as the DNA substrate for the footprinting experiments. Individual strands of the DNA were ^{32}P-labeled. After the protein-DNA complexes were treated with hydroxyl radicals, the DNA were resolved on a 8% sequencing gel. The footprints of both the wt *Fok*I and the 41-kDa MP are shown in Fig. 5 and they reveal very similar patterns. On the 5′-GGATG strand of the substrate, it appears that both the wt *Fok*I and the 41-kDa MP make contact with DNA at three locations (Fig. 5A). On the 5′-CATCC strand, they seem to interact with the DNA at a single location (Fig. 5B). Partial protection of the nt bordering the recognition site on the 3′ end was also observed. The positions of these interactions with respect to the *Fok*I recognition site are illustrated in Fig. 5C. Both proteins appear to interact with only parts of the recognition site on both strands; however, on the 5′-GGATG strand they make additional contacts outside

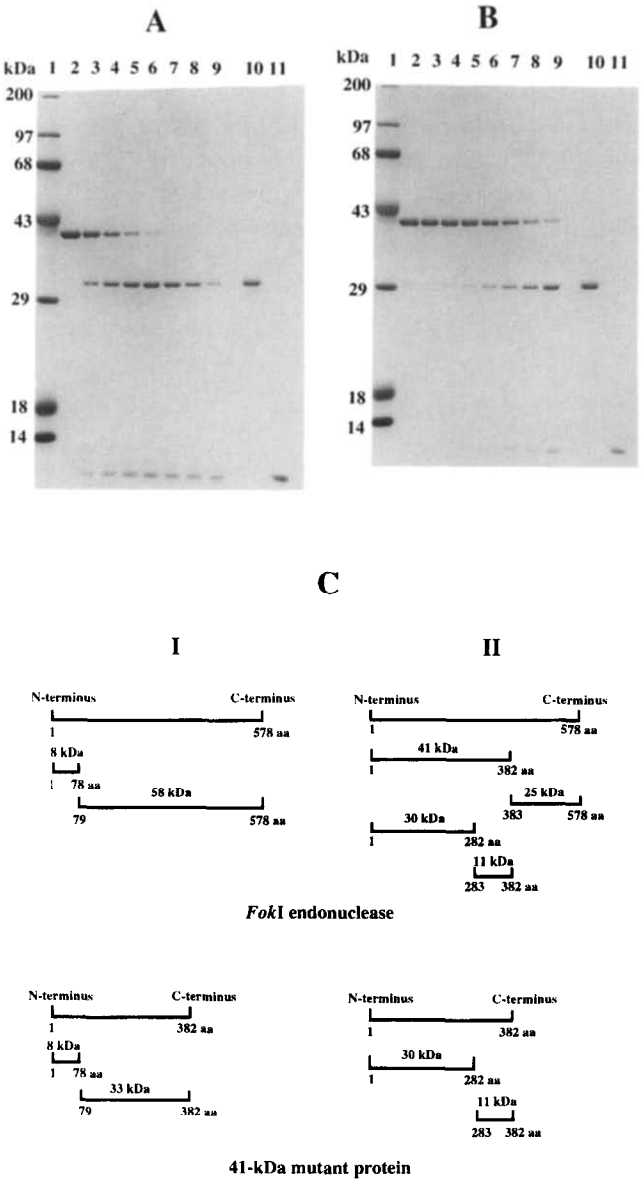

Fig. 4. 0.1% SDS-12% PAGE profiles of trypsin digestion of the 41-kDa MP. (**A**) Trypsin digestion of the 41-kDa protein in the absence of a DNA substrate. The 41-kDa MP (200 μg) in a volume of 200 μl containing 10 mM Tris·HCl/50 mM NaCl was digested with trypsin at 25°C. The ratio of trypsin to the 41-kDa MP was 1:50 by weight. (**B**) Trypsin digestion of the 41-kDa MP in the presence of the 15-mer oligo duplex (see Fig. 2 legend). The molar ratio of the 41-kDa MP to the oligo duplex was 1:2.5. The 41-kDa MP (200 μg) was preincubated with the oligo duplex in a 180 μl reaction volume containing 10 mM Tris·HCl/50 mM NaCl, pH 8.0, at 25°C for 1 h before adding trypsin (0.2 mg/ml, 20 μl). For both (**A**) and (**B**), aliquots (28 μl) from the digestion mixtures were removed at different time intervals and quenched with excess trypsin inhibitor antipain. Lanes (for **A** and **B**): 1, protein standard; 2, 41-kDa MP; 3, 2.5 min (of trypsin digestion); 4, 5 min; 5, 10 min; 6, 20 min; 7, 40 min; 8, 80 min; 9, 160 min. Lane 10 and 11 in (**A**), the HPLC-purified 33-kDa and 8-kDa tryptic fragments respectively. Lane 10 and 11 in (**B**), the HPLC-purified 30-kDa and 11-kDa tryptic fragments respectively. The N-terminal aa sequences of the HPLC-purified tryptic fragments are shown in Table I. (**C**) Tryptic map of *Fok*I ENase and the 41-kDa mutant protein. (**I**) fragmentation pattern in absence of the DNA substrate; (**II**) fragmentation pattern in presence of the DNA substrate. Numbers indicate the aa locations in the *Fok*I protein sequence.

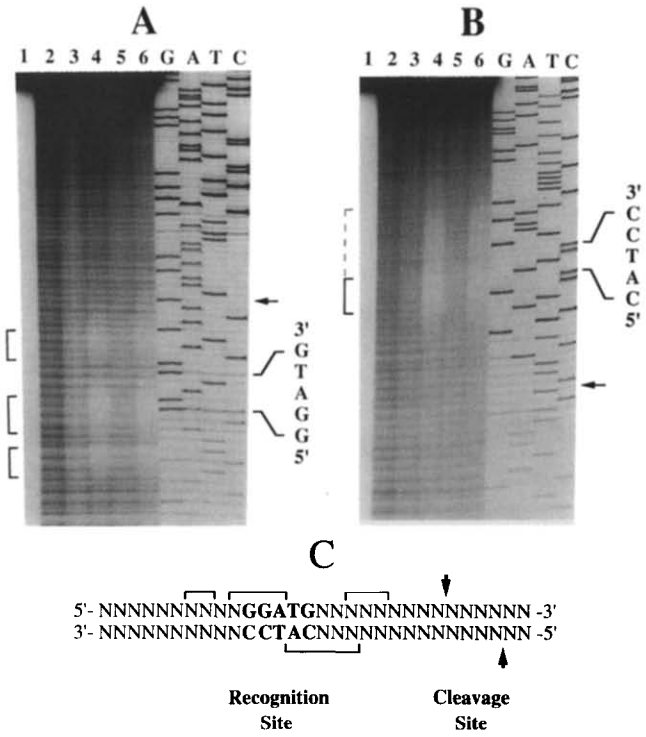

Fig. 5. Hydroxyl radical footprinting of the *Fok*I-DNA complex and the 41-kDa MP-DNA complex. (**A**) Autoradiograph of 100-bp pTZ19R fragment containing ^{32}P-label on the 5′-GGATG strand. (**B**) Autoradiograph of the same fragment containing ^{32}P-label on the 5′-CATCC strand. Lanes: 1, untreated DNA substrate; 2, DNA substrate cleaved by hydroxyl radicals; 3 and 4, hydroxyl radical cleavage pattern of DNA substrate in the presence of 7.5 pmoles and 15 pmoles *Fok*I; 5 and 6, hydroxyl radical cleavage pattern of the DNA substrate in the presence of 9 pmoles and 18 pmoles 41-kDa MP. The protected regions are marked by brackets. The partially protected nt bordering the recognition site on the 3′ end of 5′-CATCC strand is marked by the dashed line. Arrows indicate the cleavage sites of *Fok*I. (**C**) Illustration of contact regions of the DNA substrate made by the wt *Fok*I and the 41-kDa MP. **Methods: Preparation of DNA substrates.** A 100-bp DNA fragment containing a single *Fok*I recognition sequence was amplified from pTZ19R by PCR. Oligo primers, 5′-CGCAGTGTTATCACTCAT and 5′-CTTGGTTGAGTACTCACC were used to generate the substrates. Individual strands within the substrates were radiolabeled by using the corresponding ^{32}P-labeled primers during PCR. Thus two identical substrates differing only in the labeled strand were prepared. The products were purified from a low-melting-point 1% agarose gel, ethanol precipitated and resuspended in TE buffer. **Hydroxyl radical footprinting.** We followed procedures as described by Tullius et al. (1987). Hydroxyl radicals were generated by the Fenton reaction of hydrogen peroxide with the complex of iron(II) and EDTA. A typical reaction contained the end-labeled 100-bp pTZ19R fragment (40000 cpm, 10 pmoles) and the *Fok*I or the 41 kDa mutant protein in 70 μl of binding buffer (10 mM Tris·HCl/50 mM NaCl, pH 8.0). The mixture was incubated at 25°C for 1 h to allow complexation of protein with DNA. The cutting was initiated by mixing 10 μl 20 mM Na·ascorbate/10 μl iron(II)(1 mM)-EDTA(2 mM)/10 μl 0.03% hydrogen peroxide on the side of the Eppendorf tube, followed by addition to the protein-DNA solution. The reaction was allowed to proceed for 4 min at 25°C and then quenched by adding 30 μl 0.1 M thiourea (an efficient hydroxyl radical scavenger)/2 μl 0.2 M EDTA. To remove the protein, the reaction mixtures were extracted with phenol, phenol:chloroform and chloroform, and ethanol precipitated. The DNA pellets were dissolved in formamide-dye mixture and resolved by denaturing 8% PAGE with 7 M urea.

TABLE I

N-terminal sequences of the 41-kDa MP fragments from trypsin digestion

Fragment[a]	N-terminal sequence[b]	DNA substrate[c]
8 kDa	VSKIRTFGWV	–
33 kDa	SEAPCDAIIQ	–
30 kDa	VSKIRTFGWV	+
11 kDa	FTRVPKRVYW	+

[a,c] Refer to Fig. 4C and to the legend.
[b] See complete aa sequence of *Fok*I ENase published by Looney et al. (1989).

the recognition site. The DNA at the cleavage site is not protected by *Fok*I, indicating that the enzyme does not make any additional contact of the type detected by hydroxyl radicals with the DNA around this region.

(e) Conclusions

(*1*) Two C-terminal deletion mutants (41-kDa and 30-kDa) of the *Fok*I ENase have been constructed by using PCR and expressed in *E. coli*. The MP have been purified to homogeneity.

(*2*) The 41-kDa MP binds DNA in a sequence-specific manner like the wt enzyme. The 30-kDa MP can no longer bind DNA.

(*3*) In the presence of the DNA substrate, the 41-kDa MP is cleaved by trypsin into a 30-kDa N-terminal fragment and a 11-kDa C-terminal fragment. Addition of HPLC-purified 11-kDa fragment to the 30-kDa MP reconstitutes the recognition domain of *Fok*I and restores its sequence-specific binding of the DNA.

(*4*) Both the wt *Fok*I and the 41-kDa MP bind the DNA substrate with similar affinity; and they both interact with DNA in a similar way as revealed by hydroxyl radical footprinting experiments.

(*5*) Our study further supports the presence of two separate domains within *Fok*I ENase–one for sequence-specific recognition and the other for endonuclease activity. The modular structure of the enzyme suggests that it may be feasible to construct chimeric ENases of different sequence specificity by linking other DNA-binding proteins to the cleavage domain of *Fok*I ENase.

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REFERENCES

Kaczorowski, T., Skowron, P. and Podhajska, A.J.: Purification and characterization of the *Fok*I restriction endonuclease. Gene 80 (1989) 209–216.

Kim, S.C., Podhajska, A.J. and Szybalski, W.: Cleaving DNA at any predetermined site with adapter-primers and class-IIS restriction enzyme. Science 240 (1988) 504–506.

Kita, K., Kotani, H., Sugisaki, H. and Takanami, M.: The *Fok*I restriction-modification system. J. Biol. Chem. 264 (1989a) 5751–5756.

Kita, K., Kotani, H., Hiraoka, N., Nakamura, T and Yonaha, K.: Overproduction and crystallization of *Fok*I restriction endonuclease. Nucleic Acids Res. 17 (1989b) 8741–8752.

Li, L., Wu, L.P. and Chandrasegaran, S.: Functional domains in *Fok*I restriction endonuclease. Proc. Natl. Acad. Sci. USA 89 (1992) 4275–4279.

Li, L. and Chandrasegaran, S.: Alteration of the cleavage distance of *Fok*I restriction endonuclease by insertion mutagenesis. Proc. Natl. Acad. Sci. USA 90 (1993) 2764–2768.

Looney, M.C., Moran, L.S., Jack, W.E., Feehery, G.R., Benner, J.S., Slatko, B.E. and Wilson, G.G.: Nucleotide sequence of the *Fok* restriction-modification system: separate strand-specificity domains in the methyltransferase. Gene 80 (1989) 193–208.

Podhajska, A.J. and Szybalski, W.: Conversion of the *Fok*I endonuclease to a universal restriction enzyme: cleavage of phage M13mp7 DNA at predetermined sites. Gene 40 (1985) 175–182.

Skoglund, C.M., Smith, H.O. and Chandrasegaran, S.: Construction of an efficient overproducer clone of *Hin*fI restriction endonuclease using the polymerase chain reaction. Gene 88 (1990) 1–5.

Skowron, P., Kaczorowski, T., Tucholski, J. and Podhajska, A.J.: Atypical DNA-binding properties of class-IIS restriction endonucleases: evidence for recognition of the cognate sequence by a *Fok*I monomer. Gene 125 (1993) 1–10.

Sugisaki, H. and Kanazawa, S.: New restriction endonucleases from *Flavobacterium okeanokoites* (*Fok*I) and *Micrococcus luteus* (*Mlu*I). Gene 16 (1981) 73–78.

Szybalski, W.: Universal restriction endonucleases: designing novel cleavage specificities by combining adapter oligodeoxynucleotide and enzyme moieties. Gene 40 (1985) 169–173.

Szybalski, W., Kim, S.C., Hasan, N. and Podhajska, A.J.: Class-IIS restriction enzymes-a review. Gene 100 (1991) 13–26.

Tullius, T.D., Dombroski, B.A., Churchill, M.E.A. and Kam, L.: Hydroxyl radical footprinting: a high-resolution method for mapping protein-DNA contacts. Methods Enzymol. 155 (1987) 537–558.