Sequence-specific DNA glycosylase found in a restriction-modification system

K. Miyazono1, Y. Furuta2,3, M. Watanabe-Matsui2, T. Miyakawa1, T. Ito1, I. Kobayashi2,3,4, M. Tanokura1

1The University of Tokyo, Department of Applied Biological Chemistry, Tokyo, Japan, 2The University of Tokyo, Department of Medical Genome Sciences, Tokyo, Japan, 3The University of Tokyo, Institute of Medical Science, Tokyo, Japan, 4The University of Tokyo, Graduate Program in Biophysics and Biochemistry, Tokyo, Japan

Restriction-modification systems consist of genes that encode a restriction enzyme and a cognate modification methyltransferase. It was believed that restriction enzymes are sequence-specific endonucleases that introduce double-strand breaks at specific sites by catalyzing the cleavages of phosphodiester bonds. R.PabI is a type II restriction enzyme from a hyperthermophilic archaea Pyrococcus abyssi that recognizes 5'-GTAC-3' sequence and cleaves DNA duplexes without the addition of a divalent cation. The structural and mutational analyses of R.PabI in our previous work showed that R.PabI forms a homodimer and has a novel DNA-binding fold called a “half-pipe,” which consists of a highly curved anti-parallel β-sheet. Because the structure of R.PabI shares no structural similarity to any other protein, the structural basis of the sequence-recognition and DNA-cleavage mechanisms remained unclear. In this study, we report the crystal structure of R.PabI in complex with a DNA duplex containing the R.PabI recognition sequence. The structure of the R.PabI-DNA complex shows that R.PabI unwinds a DNA duplex at a 5'-GTAC-3' site and flips the guanine and adenine bases out of the DNA helix to recognize the sequence. The electron-density map of the R.PabI-DNA complex shows that R.PabI releases adenine bases from the R.PabI recognition sequence. Biochemical assays using HPLC and MALDI-TOF MS spectrometry also support the observation that R.PabI releases adenine bases by hydrolysis. These results show that R.PabI is not an endonuclease but a sequence-specific adenine DNA glycosylase. R.PabI is the first example of a restriction enzyme that shows DNA glycosylase activity. Mutational analysis reveals the active site of the adenine DNA glycosylase activity of R.PabI. The two opposing apurinic/apyrimidinic (AP) sites generated by R.PabI are cleaved by heat promoted β elimination and/or by endogenous AP endonucleases of host cells to introduce a double-strand break.


Keywords: Restriction enzyme, DNA glycosylase, crystal structure