Incipient complex formation between AP endonucleases and DNA containing AP site: A vital role of the tryptophan residue

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ABSTRACT

To elucidate whether the tryptophan residues in the vicinity of the catalytic site are involved in AP site recognition and are critical for AP endonuclease activity, the AP endonucleases of the four subtypes in the ExoIII AP endonuclease family were characterized and compared the positions of the tryptophan residues. The positions of the catalytic amino acid residues, corresponding to Glu-34, Asp-229, and His-259 of ExoIII, are strictly conserved. On the other hand, the positions of the tryptophan residues, which are critical to the incipient complex formation, do not exist at a fixed position. There are four patterns at the position of the essential tryptophan residue.

RESULTS AND DISCUSSION

To get further support of the AP site recognition mechanism of ExoIII family AP endonucleases, the putative AP endonuclease of C. glutamicum (CglAPE2) was chosen. Amino acid sequence of CglAPE2 is highly homologous to E. coli ExoIII. First, to investigate whether this protein have AP endonuclease activity, CglAPE2 was overexpressed as hexahistidine tagged protein in E. coli. The recombinant protein was purified from E. coli cell extracts. AP endonuclease assay was conducted using this purified protein and the oligonucleotide duplex containing an AP site. Since it is known that ExoIII cleaves DNA containing a 1", 2"-dideoxyribofuranose residue, which is an alkaline-stable AP site, the duplex containing this analogue residue was used to detect the AP endonuclease activity.

Fig. 1 Products from ds-DNA containing an AP site (H). lane 1, no enzyme; lane 2, ExoIII (E. coli); lanes from 3 to 6, cglAPE2 (0.5, 1, 2, and 5 pmol) Phosphatase labeling was performed at 5' end of the oligonucleotide containing an AP site. Product 1 was produced by AP endonuclease activity. Products 2 were produced by exonuclease activity of the multifunctional AP endonuclease from product 1.

The reaction mixtures were incubated at 30 °C for 10 minutes, and then electrophoresed in a 7 M urea denaturing polyacrylamide gel. As shown in Fig. 1, the patterns of reaction products (lanes 3 to 6) generated by CglAPE2 were significantly different from that of ExoIII (lane 2). When
ExoIII was added to the reaction mixture, the product generated by 5' side cleavage of AP site (AP endonuclease activity) was appeared as a major product (Fig. 1, lane 2, product 1). Products smaller than the product 1 (Fig. 1, lanes 3 to 6, products 2) were generated by exonucleolytic cleavage of AP endonuclease product from 3' end to 5' side. In the case of CglAPE2, the bands, which have the same mobilities as products 2, were mainly detected. These bands clearly indicated that CglAPE2 has comparatively strong 3'-5' exonuclease activity.

To determine whether the tryptophan residue (W208) is involved in the AP site recognition, site-directed mutagenesis of this CglAPE2 was performed. If the substituted tryptophan residue contributes to the AP site recognition, the mutant proteins will be unable to recognize an AP site, resulting in reduction of the AP endonuclease activity and binding ability to AP-DNA. For this purpose, the W208S, W208S/Y207W, W208S/M221W, Y207W, and M221W mutants of CglAPE2 were constructed. Trp-208 and Met-221 of CglAPE2 correspond to Trp-212 and Leu-226 of ExoIII, respectively. A serine substitution of the Trp-208 resulted in the loss of AP endonuclease activity (Fig. 2).

![Fig. 2](image)

**Fig. 2** The AP endonuclease and 3'-5' exonuclease activities of the CglAPE2 mutants. The site-directed mutants were named from the mutated amino acid residue (one-letter amino acid notation + position) plus the substituted amino acid (one-letter amino acid notation), e.g. Y207W, a mutant having substitution of Try for Trp at position 207. Product 1 was produced by AP endonuclease activity. Products 2 were produced by exonuclease activity of the multifunctional AP endonuclease CglAPE2 from product 1.

### CONCLUSION

Recently, we have speculated the AP site recognition mechanism of the ExoIII family AP endonuclease. The two AP endonucleases, *E. coli* ExoIII and human APE1, were used to clarify this mechanism. It has been speculated that the tryptophan residue, which protrudes from the surface in the vicinity of the catalytic site, is an essential player in the AP site recognition mechanism. Such a tryptophan residue is Trp-212 in *E. coli* ExoIII, and Trp-280 in human APE1. It has been suggested that intercalation of the indole ring of the tryptophan residue into the space produced by deletion of the DNA base is the mechanism of AP site recognition. The positions of the tryptophan residues, which are critical to the incipient complex formation, do not exist at a fixed position. It has been elucidated that there are four patterns (Fig. 3) at the position of the essential tryptophan residue.

![Fig. 3](image)

**Fig. 3** Sequence alignment of the vicinities of the active sites of AP endonucleases. The tryptophan residues, which are critical to AP endonuclease activities and to incipient complex formation, are shown in the outside of an AP endonuclease shown as an oval. A tryptophan residue in the inside of the oval does not play an important role, however the tryptophan residue exists in the vicinity of the active site.

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### REFERENCES


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