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Influence of orphan base and sequence context on the processing of AP sites by AP lyases in Arabidopsis thaliana

M. Jordano-Raya[1,2,3], R.R. Ariza[1,2,3], T. Roldán-Arjona[1,2,3] and D. Córdoba-Cañero[1,2,3]

[1] Department of Genetics, University of Córdoba, Córdoba, Spain
[2] Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Córdoba, Spain
[3] Reina Sofía University Hospital, Spain
E-mail: b52joram@uco.es

Abasic (apurinic/apyrimidinic, AP) sites are ubiquitous DNA lesions arising by spontaneous loss of a nitrogenous base due to the susceptibility of the N-glycosidic bond to undergo hydrolysis reactions. Moreover, AP sites are also enzymatically generated as intermediates during the Base Excision Repair (BER) pathway, in which DNA glycosylases catalyze the excision of modified bases from DNA. AP sites may be processed by either AP endonucleases or AP lyases, but the relative roles of these two types of enzymes are not well understood. We hypothesized that the sequence flanking the AP site and the orphan base opposite the lesion may determine the enzyme responsible for its processing and the repair efficiency.

We analyzed the activity of the major Arabidopsis AP lyase (FPG) on DNA substrates containing an abasic site opposite C or G in different sequence contexts. AP sites opposite G are common intermediates during the repair of deaminated cytosines, whereas AP sites opposite C frequently arise from oxidized guanines. We found that FPG shows a preference for AP sites opposite C, but such specificity is modulated by the DNA sequence context surrounding the lesion.

To identify possible mechanisms responsible for the opposite base preference displayed by FPG we performed DNA binding assays with different DNA substrates, as well as with reaction products. We found that FPG binds DNA with high affinity regardless of the presence of and AP site, suggesting that the enzyme binds DNA non-specifically and slides randomly in search for its target. We have also observed that FPG binds DNA substrates containing AP sites with the same affinity independently of the base opposite the lesion. However, FPG remains bound with higher affinity to its incised reaction product when G is the orphan base. This result suggests that the higher activity of FPG for AP sites opposite G is not due to increased affinity for the substrate, but to decreased product dissociation. Our results shed light on the role of AP lyases in the processing of a ubiquitous DNA lesion.

Keywords:
Base Excision Repair, AP sites, AP lyase, FPG.