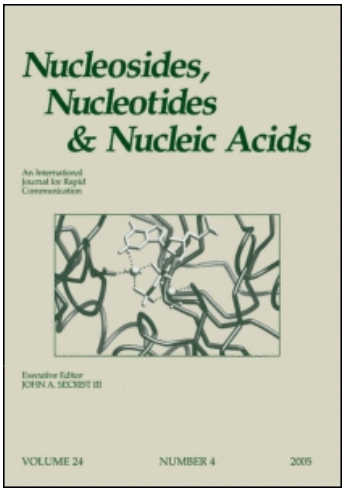

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HIGHER BINDING AFFINITY OF DUPLEX OLIGODEOXYNUCLEOTIDES CONTAINING 1,2-DIDEOXY-D-RIBOSE TO THE N6-ADENINE DNA METHYLTRANSFERASE M·*TAQ*I SUPPORTS A BASE FLIPPING MECHANISM

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ABSTRACT: The N6-adenine DNA methyltransferase M·*Taq*I binds duplex oligodeoxynucleotides containing 1,2-dideoxy-D-ribose at the target or 3' neighbouring position with 200fold and 60fold higher affinity, respectively, compared to the natural substrate. These results are explained thermodynamically by a base flipping mechanism of M·*Taq*I.

The DNA methyltransferase (Mtase) from *Thermus aquaticus* (M·*Taq*I) catalyses the transfer of the activated methyl group from the cofactor *S*-adenosyl-L-methionine to the N6 position of adenine within the double-stranded 5'-TCGA-3' DNA sequence. M·*Taq*I is a very interesting target for structure-function studies, because its structure in complex with the cofactor has been elucidated by X-ray crystallography[1]. However, model building of the ternary complex between M·*Taq*I, cofactor and B-DNA showed that the distance between the methyl group donor and the acceptor is too large for a direct methyl group transfer[2], and a base flipping mechanism, as observed in the three-dimensional structure of the C5-cytosine DNA Mtase M·*Hha*I in complex with DNA[3], was suggested.

Recently, duplex oligodeoxynucleotides (ODNs) containing a mismatched or deleted target base were found to bind with higher affinity to the C5-cytosine DNA Mtase M·*Hha*I compared to the natural substrate[4]. We have investigated binding of the duplex ODNs **1**, **2** and **3** (TABLE 1) containing 1,2-dideoxy-D-ribose (a stable abasic site) to the N6-adenine DNA Mtase M·*Taq*I in a gel mobility retardation assay (FIG. 1). This assay makes use of the observation that many DNA-protein complexes do not dissociate in the pores of a polyacrylamide gel during electrophoresis under native conditions. The duplex ODN **1** containing 1,2-dideoxy-D-ribose at the target position binds to M·*Taq*I with about 200fold higher affinity than the regular duplex ODN **4** (FIG. 1 A and D). This result can be explained thermodynamically on the basis of a base flipping mechanism (FIG. 2). Upon

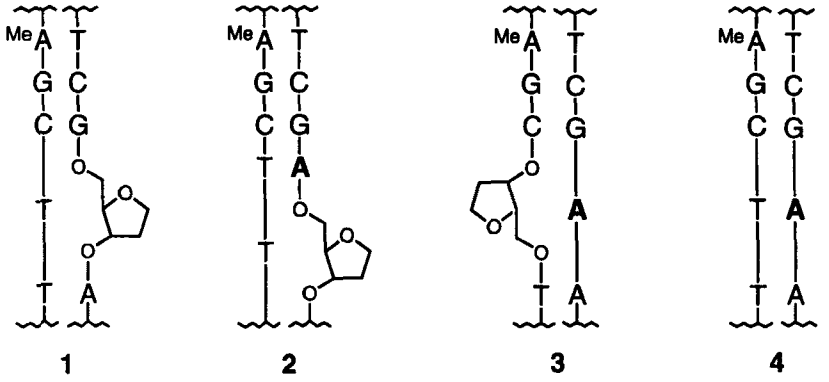

TABLE 1. Sequences of duplex oligodeoxynucleotides (ODNs) used in this study and relative affinities to M·*Taq*I[a]

5'-GCTGTTGAGATCCAGT T C G **x** yGTAACCCACTCGTGC-3'
3'-CGACAACTCTAGGTCA A^{Me} G C z TCATTGGGTGAGCACG-5'

x	y	z	duplex ODN	relative affinity
Δ	A	T	**1**	200
A	Δ	T	**2**	60
A	A	Δ	**3**	0.4
A	A	T	**4**	1

[a]The target position of M·*Taq*I is printed in bold, and the following symbols are used for modifications: Δ = 1,2-dideoxy-D-ribose and A^{Me} = 6-methyl-2'-deoxyadenosine.

binding of **1** the enzyme does not need to pay the energetic cost to disrupt the target base pair, and it can not gain energy by binding an extrahelical target base. However, it can bind the unpaired partner thymine directly, which should lead to an increased overall binding affinity. Similarly, a 60fold higher binding affinity is observed for the duplex ODN **2** (FIG. 2 B and D), which contains 1,2-dideoxy-D-ribose at the 3'-neighbouring position of the target adenine. Here it should be easier to flip the target adenine, because the stacking interaction to its 3'-neighbour is missing, and again an increased overall binding

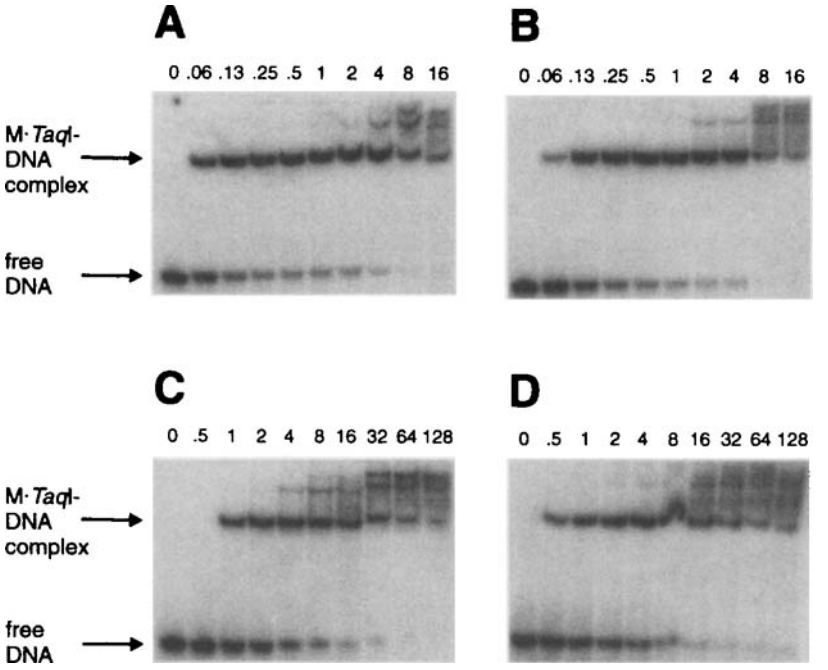

FIG. 1. Gel mobility retardation analysis of the binding of the N6-adenine DNA Mtase M·*Taq*I to duplex ODNs containing 1,2-dideoxy-D-ribose. ODNs were ^{32}P-labelled and hybridised with their complementary strands. Radioactive bands were visualised using a phosphoimager. A, duplex ODN **1** with 1,2-dideoxy-D-ribose at the target position of M·*Taq*I; B, duplex ODN **2** with 1,2-dideoxy-D-ribose located 3' to the target adenine; C, duplex ODN **3** containing 1,2-dideoxy-D-ribose at the partner position; D, regular duplex ODN **4**. The protein concentrations (nM) are indicated at the top of each gel. The concentrations of the duplex ODNs were 0.01 nM (**1** and **2**) and 1 nM (**3** and **4**).

affinity is expected. With the duplex ODN **3**, which contains 1,2-dideoxy-D-ribose at the partner position, a slightly decreased overall binding affinity compared to **4** is observed (FIG. 1 C and D). In the duplex ODN **3** it should be easier to flip the target adenine because the stabilising Watson-Crick hydrogen bonds are missing. However, the enzyme can no longer bind the unpaired partner base. Thus, a higher, lower or even unchanged overall binding affinity could result depending on the sum of the energies for these interactions.

A 7fold and 4fold higher binding affinity of duplex ODNs containing 1,2-dideoxy-D-ribose at the target position was also observed for the N6-adenine DNA Mtases M·*Eco*RV[5] and M·*Eco*RI[6], respectively. Thus, a common base flipping mechanism for N6-adenine DNA Mtases and C5-cytosine DNA Mtases seems very likely. In addition,

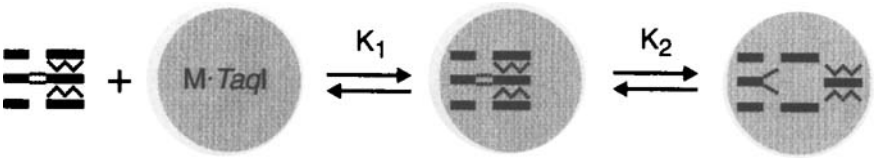

FIG. 2. Simple two-step binding mechanism for M·*Taq*I, in which base flipping occurs after initial binding to the target site. The solid lines represent the nucleobases within the DNA helix, and the dashed lines indicate interactions of the target base pair within the DNA and the DNA-M·*Taq*I complex. The overall binding affinity equals K_1 (1 + K_2) and thus will be influenced by the equilibrium K_2 between the extra- and innerhelical target base within the DNA-M·*Taq*I complex.

these results suggest that ODNs containing 1,2-dideoxy-D-ribose at the target position could be used as specific inhibitors for DNA Mtases.

EXPERIMENTAL

1,2-Dideoxy-D-ribose and 6-methyl-2'-deoxyadenosine phosphoramidites were purchased from Glen Research. Synthesis, purification, ^{32}P-labelling and annealing of ODNs as well as preparation of M·*Taq*I and the gel mobility retardation assay were performed as descibed before[7]. Bands corresponding to the free duplex ODNs were quantified using a phosphoimager, and the obtained data were fitted to the real solution of the quadratic binding equation for one binding site.

REFERENCES

(1) Labahn, J.; Granzin, J.; Schluckebier, G.; Robinson, D. P.; Jack, W. E.; Schildkraut, I.; Saenger, W. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 10957-10961.
(2) Schluckebier, G.; Labahn, J.; Granzin, J.; Schildkraut, I.; Saenger, W. *Gene*, **1995**, *157*, 131-134.
(3) Klimasauskas, S.; Kumar, S.; Roberts, R. J.; Cheng, X. *Cell*, **1994**, *76*, 357-369.
(4) Yang, A. S.; Shen, J.-C.; Zingg, J.-M.; Mi, S.; Jones, P. A. *Nucleic Acids Res.*, **1995**, *23*, 1380-1387; Klimašauskas, S.; Roberts, R. J. *Nucleic Acids Res.*, **1995**, *23*, 1388-1395.
(5) Cal, S.; Connolly, B. A. *J. Biol. Chem.*, **1997**, *272*, 490–496.
(6) Allan, B. W.; Beechem, J. M.; Lindstrom, W. M.; Reich, N. O. *J. Biol. Chem.*, **1998**, *273*, 2368-2373.
(7) Holz, B.; Klimasauskas, S.; Serva, S.; Weinhold, E. *Nucleic Acids Res.*, **1998**, *26*, 1076-1083.