Translocation-Independent Dimerization of the *Eco*KI Endonuclease Visualized by Atomic Force Microscopy

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ABSTRACT Bacterial type I restriction/modification systems are capable of performing multiple actions in response to the methylation pattern on their DNA recognition sequences. The enzymes making up these systems serve to protect the bacterial cells against viral infection by binding to their recognition sequences on the invading DNA and degrading it after extensive ATP-driven translocation. DNA cleavage has been thought to occur as the result of a collision between two translocating enzyme complexes. Using atomic force microscopy (AFM), we show here that *Eco*KI dimerizes rapidly when bound to a plasmid containing two recognition sites for the enzyme. Dimerization proceeds in the absence of ATP and is also seen with an *Eco*KI mutant (K477R) that is unable to translocate DNA. Only monomers are seen when the enzyme complex binds to a plasmid containing a single recognition site. Based on our results, we propose that the binding of *Eco*KI to specific DNA target sequences is accompanied by a conformational change that leads rapidly to dimerization. This event is followed by ATP-dependent translocation and cleavage of the DNA.

INTRODUCTION

Most bacteria produce at least one DNA restriction and modification system by which the bacterium is able to destroy invading DNA from a bacteriophage, while at the same time modifying its own DNA to prevent destruction. The foreign DNA molecules are degraded or cut through the action of host-specific enzymes, endonucleases, which cleave double-stranded DNA after binding to specific target sequences lacking an appropriate pattern of methylation. Protection of the host's own genome is maintained by the methyltransferase activity of the system, which provides individual key residues with methyl groups (Modrich, 1979; Yuan, 1981; Bickle, 1993; Bickle and Kruger, 1993).

Type I restriction/modification enzymes combine both activities in one large protein complex and can be regarded as "smart" molecular machines, as they are capable of performing multiple actions in response to environmental changes. An archetype is the type IA enzyme *Eco*KI, a large, bifunctional protein complex with the ability to detect the methylation state of its DNA target (sK) site and switch between alternative activities accordingly. Binding to unmodified recognition sequences triggers a series of events resulting in degradation of the DNA molecule. DNA with hemimethylated target sites is rapidly modified to fully methylated DNA, with *S*-adenosyl-methionine (SAM) acting as both a cofactor and a methyl donor. Modified DNA is not a substrate for the type I enzymes, and the bound protein quickly dissociates from it. The operation strategy of *Eco*KI requires three subunits, R (134 kDa), M (59 kDa), and S (51 kDa), which are responsible for restriction, modification, and sequence specificity, respectively, forming a 437-kDa enzyme with a stoichiometry of $R_2M_2S_1$ (Dryden et al., 1997).

The restriction endonuclease reaction of type I enzymes is complex, involving ATP-driven translocation and the double-strand cleavage of DNA at remote sites up to several thousands of base pairs from the sK sequence. Electron microscopy has revealed loops of DNA attached to the protein, which remains bound at its sK site (Rosamund et al., 1979; Yuan et al., 1980; Endlich and Linn, 1985). Cleavage occurs on both sides of the sK sequence, and most of the breaks are found approximately halfway between the target sites. The double-stranded cut is believed to be the result of cooperation between two bound *Eco*KI molecules (Shulman, 1974; Studier and Bandyopadhyay, 1988; Szczelkun et al., 1997). Studier and Bandyopadhyay (1988) proposed that cleavage sites are reached when two enzyme molecules collide after ATP-driven translocation of the DNA. In contrast, we have recently provided evidence for an ATP-independent dimerization occurring between two specifically bound enzyme molecules (Ellis et al., 1999). A combination of improved technique and instrumentation has now allowed us to produce images of higher quality and resolution in support of these observations. Here we also show dimerization of the *Eco*KI mutant K477R, which is unable to translocate and cleave DNA. Our observations are incorporated into a model for the cleavage pathway of the *Eco*KI enzyme. We describe how this model can be related to previously reported cleavage patterns of type I restriction/modification systems.

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Drs. Berge and Ellis made an equal contribution to this work.

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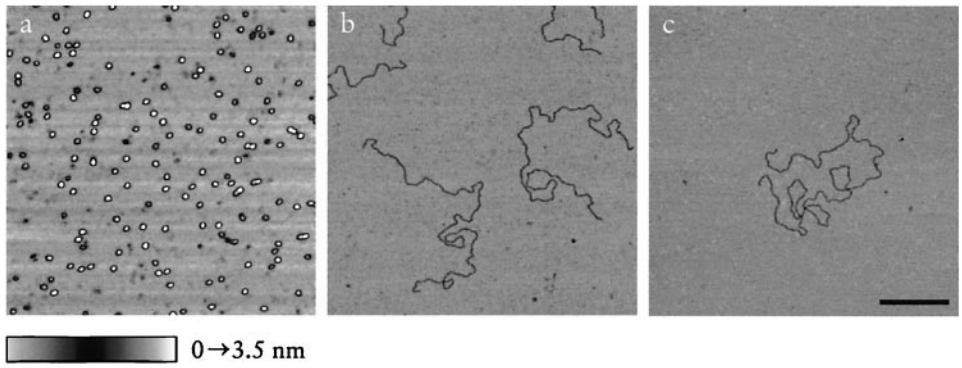

FIGURE 1 Pure *Eco*KI and linear DNA substrates imaged by tapping mode AFM in air. (*A*) *Eco*KI at a concentration of 12 nM adsorbed in the presence of 200 μM SAM. (*B*) Linearized pBRSK15 plasmid (1 sK site, 4.36 kb). (*C*) Linearized pRH3 plasmid (2 sK sites, 6.16 kb). All samples were imaged on poly-L-lysine-coated mica. Scale bar: 200 nm. A color height scale is shown at the bottom.

MATERIALS AND METHODS

Sample preparation

Plasmid DNA with unmodified sK sites was prepared from the modified *E. coli* strain DH5α, from which the *Eco*KI system has been deleted. Linear substrates were produced by cutting the plasmids with *Pvu*II (Sigma, St. Louis, MO) and purified by standard procedures (Sambrook et al., 1989). Proteins were prepared as described (Dryden et al., 1997; Davies et al., 1998). Binding of *Eco*KI and *Eco*KI K477R to the DNA substrates was carried out in 1× buffer A (33 mM Tris-acetate, pH 7.9, 10 mM Mg-acetate, 66 mM K-acetate, and 0.5 mM dithiothreitol) (Boehringer Mannheim GmBH, Mannheim, Germany) at a stoichiometric protein-to-sK ratio of 4:1. All samples were supplied with 200 μM SAM (Sigma) and incubated at 37°C for 10 min. The samples were diluted to a final DNA concentration of 0.1 nM in 1× buffer A supplied with 200 μM SAM, and 50 μl droplets were added to poly-L-lysine (Sigma)-coated muscovite mica (Goodfellow, Cambridge, UK). After 10 min, the sample was rinsed with MilliQ water (Millipore System, Bedford, MA) and air-dried. Dissociation of *Eco*KI into smaller subunits does not occur at concentrations above 20 nM (Dryden et al., 1997), and the dissociation constant is even lower when SAM is present. Pure protein was therefore prepared for imaging by diluting the enzyme to a concentration of 5 nM in the presence of 200 μM SAM and immediately binding it to mica as described above.

Atomic force microscopy imaging

Imaging was performed with a multimode atomic force microscope (Digital Instruments, Santa Barbara, CA). Samples were imaged in air, using tapping mode with a root mean square amplitude of 0.7 V (~9 nm) and a drive frequency of ~300 kHz. Commercially available silicon cantilevers with a specified spring constant of 42 N/m were used (NCH Pointprobes; Nanosensors, Wetzlar-Blankenfeld, Germany).

To ensure that the same resolution was obtained in each image, the status of the tip was constantly assessed, using DNA as a standard. B-form DNA imaged in air by AFM, using the specified tips, typically yielded a diameter of ~8 nm due to tip convolution. Regular measurements of the diameter of the DNA ensured that the change in probe geometry was minimal.

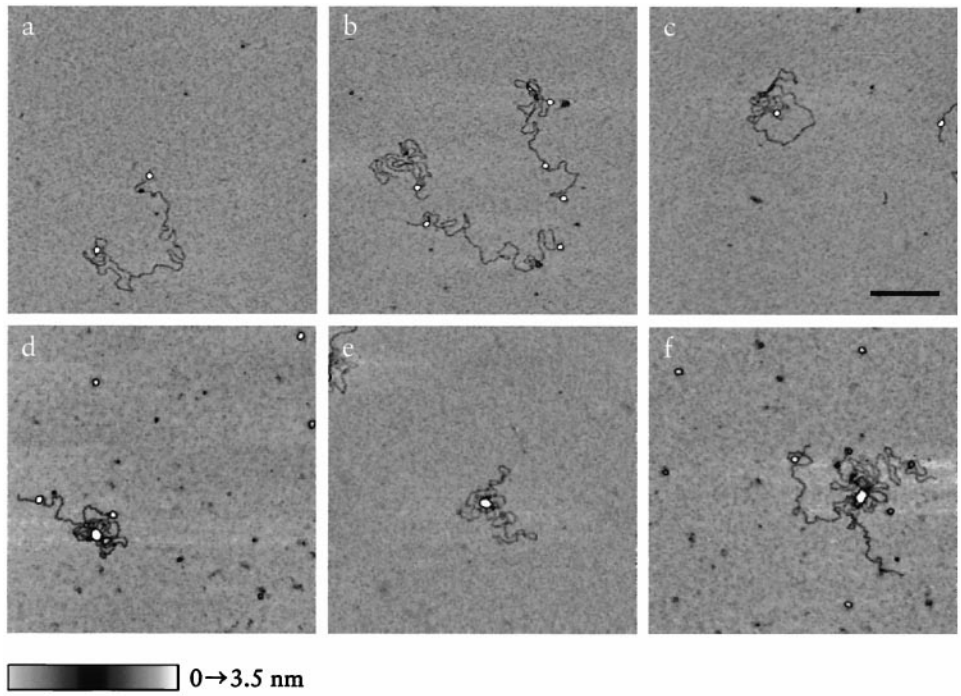

FIGURE 2 *Eco*KI binding to DNA. (*A–C*) *Eco*KI binding to pBRSK15 (1 sK site). Several *Eco*KI complexes are found attached along single DNA molecules, indicating a degree of nonspecific binding. Dimeric complexes are not seen. (*D–F*) *Eco*KI binding to pRH3 (2 sK sites). Images reveal large central structures bound to the plasmids, approximately twice the size of the proteins bound to pBRSK15. Note that only a single dimer is seen to be attached to the DNA molecule. On rare occasions, intermolecular dimers between two separate DNA strands were seen (e.g., *F*). Scale bar: 200 nm.

Molecular volume calculation

The molecular volume of the protein particles was determined from particle dimensions based on AFM images, using the following equation:

$$V_m = (\pi h/6)(3r^2 + h^2)$$

where h is the particle height and r is the radius at half-height (Schneider et al., 1995). The equation treats the protein molecules as spherical segments. Molecular volume based on molecular weight was calculated using the equation

$$V_c = (M_0/N_0)(V_1 + dV_2)$$

where M_0 is the protein's molecular mass, N_0 is Avogadro's number, V_1 and V_2 are the partial specific volumes of protein and water (0.74 cm^3/g and 1 cm^3/g, respectively), and d is the extent of protein hydration (0.4 mol H_2O/mol protein) (Edstrom et al., 1990).

RESULTS AND DISCUSSION

Atomic force microscopy was used to visualize pure complexes of *Eco*KI adsorbed onto poly-L-lysine-coated mica in the presence of the cofactor SAM. Images revealed a homogeneous population of protein particles with a molecular density sufficiently sparse to allow cross-sectional measurements of individual proteins (Fig. 1 *A*). The particle height was 4.10 ± 0.06 nm, and the diameter estimated at half-height was 21.25 ± 0.15 nm (n = 106, mean ± SE). Regarding the protein particles as spherical segments (Schneider et al., 1995, 1998) and using these dimensions, we determined the molecular volume of a single particle to be 771 ± 17 nm^3, in close agreement with the value derived from the molecular mass of the enzyme (747 nm^3). This result indicates that the free enzyme complex behaves like a monomeric particle in the absence of DNA.

To determine the dimensions of individual *Eco*KI particles bound to DNA, the enzyme was incubated with linearized pBRSK15 (4.36 kb), a plasmid with only a single sK site at 1668 bp (Fig. 1 *B*). Linear DNA substrates were preferred to circular supercoiled plasmids because their conformation as viewed by AFM is more open and allows DNA-protein complexes to be recognized unambiguously.

Binding of *Eco*KI was carried out using a molar excess of enzyme over DNA, in the presence of SAM but in the absence of ATP. AFM images show bound protein particles attached along the plasmids (Fig. 2, *A–C*), indicating binding to DNA in addition to binding to the preferred sK site. It has previously been shown that *Eco*KI in the absence of ATP, with or without SAM, is able to bind tightly, even to DNA lacking the recognition sequence (Powell et al., 1998). These particles had a diameter at half-height of 19.91 ± 0.31 nm and a height of 4.16 ± 0.07 nm (n = 65), giving an estimated molecular volume of 685 ± 15 nm^3. This change in volume between free enzyme particles and enzymes bound to DNA is significant ($p < 0.01$) and consistent with other studies that reveal a tighter complex upon binding to DNA (Bickle et al., 1978; Powell et al., 1998). Dimeric complexes were not observed on individual DNA strands, suggesting that binding of the protein to any DNA substrate is not sufficient to induce dimerization.

The plasmid pRH3 (6.16 kb) has two sK sites for *Eco*KI, located at 3458 bp and 5831 bp (Fig. 1 *C*). Images of DNA-enzyme complexes revealed large central structures representing bound proteins (Fig. 2, *D–F*). The structures had a diameter at half-height of 30.40 ± 0.74 nm and a height of 4.16 ± 0.10 nm (n = 28). The molecular volume calculated with these values was 1577 ± 51 nm^3 (Fig. 3). This is approximately twice the volume of unbound *Eco*KI particles, suggesting that dimerization of the protein complexes bound to sK sites had occurred. No more than one dimeric complex was observed on a single DNA molecule, indicating that dimerization requires binding to two separate sK sites. Single *Eco*KI molecules were regularly observed on extended lengths of DNA, whereas dimers were only found on compact DNA structures.

A previous electron microscopic study demonstrated a bimodal distribution of particle sizes on DNA (Bickle et al., 1978). It was proposed that the larger particles were single molecules of *Eco*KI, whereas the smaller particles were

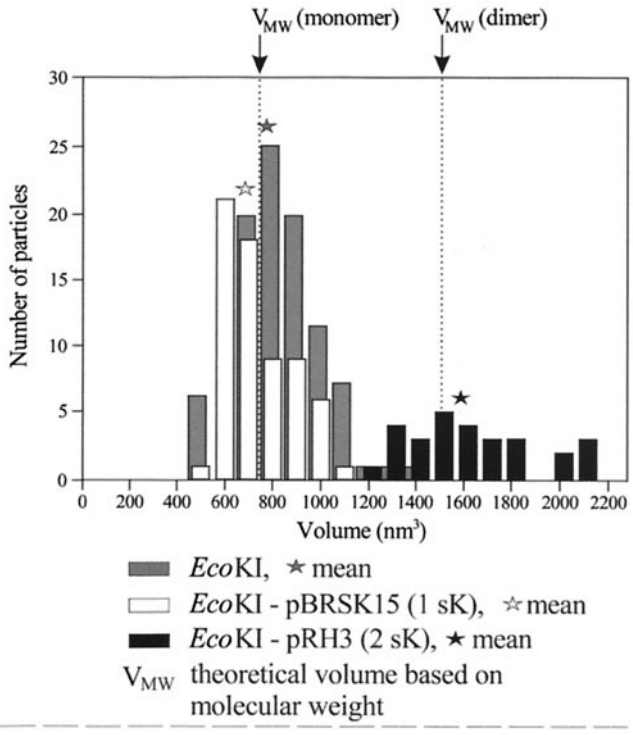

FIGURE 3 Effect of binding to DNA on the volume of *Eco*KI particles. The histogram shows particle volume of free *Eco*KI complexes (*gray*) and *Eco*KI bound to single-site pBRSK15 (*white*) and double-site pRH3 (*black*) plasmids. The corresponding mean volumes are marked by stars. Calculations were based on dimensions determined from the AFM images (see Materials and Methods). The theoretical volumes of monomeric and dimeric complexes indicated are based on the molecular mass of *Eco*KI (437 kDa).

single molecules after the loss of several subunits. Given the large size of the particles observed, it is feasible that the bimodal distribution represented monomers and dimers of *Eco*KI bound to DNA, as suggested by the results of the present study.

Interestingly, intermolecular dimerization was occasionally observed between two enzymes bound to sK sites on two different DNA substrates (Fig. 2 *F*). This was also seen in preparations with pBRSK15, although again this was a rare event. Because binding to specific sK sites is essential for the dimerization process, intermolecular dimerization is expected to be a less frequent event than intramolecular dimerization. The higher degree of mobility and conformational freedom associated with binding to two different DNA molecules provides fewer opportunities for protein-protein contact at the concentrations used in this preparation.

The *Eco*KI K477R nuclease (Davies et al., 1998; Webb et al., 1996) has a single amino acid substitution in the R subunit and shows no ATPase, translocation, or cleavage activities, although it can still exhibit an ATP-induced change in its DNA footprint identical to that of the native enzyme. This protein is therefore ideal for testing whether dimerization is linked to ATP-dependent translocation. AFM images of *Eco*KI K477R bound to the linearized plasmid pBRSK15 (1 sK site) show single protein particles attached to the DNA (Fig. 4, *A–C*), as observed with the wild-type protein. Dimeric protein complexes were not observed. If dimerization is a result of translocation, as previously proposed (Studier and Bandyopadhyay, 1988), the mutant enzyme should not be able to form dimeric complexes on pRH3. However, protein dimers were observed after incubation with pRH3 (2 sK sites), giving support to our proposal that dimerization is a translocation-independent process (Fig. 4, *D–F*). Again, intermolecular dimeric complexes (e.g., Fig. 4 *F*) were found in both samples at a low frequency.

A model for the operation of *EcoK*I

Based on the images presented here, we propose the following model for the cleavage pathway of *Eco*KI (Fig. 5). Binding of *Eco*KI to the DNA substrate rapidly induces dimerization of the protein particles, presumably as a result of conformational changes accompanying their interaction with specific sK sites. Dimerization occurs even in the absence of ATP and is therefore not linked to the translocation process, contrary to previous suggestions (Studier and Bandyopadhyay, 1988). Hydrolysis of ATP allows translocation of DNA from both sides of the enzyme complex, while the complex remains bound specifically to the sK sites. This process results in the formation of extruding and contracting loops of DNA, as previously observed by AFM (Ellis et al., 1999). In the case of a linear plasmid, translocation of the DNA loop trapped between the dimeric complex is expected to stall as a result of extensive supercoiling, resulting in a primary cut approximately halfway between target sites. This cleavage pattern has already been reported for both the type IA enzyme *Eco*KI (Dryden et al., 1997) and the type IC enzyme *Eco*R124II (Dreier et al., 1996). The two free ends of the DNA molecule are translocated at the same rate as the DNA loop contained between

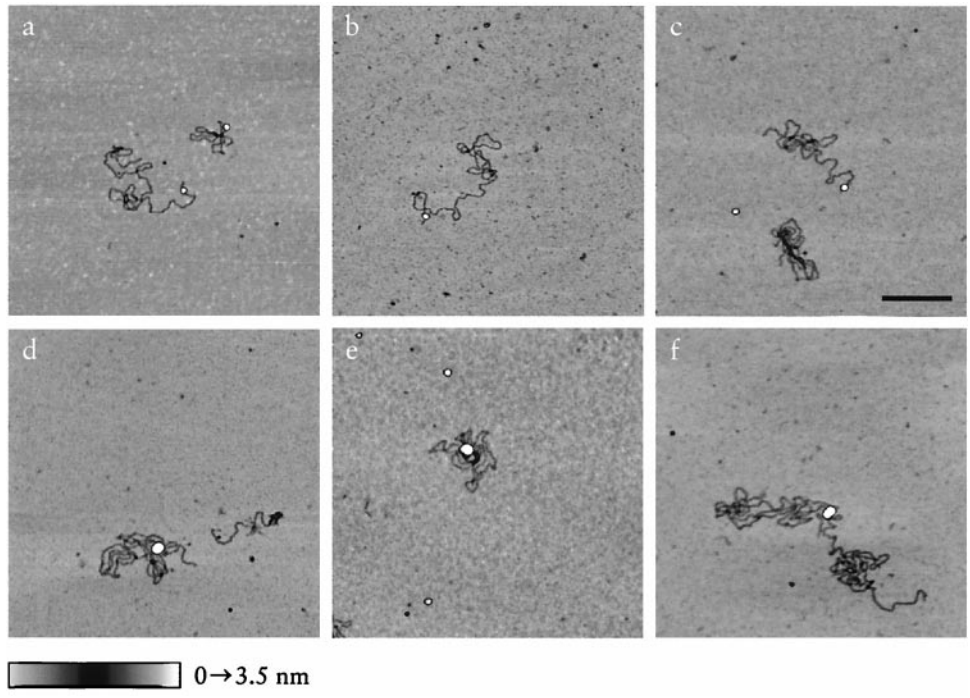

FIGURE 4 *Eco*KI K477R mutant binding to pBRSK15 and pRH3. *(A–C)* Binding to pBRSK15. Single protein particles are observed to be attached along the linear DNA substrates. *(D–F)* Binding to pRH3. The mutant, like the wild-type enzyme, dimerizes when bound to the plasmid. Scale bar: 200 nm.

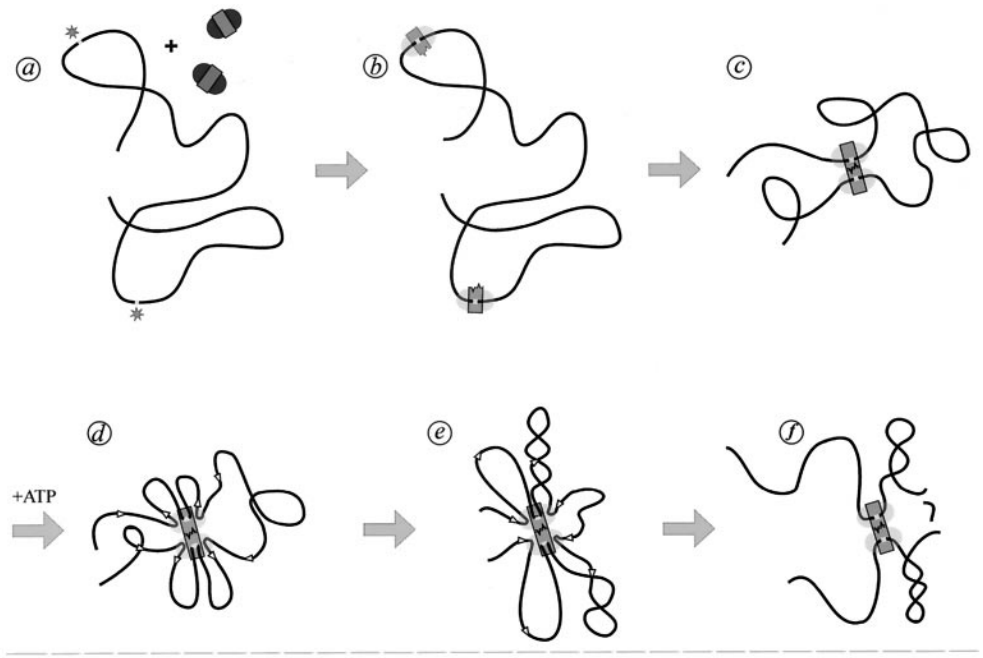

FIGURE 5 Model for the operation of *Eco*KI on linear DNA containing two target sites. (*A*) The DNA substrate has two target sK sites for *Eco*KI. The enzyme has a methylase core (M_2S_1) flanked by two restriction subunits (R). (*B*) Binding to the sK sites induces a conformational change in the enzyme. (*C*) As a result of this change, the specifically bound enzyme particles rapidly form a dimeric complex. (*D*) Addition of ATP initiates a translocation process that causes the extrusion or contraction of loops of DNA from the complex. (*E*) Extensive supercoiling of the emerging DNA loops or a block of further translocation of contracting loops produces a topological barrier, which finally stalls translocation. (*F*) The R subunits now cleave the DNA, either adjacent to the sK sites or at a distant position near the base of the translocated loop. Note that the enzyme complex remains bound to the sK sites throughout the process.

the target sites after dimerization. These ends will eventually slide through the enzyme complex without causing any topological strain and thereby escape cleavage by the R subunit. ATP hydrolysis has been found to continue throughout the process, even after DNA cleavage is completed (Yuan et al., 1980).

According to our model, *Eco*KI processes circular DNA molecules that contain two recognition sites that are much the same as those of linear plasmids. The translocation process leads to supercoiling of the extruding DNA loops, while the contracting DNA is stretched taut between the dimeric *Eco*KI complexes. This forms a topological barrier that stalls the translocation process leading to cleavage of the DNA.

One prediction of the described model is that dimerization can occur between any two occupied sK sites. Intermolecular dimeric complexes were observed at a low frequency on all DNA substrates used throughout this study, consistent with the prediction that protein contacts between enzymes specifically bound to sK sites on separate DNA molecules would be less frequent than contacts between enzymes bound to sK sites on a single substrate.

When *Eco*R124II and another type IC enzyme, *Eco*DXXI, each of which recognizes a different specific DNA target sequence, bind to linear DNA containing one copy of each sequence, it is observed that the two enzymes can cooperate to cleave the DNA, which is refractory to cleavage when only one enzyme is present (Dreier et al., 1996; Janscak et al., 1999). Because these enzymes are members of the same type I family and possess considerable sequence identity, dimerization between them is quite likely. In contrast, it is more difficult to imagine dimerization between the type IA enzyme *Eco*KI and either *Eco*AI (type IB) or *Eco*R124II (type IC), because these pairs of enzymes belong to different families (Bickle and Kruger, 1993; King and Murray, 1994) and therefore share only limited sequence identity (Dryden et al., 1995; Sturrock and Dryden, 1997; Davies et al., 1999; Janscak et al., 1999). Nevertheless, these enzyme pairs are also able to cooperate in cleaving linear DNA containing one target sequence for each enzyme (Janscak et al., 1999). Further experiments are clearly required to resolve the mechanism of cleavage in this case. Despite this potential complication, the results presented here, together with data from dynamic light scattering experiments (Dryden et al., unpublished results), clearly indicate that *Eco*KI can dimerize on sufficiently long pieces of DNA containing two target sequences before DNA translocation and cleavage.

It is possible that the mechanism of "dimerization before translocation" could be used by other type I restriction enzymes such as *Eco*AI and *Eco*R124II. Furthermore, an increasing number of unusual restriction endonucleases are

being found to require either binding at two sites to produce cleavage at one site (e.g., *Nae*I (Topal et al., 1991) and *Eco*RII (Reuter et al., 1998)) or cutting at both sites in a concerted manner (e.g., *Sfi*I (Embleton et al., 1999), *Bcg*I (Kong and Smith, 1998), and type III restriction enzymes (Meisel et al., 1995)). We suggest, therefore, that the type I restriction enzymes are archetypes of the large and diverse class of protein assemblies, the function of which depends on the ability to interact simultaneously with two or more sites on a DNA molecule (Rippe et al., 1995).

CONCLUSION

AFM has been used to investigate the binding of the type I restriction/modification enzyme *Eco*KI to various DNA substrates. Images reveal a dimerization of protein particles bound at specific sites in the absence of ATP, a phenomenon that is also seen with an *Eco*KI mutant that is unable to translocate DNA. Based on these results, we present a new model for the cleavage pathway of *Eco*KI involving a rapid dimerization as the result of a conformational change induced by specific binding to the recognition sites, followed by the ATP-dependent translocation and cleavage of the DNA.

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REFERENCES

Bickle, T. A. 1993. The ATP-dependent restriction endonucleases. *In* Nucleases. E. M. Linn, R. S. Lloyd, and R. J. Roberts, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 89–109.

Bickle, T. A., C. Brack, and R. Yuan. 1978. ATP-induced conformational changes in the restriction endonuclease from *Escherichia coli* K-12. *Proc. Natl. Acad. Sci. USA.* 75:3099–3103.

Bickle, T. A., and D. H. Kruger. 1993. Biology of DNA restriction. *Microbiol. Rev.* 57:434–450.

Davies, G. P., I. Martin, S. S. Sturrock, A. Cronshaw, N. E. Murray, and D. T. F. Dryden. 1999. On the structure and operation of type I restriction enzymes. *J. Mol. Biol.* 290:565–579.

Davies, G. P., L. M. Powell, J. L. Webb, L. P. Cooper, and N. E. Murray. 1998. *Eco*KI with an amino acid substitution in any one of seven DEAD-box motifs has impaired ATPase and endonuclease activities. *Nucleic Acids Res.* 26:4828–4836.

Dreier, J., M. P. MacWilliams, and T. A. Bickle. 1996. DNA cleavage by the type IC restriction-modification enzyme *Eco*R124II. *J. Mol. Biol.* 264:722–733.

Dryden, D. T. F., L. P. Cooper, P. H. Thorpe, and O. Byron. 1997. The in vitro assembly of the *Eco*KI type I DNA restriction/modification enzyme and its in vivo implications. *Biochemistry.* 36:1065–1076.

Dryden, D. T. F., S. S. Sturrock, and M. Winter. 1995. Structural modelling of a type I DNA methyltransferase. *Nature Struct. Biol.* 2:632–635.

Edstrom, R. D., M. H. Meinke, R. Yang, X. Yang, V. Elings, and D. F. Evans. 1990. Direct visualization of phosphorylase-phosphorylase kinase complexes by scanning tunneling and atomic force microscopy. *Biophys. J.* 58:1437–1448.

Ellis, D. J., D. T. F. Dryden, T. Berge, J. M. Edwardson, and R. M. Henderson. 1999. Direct observation of DNA translocation and cleavage by atomic force microscopy. *Nature Struct. Biol.* 6:15–17.

Embleton, M. L., S. A. Williams, M. A. Watson, and S. E. Halford. 1999. Specificity from the synapsis of DNA elements by the *Sfi*I endonuclease. *J. Mol. Biol.* 289:785–797.

Endlich, B., and S. Linn. 1985. The DNA restriction endonuclease of *Escherichia coli* B. II. Further studies of the structure of DNA intermediates and products. *J. Biol. Chem.* 260:5729–5738.

Janscak, P., M. P. MacWilliams, U. Sandmeier, V. Nagaraja, and T. A. Bickle. 1999. DNA translocation blockage, a general mechanism of cleavage site selection by type I restriction enzymes. *EMBO J.* 18: 2638–2647.

King, G., and N. E. Murray. 1994. Restriction enzymes in cells, not Eppendorfs. *Trends Microbiol.* 2:465–469.

Kong, H., and C. L. Smith. 1998. Does *Bcg*I, a unique restriction endonuclease, require two recognition sites for cleavage? *Biol. Chem.* 379: 605–609.

Meisel, A., P. Mackeldanz, T. A. Bickle, D. H. Kruger, and C. Schroeder. 1995. Type III restriction endonucleases translocate DNA in a reaction driven by recognition site-specific ATP hydrolysis. *EMBO J.* 14: 2958–2966.

Modrich, P. 1979. Structures and mechanisms of DNA restriction and modification enzymes. *Q. Rev. Biophys.* 12:316–369.

Powell, L. M., D. T. F. Dryden, and N. E. Murray. 1998. Sequence-specific DNA binding by *Eco*KI, a type IA DNA restriction enzyme. *J. Mol. Biol.* 283:963–976.

Reuter, M., D. Kupper, A. Meisel, C. Schroeder, and D. H. Krueger. 1998. Cooperative binding properties of restriction endonuclease EcoRII with DNA recognition sites. *J. Biol. Chem.* 273:8294–8300.

Rippe, K., P. H. von Hippel, and J. Langowski. 1995. Action at a distance: DNA-looping and initiation of transcription. *Trends Biochem. Sci.* 20: 500–506.

Rosamund, J., B. Endlich, and S. Linn. 1979. Electron microscopic studies of the mechanism of action of the restriction endonuclease of *Escherichia coli* B. *J. Mol. Biol.* 129:619–635.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Schneider, S. W., G. Folprecht, G. Krohne, and H. Oberleithner. 1995. Immunolocalization of lamins and nuclear pore complex proteins by atomic force microscopy. *Pflügers Arch.* 430:795–801.

Schneider, S. W., J. Lärmer, R. M. Henderson, and H. Oberleithner. 1998. Molecular weights of individual proteins correlate with molecular volumes measured by atomic force microscopy. *Pflügers Arch.* 435: 362–367.

Shulman, M. J. 1974. Model for wandering restriction enzymes. *Nature.* 384:379–383.

Studier, F. W., and P. K. Bandyopadhyay. 1988. Model for how type I restriction enzymes select cleavage sites in DNA. *Proc. Natl. Acad. Sci. USA.* 85:4677–4681.

Sturrock, S. S., and D. T. F. Dryden. 1997. A prediction of the amino acids and structures involved in DNA recognition by type I DNA restriction and modification enzymes. *Nucleic Acids Res.* 25:3408–3414.

Szczelkun, M. D., P. Janscak, K. Firman, and S. E. Halford. 1997. Selection of non-specific cleavage sites by the type IC restriction endonuclease *Eco*R124I. *J. Mol. Biol.* 271:112–123.

Topal, M. D., R. J. Thresher, M. Conrad, and J. Griffith. 1991. *Nae*I endonuclease binding to pBR322 DNA induces looping. *Biochemistry.* 30:2006–2010.

Webb, J. L., G. King, D. Ternent, A. J. B. Titheradge, and N. E. Murray. 1996. Restriction by *Eco*KI is enhanced by cooperative interactions between target sequences and is dependent on DEAD box motifs. *EMBO J.* 15:2003–2009.

Yuan, R. 1981. Structure and mechanism of multifunctional restriction endonucleases. *Annu. Rev. Biochem.* 50:285–315.

Yuan, R., D. L. Hamilton, and J. Burckhardt. 1980. DNA translocation by the restriction enzyme from *E. coli* K. *Cell.* 20:237–244.