



Interaction of *EcoP15I* DNA Methyltransferase with Oligonucleotides Containing the Asymmetric Sequence 5'-CAGCAG-3'

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EcoP15I DNA methyltransferase (Mtase) recognizes the asymmetric sequence CAGCAG and catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to the second adenine residue. We have investigated the DNA binding properties of *EcoP15I* DNA Mtase using gel mobility shift assays. *EcoP15I* DNA Mtase binds approximately threefold more tightly to DNA containing its recognition sequence, CAGCAG, than to non-specific sequences in the absence or presence of cofactors. Interestingly, in the presence of ATP the discrimination between specific and non-specific sequences increases significantly. These results suggest for the first time a role for ATP in DNA recognition by type III restriction-modification enzymes. In addition, we have shown that bromodeoxyuridine-containing oligonucleotides form complexes with *EcoP15I* DNA Mtase that are crosslinked upon irradiation. More importantly, we have shown that the crosslink site is at the site of DNA binding, since it can be suppressed by an excess of unmodified oligonucleotide. *EcoP15I* DNA Mtase exhibited Michaelis-Menten kinetics with both unmodified and bromodeoxyuridine-substituted DNA, with a higher specificity constant for the latter. Furthermore, gel mobility shift assays showed that proteolyzed *EcoP15I* DNA Mtase formed a specific complex with DNA, which had similar mobility as the native protein-DNA complex. Taken together these results form the basis for a detailed structure-function analysis of *EcoP15I* DNA Mtase.

Keywords: DNA methyltransferase; type III restriction modification; *S*-adenosyl-L-methionine; DNA-protein interactions

1. Introduction

Sequence-specific recognition of DNA by proteins is involved in many of the fundamental processes that occur inside cells including replication, packaging, recombination, restriction, DNA repair and transcription. Proteins that interact with particular target sequences in DNA may show sequence selectivities ranging from stringent to fairly permissive, depending on the requirements imposed by their functions. Restriction and modification enzymes are attractive model systems for studying the mechanisms of interactions of proteins with specific DNA sequences. The restriction endonuclease and the cognate methyltransferase (Mtase†) recognize the same DNA sequence although each

catalyzes quite a different reaction (Wilson & Murray, 1991). Perhaps the best studied enzymes are the *EcoRI* and *EcoRV* endonucleases, on which a variety of studies have been carried out. Crystallographic lattices of the complexes of *EcoRI* and *EcoRV* endonucleases containing their respective recognition sequences have shown that the specific binding is facilitated by a small number of specific hydrogen bonds and van der Waals interactions between functional groups of amino acid side-chains and nucleotide bases (Rosenberg, 1991; Winkler, 1992).

In contrast to the restriction endonucleases, few comparable studies of DNA binding by DNA Mtases have been reported (Bergerat & Guschlbauer, 1990; Dubey & Roberts, 1992; Taylor *et al.*, 1993; Powell *et al.*, 1993). None of the known DNA binding motifs has been detected by inspection of the protein sequences of several Mtases and yet exquisite specificity in recognizing DNA is achieved. We have focussed on the *EcoP15I* DNA methyltransferase (*EcoP15I* DNA Mtase) as a model system to study protein-DNA interactions. *EcoP15I* DNA Mtase is part of the type III restriction-modification system (Bickle & Krüger, 1993). The type III restriction and modification

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† Abbreviations used: Mtase, methyltransferase; AdoHcy, *S*-adenosyl-L-homocysteine; AdoMet, *S*-adenosyl-L-methionine; BrdUrd, bromodeoxyuridine; DMS, dimethylsulphide; K_d , dissociation constant; R-M, restriction-modification.