Study of Deamination Kinetics of 5-methylated Cytosine-Containing Pyrimidine Dimers in Oligodeoxynucleotides by Nuclease P1 Enzyme Coupled Tandem Mass Spectrometry

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Introduction

Recent work indicates that the covalent addition of a methyl group to cytosine in DNA plays an important role in sunlight-induced mutagenesis. It has been found that the presence of 5-methylcytosine bases enhances the extent of cyclobutane pyrimidine dimers (CPD) formation by UVB or sunlight at 5'-CCG and 5'-TCG sequences in the p53 gene relative to UVC irradiation. Based on its abundance, slow repair and distinct mutagenicity, the CPD has been considered the most mutagenic lesion. The CPD undergoes deamination, and that may account for UV-induced C→T and CC→TT transition mutations linked to skin cancer. After the deamination-bypass mechanism was proposed, a number of studies have been made to determine the rates and mechanism of deamination in vitro and vivo. We report here the results of a quantitative enzyme-coupled tandem mass spectrometry method to monitor the deamination kinetics of the cis-syn cyclobutane dimers containing 5-methylated cytosine in both single- and double-strand DNAs.

Methods

Oligodeoxynucleotides (ODNs) containing 5-methylcytosine were obtained from IDT (Integrated DNA Technologies, INC). Duplex hairpin DNAs were annealed by heating 1-mM solutions of ODNs to 90°C prior to use. Melting temperature curves were obtained to confirm the duplex formation at 37°C. Single-strand and double-strand DNAs containing cis-syn dimers were prepared by irradiating nitrogen-purged 30-μM aqueous solutions of ODNs in Pyrex NMR tubes with 450 W mercury arc light for 2 or 3 h in an ice-water bath. The cis-syn dimer-containing ODN solutions were incubated in 10-mM ammonium acetate buffer (pH = 6.8 at T = 25°C), then deamination reactions were carried out at T = 37°C for various times. The deaminated samples were digested with nuclease P1 for 3 min at room temperature. Typically, 1 μl of a 1-unit/μl aqueous solution of nuclease P1 was added to a 30-μl aliquot of ODNs. Then, the nuclease P1 digestion solution was analyzed by ESI coupled to MS/MS (Finnigan LCQ Classic). MS/MS data were acquired on the selected [M-H]- ions. To select both the deaminated and undeaminated components for fragmentation, the mass width for precursor selection was set at 5-6 m/z units. Figure 1 is an example that shows the "zoom-scan" MS/MS of 803 m/z ion for the deamination of hairpin GCG CGT AmC[c,s]T ATC GCG at various time points.

Results and Discussions

The fractions of deaminated and undeaminated species in the mixtures were calculated from peak areas obtained by integrating the ion chromatogram of one unit wide after correcting for contributions from isotopic abundances. Rate constants of deamination of cis-syn dimers were extracted from plots of ln (fraction of undeaminated species) vs time. In this study, the deamination of 5-methylated cytosine-containing CPDs was examined as functions of sequence context (mCT site vs TmC site), flanking base (adenines vs guanines) and secondary structure (single strand vs double strand). The obtained results were tabulated in table 1.

The results showed that duplex formation inhibits deamination of methylated cis-syn dimers at both TmC and mCT sites flanked by adenines. The inhibition of deamination in the duplex may be due to a conformation of the flanking adenines, which block the attack of water on the 5-methylcytosine. For cis-syn dimers of a mCT site, the rate of the deamination is 2.5-fold slower in double-stranded ODNs than that in single-strand ODNs, whereas the deamination rate of cis-syn dimers at TmC sites is about 6-fold slower in double-stranded ODNs than in single-strand ODNs.

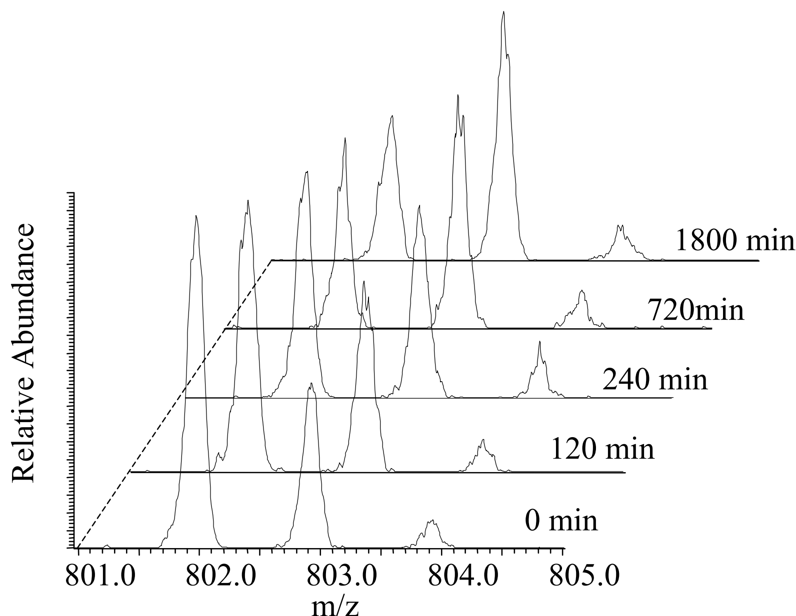
Replacing flanking As by Gs increases the rate of deamination at both mCT and TmC sites in single-strand and double-strand DNAs. This effect may originate from the ability of guanine to assist the addition of water to the 5-methyl cytosine, and that this is slightly enhanced for the 5'-mC and slightly diminished for the 3'-mC. Extensive study is needed to understand the effect of flanking guanines on the deamination of the 5-methylated cytosine in CPDs.

By using enzyme-coupled with tandem mass spectrometry method, we were able to determine the rates of deamination of the CPDs and study partially the effects of the flanking sequence, sequence context and secondary structure on the rates of deamination.

Table 1: Summary of the kinetics of 5-methylated cytosine-containing CPDs

		37°C	
		k (min^{-1})	**$t_{1/2}$ (min)**
Flanking Adenines	GAGT**AmC[c,s]TA**TGAG	0.000925 ± 22%	750
	GAGT**AT[c,s]mCA**TGAG	0.00165	420
	Hairpin GCGCGT**AmC[c,s]TA**TCGCG	0.00076 ± 19%	1760
	Hairpin GCGCGT**AT[c,s]mCA**TCGCG	0.000266 ± 23%	2600
Flanking Guanines	GT**GmC[c,s]TG**GCAC	0.00106 ± 15%	650
	GT**GT[c,s]mCG**GCAC	0.00416 ± 4%	170
	Hairpin CACGT**GmC[c,s]TG**GCACCAC	0.00178 ± 12%	390
	Hairpin GT**GT[c,s]mCG**GCACCAC	0.00218 ± 18%	320

Figure 1: The "zoom scan" MS/MS of 803 m/z ion for the deamination of hairpin GCG CGT AmC[c,s]T ATC GCG at various time points.



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