Distorting Duplex DNA by Dimethylenesulfone Substitution: A New Class of "Transition State Analog" Inhibitors for Restriction Enzymes

Monika O. Blättler,[§] Christian Wenz,[‡] Alfred Pingoud,[‡] and Steven A. Benner*,[§]

Department of Chemistry, University of Florida Gainesville, Florida 32611 Institute for Biochemistry Justus-Liebig-University, Heinrich-Buff-Ring 58 D-35392 Giessen, Germany

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After they bind (but before they cleave) duplex DNA, some restriction enzymes (such as *Eco*RV[1,2] and *Eco*RI[3]) distort the duplex. The distorted duplex is not, of course, in its ground-state conformation; it requires "binding energy" to bend DNA.[4] Thus, an analogue of DNA that generates this distortion in the unbound state (without altering other features of the substrate that are recognized by the enzyme) should bind to these restriction enzymes with a higher affinity than the DNA substrate itself. This is, of course, the principle underlying transition-state analogues generally, which approximate in structure the "distorted" transition state (or a distorted high-energy intermediate) for an enzymatic reaction.

Recently, we noted that duplex nucleic acid having a dimethylene sulfone unit replacing a phosphate has a distorted backbone conformation[5] reminiscent of the distortion produced by restriction enzymes and other proteins that bend DNA when they bind. In particular, the twist observed in a duplex built from the dinucleotide analogue r($G_{SO_2}C$) is a low 20.8° (instead of 34.7°), similar to the twist observed between the central four base pairs in DNA carrying the recognition sequence bound to *Eco*RV (19.3−23.0°) (Table 1). Thus, a DNA duplex having a dimethylene sulfone substitution joining these base pairs should be "pre-distorted" in its ground state and, therefore, bind more tightly to *Eco*RV than the cognate DNA substrate itself.

For such an oligonucleotide analogue to be an effective inhibitor of the enzyme, the sulfone group must also mimic interactions that the phosphate group has with the enzyme itself. *Eco*RV makes many contacts to the phosphate groups in its recognition sequence, both directly and via water molecules.[1,2,6] As the sulfone S═O bond has a high dipole moment, the sulfone should accept hydrogen bonds, although perhaps not as well as phosphate, where each oxygen bears a charge of ca. −0.5. Solvation of the sulfone group in the G_{SO_2C} duplex[5] is rather similar to solvation of the phosphate group in the $G_{PO_2^-C}$ duplex,[7] providing experimental evidence suggesting that the sulfone group might adequately serve as a neutral phosphate mimic.

Table 1. Distortion of DNA from Idealized Structure by *Eco*RV and in the Linkage Modified by the Dimethylene Sulfone Group

linkage	twist (deg)[a]	K_i for sulfone -substituted chimera
$G^{-5}-G^{-4}$	32.5/40.1	
$G^{-4}-G^{-3}$	33.3/32.9	
$G^{-3}-A^{-2}$	35.4/36.6	
$A^{-2}-T^{-1}$	19.3/19.8	ca. 20 nM[b]
$T^{-1}-A^{1}$	23.0/16.0	
$A^{1}-T^{2}$	19.9/19.8	25 pM[c]
$T^{2}-C^{3}$	37.4/36.6	
$C^{3}-C^{4}$	40.4/32.9	
$C^{4}-C^{5}$	29.6/40.1	
ideal A-DNA[18]	32.4	
ideal B-DNA[14]	36.0	
r(GpC)[7]	34.7	
$r(GSO_2C)$[5]	20.8	

[a] Twist is defined by the relative angle of the C1′−N bonds in consecutive bases in the strand; the two values given refer to different values in the two subunits of the noncrystallographic dimer (from ref 1). [b] For duplex-incorporating **1** ACCAGAATTCGGATCCA-GA_{SO_2}TATCGCCA; the recognition site is underlined. Cleavage occurs between TA in the recognition site. [c] For duplex-incorporating **2** ACCAGAATTCGGATCCA$GATA_{SO_2}$TCGCCA; the recognition site is underlined. Cleavage occurs between TA in the recognition site.

To learn whether a potent inhibitor of *Eco*RV could be obtained by introducing a dimethylenesulfone linker into its restriction site to replace a phosphate at a position where the twist is low, two chimeric DNA analogues were synthesized, the first with a dimethylene sulfone linker replacing a phosphodiester group between the first AT unit in the *Eco*RV recognition site (underlined) (ACCAGAATTCGGATCCAGA_{SO_2}TATCGCCA, **1**), the second with a dimethylene sulfone linker replacing a phosphodiester group between the second AT unit in the *Eco*RV recognition site (ACCAGAATTCGGATCCA$GATA_{SO_2}$TCGCCA, **2**). These are the linkages with the lowest twists in the *Eco*RV restriction site when bound to the restriction enzyme (twists ≈ 20°, Table 1).[1,6]

Iodide **7** of the 5′-homologated analogue of thymidine was synthesized by the method of Baeschlin et al. (Scheme 1).[8] A protected derivative of the 3′-homologated 2′-deoxyadenosine **8** was prepared by the procedure of Sanghvi et al.[9] and then converted by a four-step procedure to thiol **12**. Thiol **12** and iodohomothymidine **7** were then coupled, and the product was converted to the 5′-tritylated derivative of the $A_{SO_2}T$ phosphoramidite **16**. This was incorporated into chimeric oligonucleotides by standard solid-phase automated oligonucleotide synthesis.

Neither the modified nor unmodified strand of duplexes containing **1** and **2** were substrates for *Eco*RV. The duplexes were, however, inhibitors of the cleavage of the 5′-radiolabeled self-complementary *Eco*RV substrate GATCGACGATATCGTC-GATC (20 mM Tris/HCl, pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, and 100 mg/mL BSA, at 21 ± 1 °C).[10] Initial rates k_{obsd} were determined under steady-state conditions from the linear part of the progress curves. Products were resolved by chromatography[11] on DEAE−cellulose (Machery-Nagel) and quantitated using an InstantImager (Canberra Packard). For duplexes containing **1**,

‡ Justus-Liebig-University.
§ University of Florida.

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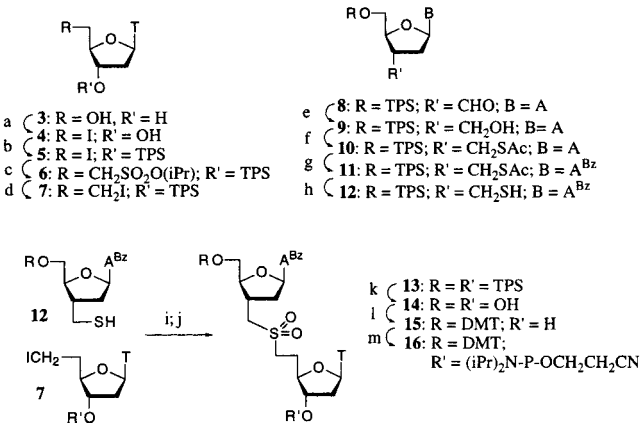
Scheme 1[a]



[a] Key: (a) PPh_3/I_2/pyridine; (b) triphenylsilyl (TPS) chloride/CH_2Cl_2/imidazole (78% over 2 steps); (c) (^{i}Pr)OSO_2CH_2Li/THF/hexamethylphosphoric triamide (74%); (d) PPh_3/I_2/benzene/Δ (55%); (e) $NaBH_4$/EtOH (aqueous, 80%); (f) AcSH/DIAD/PPh_3/THF (79%); (g) BzCl/4-(*N*,*N*-dimethylamino)pyridine (DMAP)/pyridine; (h) NaOH/pyridine/EtOH (49% over 2 steps); (i) Cs_2CO_3/THF/DMF; (j) oxone/THF/MeOH/NaOAc; (k) pyridine/HF (69% over 3 steps); (l) DMTCl/DMAP/TEA/pyridine (66%); (m) $((^iPr)_2N)_2POCH_2CH_2CN$/diisopropylammonium tetrazolide/CH_3CN/DMF (94%); TPS = *tert*-butyldiphenylsilyl group; DMT = 4,4′-dimethoxytrityl; T = thymine; A = adenine; Ac = acetyl; Bz = benzoyl.

2, and a control oligodeoxynucleotide lacking an *Eco*RV site (GATCGACGAGCTCGTCGATC), concentrations of 100, 1, and 1000 nM (respectively) were required to observe greater than 50% reduction of the initial cleavage velocity.

Values for k_{cat} and K_m for the substrate were determined from data collected without inhibitor. Kinetics simulations (Microsoft EXCEL) were then performed for data collected with inhibitors assuming competitive, noncompetitive, and uncompetitive inhibition. Under a competitive inhibition model, a best fit to the experimental data was obtained with K_{EI} (enzyme−inhibitor disassociation constants) values of 21 nm and 190 nM for the duplex incorporating **1** and the control. Further kinetic analysis, at 0.1−30 nM [substrate] and 0, 0.1, and 0.3 nM [inhibitor], was done for the best inhibitor, containing strand **2**. Values for the kinetic parameters k_{cat} and K_m and the inhibition constants K_{EI} and K_{EIS} for competitive, noncompetitive, and uncompetitive inhibition were obtained by fitting. Both noncompetitive and uncompetitive mechanisms gave a mean residual roughly three times larger than for competitive inhibition, which gave an inhibition constant of 25 pM. A free fit gave no significant improvement of the mean residual relative to the fit obtained if competitive inhibition is assumed. Consequently, the data indicate that the DNA duplex having the recognition sequence $GATAT_{SO_2}$-TC (**2**) is a competitive inhibitor of *Eco*RV.

The remarkably low inhibition constant of **2** (25 pM) might be compared with the 2 nM K_M for a standard 20-mer DNA sequence lacking the dimethylene sulfone modification.[10] The tight binding of the duplex having the $GATA_{SO_2}TC$ recognition site to the *Eco*RV enzyme suggests that the distortion induced by the dimethylene sulfone unit allows the chimeric duplex DNA to fit in the active site without consuming "intrinsic" binding energy to distort the structure from its ground-state conformation.[4] This makes the duplex a "transition-state analog" (or, perhaps more precisely, a "high-energy intermediate analog") for *Eco*RV, but of a special sort, where the geometric distortion is distributed over many atoms. This result might be compared with the enhanced binding of nicked DNA,[12] DNA duplexes containing mismatches,[13] or DNA duplexes distorted by cross-links[14] for proteins that bend (but do not cleave) DNA.

Furthermore, these results show that the sulfone unit is an adequate mimic for phosphate in its hydrogen-bonding potential, at least for the second AT linkage. The difference between the K_i values for **1** and **2** might be explained by the differences between the hydrogen-bonding network in which the corresponding phosphates participate, or differences in the detailed geometry in the linkages. In a DNA−*Eco*RV complex soaked with Mg^{2+}, the phosphate replaced by a dimethylene sulfone unit in **1** forms a hydrogen bond with the side chain of Ser 112 and three water molecules, which interact directly or via water with hydrogen-bonding groups from Asn 185, Thr 186, Thr 187, Asn 188, and the next phosphate in the chain.[1,6] The phosphate replaced by a dimethylene sulfone in **2**[6] is involved in a less extensive hydrogen-bonding network. These differences may explain the greater tolerance of the enzyme for sulfone substitution at the second position. Alternatively, the dimethylene sulfone unit may mimic the distorted geometry of the second AT linkage better than the first. Interestingly, the fact that the chimera containing **2** is not a substrate for the restriction endonuclease is consistent with the proposal that the phosphate group 3′ to the phosphate being cleaved helps activate the attacking water[15] (however, see also ref 2).

Many proteins that bind DNA distort it by bending and/or unwinding. These include restriction enzymes, transcription factors, and other regulatory elements. DNA analogues that are "pre-distorted" by a backbone substitution should therefore be general tools for blocking a variety of protein−nucleic acid interactions. Furthermore, neutral analogues for phosphate are frequently sought,[16] and the dimethylene sulfone group should be considered as a potential candidate. Finally, short oligonucleotide analogues, if they have the correct geometries and correspondingly high affinities, could have value as biological reagents, alternatives to "antisense" and "antigene" oligonucleotides and their analogues, which typically must be 16−30 nucleotides in length to exhibit useful selectivity (for a review, see ref 17).

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Supporting Information Available: Experimental details, one table, and three figures (9 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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