Molecular design of hairpin pyrrole-imidazole polyamides possessing sequence specific DNA alkylating moiety

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ABSTRACT

By conjugating pyrrole(Py)/imidazole(Im) hairpin polyamides with CBI, a stable alkylating moiety, we have developed a new type of DNA-sequence-specific alkylating agent. The sequence specificity and alkylation efficiency of the CBI conjugates were analyzed with high-resolution denaturing gel electrophoresis using linear 450- and 1000-bp DNA. The results demonstrate that CBI conjugates selectively alkylate adenine in specific sequences, whereas the previous type of alkylating polyamide, which contains segment A of DU-86, alkylates both adenine and guanine.

INTRODUCTION

The development of biological agents that can specifically regulate the expression of particular genes is currently topical in therapeutic applications. The introduction of ribozymes and RNAi into mammalian cells has shown that such antisense agents can regulate or silence the expression of specific genes. In another approach, antigene agents like PNA, LNA, and Py-Im polyamides¹,² can directly target genomic DNA, which exists on rather upstream of general central dogma than mRNA. Dervan et al. demonstrated that Py-Im polyamides designed to bind to the promoter region of HIV-1 inhibited the transcription of HIV-1 in a cell-free system.³ These polyamides are designed to prevent the access of transcription factors (TF) to their recognition sequence, because polyamides binding to sequences in the coding region are
immediately removed from template DNA by the transcribing RNA polymerase and consequently cannot inhibit transcription. Because gene expression is generally controlled by a combination of multiple common transcription factors, the inhibition of gene expression by the binding of Py–Im polyamides to regulatory sequences potentially limits the design of the polyamides. That is, because the target sequence must be unique to a specific gene, it must contain part of the recognition sequence for the transcription factor together with unique flanking sequences.

DNA-alkylating Py–Im polyamides are expected to regulate the expression of specific genes even if they target a sequence in the coding region. We recently reported a series of Py–Im hairpin polyamides conjugated with segment A of DU-86.4 They efficiently and sequence-specifically alkylated DNA in nanomolar concentrations, resulting in the specific inhibition of mRNA expression in the GFP coding region.5 Herein, we report the specific DNA-alkylation activity of CBI hairpin conjugates.

RESULTS AND DISCUSSION
CBI is a well-studied DNA alkylating unit, and its synthetic route has been established.6–8 To assess the alkylation ability of CBI tethered to Py–Im polyamides, compound 2 was synthesized. The sequence-specific alkylation of 1 and 2 were compared using a 1000-bp linear dsDNA fragment. Py–Im polyamide 2 was expected to show the same DNA-alkylation activity as the previously reported alkylating polyamide 1, which alkylates at the purine of 5'- (T/A)GCCPu-3' with high efficiency. However, compound 2 specifically alkylated at the adenine of 5'- (T/A)GCCA-3'. These results suggest that the DU-86 and CBI alkylating moieties discriminate adenine and guanine in a target sequence. Because the expansion of the recognition sequence is important in targeting a unique sequence in a specific gene, this adenine selectivity of the CBI moiety might enhance the efficacy of alkylating Py–Im polyamides as antigene agents.

REFERENCE