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'Interactive' recognition in *Eco*RI restriction enzyme-DNA complex

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ABSTRACT

A solution study of interaction between DNA and EcoRI restriction enzyme shows that there is a definite distortion of DNA in the specific recognition complexes but no measurable DNA distortion in the non-specific interaction.

INTRODUCTION

Interaction between DNA and protein, which plays an important role in all living cells, can be divided into two categories. In specific recognition, a protein recognizes a particular DNA sequence several orders of magnitude tighter than in non-specific recognition, where the protein recognizes primarily the general features with little or no specificity for a particular base sequence. Interaction between the EcoRI restriction enzyme (1, 2) and DNA provides a good example of both recognitions.

In specific recognition the mechanism of how a protein finds a particular sequence of DNA is under intense study. The current view is that, initially, protein binds to DNA non-specifically, and then finds a cognate DNA sequence by sliding, short hopping and/or inter-strand jumping (3, 4, 5, 6). The question we would like to address is what happens to DNA after a protein binds to its cognate DNA sequence in solution. Two consequences can arise. In one the protein may simply stop at the cognate DNA sequence without substantial conformational changes in DNA and/or protein (we'll call this "passive recognition"), and in the other, there may be an interactive process of recognition or selection between protein and DNA resulting in a conformation of DNA and/or protein different from the average conformation in free solution ("interactive recognition"). These two processes are schematically shown in Figure 1. (The same question can be asked for non-specific interaction as well.) Depending on the function of the protein subsequent to the recognition step, additional conformational changes of DNA and/or protein may occur.

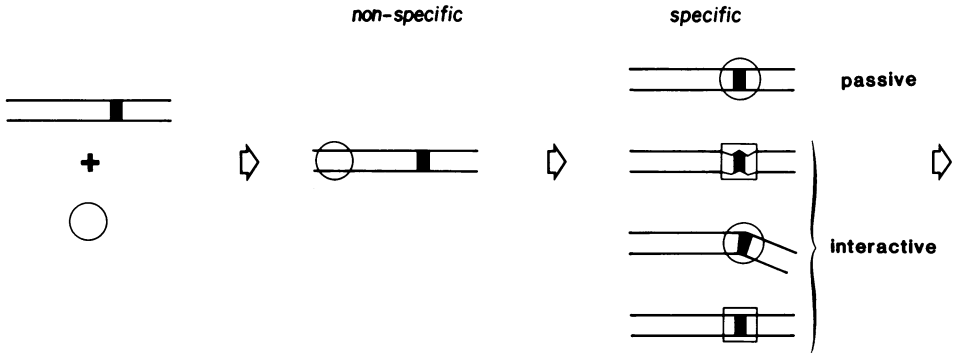

Figure 1. Schematic drawing to show the distinction between "passive" and "interactive" recognition. Initially, a protein is assumed to bind to DNA non-specifically without any conformational changes on DNA or protein. The protein then slides or short-hops along the DNA and finds a cognate DNA sequence. In passive recognition, the protein simply stops at the cognate site in the average conformation of free DNA. In interactive recognition, the conformation of DNA and/or protein in the recognition complex is different from the average conformation of free DNA and/or protein.

We report our observation that there is a definite distortion of DNA in the specific recognition complex between EcoRI endonuclease and DNA but no detectable distortion of DNA in the non-specific recognition complex. As a measure of conformational change on DNA, the angle of topological unwinding (or winding) of circular plasmid DNA was measured by electrophoresis on agarose gel according to the methods developed by Keller and Wandell (7) and exploited so well by various investigators (for examples, see 8, 9).

AMPLIFICATION OF SMALL DISTORTION

In the initial recognition step of EcoRI restriction enzyme with its cognate DNA, the magnitude of the conformational changes, if any, is likely to be small. To amplify the signal from small topological winding/unwinding of DNA, we have constructed a plasmid, pRK112-8 (19), containing 19 EcoRI sites (see Figure 2). Each EcoRI site within the cluster of 6 sites is separated by a 34 base-pairs long synthetic DNA fragment (10), thus the center-to-center distance between two adjacent EcoRI sites is 41 base-pairs. Since plasmids containing tandem sequences are often unstable, we introduced a "spacer sequence" (0.85 KB fragments) between clusters to stabilize the plasmids. Furthermore, we chose the plasmid that has the head-to-tail joining between clusters, an orientation known to minimize deletion. The stability of this plasmid is manifested by one set

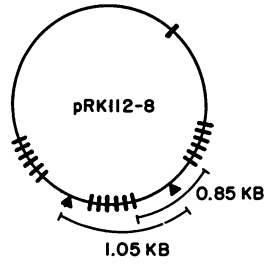

Figure 2. The plasmid pRK112-8 has 19 EcoRI sites, indicated by short bars. Any two adjacent EcoRI sites within the cluster of six sites is separated by a 34 base-pair long synthetic DNA. The size of this plasmid is 7.5 KB (19).

of sharp, unique, and predictable restriction fragments for each of several restriction enzymes, even after being stored for over a year. For our purpose, the relative orientation of each cluster with respect to each other in the plasmid is not important since the handedness of winding or unwinding of DNA is independent of the orientation of the inserts.

EXPERIMENTS AND RESULTS

All the plasmid DNAs are prepared by buoyant density methods (11). To test for topological changes induced by EcoRI restriction enzyme, 0.3 μg of supercoiled or pre-relaxed plasmid DNA was mixed with various concentrations of the enzyme (prepared according to Modrich and Zabel [11]) in 15 μl of Buffer A (100mM Tris-HCl pH7.4, 80mM NaCl, 2 mM DTT, and 100 μg/ml bovine serum albumin) and incubated at 37°C for 30 minutes. In the absence of Mg ions, the enzyme binds the cognate DNA sequence specifically without cleaving it (12). The sample was then transferred to a 4°C bath and one unit (the amount of topoisomerase I required to totally relax one μg of supercoiled DNA at 4°C in 1 hour in buffer A) of HeLa topoisomerase I was added and incubated for 2 hours. One more unit of the topoisomerase was added to make sure relaxation was complete and incubated for one more hour. The reaction was terminated by bringing the solution to 20mM EDTA, 24% sucrose, 5% sodium dodecyl sulfate, and 0.075% bromophenol blue. The DNA was subjected to electrophoresis at 37° for 20 hours in an 0.8% agarose gel in 40mM Tris-HCl (pH7.9), 20mM sodium acetate, 1mM EDTA. The voltage gradient was 1.5V/cm. The gels were stained in a 2 μg/ml ethidium bromide solution, illuminated with a short-wave ultraviolet light and photographed on Polaroid Type 665 positive-negative film. The negatives were traced on

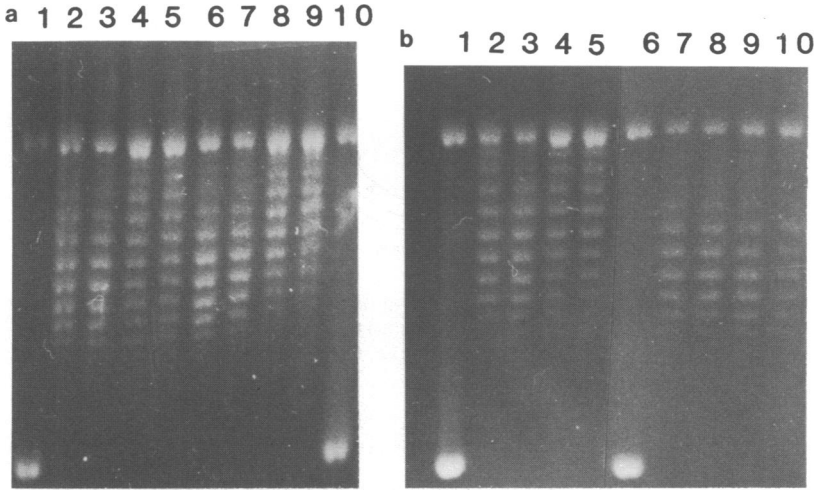

Figure 3. Topoisomer distribution patterns obtained by gel electrophoresis at 37°C. All the nicking/closing reactions were done at 4°C. (a) Lanes 1 and 10 contain supercoiled (bottom band) and nicked (top band) pRK112-8. lanes 2-5 are pre-relaxed pRK112-8 DNAs that are nicked and closed in the presence of 0, 0, 7, and 10 molar equivalents of EcoRI restriction enzyme dimer per site. Lanes 2 and 3 are control lanes. Lanes 6-9 are the same as lanes 2-5 except that the starting DNAs are supercoiled pRK112-8. (b). Lanes 1 and 6 contain supercoiled (bottom band) and nicked (top band) pRK112-8. Lanes 2-5 are supercoiled pRK112-8 DNAs that are nicked and closed in the presence of 0, 0, 7 and 10 molar equivalents of EcoRI restriction enzyme dimer per restriction site. Lanes 7-10 are the same as lanes 2-5 except that the DNAs were methylated by EcoRI methylase.

a Joyce Loebl microdensitometer.

The association constant between the endounclease and DNA in the absence of Mg^{++} ions, and in a comparable ionic strength at 37°C is 1.9×10^{11} M^{-1} for pBR322 with one EcoRI site, and 3.9×10^{11} M^{-1} at 4°C (13). At the saturation point, under our experimental condition, more than 99.9% of EcoRI sites should be occupied by the endonuclease.

As shown in Fig. 3a (lanes 6-9), the enzyme binding induced a significant shift in band pattern toward the direction corresponding to topological unwinding of the DNA (the same direction as the band pattern of heat-induced unwound DNA) The same results were obtained when pRK112-8 was pre-relaxed prior to the addition of EcoRI restriction enzyme and the subsequent treatments with the HeLa topoisomerase I (Figure 3a, lanes 2-5). This indicates that the band pattern shift observed is due to EcoRI restriction enzyme binding and not to inhibition of the HeLa topoisomerase I

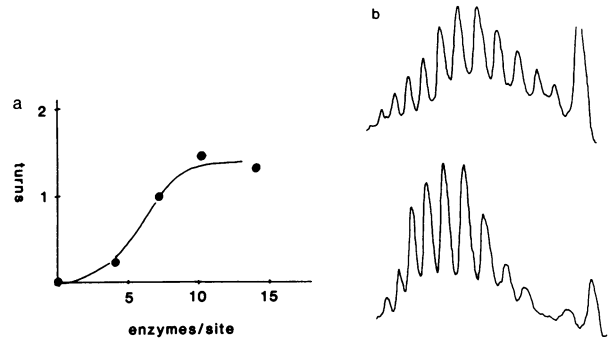

Figure 4 (a). Under our experimental conditions, addition of the EcoRI restriction enzyme at the molar ratio of about 8 dimers per site in solution saturates all the EcoRI sites on DNA. Further addition of the restriction enzyme does not shift the band pattern until the point where the topoisomerase I activity is inhibited. One example of the saturation behavior is shown here. (b). An example of a microdensitometer tracing of pRK112-8 DNAs that were nicked and closed in the presence of 0 (bottom figure) and 10 (top figure) molar equivalents of the restriction enzyme dimer per site. The right-most peaks correspond to the nicked DNA at the top of the gel lanes scanned. To locate the center of the Gaussian distribution of the peaks in each scan, individual peaks from the microdensitometer tracing were cut out and weighed, and the weighted moments for all peaks were summed.

by EcoRI restriction enzyme nor to the supercoil state of the DNA. With pBR322, which contains one EcoRI site, there was no detectable shift induced in the band pattern (results not shown). Furthermore, when the pRK112-8 DNA was methylated by EcoRI methylase there was also no detectable band shift (Figure 3b, lanes 7-10) consistent with the observation that EcoRI restriction enzyme does not specifically recognize the restriction site which is premethylated by EcoRI methylase although the enzyme still binds to DNA non-specifically (13).

An average of eight experiments showed that there is DNA unwinding of 1.31 topological turns per plasmid pRK112-8 at saturating amounts of the restriction enzyme. This corresponds to an average topological unwinding of 25^{o} per EcoRI restriction site with a standard deviation of 5^{o}. Examples of a titration curve and a densitometer scan of a band pattern are shown in Figure 4.

In order to determine the number of EcoRI restriction enzyme molecules that can bind simultaneously to their specific sites in each cluster of six sites, we used a method described by Fried and Crothers (14). For this purpose a 1.05 KB HaeIII fragment containing six EcoRI restriction sites

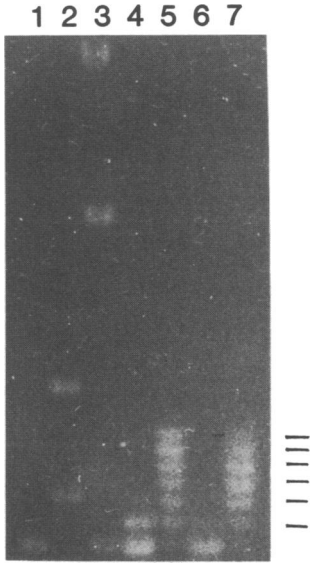

Figure 5. Binding of EcoRI restriction enzyme to a 1.05 KB fragment containing 6 EcoRI sites. Lanes 1, 4, and 5 are 1.05 KB fragments with 0, 4, and 10 molar equivalents of EcoRI enzyme dimers per site. Lanes 6 and 7 are the same as 4 and 5, with reduced amount of DNA. Lanes 2 and 3 are DNA size markers. Lanes 5 and 7 show six discrete bands in addition to the 1.05 KB band. They are arranged in a logarithmic fashion.

and an 0.85KB spacer fragment were used (see pRK112-3 in Figure 2). The 0.85 KB fragment has practically the same sequence as the 1.05KB fragment but lacks the EcoRI restriction sites. To 0.1 ug of DNA in buffer A, EcoRI restriction enzyme at two different concentrations (0.285 μM and 0.65 μM), which are equivalent to four and ten EcoRI restriction enzyme dimers per EcoRI restriction site, were added. The samples were very gently mixed by hand and incubated at 37°C for one hour. To the sample, 2.5 ul of a 10 μg/ml xylene cyanol FF solution in 5% glycerol was added and loaded immediately on a 1% TBE (45mM Tris HCl, pH8.3, 45mM Boric Acid, 1.25mM EDTA) agarose gel and electrophoresis run at 5V/cm for 10 hours.

As shown in Figure 5, upon addition of saturating amounts of EcoRI restriction enzyme to the 1.05KB fragment, six discrete bands can be resolved that migrate slower than the starting DNA fragment in a logarithmic fashion. The simplest interpretation is that the six bands correspond to DNA with one to six bound enzymes. This shows that all six sites on the 1.05 KB fragment are simultaneously available for the enzyme

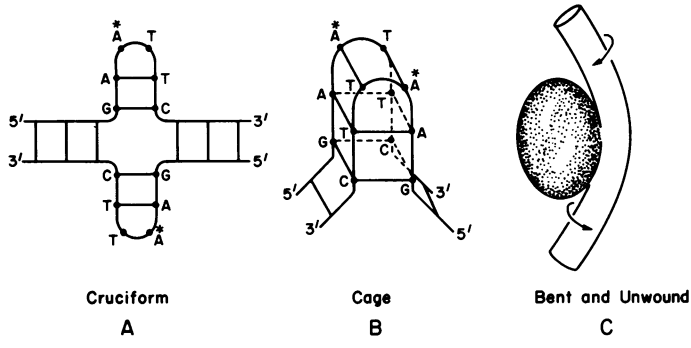

Figure 6. Models for DNA conformation in EcoRI restriction enzyme-DNA complex. (a) Cruciform. (b) Cage model. (c) Bent and unwound model.

binding. On the other hand, with the 0.85KB fragment (which has no EcoRI site) no such discrete bands are observed. Instead only a smeared band due to non-specific binding could be detected (data not shown).

DISCUSSION

The average topological unwinding angle of 25° rules out two models proposed for the EcoRI restriction enzyme-DNA complex: the cage model (Fig. 6) in which six base-pairs of the restriction sequence form a four-stranded cage (15, 16), with a net increase of total hydrogen bonds, and the cruciform model (Figure 6; 17, 18). Such cage or cruciform structure should produce unwinding of close to 180° per site, far too large compared to the observed values.

We have previously shown that in the lactose operator-repressor system, a sequence specific binding of lac repressor to lac operator unwinds DNA by 55° per site, but no unwinding was observed for the non-specific binding of lac repressor (19). Thus in both recognition systems we have studied, the specific recognition appears to induce a conformational change of DNA (interactive recognition) corresponding to duplex unwinding and/or bending that causes negative supercoiling (Figure 6c) while non-specific binding does not.

Since the submission of our manuscript, Frederick et al. showed that the DNA in a crystalline complex between EcoRI endonuclease and a short duplex DNA is bent and unwound (20), which is consistent with our observation in solution.

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