Sequence-Specific DNA Glycosylase Found in a Restriction-Modification System

A restriction-modification system consists of genes that encode a restriction enzyme and a cognate methyltransferase. Thus far, it was believed that restriction enzymes are sequence-specific DNA endonucleases. Here we report for the first time that one of the restriction enzymes, R.PabI, is not an endonuclease but a sequence-specific adenine DNA glycosylase based on the crystal structure and enzymatic activity. R.PabI captures a 5'-GTAC-3' sequence and removes adenine bases to generate opposing AP sites. The AP sites are cleaved by heat-promoted β elimination, and/or by endogenous AP endonucleases of host cells to introduce a double-strand break.

Restriction enzymes recognize specific DNA sequences and introduce strand breaks unless the sequences are methylated by the cognate methyltransferases. In prokaryotic cells, restriction-modification systems limit transfers of exogenous DNA and protect themselves from infections of phages. Thus far, it was believed that restriction enzymes are sequence-specific endonucleases that leave the 3'-OH end and 5'-phosphate end. Most of the type II restriction enzymes, which are frequently used in the field of biotechnology, have a PD-(D/E)XK motif in their active sites and use Mg^{2+} ions for their enzymatic activities. Other type II restriction enzymes belong to the phospholipase D (PLD) superfamily, the HNH superfamily, and the GIY-YIG superfamily. In addition to these restriction enzyme superfamilies, we found a half-pipe superfamily as represented by R.PabI from a hyperthermophilic archaea *Pyrococcus abyssi*.

R.PabI is a type II restriction enzyme that recognizes a 5'-GTAC-3' sequence and cleaves double-stranded DNAs at high temperature without the addition of a divalent cation similar to the PLD superfamily, although R.PabI shows no sequence similarity to the PLD superfamily. Our previous report showed that R.PabI forms a homodimeric structure and has a novel DNA-binding fold called a "half pipe," which consists of a highly conserved anti-parallel β-sheet [1]. Among the thousands of confirmed and hypothetical restriction enzymes identified, only a few proteins are predicted to have the half-pipe fold. To reveal the sequence recognition and DNA cleavage mechanisms of R.PabI, we determined the crystal structure of the R.PabI-DNA complex by X-ray crystallography using synchrotron radiation at the Photon Factory AR-NE3A beamline [2].

The structure of the R.PabI-DNA complex is shown in Fig. 1. R.PabI bends the double-stranded DNA approximately 90° at the 5'-GTAC-3' sequence and the highly expanded minor groove side of the bent DNA faces the half-pipe region. The 5'-GTAC-3' duplex is unwound by the binding of R.PabI and the bases are captured between the core region and the β8-β9 loop of R.PabI. The most striking feature of the R.PabI-DNA complex determined in this study is that the *N*-glycosidic bond between the adenine base and deoxyribose of Ade11 is cleaved (Fig. 1, right panel). This suggests that R.PabI has catalyzed the cleavage of the *N*-glycosidic bond of Ade11 similar to DNA glycosylases, although R.PabI shows no sequence similarity to DNA glycosylases. The DNA glycosylase activity of R.PabI was confirmed by denaturing polyacrylamide gel electrophoresis (denaturing PAGE), high performance liquid chromatography (HPLC) and matrix-assisted laser desorption-ionization time-of-flight mass (MALDI-TOF MS) spectrometry. Mutation analyses of R.PabI showed that the DNA glycosylase activity of R.PabI is catalyzed by two highly conserved residues, Tyr68 and Asp214.

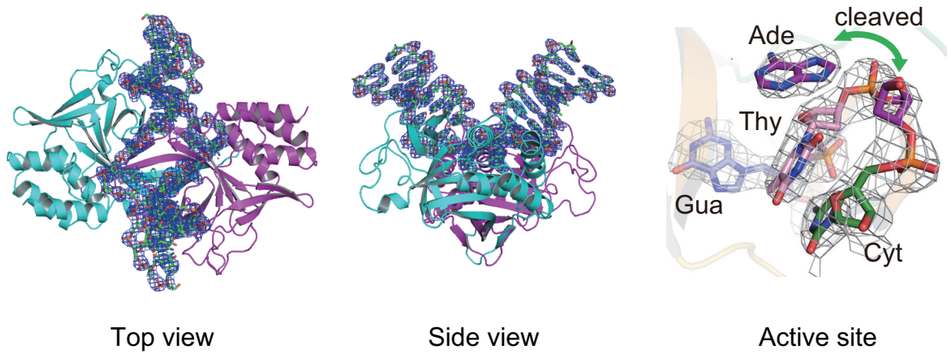

Figure 1: Structure of the R.PabI-DNA complex.

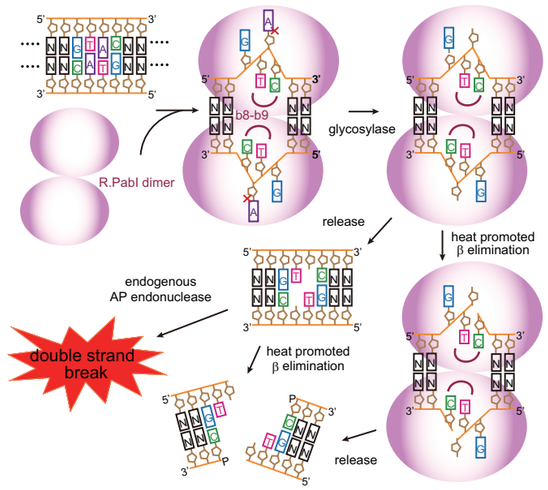

Figure 2: DNA cleavage mechanism by R.PabI.

Although the structural and biochemical analyses of R.PabI indicate that R.PabI is an adenine DNA glycosylase, R.PabI cleaves the DNA duplex at the specific site in a manner similar to restriction endonucleases. The opposing AP sites generated by R.PabI may be cleaved by two different mechanisms to introduce a double-strand break (Fig. 2). First, the R.PabI product is cleaved by heat-promoted β elimination. Because R.PabI is an enzyme from hyperthermophilic archaea, R.PabI shows DNA-cleavage activity at high temperature (60–90°C). Under these conditions, the opposing AP sites generated by R.PabI will be cleaved by heat-promoted β elimination. Second, the R.PabI product is cleaved by AP endonucleases of host cells. This cooperating mechanism would be important for R.PabI homologues from mesophiles to cleave exogenous DNA.

Because the DNA fragments cleaved by the effect of R.PabI lose adenine bases at the cleavage site, it would be hard to repair the R.PabI products by DNA ligase, unlike in the case of DNA fragments produced by typical restriction endonucleases. This feature may make the restriction-modification system using DNA glycosylase activity more powerful in attacking DNA than the typical restriction-modification system. Because R.PabI does not share any structural similarity with known DNA glycosylases, the half-pipe superfamily represented by R.PabI is a new superfamily of DNA glycosylases.

REFERENCES

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BEAMLINE

AR-NE3A

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