Solubility engineering of the \textit{HhaI} methyltransferase

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DNA methylation is involved in epigenetic control of numerous cellular processes in eukaryotes, however, many mechanistic aspects of this phenomenon are not yet understood. A bacterial prototype cytosine-C5 methyltransferase, \textit{M.HhaI}, serves as a paradigm system for structural and mechanistic studies of biological DNA methylation, but further analysis of the 37 kDa protein is hampered by its insufficient solubility (0.15 mM). To overcome this problem, three hydrophobic patches on the surface of \textit{M. HhaI} that are not involved in substrate interactions were subjected to site-specific mutagenesis. Residues M51 or V213 were substituted by polar amino acids of a similar size, and/or the C-terminal tetrapeptide FKPY was replaced by a single glycine residue (\textit{\textsuperscript{324}G}). Two out of six mutants, \textit{\textsuperscript{324}G} and V213S/\textit{\textsuperscript{324}G}, showed improved solubility in initial analyses and were purified to homogeneity using a newly developed procedure. Biochemical studies of the engineered methyltransferases showed that the deletion mutant \textit{\textsuperscript{324}G} retained identical DNA binding, base flipping and catalytic properties as the wild-type enzyme. In contrast, the engineered enzyme showed (i) its significantly increased solubility (>0.35 mM), (ii) high-quality 2D-[\textsuperscript{15}N,\textsuperscript{1}H] TROSY NMR spectra, and (iii) \textsuperscript{15}N spin relaxation times evidencing the presence of a monomeric well-folded protein in solution.

\textbf{Keywords:} DNA cytosine methyltransferase/fluorescence spectroscopy/NMR/protein engineering/protein solubility

\textbf{Introduction}

The post-replicative modification of cytosine and adenine residues in DNA plays an important role in both prokaryotes and eukaryotes. DNA methylation is performed by DNA methyltransferases (MTases) using the methyl group donor S-adenosyl-L-methionine. In higher organisms, methylation at the C5 position of cytosine is solely observed. DNA methylation is involved in controlling many cellular processes such as gene repression, X-chromosome inactivation, genome imprinting and replication timing (Vertino, 1999). 5-Methylcytosine is a key determinant of epigenetic regulation and is essential for normal development of animal and plant species. Ablations in cytosine-5 methylation correlate with human genetic disease and, therefore, the MTases are potent candidate targets for developing new therapies (Szyf, 1998). In prokaryotes, DNA MTases often serve as components of restriction-modification systems (Dryden, 1999). Their role in regulation of certain essential genes in pathogenic bacteria has led to their use as targets for developing novel antibiotic drugs (Wahnon \textit{et al.}, 2001).

\textit{M.HhaI} is a DNA cytosine-5 methyltransferase from bacterium \textit{Haemophilus haemolyticus}, which recognizes the tetranucleotide sequence 5\textsuperscript{\prime}–GCGC-3\textsuperscript{\prime} and methylates the inner cytosine (bold face). With 327 residues (37 kDa), it is one of the smallest representatives of a homologous family of enzymes (Cheng and Roberts, 2001). The \textit{HhaI} MTase became a paradigm for structural studies after the discovery of an unusual reaction intermediate, whereby an enzyme completely flips its target nucleotide out of the DNA helix and into the catalytic site for methylation (Klimašauskas \textit{et al.}, 1994). Numerous subsequent studies showed that DNA base flipping is a common and crucial event in enzymatic DNA modification and DNA repair (Roberts and Cheng, 1998; Hollis \textit{et al.}, 2000; Mol \textit{et al.}, 2000; Goedecke \textit{et al.}, 2001).

Substantial efforts have since been devoted to elucidate the mechanisms of base flipping by DNA modifying enzymes. The \textit{HhaI} MTase has been extensively examined by employing a variety of methods including mutagenesis, kinetic analysis, fluorescence and NMR spectroscopy, isothermal calorimetry and X-ray crystallography (Wang \textit{et al.}, 2000; Cheng and Roberts, 2001; Vilkaitis \textit{et al.}, 2001; Swaminathan \textit{et al.}, 2002; Varnai and Lavery, 2002; Zhou \textit{et al.}, 2002). A series of crystal structures for \textit{M.HhaI}-DNA complexes revealed valuable details of interactions at atomic resolution. However, they all showed the target base in its final position outside the helix (O’Gara \textit{et al.}, 1998; Vilkaitis \textit{et al.}, 2000). \textsuperscript{19}F-NMR studies in solution identified important base-flipping intermediates (Klimašauskas \textit{et al.}, 1998), but technical limitations (see below) precluded thorough structural characterization by NMR. Crucial aspects of the reaction mechanism still remain obscure and to advance understanding of the base-flipping process requires structural characterization of initial and intermediate conformers of the reaction complex. To obtain further insights into the mechanism of base flipping we decided to initiate an NMR structural study of macromolecular interactions and dynamics of the \textit{HhaI} methyltransferase–DNA system in solution. However, our extensive experience, including previous \textsuperscript{19}F-NMR studies (Klimašauskas \textit{et al.}, 1998), revealed an insufficient solubility of the enzyme under reaction conditions (0.15 mM in a buffer containing 50 mM NaCl). Increased salt concentration or other additives help somewhat to maintain the \textit{HhaI} MTase in solution, but variation of the ionic strength also interferes with the enzyme’s interaction with DNA. Moreover, owing to poor behavior at high concentrations, in certain cases problems were encountered when studying MTase–DNA interactions with isothermal calorimetry, stopped-flow fluorescence or even gel shift analysis (see below).

Here we report the structure-based rational design of func-