APE1 Exonuclease Distinguishes Various DNA Substrates by an Induced Space-Filling Mechanism

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Apurinic/apyrimidinic endonuclease 1 (APE1) is a well-known endonuclease specifically targeting an AP site to initiate base excision repair. Interestingly, APE1 also bears 3′-to-5′ exonuclease activity that shows very different catalytic properties and cellular functions. The 3'-to-5' exonuclease activity of APE1 is responsible for processing matched/mismatched terminus of duplex DNA in various DNA repair pathways, as well as for nucleoside analogs removal associated with drug resistance. Due to the limited information of APE1's exonucleolytic catalysis, its fundamental roles in various DNA repair pathways and in drug resistance are poorly understood. In addition, how APE1 exonucleolytically recognizes and processes the terminus of duplex DNA without base preference remain unclear. We determined the first two APE1-dsDNA complex structures, which displayed a dsDNA end-binding mode. Integration of our structures, biochemical assays, and molecular dynamics simulation reveals the general rules of APE1 in handling various dsDNA substrates. The DNA binding-induced RM (Arg176 and Met269) bridge formation in active site and DNA-binding modes transition between matched and mismatched termini of dsDNA compose the exquisite machinery for substrate selection, binding, and digestion. Our studies pave the way for understanding the dsDNA terminal-processing-related cellular functions and drug resistance mechanisms of APE1.

[1] Liu, T.C.; Lin, C.T.; Chang, K.C.; Guo, K.W.; Wang, S.Y.; Chu, J.W. & Hsiao, Y.Y. (2021). *Nat Commun.* 12, 601.

[2] Liu, T.C.; Guo, K.W.; Chu, J.W. & Hsiao, Y.Y. (2021). *Comput. Struct. Biotechnol. J.* 19, 3682-3691

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