Sequence-Selective Inhibition of Restriction Endonucleases by the Polyintercalator Bis(methidium)spermine

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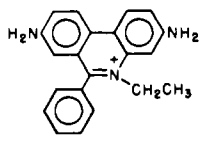
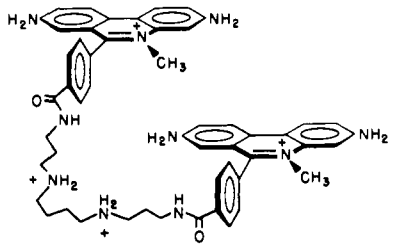
The design of site-specific probes of double helical nucleic acid is complicated by the fact that the critical recognition features of a defined base sequence are still not well understood.[1-4] We recently reported that the nucleic acid binding affinity of the polyintercalator[5-9] bis(methidium)spermine (BMSp)[5] was substantially increased over that of the corresponding monomer, ethidium bromide (EB).[10] In addition, from binding studies with nucleic acid homopolymers and copolymers, BMSp was found to have substantially increased sequence specificity.[11] If this sequence-selective binding is found in *heterogeneous* DNA, BMSp might selectively inhibit DNA-dependent enzymes. To test for selectivity with a noncovalently binding molecule, a "kinetic sampling" assay was devised.[12-15] We report that the dimer, BMSp, selectively inhibits restriction sites on the pBR322 plasmid,[16] while the monomer, EB, shows no selective action in competition with any of the restriction enzymes utilized.



EB



BMSp

Table I

restriction endonuclease[a]	PBR322 plasmid position[b]	order of reactivity[c]
Rsa I	164	2
	2281	3
	3846	1
Bgl I	928	1
	1162	2
	3481	2
Mst I	260	3
	1355	1
	1453	2
	3587	4

[a] Recognition sites: Rsa I, GTAC; Bgl I, GCCNNNNNGGC; Mst I, TGCGCA. [b] Base pair position from Eco RI cleavage position as zero. [c] Determined from partial digestion fragments by gel electrophoresis on 5% polyacrylamide.

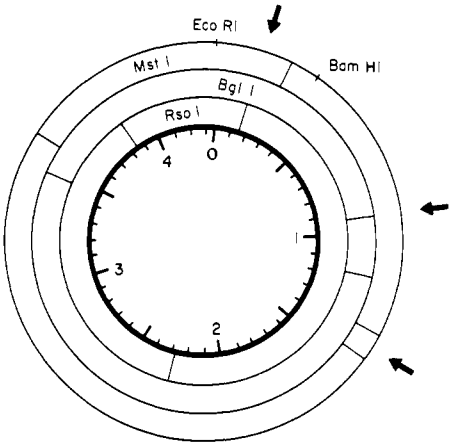


Figure 1. Restriction map of pBR322 for Rsa I, Bgl I, and Mst I. The Eco RI cut defines the zero position on the plasmid. Arrows indicate sites corresponding to BMSp inhibition.

Complete cleavage of closed circular DNA by a sequence-specific endonuclease produces a unique set of DNA fragments that can be characterized by gel electrophoresis. Although the restriction sites of a given enzyme are identical, each site is uniquely located on the plasmid. Consequently, the rates of cleavage are not equal, and the order of reactivity may be determined by monitoring the appearance of partial and final fragments. Site-selective inhibition by some DNA binding molecule is then reported as a change in the partial restriction pattern, whereas nonselective inhibition would only affect the rate of digestion and not the order of cleavage.

The restriction enzymes, Rsa I,[17] Bgl I,[18-21] and Mst I,[22] were chosen because they produce a small number of final DNA fragments (3, 3, and 4, respectively). Accordingly, Rsa I and Bgl I could only produce four partial digestion fragments, a three-fragment linear (Rsa I/Bgl I = 4361 bp;bp = base pair) and three two-fragment partials (Rsa I = 3682, 2796, and 2244 bp; Bgl I = 4127, 2553, and 2042 bp), while Mst I could possibly produce nine partial digestion fragments, a four-fragment linear (4361 bp), four three-fragment partials (approximately 4263, 3327, 3266, and 2227 bp), and four two-fragment bands (approximately 3168, 2232, 2129, and 1193 bp).[23]

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The intercalator free fingerprints of Rsa I and Bgl I show all the partial fragments; however, Rsa I produces a weak 2244-bp band, whereas the fragments produced by Bgl I appear equally. From these fingerprints the relative cutting order may be assigned for each set of restriction sites. Using the Eco RI cleavage site to define the zero point, the order for Rsa I is 3846 > 164, 2281 and for Bgl I is 928 > 1162,3481. Finally, Mst I displays a prominent set of partial bands, the 4263-, 3266-, and 3168-bp fragments, which correlates with an order of reactivity for Mst I of 1355 > 1453 > 260 > 3587 (Table I).

In a typical run, BMSp (or EB), DNA, and assay buffer (50 mm of tris/HCl, pH 7.4, 6 mm of NaCl, 6 mm of dithiothreitol, 6 mm of $MgCl_2$, and 100 μg/mL bovine serum albumin) were mixed and allowed to equilibrate for 1 h. The restriction enzyme was added at time 0. At the proper intervals a 10-μL sample was withdrawn and terminated with 5 μL of 5% sodium dodecyl sulfate. The ethidium and intercalator free samples were then prepared for electrophoresis. Because BMSp can affect the mobility of DNA, BMSp was removed on a BioRad AG 50W-X8 column before preparing the samples for electrophoresis.[24] The samples were then electrophoresed in 5% polyacrylamide.

The addition of EB at 30% saturation ([EB]/[BP] = 0.16) induces no changes in the Rsa I and Bgl I fingerprints. There is only a reduction in the rate of restriction; therefore, no selective action is indicated. Conversely, the addition of BMSp at 30% saturation ([BMSp]/[BP] = 0.08) alters the fingerprints of all three enzymes. The 2244-bp partial of Rsa I disappears. The order of appearance of the 2553- and 2042-bp partials of Bgl I is reversed (the 2042-bp fragment now appears before the 2553-bp fragment), and Mst I shows a new set of prominent partials. The Mst I 2232/2227- and 2129-bp fragments appear in preference to the 4263, 3266, and 3168-bp partials. These changes correspond to inhibition at 164 for Rsa I, at 928 for Bgl I, and at 1355/1453 for Mst I (Figure 1).

Why BMSp can selectively compete with a restriction enzyme while EB shows no selective effects may simply be a demonstration of the enhanced sequence selectivity of a dimer (BMSp) over a monomer (EB).[11] Alternatively, BMSp and EB may both bind selectively, but only BMSp competes effectively with the restriction enzyme as a consequence of the relative binding affinities of the enzyme, BMSp, and EB.[10,11] BMSp seems to preferentially inhibit restriction sites in the first third of the plasmid.[25] This corresponds to the BMSp saturation (30%) and suggests that the entire region, base pairs 164-1453, may be the preferred binding site of BMSp. A third interpretation is nonselective binding with sequence selective alteration of the plasmid DNA by *BMSp* (but not *EB*) which inhibits enzyme cleavage. Previous evidence that BMSp and EB bind rAdT in preference to dAdT suggests that the specificity of the intercalators may be due to the preferential binding of specific conformations of the nucleic acid double helix.[10d,e,11,26]

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Registry No. BMSp, 79919-41-8; EB, 1239-45-8.

Supplementary Material Available: Electrophoretic data for Rsa I, Bgl I, and Mst I (2 pages). Ordering information is given on any current masthead page.

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Titanacyclobutane: Structural Considerations

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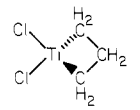
Metallocyclobutane complexes have been implicated in several organometallic reactions including olefin dimerization,[1] polymerization,[2] and metathesis.[3] Indeed, such complexes have been

1

isolated,[4,5] and a few platinum complexes[5] have been structurally characterized. However, for early transition metals where the catalytic processes occur, the structural characterization is less complete.[6]

Herein we report the structure for a simple metallocyclobutane obtained from ab initio theoretical studies and provide relative



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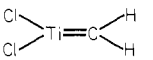
energetics for its equilibrium with free ethylene plus the metal alkylidene complex.



3

For the metallocyclobutane **2** we find[7] a planar, symmetric ring

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(7) The structural calculations for **2-4** were ab initio Hartree-Fock. All electrons of the Ti, C, and H were considered by using valence double-ζ basis sets (the H was unscaled). The Cl was described by a molecularly optimized minimum basis[8] using the SHC effective potential[9] to replace the core electrons. Generalized valence bond (GVB) calculations were carried out at the optimized geometry to obtain the orbitals in Figure 1. Constraints in the geometric optimizations were $\angle ClTiCl = 142°$ for **2** and $R_{TiCl} = 2.238$ Å for **2** and **3** (based on the calculated geometry for **4**; in addition, the CH_2 plane of **2** was taken to pass through the diagonally opposite atom; A. K. Rappé, Ph.D. Thesis, California Institute of Technology, October 1980.

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