No. 78

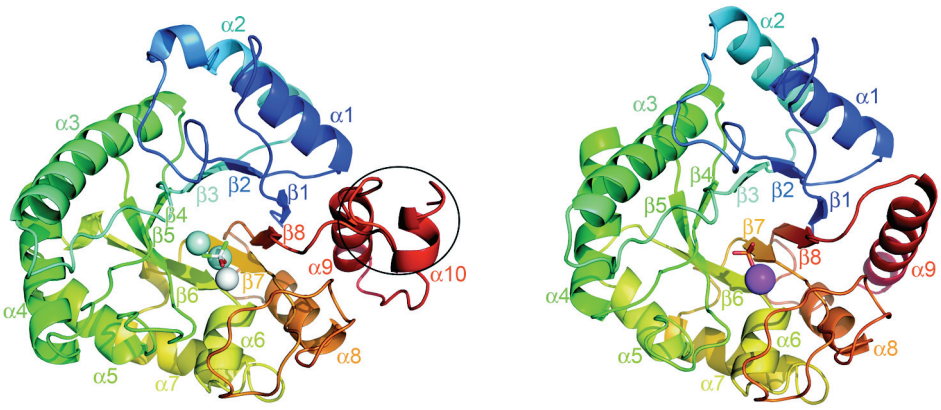
An additional C-terminal loop in endonuclease IV, an apurinic/apyrimidinic endonuclease, controls binding affinity to DNA

Ryuichi Asano[1], Hirohito Ishikawa[1], Shuhei Nakane[1], Noriko Nakagawa[1,2], Seiki Kuramitsu[1,2] and Ryoji Masui[1,2]

[1]Dept. Biol. Sci., Grad. Sch. Sci., Osaka Univ., [2]RIKEN SPring-8 Center, Harima Inst.

e-mail: niur@bio.sci.osaka-u.ac.jp

The apurinic/apyrimidinic (AP) site is one of the most common DNA lesions *in vivo* and is produced by spontaneous hydrolysis, chemical toxins, radiation, or DNA glycosylases that remove abnormal DNA bases. The AP site prevents transcription and translation, and thus must be repaired by DNA repair pathways, including base excision repair. In base excision repair, AP endonuclease recognizes the AP site and cleaves the DNA backbone at the 5′-side of the AP site to generate a 5′-deoxyribose phosphate group and a free 3′-hydroxyl end for DNA polymerase repair synthesis. AP endonucleases are classified on the basis of their structure as members of either the exonuclease III family or the endonuclease IV (EndoIV) family. EndoIV is further classified as either long or short type. The presence of an additional short sequence at the C-terminus of a long type EndoIV may affect the molecular function of the enzyme. To date, however, the tertiary structure of the long type EndoIV has not been determined. In this study, we chose thermophilic representatives of each type of EndoIV: *Geobacillus kaustophilus* EndoIV (gkEndoIV) is that of long type, while *Thermus thermophilus* HB8 EndoIV (ttEndoIV) is that of short type. In these species, gkEndoIV and ttEndoIV are the only AP endonucleases present. We determined the tertiary structures of gKEndoIV and ttEndoIV by X-ray crystallography. gkEndoIV (the long type) had a higher affinity for double-stranded DNA containing an AP site analogue than ttEndoIV (the short type). Structural analysis of the two different EndoIVs suggested that a C-terminal DNA recognition loop present only in the long type contributed to its high affinity for AP sites. A mutation analysis showed that Lys267 in the C-terminal putative DNA recognition loop plays an important role in DNA binding. These results suggested that the C-terminal additional loop in a long type EndoIV contributed to the high binding affinity for DNA containing an AP site.



The overall structures of gkEndoIV (left) and ttEndoIV (right)