Imaging DNA Loops Induced by Restriction Endonuclease EcoRII

A SINGLE AMINO ACID SUBSTITUTION UNCOUPLES TARGET RECOGNITION FROM COOPERATIVE DNA INTERACTION AND CLEAVAGE

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EcoRII is a type IIE restriction endonuclease characterized by a highly cooperative reaction mechanism that depends on simultaneous binding of the dimeric enzyme molecule to two copies of its DNA recognition site. Transmission electron microscopy provided direct evidence that EcoRII mediates loop formation of linear DNA containing two EcoRII recognition sites. Specific DNA binding of EcoRII revealed a symmetrical DNase I footprint occupying 16–18 bases. Single amino acid replacement of Val258 by Asn yielded a mutant enzyme that was unaffected in substrate affinity and DNase I footprinting properties, but exhibited a profound decrease in cooperative DNA binding and cleavage activity. Because the electrostatics of the mutant enzyme-DNA complexes was significantly higher than that of the wild-type, we investigated if mutant V258N binds as a monomer to the substrate DNA. Analysis of the molecular mass of mutant V258N showed a high percentage of protein monomers in solution. The dissociation constant of mutant V258N confirmed a 350-fold decrease of the enzyme dimerization capability. We conclude that Val258 is located in a region of EcoRII involved in homodimerization. This is the first report of a specific amino acid replacement in a restriction endonuclease leading to the loss of dimerization and DNA cleavage while retaining specific DNA binding.

Studies on the molecular reaction mechanism of restriction endonucleases (ENases) in the last decade revealed the existence of a subset of unconventional type II ENases. The enzymatic activity of these ENases depends on simultaneous binding of two copies of the recognition site (reviewed in Ref. 1). Initially observed for EcoRII (2), the need for a second (effector) site was also confirmed for several other ENases (3, 4). These ENases were named as type IIE. Cooperatively interacting type IIE ENases share characteristic molecular features with transcription regulators, recombinases, and DNA repair enzymes (5). Experimental results even support the idea of evolutionary relationships to other cellular DNA-binding protein families (6, 7).

Comprehensive studies have also been performed on two type II enzymes, SfiI and Cfr10I. These enzymes interact simultaneously with two DNA sites like type IIE ENases but differ in the quaternary structure. SfiI and Cfr10I are composed of four identical subunits (8–10).

Detailed investigations on the substrate requirements of EcoRII uncovered a cooperative reaction mechanism (11–13). Functional cooperativity can occur between two sites on a single DNA molecule (in cis). This is thought to represent the preferred mode of action. However, depending on the substrate length and concentration, the enzyme can also interact with two sites from different DNA molecules (in trans). One dimeric EcoRII enzyme molecule is sufficient to bind cooperatively two DNA sites (12, 14). These sites can be cleaved simultaneously in one binding event. Premature dissociation of enzyme-substrate complexes releases partial products (14). EcoRII cleavage activity is inversely correlated to the distance between two sites of a linear DNA molecule (14). No cleavage occurred when the spacing between two sites exceeded a critical length of 1000 bp. Overall, these biochemical studies suggest that EcoRII takes advantage of DNA bending or looping to achieve cooperativity in cis (11–14).

Using a membrane-bound EcoRII-derived peptide scan, we recently identified two separate DNA-binding regions I (aa 88–102) and II (aa 256–273) in the primary amino acid sequence of EcoRII (15). We hypothesize that regions I and II participate in forming one of the two equivalent substrate binding sites of EcoRII, because a mutation introduced in DNA-binding region I abolished substrate binding of the complete enzyme dimer (15).

Here we answer the question of whether one substrate binding site is formed by an EcoRII monomer or includes contributions of DNA-binding regions from both monomers. The direct observation of EcoRII-induced DNA loops supports earlier biochemical inferences. The experimental data confirm the proposed two-site cooperative reaction mechanism of EcoRII.

**EXPERIMENTAL PROCEDURES**

**Transmission Electron Microscopy**—A DNA fragment of 1202-bp length containing two EcoRII recognition sequences, was obtained by XmnI and AflIII digestion of pUC18 DNA. The DNA fragment was purified by gel extraction. DNA-binding protein reactions were performed at room temperature for 10 min in a 10-μl reaction volume containing 40 fmol of DNA and 40 fmol of enzyme in 25 mM Tris-HCl, pH 6.8; 1 mM Ca(OAc)2; 100 mM KOAc; 2 mM dithiothreitol with or without 5 mM Mg(OAc)2. Complexes were fixed and directly adsorbed to
fresly cleaved mica as described (16). Micrographs were taken using a Philips CM100 electron microscope at 100 kV and a primary magnification of 15,500×. To determine the contour length, measurements were carried out on projections of negatives using a digitizer (LM4, Bruhl, Nuremberg, Germany).

Determination of Cleavage Activity of V258N in Comparison to wt EcoRII—We analyzed the time dependence of the cleavage activity of both enzymes by incubating 1.2 pmol of BanHI-linearized pBR322 Dcm DNA with 3.6 pmol of the respective enzyme in a reaction volume of 160 μl containing 1× universal buffer (Stratagene) at 37 °C. Aliquots were removed over 2 h and immediately placed at −20 °C. Cleavage products were run on 0.8% agarose gels and quantitated by Phoretix 1D Software (Biostep Labor- und Systemtechnik GmbH, Jahnsdorf, Germany). Cleavage efficiency was determined as the quotient of uncleaved substrate and total DNA per lane. Cleavage activity in trans was analyzed at 37 °C for 30 min after incubating 4 pmol of a one-site oligonucleotide duplex (30 bp) with 2 pmol of EcoRII wt and 2 pmol of EcoRII V258N, respectively, containing 1× universal buffer (Stratagene) in a 20-μl reaction volume. Cleavage products were separated by a 15% polyacrylamide gel containing 7 m urea.

CD Spectroscopy—CD measurements were performed on a Jasco J-600 spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan) using a 1-ml cuvette with 0.2-mm path length. Protein preparations were of ≥95% purity, and the concentration was around 0.29 mg/ml. Ten measurements were taken at 250- to 195-nm wavelength using a scan rate of 10 nm/min and a step resolution of 0.2 nm. Data were analyzed using k2D software (17,18).

Two-dimensional Gel Electrophoresis—First dimension native electrophoresis was performed according to previous studies (19,20) using 20 μg of EcoRII wt and 20 μg of V258N protein on a 5–18% linear acrylamide gradient gel. Marker proteins were lactate dehydrogenase (140 and 280 kDa), hexokinase (99 kDa), conalbumin (78 kDa), albumin (66 and 132 kDa), and β-lactoglobulin (35 kDa). Seven independent experiments were performed, each including a molecular mass calibration. The calibration curve fit a logarithmic function. Second dimension 12% SDS-PAGE was run according to the procedure described previously (19).

Analytical Ultracentrifugation—Molecular mass analyses were carried out in an XL-A type analytical ultracentrifuge (Beckman) with UV absorbance scanner optics. The sedimentation equilibrium was analyzed using externally loaded six-channel centerpieces of 12-mm optical path length usually filled with 70 μl of each protein solution. Three of these cells were used to analyze different samples in one run simultaneously. Sedimentation equilibrium was reached after 2 h of overspeed at 18,000 rpm, followed by an equilibrium speed of 14,000 rpm at 10 °C for 24–30 h. The radial absorbancies of each compartment were scanned at 275, 280, and 285 nm, using the molecular absorption coefficients determined with EcoRII wt. Average molecular mass was calculated by simultaneously fitting the sets of three radial distribution curves as described previously (21).

RESULTS

EcoRII Mutant Enzyme V258N—The primary amino acid sequence of EcoRII harbors two distinct DNA-binding regions (I, aa 88–102 and II, aa 256–273) (15). Mutational analyses supported the significance of these two DNA-binding regions (15). Searching for similar peptides in other ENases, we found a strong local homology of EcoRII-binding region II with SsoII (aa 110–127) and some sequence conservation with other ENases that have terminal C:G or G:C base pairs in their recognition sequences in common (15). Additional support for the importance of DNA-binding region II is provided by investigations on the newly discovered EcoRII-homoioschizomer PspGI that showed significant similarity to EcoRII binding site II in a segment of 87 aa residues (22).

Based on these findings, we examined the role of single amino acid residues of EcoRII DNA-binding region II in target-site recognition and catalytic activity. Taking into account the similarity between EcoRII (CCWG) and SsoII (CCNG), we exchanged non-conserved amino acids within the homologous region. One of these positions was the non-polar Val228 in EcoRII, which was substituted by a polar Asn residue as found in the corresponding amino acid position in SsoII. The resulting EcoRII mutant enzyme V258N was expressed with N-terminal His6-tag and purified to 95% homogeneity following a procedure described previously (14).

EcoRII-induced Loop Formation Visualized by Transmission Electron Microscopy—To prove the ability of EcoRII to cooperate with two copies of its recognition site, we analyzed complexes of the enzyme with different DNA substrates by means of transmission electron microscopy. The interaction of EcoRII with a 1202-bp linear DNA substrate containing two EcoRII recognition sites 191 bp apart gave rise to DNA loop formation (Fig. 1a). The asymmetric location of the two EcoRII sites in this particular DNA molecule allowed us to distinguish one DNA end from the other. In contrast, the binding of EcoRII to a single site substrate did not induce DNA looping (data not shown). We analyzed 90 of the looped DNA molecules by measuring the contour length of the three respective regions (short DNA end, DNA loop, long DNA end). Thus, we determined that DNA loop formation occurred specifically between the EcoRII recognition sites (Fig. 1b). However, we also observed EcoRII protein complexes bound to the DNA molecule without loop formation (Fig. 1a).

The frequency of loop formation by wt EcoRII and mutant V258N was determined in the presence or absence of Mg2+ (Fig. 1c). In the presence of Mg2+, wt EcoRII formed loop structures with 18.4% of the total DNA molecules, but the mutant V258N formed loop structures only with 3.3%. Without Mg2+, 25.4% of the DNA molecules formed loops with wt EcoRII and 6% with the mutant enzyme. The reduction of DNA loop formation in the presence of Mg2+ can be due to increased specificity of DNA binding.

From these data it became obvious that the single amino acid substitution of Asn for Val228 led to a significantly reduced interaction between two recognition sites. The decreased DNA loop formation by the mutant V258N compared with wt EcoRII is independent of the addition of Mg2+ (Fig. 1c). In the presence of Mg2+, wt EcoRII formed loop structures with 18.4% of the total DNA molecules, but the mutant V258N formed loop structures only with 3.3%. Without Mg2+, 25.4% of the DNA molecules formed loops with wt EcoRII and 6% with the mutant enzyme. The reduction of DNA loop formation in the presence of Mg2+ can be due to increased specificity of DNA binding.

DNase I Footprinting Analyses of wt EcoRII and V258N—To define the DNA region mediating the specific wt EcoRII- and V258N-substrate interaction, DNase I footprinting analyses were performed using a DNA substrate containing two EcoRII recognition sites 82 bp apart. Fig. 2 shows the footprinting patterns of wt EcoRII and mutant V258N either at the CCAGG sequence or at the CCTGG sequence of two EcoRII recognition sites (Fig. 2, a and b). With increasing concentrations of wt EcoRII and mutant V258N, respectively, DNase I digestion of the DNA seems to be blocked to a similar extent in the A and T strand of both recognition sites. The footprints were detectable at an enzyme-to-site ratio of 10 in both cases (lanes 6 and 10). DNase I footprints due to specific DNA binding of EcoRII are 16–18 bp long. Footprints were located symmetrically in both
strands of one recognition site (data not shown). Mutant V258N revealed the same DNase I footprinting pattern, demonstrating that specific recognition of the double stranded EcoRII sequence was not altered by the introduced amino acid replacement.

Comparison of DNA Binding, Cleavage Properties, and Secondary Structure Distribution—Substituting Asn for Val258 led to changes in the protein-DNA complex. Complexes formed by mutant V258N enzyme exhibited significantly higher electrophoretic mobility than those complexes formed by the wt enzyme (Fig. 3a). To verify that formation of the faster migrating complex relies on the specific interaction of mutant V258N with the EcoRII recognition sequence, competition experiments were performed. A 1000-fold molar excess of unlabeled unspecific oligonucleotides did not reduce complex formation. In contrast, the DNA-protein complexes completely disappeared due to the addition of the same concentration of unlabeled specific competitor DNA. Because of the increased electrophoretic mobility, we expected significant deviations in the molecular composition of the V258N protein-DNA complex. Using constant low substrate concentration and increasing excess of the respective protein, we calculated the $K_{D(app)}$ according to a previous study (15). The $K_{D(app)}$ of both wt EcoRII and mutant V258N was 5 nM, indicating that the DNA-binding affinity was unaffected by the amino acid replacement.

The DNA cleavage activity of wt EcoRII and V258N protein was assayed on linearized pBR322 DNA. In contrast to the unchanged substrate recognition shown by $K_{D(app)}$ values and DNase I footprints, the cleavage activity of V258N reached only approximately 16% of the wt enzyme activity (Fig. 3b). In addition, we compared the cleavage reactions of both proteins using 30-bp oligonucleotide substrates harboring a single recognition site. In contrast to wt EcoRII, V258N was incapable of cleaving these substrates that require DNA binding in trans (data not shown).

CD spectra of wt EcoRII and mutant V258N did not reveal remarkable changes in the secondary structure distribution. The percentages of $\alpha$-helix and $\beta$-sheet structures were 27 and 23% for EcoRII wt and 31 and 10% for the V258N mutant enzyme, respectively.

Analysis of Molecular Mass and Oligomeric Status of Wt EcoRII and V258N by Two-dimensional PAGE—To gain insight into the altered composition of the V258N enzyme-substrate complex, we examined both enzymes by two-dimensional PAGE. In the denaturing SDS-PAGE, EcoRII wt and mutant V258N migrated as one main band of 46 kDa corresponding to the molecular mass of an EcoRII monomer (Fig. 4a). In contrast, native electrophoresis of the wt and the mutant enzyme elucidated remarkable differences in the composition of both proteins.
protein solutions (Fig. 4b). The apparent molecular masses of wt EcoRII and V258N mutant bands were estimated by direct fit to a logarithmic calibration curve. EcoRII wt mainly consisted of a component with an apparent molecular mass of 104.7 kDa (band 2), coinciding with the homodimer, and of two minor protein fractions of about 240 kDa (band 1) and 59.5 kDa (band 3). These could correspond to a tetrameric and monomeric protein structure, respectively. The mutant protein solution showed three bands of similar intensity to each other with apparent molecular masses of 156.7, 101.5, and 59.1 kDa. These mutant V258N bands are in agreement with the theoretical molecular mass of the protein trimer, dimer, and monomer, respectively. The EcoRII specificity of the described protein bands has been verified by Western blot (data not shown). We analyzed natively separated protein bands in the second dimension by SDS-PAGE (Fig. 4c). Thus, we confirmed that the bands of the wt and the mutant enzyme, respectively, resolved in the first dimension, displayed a molecular mass corresponding to that of an EcoRII monomer. In response to the amino acid replacement Val to Asn, enzyme dimerization was strongly destabilized and the dimer-tetramer equilibrium of wt EcoRII (with a pronounced preference for the dimeric form) was shifted to a monomer-dimer equilibrium.

Analytical Ultracentrifugation—To characterize the oligomeric nature of the wt EcoRII and the mutant enzyme in solution under equilibrium conditions and to evaluate the affinity of the protein subunits, we determined the concentration dependence of the average molecular mass by sedimentation equilibrium experiments. Fig. 5 shows extrapolated graphs of the concentration dependence of the average molecular mass of the wt EcoRII and the mutant assuming a monomer-dimer equilibrium. Whereas the molecular mass of wt EcoRII did not differ with protein concentration and was determined to be 92 kDa consistent with the homodimeric form, the molecular mass of V258N depended on the protein concentration. In the examined protein concentration range of 35–100 μg/ml, molecular mass values varied from 66 to 77 kDa. These values lie between the EcoRII monomer (46 kDa) and dimer (92 kDa), thus, supporting a state of equilibrium of the monomeric and the dimeric form of the mutant.

Assuming a monomer-dimer equilibrium, the dimer dissociation constants $K_D$ of V258N and wt EcoRII were calculated from the concentration dependence of the molecular mass to be 0.99 (± 0.06) μM and 2.89 (± 1.08) nM, respectively. This indicates a 350-fold decrease in dimer stability for the V258N mutant.

R261S, another mutant within DNA binding site II, showed two enzyme-specific bands in native gel electrophoresis corresponding to the molecular mass of monomer and dimer (not shown). Analytical ultracentrifugation confirmed a monomer-dimer equilibrium of R261S with a $K_D$ for protein dimerization of 77.8 (± 9.5) nM (Fig. 5). This represents a 25-fold destabilization of the protein dimer.

Sedimentation equilibrium experiments validated the prediction that substitution of Asn for Val substantially destabilizes the dimeric protein structure. In addition, one can assume that Arg plays a significant role in EcoRII dimerization.

DISCUSSION

Type II ENases are homodimers in solution and interact as such with their substrate (23, 24). Each of the identical monomeric subunits mediates half of the specific protein-DNA contacts and possesses one catalytic center to catalyze the phosphodiester bond hydrolysis of one single strand of the double stranded DNA recognition site.

EcoRII also exists as a homodimer in solution but, unlike most type II ENases, must bind two recognition sites simultaneously, either in cis or in trans, for effective DNA cleavage (1). Stoichiometric data indicated a functionally active complex composed of one EcoRII dimer and two DNA recognition sites (12, 14). Interaction with two target DNA sites has also been described for the type II ENases SfiI and Cfr10I, except that both of these are functionally active as tetramers. SfiI and Cfr10I cleave the double stranded DNA of two recognition sites in a concerted reaction. This corresponds to the cleavage of a single strand by each subunit of the tetramer (10, 25). One catalytic center per enzyme subunit seems to be a common theme among type II ENases, raising the question of how the EcoRII homodimer achieves the hydrolysis of two double stranded DNA recognition sites.

We observed that two cooperating EcoRII recognition sites in a linear DNA molecule are cleaved regardless of the Lac repressor blocking the continuity of the intervening DNA. Furthermore, EcoRII cleavage diminishes with the increasing dis-
distance of the two cooperating recognition sites in cis due to decreased local concentration of recognition sites (14). These two facts combined with a well characterized substrate cooperativity (11–13) support the hypothesis that EcoRII communicates between remote recognition sites on one DNA molecule via loop formation.

Transmission electron microscopy was used to visualize the capability of the dimeric enzyme to form specific DNA loops after binding at two sites simultaneously. The proportion of approximately 20% of loops formed by the wt EcoRII is consistent with the cleavage efficiency of 23.8% for a DNA substrate with two sites separated by the same distance (191 bp) (14). In this cleavage assay conditions allowed EcoRII interaction with the target sites in cis exclusively. Therefore, the low cleavage efficiency can be attributed to the decreased loop stability and loop formation probability due to increasing site distance (14). The reduced loop formation by the monomeric mutant V258N enzyme indicates that induction of DNA looping by EcoRII directly depends on it being an intact dimer. We assume that a residual portion of dimeric V258N mutant species mediated the observed loops. Cleavage efficiencies exerted by both enzymes mirror the loop formation frequencies displayed by wt EcoRII and V258N. These results illustrate that the complex of the EcoRII dimer with two copies of the recognition sequence represents the active form and is, likewise, the precondition for the concerted hydrolysis of four phosphodiester bonds. So far, direct electron microscopic evidence for a type IIE enzyme-induced DNA loop formation was exclusively available for NaeI (26). Very recently Siksnys et al. (10) visualized that the reaction of the tetrameric type II ENase Cfr 10I included an intermediate DNA loop structure, and Wentzell and Halford (27) suggested the same for the SfiI tetramer after investigating DNA cleavage rates of plasmids with differently spaced sites.

The DNase I footprinting patterns of EcoRII symmetrically spanned 16–18 bases of both DNA strands of one recognition site, as one would expect for a 2-fold rotational symmetry of EcoRII similar to most type II ENases that bind symmetrically to the DNA helix. Neither the wt nor the mutant V258N enzyme seem to discriminate between the A or T strand. This is consistent with the facts that EcoRII accepts central A/A and TT mismatches within its recognition sequence (28) and that the recognition site orientation does not influence EcoRII cleavage activity (14). The protected DNA region had been estimated earlier for EcoRII to be 21 ± 1 bp using dissociation kinetics of the protein bound to synthetic concatemeric DNA substrates (29). Our DNase I footprinting data reduced the protected DNA region to 16–18 bp. The mutant V258N enzyme revealed no differences in its DNA recognition properties when compared with wt EcoRII, confirming that the binding capacity for a double stranded EcoRII recognition site resides in the monomeric subunit of the enzyme. Bands resulting from hypersensitivity toward DNase I cleavage around the footprint were possibly consequences of structural deformation, such as DNA bending or kinking, induced by the binding of the enzyme. Such distortions are thought to be a structural basis of cooperativity between DNA target sites at a short distance (30).

The monomeric character of the mutant enzyme V258N was independently documented by molecular mass determinations and oligomeric state analyses using analytical ultracentrifugation and two-dimensional gel electrophoresis. We could verify that V258N exists in a monomer/dimer equilibrium with distinct concentration dependence. The protein dimerization was destabilized 350-fold compared with the wt. Another mutant of EcoRII, R261S, located only three amino acids farther away in the C-terminal direction, also showed decreased dimer stability, but the weakening was not as severe as that for V258N. This led us to conclude that both amino acids contribute to a putative dimer-interface region and that multiple amino acid interactions are responsible for oligomerization. The same experimental procedures confirmed the dimeric character of EcoRII wt in solution and distinguished it from functional active tetrameric ENases such as Cfr 10I and SfiI.

The determined molecular masses and oligomeric states supported our assumption that the V258N mutant specifically binds to the DNA as a monomer, whereas the wt enzyme binds in its dimeric form. A proteolytic C-terminal fragment of NaeI (aa 169–317), another type IIE ENase, was shown to be monomeric in solution but still capable to form complexes with cognate double stranded DNA (31). Together with the NaeI crystal structure (32), these data could support the idea that a monomeric subunit of type IIE ENases comprises the DNA recognition elements for a single recognition site.

Cleavage activity of V258N mutant enzyme on linearized pBR322 DNA molecules decreased to 16% of wt activity. Because DNA binding affinity as well as secondary structure distribution of V258N remained nearly unaffected, we consider the destabilization of protein-protein interactions at the dimer interface to be responsible for the reduced capability to interact cooperatively. Moreover, cleavage in trans using oligonucleotide duplexes containing one recognition site could not be observed for the mutant V258N. We assume this is due to the weak V258N protein-protein contact that, although allowing a
EcoRII and catalysis are tightly interwoven. However, it contradicts a hypothesis of a catalytic inactive enzyme monomer-DNA complex and demonstrate that amino acids essential for dimerization in independent DNA substrates. These data strengthen our hypothesis. In contrast to the monomeric ENase variant that retains the property of specifically recognizing the double stranded recognition site. It has been suggested that the RII mutant V258N is still able to bind specifically to the recognition sequence and that an RII can hydrolyze the two phosphodiester bonds of one recognition site in one event (36), two catalytic sites in one monomer could be postulated. Cleavage activation due to the essential binding of a second recognition site should be reflected in a network of amino acid residues associated with DNA recognition, protein dimerization, and DNA hydrolysis. This hypothesis has to be proven in further structural analyses.

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Fig. 5. Dependence of the molecular mass on the protein concentration analyzed by analytical ultracentrifugation. Molecular mass analyses were carried out as described under “Experimental Procedures.” Open circles, mutant V258N; open squares, mutant R261S; and filled circles, wt EcoRII.

are underlined) (15).3 Recently it has been reported that the monomeric PI-SceI endonuclease achieves specific hydrolysis of both DNA strands by two active sites per monomer (35). These results encourage further investigation on the number and architecture of EcoRII active site(s).

To summarize, we conclude that one monomer of the homodimeric EcoRII alone possesses the structural elements for DNA recognition. Subsequent to the primary binding event at one recognition site, the second subunit cooperatively binds to another recognition site culminating in the formation of the catalytic active complex that, after a conformational change, triggers the cleavage reaction. Thus, the dimeric status of the EcoRII ENase is an essential prerequisite for cooperative substrate binding. We assume that each monomeric subunit contributes one DNA-binding cleft, which is comprised of the DNA-binding regions I and II interwoven with each other and with catalytic residues and which is able to hydrolyze two single strands. Hence, four DNA strands of two coordinated recognition sites can be cleaved in a concerted reaction. Considering that an EcoRII monomer is sufficient to bind specifically to the recognition sequence and that EcoRI can hydrolyze the two phosphodiester bonds of one recognition site in one event (36), two catalytic sites in one monomer could be postulated. Cleavage activation due to the essential binding of a second recognition site should be reflected in a network of amino acid residues associated with DNA recognition, protein dimerization, and DNA hydrolysis. This hypothesis has to be proven in further structural analyses.

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