Differences in the Kinetic Properties of *BamHI* Endonuclease and Methylase with Linear DNA Substrates*

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**BamHI** endonuclease and methylase were found to exhibit a kinetic preference for linear pBR322 DNA substrates containing the recognition site in a central location. The $K_m$ values for substrates having the recognition site in a terminal location were approximately 3-fold greater than those with a centrally located site. This phenomenon may be partially due to facilitated transfer of the enzymes to the recognition site over nonspecific flanking sequences. The exploitation of facilitated transfer by these enzymes has been inferred from studies demonstrating kinetic preferences for longer DNA substrates. The reaction rates of the endonuclease were 9-fold greater with full-length pBR322 DNA than with a 74-base pair derivative. The methylase exhibits a kinetic preference for longer substrates but only under conditions of comparatively higher DNA concentrations. In addition, the methylase has the property of increasing long chain preference with increasing salt concentrations up to 120 mM. Increasing salt concentrations decreased the endonuclease's preference for longer substrates. Nonspecific inhibition studies revealed qualitative and quantitative differences between the two enzymes under catalytic conditions. These studies suggest that *BamHI* endonuclease and methylase interact with nonspecific DNA in different ways.

The restriction-modification system of *Bacillus amyloliquefaciens* H consists of an endonuclease and a methylase that recognize the duplex symmetrical sequence 5'-GGATCC-3'. The endonuclease catalyzes double-stranded cleavage between the guanines, and the methylase catalyzes the transfer of the methyl group of S-adenosyl-L-methionine to the C$^5$ position of the internal cytosines (1, 2). Methylation prevents cleavage by the endonuclease. We have purified both enzymes to apparent homogeneity and have investigated their structural and catalytic properties (3–6). The cognate sequence specificity of these two different proteins has prompted several comparative studies in our laboratory.

There are several structural features of DNA that could affect the reaction rates of a restriction endonuclease and its cognate methylase. Perturbations in secondary structure such as Z-DNA and cruciforms have been found to adversely affect the reaction rates of certain restriction-modification enzymes (7, 8). The nature of flanking sequences immediately adjacent to the recognition site appear to influence the kinetic behavior of EcoRI, *BamHI*, and *PstI* endonucleases and dam methylase (9–12). However, nonspecific flanking DNA that is located over 1000 base pairs away from a recognition site also has large effects on the kinetic ability of EcoRI endonuclease to form specific enzyme-substrate complexes (13). These long-distance effects have been theoretically explained by thermally driven facilitated diffusion mechanisms that can involve the linear transfer of a protein along the DNA contour length, intradomain dissociation-association events, and intersegment exchange of a protein between areas of the DNA polymer brought into close proximity by conformational fluxes (14). Such intramolecular-facilitated transfer mechanisms are expected to enhance specific binding rate constants of a protein since they are inherently faster than random sampling by three dimensional diffusion. Utilization of facilitated diffusion may be of adaptive value to restriction-modification enzymes, RNA polymerases in the targeting of promoters (15), and repressors (16, 17) considering the great molar excess of nonspecific sequences and the small diffusion constants of large, solvated DNA molecules. In this article we report the effects on the reaction rates of *BamHI* endonuclease and methylase as the distance between the recognition site and a DNA terminus is changed. Some of the kinetic observations described can be qualitatively explained in terms of facilitated diffusion. However, the methylase exhibits unique behavior that differs from the endonuclease with respect to salt and substrate concentrations. Implications on the nonspecific interactions with DNA are discussed. Preliminary results on this work have been previously reported (18).

**EXPERIMENTAL PROCEDURES**

**Materials**

Bacterial alkaline phosphatase, *T. pyogenes* polynucleotide kinase, *Bal-31* nuclease, agarose, acrylamide, NACS$^2$ resins and restriction endonucleases (unless otherwise noted) were purchased from Bethesda Research Laboratories. (${\gamma}^32P$)ATP (2900 Ci/mmol) was purchased from New England Nuclear. AdoMet and ethidium bromide were from Sigma. Proteinase K was purchased from Boehringer Mannheim. All other reagents were of the highest available purity.

**Methods**

**Enzyme Preparation and Reaction Conditions**—*BamHI* endonuclease and methylase were purified to homogeneity according to published procedures (3, 6). *BglI* endonuclease was purified as previously reported (19). *BamHI* endonuclease reactions contained 50 mM Tris-HCl, pH 7.5, 10 mM MgCl$_2$, 2 mM mercaptoethanol, 100 µg/ml

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* The abbreviations used are: NACS, Bethesda Research Laboratories trademark for Nucleic Acids Chromatography System; Form I, closed circular DNA.
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RESULTS

The reaction rates of BamHI endonuclease and methylase were examined with linear pBR322 DNA substrates having the BamHI cleavage site 375 or 1921 bases from the nearest end (EcoRI and NdeI linearized, respectively). Under standard reaction conditions and a DNA concentration of 12 nM the endonuclease and methylase exhibited faster reaction rates as the recognition site was located further from an end (Figs. 1, A and D). This kinetic preference was independent of enzyme concentrations in the range of 0.03 to 2 nM. Cleavage and methylation rates with PstI- and Aval-linearized pBR322 DNA (1125 bases 5' and 1049 bases 3' to the BamHI cleavage site, respectively) were identical within experimental error and were intermediate between those rates obtained with the EcoRI- and NdeI-linearized substrates. Increasing concentrations of NaCl decreased the differences between cleavage rates (Fig. 1B) and any kinetic preference for a centrally located site was abolished at a NaCl concentration of 160 mM (Fig. 1C). In contrast, a difference of approximately 2-fold remained between methylation rates at NaCl concentrations in the range of 100 to 200 mM (Fig. 1, E and F). In addition, the overall methylation rates were fastest at a NaCl concentration of 100 mM which is a result previously observed with supercoiled substrates (6).

Initial velocity kinetics were used to investigate the substrate concentration dependence of the observed reaction rate differences in the range of 1.5 to 35 nM DNA in the absence of added NaCl. Reciprocal plots revealed that the endonuclease and methylase have $K_a$ values for NdeI-linearized pBR322 DNA of 3.6 and 3.2 nM, respectively. The $K_a$ values for EcoRI-linearized DNA were 12.5 nM for the endonuclease and 11.2 nM for the methylase. Each enzyme had the same $V_{max}$ with both substrates. These results suggest that binding is more favorable for a centrally located recognition site. No change in the methylase kinetics with AdoMet were observed when either DNA was used as the nonvarying substrate.

The reaction rate differences described for the endonuclease and methylase at low salt concentrations could originate from one or more types of facilitated diffusion. If these enzymes can locate their recognition sites by a sliding mechanism, then an enhancement of this process would be expected to occur as the recognition site is located in a more central position. This would be a manifestation of the proportionately larger target area for nonspecific binding around the BamHI site. Implicit in this assumption is that the average scanning length of the enzymes is less than 3987 and greater than 375 base pairs (the longest and shortest distances between a DNA terminus and the BamHI cleavage site on EcoRI-linearized pBR322 DNA). A sliding mechanism has been implicated in the EcoRI endonuclease reaction as judged by increases in the specific association and dissociation rate constants with increasing DNA chain length up to approximately 4000 base pairs (13). The average scanning length was determined to be 1300 base pairs. In addition, the kinetics of cleavage were faster with longer substrates. This phenomenon, along with the increases in specific association rate constants with increasing substrate length, can be a result of the larger target area for nonspecific binding and the subsequent facilitated transfer of the enzyme to the recognition site.

In order to ascertain if facilitated diffusion is kinetically evident for BamHI, endonuclease and methylase fragments of pBR322 DNA were prepared with an attempt to place the BamHI site in a central location (Table I). Under standard reaction conditions and DNA concentrations of 1 nM, the initial rate of cleavage increased with increasing substrate length (Fig. 2). However, in the presence of 160 mM NaCl the

bovine serum albumin, and 0.1% Tween 20. The methylation reaction mixture contained 0.25 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 5 mM 2-mercaptoethanol, 100 pg/ml bovine serum albumin, 0.1% Tween 20, and 35 mM AdoMet. Both enzymes were assayed at 37 °C. AdoMet was purified as previously described (6). Stock solutions of Tween 20 were made 0.0001% butylated hydroxy-toluene and sodium metabisulfite and stored in the dark.

DNA—pBR322 DNA was purified from Escherichia coli HB101 using base-acid extraction and chromatography on NACS-37 resin (20). Form I pBR322 DNA was linearized with either NdeI or EcoRI endonucleases. This produced full-length molecules with the BamHI cleavage site 1921 or 375 bases from the nearest end. The protein in all preparative restriction digests was removed by the addition of proteinase K to a concentration of 100 μg/ml (incubated for 0.5 h at 37 °C) and phenol-chloroform extraction. The DNA was ethanol precipitated in the presence of 2.5 M ammonium acetate and dried under vacuum. The DNA was subsequently purified by chromatography on NACS-57 resin. Restriction fragments 1000 base pairs or greater were isolated from agarose gels by electroelution. Smaller fragments were isolated from 8% polyacrylamide gels. The DNA fragments were filtered through Millipore G-50 0.22 μm units (Millipore), concentrated, and purified on NACS-57 resin. DNA was prepared for gel electrophoresis at 366 nm after ethidium bromide staining. DNA fragments were also end-labeled with [32P]dATP and the unincorporated ATP was removed on NACS-57 resin. Specific activities ranged from 9.6 × 106 to 1 × 107 cpm/μg DNA.

Protein and DNA Quantitation—Protein was determined by the method of Bradford (21) using bovine gamma globulin as a standard. DNA was determined at 260 nm.

**Gel Electrophoresis**—DNA cleavage products were fractionated on 0.8–1% agarose gels at 4 V/cm or 8% polyacrylamide gels in the Tris-acetate buffer system (22) at 8 V/cm for 2–4 h.

**Quantitation of Reaction Products**—Densitometric analysis was performed by a modification of published procedures (23, 24). Gels were stained in 5 μg/ml of etidium bromide for 15 min and photographed for gel at 366 nm. Gels were exposed to ultraviolet light, dried, and scanned at 595 nm in a Bio-Rad 1650 densitometer equipped with a Spectro Physics SF4270 integrator. Film response was linear from 1 to 400 ng of DNA. Film calibration was performed with Ecoli or HindIII digestion products of λ-DNA (agarose) or HaelIII digestion products of phiX174RF DNA (polyacrylamide). No significant differences in fluorescence was detected between known amounts of various fragments of pBR322 DNA generated by combinations of BamHI, EcoRI, Aval, and NdeI endonucleases as judged by calibration curves. Quantitation was performed by calculating the percent of a given species of DNA in the total digest or by comparison to known amounts of restriction fragments run in parallel. Alternatively, radioactive cleavage products were excised from gels and counted in a Packard liquid scintillation spectrometer. Both methods of analysis showed good agreement.

**Enzyme Assays**—Ten-microliter aliquots from reactions containing BamHI endonuclease and full-length linear pBR322 DNA were placed at 85 °C for 1 min. Time course studies indicated that this temperature irreversibly deactivated catalytic amounts of the endonuclease within 10 s. BamHI endonuclease and methylase reactions containing EcoRI-linearized DNA were subjected to secondary digests with 5 units of Aval endonuclease or 0.3 μg of DNA at 45° for 0.5 h. This produced fragments that could be readily fractionated and quantitated. BamHI methylase reactions were quenched with MgCl2 to a final concentration of 25 mM. Twenty units of BamHI endonuclease were added per μg of DNA, and the mixture was incubated for 1 h at 37 °C. Controls were identical, except no methylase was present, and showed complete cleavage of DNA by BamHI and Aval endonuclease at all salt concentrations and with all types of linear substrates used in these studies. Unit assays showed that the activity of BamHI and Aval endonuclease was not affected in the presence of 35 mM AdoMet, BamHI methylase, and MgCl2. The initial velocity of BamHI methylation was linear with respect to enzyme concentration from 0.03 to 1.2 nM in the presence of saturating substrates (25 nM Form I pBR322 DNA and 35 mM AdoMet). The velocity of endonuclease cleavage was linear with enzyme concentration from 0.03 to 1 nM in the presence of 25 nM pBR32 DNA. The velocity of cleavage and methylation was linear for at least 10 min in reactions having DNA/enzyme concentration ratios of 4 or greater.
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**FIG. 1.** Differences in reaction rates of BamHI endonuclease and methylase as related to the relative location of the recognition site on linear DNA. Reaction conditions and DNA quantitation were performed as described under "Methods". The time course of cleavage and methylation was followed in reactions containing pBR322 DNA that had been linearized with EcoRI or NdeI endonucleases, placing the BamHI cleavage site 375 (O) or 1921 (O) bases from the nearest end, respectively. The DNA concentration was 12 nM and the concentrations of endonuclease and methylase were 0.4 and 0.25 nM, respectively. The time course of endonuclease cleavage under standard reaction conditions (A), 100 mM NaCl (B), and 160 mM NaCl (C). The time course of methylation under standard reaction conditions (D), 100 mM NaCl (E), and 200 mM NaCl (F).

**TABLE I**

| Fragment length | Restriction digests | Distance of the BamHI cleavage site from termini |
|-----------------|---------------------|-----------------------------------------------|
| 74              | TagI-HaeII          | 36,38                                         |
| 376             | SphI-EcoRV          | 190,186                                       |
| 650             | EcoRI-SalI          | 375,275                                       |
| 870             | DdeI-AvaII          | 447,423                                       |
| 1000            | DdeI-BglI           | 447,553                                       |
| 1500            | AvaI-PstI           | 740,769                                       |
| 2278            | NdeI               | 1129,1049                                     |
| 4362            |                     | 2441,1921                                     |

*This fragment was produced by the digestion of the 2278-base pair fragment with Bal-31 nuclease.*

**FIG. 2.** Kinetic behavior of BamHI endonuclease with DNA substrates of different lengths. The initial velocity of BamHI endonuclease was examined in reactions containing 0.2 nM enzyme and 1 nM pBR322 DNA fragments in the range of 74-4362 base pairs. The reactions were run under standard conditions (O) or 150 mM NaCl (O). The kinetic preference for longer substrates was markedly smaller as exemplified by a reduction in the velocity ratio of the 4362:74-base pair substrates from 9.0 to 1.9 (Fig. 2). The decrease in the kinetic preference for longer substrates at higher concentrations of salt is consistent with the concomitant decrease in the electrostatic component of nonspecific binding (13, 14). The possibility of different inhibitory impurities in the DNA fragment preparations was tested by mixing equimolar amounts of the 74- and 4362-base pair substrates in the same reaction. A kinetic preference was again observed, with a ratio of long/short cleavage rates of 7.8 at 1 nM DNA. Furthermore, a kinetic preference for the longer substrate was observed over a 10-fold concentration range with a long/short ratio of approximately 5.0 at 12 nM DNA. NaCl concentrations of 160 mM eliminated long chain preference at both substrate concentrations in these mixing experiments.

The kinetic behavior of BamHI methylase with substrates of various lengths differed from the endonuclease under similar reaction conditions. The velocity of methylation decreased in the range of 74 to 1,000 base pairs but increased in the range 1,500-4,362 base pairs (Fig. 3A). Mixing experiments with selected fragments revealed similar kinetic preferences: 74:1,500 = 4.3; 74:4,362 = 2.6; 4,362:1,000 = 2.8. It is tempting to explain the decrease in methylation rates between the 74- and 1,000-base pair fragments (Fig. 3A) in terms of diffusion-controlled mechanisms. In this context, the decrease in the free diffusion constants with increasing substrate length could kinetically dominate initial association events between enzyme and DNA in the absence of facilitated diffusion mechanisms. The native molecular weight of BamHI methylase is approximately 65,000 (6). Assuming a spherical shape, the diffusion constant is in the vicinity of $6 \times 10^{-7}$ cm$^2$/s. Estimates for the translational frictional coefficient of the 74-base pair fragment were based on a rigid rod configuration while those for the 1000-base pair fragment were based on a random coil. In both calculations a nondraining model was assumed.
**Fig. 3. Kinetic behavior of BamHI methylase with DNA substrates of different lengths.** The initial velocity of BamHI methylase was examined in reactions containing pBR322 DNA fragments in the range of 74-4362 base pairs. A, the concentrations of DNA and enzyme were 1 and 0.15 nM, respectively. The reactions contained no added NaCl. B, as in A except the NaCl concentration was 200 mM. C, the concentrations of DNA and enzyme were 12 and 0.5 nM, respectively. Reactions were performed under standard reaction conditions (○) or in the presence of 100 mM NaCl (△). D, initial velocity ratios of the 4362- to 376-base pair substrates with increasing NaCl concentration. Each reaction contained both fragments at a concentration of 12 nM. The enzyme concentration was 0.5 nM.

Kinetics might be observed with 2- or 3-fold differences between the diffusion constants of the enzyme and DNA. However, larger differences would probably be masked in these studies because of the comparatively fast diffusion rate of the enzyme. Therefore, it seems unlikely that the decrease in methylation rates are due to substrate diffusion alone.

The increases in methylation velocity with substrates 1500 base pairs and longer (Fig. 3A) suggest that facilitated diffusion is a contributing factor. At NaCl concentrations of 200 mM and a DNA concentration of 1 nM, these increases in reaction rates were eliminated without changing the trend of velocity decreases from 74 to 1000 base pairs (Fig. 3B). At DNA concentrations of 12 nM, in the absence of added salt, a comparatively small methylation preference for fragments larger than 74 base pairs was observed (Fig. 3C). An increase in the NaCl concentration to 100 mM increased the preference for longer substrates at these higher DNA concentrations (Fig. 3C). This preference was also observed at NaCl concentrations as high as 200 mM. The salt dependency of methylase long chain preference at high DNA concentrations was further studied in reactions containing equimolar concentrations of the 4362- and 376-base pair substrates (Fig. 3D). An increasing preference for the longer substrate was observed with increasing the NaCl concentrations up to 120 mM.

The relative nonspecific binding affinities of these enzymes was examined by inhibition experiments. The initial velocities of cleavage and methylation with the 74-base pair substrate were assessed in the presence of different concentrations of a NACS purified 3986-base pair fragment of pBR322DNA lacking the BamHI site. The concentration of the nonspecific DNA was determined in reactions containing the 74-base pair substrate at 1 nM and various concentrations of a 3896-base pair fragment of pBR322 DNA not containing the BamHI site. The concentration of the nonspecific fragment is expressed in terms of base pairs. The percent decrease in velocity was calculated from reactions run in the absence of the nonspecific DNA.

**DISCUSSION**

The reaction rates of BamHI endonuclease and methylase are affected by the proximity of their recognition site to the end of a linear DNA molecule. These effects appear to be correlated with the distance, and not the direction (5' or 3'), of the nearest DNA terminus. This suggests that the nature of the intervening flanking sequences between the recognition site and a terminus is not responsible for the variation in reaction rates. The dependence of the reaction rates on the relative location of the recognition site can be theoretically explained in terms of facilitated transfer of the enzymes via nonspecific sequences. This assumption requires that the enzymes have an average scanning length. The average scanning length is a function of the salt concentration since it affects the nonspecific binding constant (14). The kinetic preference for a centrally located site may exist because there is a functionally greater extension of nonspecific target area...
around the BamHI palindrome in NdeI-linearized pBR322 DNA (Fig. 1). Therefore, a greater proportion of enzyme molecules would be expected to come into the vicinity of the recognition site from both flanks. Recent studies have shown that EcoRI endonuclease has a kinetic preference for centrally located recognition sites on linear DNA (25). The authors demonstrated that facilitated transfer could account for this effect. They suggest that the kinetic preference can be explained by an increased frequency of entry to the recognition site from the proportionately larger area of nonspecific target sequences.

The differences in $K_a$ values for the NdeI- and EcoRI-linearized substrates is supportive evidence for the occurrence of facilitated transfer since this process ultimately affects specific binding. However, it has been suggested that the difference in $K_a$ values is too large if a sliding mechanism was solely responsible for the differences in reaction rates. The maximum kinetic difference generated by a sliding model (14) can be calculated by the relationship:

$$\frac{\text{tanh}(qL_t) + \text{tanh}(qL_s))}{(\text{tanh}(qL_t) + \text{tanh}(qL_s))}$$

where $L_t$ and $L_s$ equal 1921 and 2441 base pairs, respectively (distances from the ends to the BamHI site on NdeI-linearized DNA); $L_0$ and $L_s$ equal 3987 and 375 base pairs, respectively (distances from the ends to the BamHI site on EcoRI-linearized DNA); $q = (k_a/D_r)^{1/2}$ with $K_c$ = nonspecific dissociation rate constant; $D_r$ = sliding rate. A rough approximation of this ratio was made by using the estimated $K_c$ of $3 \times 10^{-2} \text{ s}^{-1}$ for EcoRI endonuclease (13) and the calculated sliding rate for lac repressor of $9 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ (17). A maximum difference in association rate constants of a factor of 1/1.3 is predicted. The $K_a$ differences are on the order of 1/3. This may indicate that other factors could be important in generating the observed reaction rates. The DNA conformation in the vicinity of the BamHI site and facilitated diffusion processes in addition to sliding may play a role. However, since the true $K_c$ and sliding rate constant for the BamHI enzymes may be considerably different from the assumed values under the reaction conditions employed, the actual ratio could be significantly underestimated. The DNA substrate concentration used in some of the experiments described here (Figs. 1, 3C and D) may not have been completely conducive to kinetic conditions in which diffusion of the enzyme to the DNA is rate limiting. Experiments performed with dilute DNA concentrations (1 nM) probably meet this criterion since these substrate concentrations are approximately 4-fold below the apparent $K_a$ values and the enzyme concentrations were 5- to 10-fold below initial substrate concentrations. Both enzymes are inhibited in a competitive manner by phiX174 RF DNA (contains no BamHI site) in the range of 1-10 nM as judged by initial velocity kinetics using Form I pBR322 DNA as substrate. This suggests that association with substrate DNA is rate limiting since the added DNA slows the arrival of enzyme in a manner directly proportional to inhibitor. The competitive effect is also observable at a substrate DNA concentration of 12 nM. Theoretical models of facilitated diffusion, and their application, require that once the protein is bound to DNA the complex can be considered as a closed system. This is because facilitated diffusion is an intramolecular phenomenon. The BamHI system probably satisfies this requirement in several respects. Rates of enzyme-DNA dissociation in these systems are low, on the order of minutes and the turnover number of both enzymes are small, approximately 1.3 min$^{-1}$, at 25 nM DNA. The time frame in which catalysis occurs after specific association would not be expected to vary significantly since the BamHI sites in all fragments are identical with respect to flanking sequences within at least 35 base pairs. Variability in the stability of the specific complex might occur. It was found that the half-life of specific complexes with EcoRI endonuclease dramatically decreased with increasing substrate length, but the overall specific binding equilibrium remained unaffected (13). These compensatory changes between the specific association and dissociation rate constants might not apply to the BamHI system, particularly not to the methylase. Differences in the overall binding affinities between large DNAs and fragments 200 base pairs and smaller were found to occur with lac repressor (17). These possibilities could be involved in the kinetic profiles observed with the methylase at several DNA concentrations. Binding of AdoMet might also be affected by chain length but did not appear to be altered (on the basis of reaction rates) by using either the NdeI- or EcoRI-linearized substrates. Differences between the reaction rates with these two substrates were still apparent at concentrations of 1 nM, yielding a velocity ratio for NdeI/EcoRI-linearized DNAs on the order of 1:8. This result indicates that the differences in the kinetic properties between these DNA molecules are preserved over a 10-fold concentration range.

The increase in the velocity of endonuclease cleavage with increasing substrate length is suggestive of facilitated transfer. This could be due to the greater extension of nonspecific target area in longer fragments. At a NaCl concentration of 160 mM long chain preference and cleavage rate differences between the NdeI- and EcoRI-linearized substrates are negligible (Figs. 2 and 3C). These observations are consistent with a decrease in the electrostatic component of nonspecific binding and the subsequent decrease in facilitated transfer (13, 14). While this manuscript was in preparation, Ehbrecht et al. reported a kinetic preference of BamHI endonuclease for longer substrates (26) which has confirmed our own results. The nonlinear profile of cleavage and methylation rates with different-sized substrates (Figs. 2 and 3C) indicates that there is a limit to the range of facilitated transfer. Extrapolation of this data gives an approximation of 1350 base pairs as an upper limit to the scanning length. This value is strikingly similar to the average scanning length determined for EcoRI endonuclease at an ionic strength of 0.08 (13). However, the scanning length of BamHI endonuclease may be different if it was determined at this higher ionic strength.

BamHI endonuclease and methylase show complex inhibition profiles with the 74-base pair substrate (Fig. 4). The biphasic inhibition pattern observed with the endonuclease may indicate that the enzyme possesses two binding sites with different affinities for non-specific DNA. Saturation of the stronger site results in 20% inhibition of activity while saturation of the weaker site almost totally inhibits activity. One of these sites might be the active site and both sites would be interactive assuming this interpretation is valid. The methylase might bind two non-specific DNA fragments cooperatively as judged by the abrupt increase in inhibition at 3 $\mu$M base pairs. These competitive curves indicate that the enzymes nonspecific association with DNA are qualitatively different. This may account for some of the differences seen in the previous kinetic experiments. We are currently investigating the effects on inhibition when the length of the non-specific DNA is varied.

The kinetic behavior of the methylase at low DNA concentrations (Fig. 3, A and B) cannot be fully explained at this time. The salt sensitivity of the methylation rates with DNA fragments 1500 base pairs and longer might be indicative of facilitated transfer. The gradual increase in methylation velocities with these fragments might be involved with the
DNA, salt-induced tions of NaCl may contribute to kinetics observed in these studies utilizing different-sized fragments at the same concentration (which may affect specific and nonspecific binding) concentrations exceeding 220 mM cause severe inhibition of preliminary initial velocity studies using Form tions as high as 200 mM. This comparatively refractory behavior to high salt concentrations at high DNA concentrations indicates residual long chain preference at NaCl concentrations below their original values in the low salt case. Since salt strongly affects the nonspecific binding constant, these results suggest that a marginal decrease in nonspecific binding affinity allows for a greater amount of productive interactions that lead to sliding as opposed to the protein being trapped in an unproductive, nonspecific binding mode. A similar situation might be occurring with BamHI methylase and could explain its enhanced long chain preference at NaCl concentrations of 100–120 mM (Fig. 3, C and D). Fig. 3D indicates residual long chain preference at NaCl concentrations as high as 200 mM. This comparatively refractory behavior to high salt concentrations at high DNA concentrations is consistent with the persistence of the differences between methylation reaction rates observed with the NdeI and EcoRI-linearized substrates at 200 mM NaCl (Fig. 1F). It is difficult to assess the range of these effects since NaCl concentrations exceeding 220 mM cause severe inhibition of methylation activity. The persistence of the differences in methylation rates between the full-length substrates could be a consequence of residual nonspecific binding at high DNA concentrations. The effects of salt on the enzyme’s conformation (which may affect specific and nonspecific binding) and on its catalytic mechanism could create another dimension of kinetic consequences that have to be evaluated. Preliminary initial velocity studies using Form I pBR322 DNA indicate an increase in \( V_{\text{max}} \) with NaCl concentrations up to 100 mM. Changes in catalytic efficiency at various concentrations of NaCl may contribute to kinetics observed in these experiments. Assuming that the catalytic mechanism does not significantly change with the length of nonspecificflanking DNA, salt-induced \( V_{\text{max}} \) effects should remain the same in studies utilizing different-sized fragments at the same concentration of NaCl. As stated previously, the size of the difference between the \( K_m \) values for the NdeI- and EcoRI-linearized substrates suggests that sliding may not be totally responsible for the variation in reaction rates. Other factors that contribute to this phenomenon may be relatively insensitive to high salt concentrations. Nonspecific binding by the methylase may also involve other interactions in addition to electrostatic forces. Hydrophobic interactions are stabilized by salt and could be partially involved with nonspecific binding.

Collectively, these data imply that BamHI endonuclease and methylase differ in their nonspecific interactions with DNA. However, on the basis of initial velocity kinetics both enzymes share approximately the same binding affinity for the BamHI site. The presence of AdoMet may also affect the nonspecific interactions of the methylase. On the basis of the studies presented here, it seems reasonable to conclude that facilitated diffusion processes are at least partially responsible for the variations in reaction rates of BamHI endonuclease and methylase with changes in the position of the recognition site and substrate length. A more complete appreciation of the experimental results awaits further kinetic studies with various sized DNA fragments, the effects of AdoMet on specific and nonspecific complex formation, and substrate binding experiments. Investigations into other restriction-modification systems may demonstrate the occurrence of facilitated diffusion mechanisms. The exploitation of facilitated transfer by restriction-modification enzymes could be a general phenomenon used to expedite sequence-specific interactions with DNA.

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REFERENCES
1. Roberts, R. J., Wilson, G. A., and Young, F. E. (1977) Nature 265, 82–84
2. Hartman, S., Keister, T., and Godthaler, A. (1978) J. Mol. Biol. 124, 701–711
3. Smith, L. A., and Chirikjian, J. G. (1979) J. Biol. Chem. 254, 1003–1006
4. George, J., Blakesley, R. W., and Chirikjian, J. G. (1980) J. Biol. Chem. 255, 6621–6624
5. George, J., and Chirikjian, J. G. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2432–2436
6. Nardone, G., George, J., and Chirikjian, J. G. (1984) J. Biol. Chem. 259, 10567–10562
7. Verdiction, L., and Rich, A. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3268–3272
8. Chiswick, A. J., and Wang, J. C. (1985) Cell 35, 817–829
9. Halford, S. E., Johnsen, N. P., and Grintz, J. (1980) Biochem. J. 191, 581–602
10. Nath, K., and Azulina, B. A. (1981) in Gene Amplification and Analysis (Chirikjian, J. G., ed) Vol. I, pp. 113–129, Elsevier North Holland, NY
11. Armstrong, K., and Bauer, W. (1982) Nucleic Acids Res. 10, 995–1007
12. Stryer, M., Avraham-Haetizini, R., Beilman, A., Shlomai, J., Kaplan, F., Oppenheim, A., and Razin, A. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3278–3282
13. Jack, W. E., Terry, B. J., and Modrich, P. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 4010–4014
14. Berg, O. G., Winter, R. B., and von Hippel, P. H. (1981) Biochemistry 20, 6929–6948
15. Park, C. S., Wu, F. Y-H., and Wu, C. W. (1982) J. Biol. Chem. 257, 6950–6956
16. Winter, R. B., and von Hippel, P. H. (1981) Biochemistry 20, 6948–6960
17. Winter, R. B., Berg, O. G., and von Hippel, P. H. (1981) Biochemistry 20, 6961–6977
18. Nardone, G., George, J., and Chirikjian, J. G. (1984) Fed. Proc. 43, 1581
19. Lee, Y. H., and Chirikjian, J. G. (1979) J. Biol. Chem. 254, 6583–6594
20. Thompson, J. A., Blakesley, R. W., Doran, K., Hough, C. J., and Wells, R. D. (1983) Methods Enzymol. 100, 368–399
21. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
22. Peacock, A. C., and Dingman, C. W. (1968) Biochemistry 1, 668–674
23. Depew, R. E., and Wang, J. C. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 4277–4279
24. Rubin, R. A., and Modrich, P. (1977) J. Biol. Chem. 252, 7965–7972
25. Terry, B. J., Jack, W. E., and Modrich, P. (1985) J. Biol. Chem. 260, 13130–13137
26. Ehbrecht, H.-J., Pingoud, A., Urbanske, C., Mass, S. G., and Gualerzi, C. (1985) J. Biol. Chem. 260, 6160–6166