

**Insights into the length- and location-dependent deaminase activities of
APOBEC3B/F and the deaminase activity determinants of APOBEC3F**

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Human A3 proteins play important biological roles in the intrinsic immune system by defending against endogenous and exogenous DNA pathogens. Specifically, A3F exhibits efficient anti-HIV-1 activity by being packed into the progeny viral particles, and then interferes with reverse transcription, and mutates cytosines into uracils in nascent cDNA, resulting in blocking of viral replication. Different from A3F, A3B is involved in various cancer types, and it was revealed that aberrant expression of A3B causes a genomic DNA lesion. Here, we used the real-time NMR method, together with biochemical methods, to study the deamination characteristics of A3B and A3F, and identified the mechanism of deamination by A3B and A3F.

In chapter 1, a general introduction was given, including energy and enzymes, HIV and cancers, and the structural characteristics of A3 proteins, together with its antiviral activity and relationship with cancers, the binding interface of A3 proteins with oligonucleotides, and the movement on ssDNA.

In chapter 2, how the target sequences are searched for and deaminated by A3B-CTD was analyzed. The real-time NMR method, as well as the UDG assay, were applied to measure the length- and location-dependent deaminase activity of A3B-CTD. We found that A3B-CTD shows higher activity toward its target sequence in short ssDNA and efficiently deaminates a target sequence located near the center of the ssDNA. These properties are quite different from those of well-studied A3G, which shows higher activity toward its target sequence in long ssDNA and one located close to the 5'-end.

The unique properties of the A3B-CTD can be rationally interpreted by considering that after non-specific binding to ssDNA, A3B-CTD only slides for a relatively short distance to search for the target sequence and tends to dissociate from the ssDNA before reaching the target sequence. The possible biological significance of this was also discussed.

In chapter 3, the deaminase activity of A3F-CTD, including the influence of the DNA sequence and pH on deaminase activity, together with the roles of the amino acid residues located close to the catalytic center in deaminase activity and ssDNA binding were systematically characterized. Firstly, we optimized the target sequence and the length of the ssDNA substrate, and found that A3F-CTD efficiently deaminates 5'-TTCA/G-3' in longer ssDNA(>30 nt). Secondly, on the basis of the result of mutagenesis analysis of the amino acid residues that are close to the catalytic center, combining the structure model of the A3F-CTD:ssDNA complex, we found that some amino acid residues are responsible for the binding to ssDNA, while others are responsible for the catalytic efficiency. Thirdly, we revealed that the deaminase activity of A3F-CTD may be regulated through phosphorylation and de-phosphorylation of the S216 residue. Lastly, we investigated the pH effects on the deaminase activity of A3F-CTD and noticed that the N214H mutant shows obviously high deaminase activity at pH 5.0 and pH 7.5.

In chapter 4, the length-dependence of the deaminase activity of A3F-CTD was studied. We identified the length-dependent deamination by wt A3F-CTD and the deaminase activity of the N214H mutant. Comparing the results obtained with the UDG assay and real-time NMR method, we found that the concentration of the ssDNA substrate could affect the length-dependent deamination by A3F. In presence of a low

ssDNA concentration, A3F prefers to deaminate a target sequence in long ssDNA, while in presence of high ssDNA concentration, A3F efficiently deaminates a target sequence in short ssDNA. On the basis of the different binding affinities of N214H with different lengths of ssDNA, these unique characteristics were rationally interpreted.

In chapter 5, the deaminase activity of A3F-CTD in presence of the Vif complex was investigated using the UDG assay. The results suggested that the Vif complex efficiently inhibits the deamination by A3F-CTD *in vitro*. This finding indicates that the Vif complex neutralizes the function of A3F not only through E3 ubiquitin mediated proteasomal degradation, but also by direct interference with the deamination.

In chapter 6, a general conclusion was given.

In this study, we mainly investigated the length- and location-dependent deaminase activities of A3B and A3F, respectively, as well as the deamination properties of A3F. The results will help us to better understand the overall molecular mechanism of DNA editing by cytosine deaminases.