The biological impact of sequence-specific DNA alkylation by pyrrole-imidazole polyamides

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ABSTRACT

We have developed a series of novel DNA alkylating polyamides possessing indole linkers. Investigations using high-resolution gel electrophoresis revealed that the indole linked Py–Im polyamide alkylated at A of a targeted nine base pair matching sequence. Evaluation in human cancer cell lines revealed that the indole linked Py–Im polyamides have strong cytotoxicities. Furthermore, we showed that alkylation of the template strand of the coding region by these polyamides causes effective gene silencing.

INTRODUCTION

Minor groove-binding Py–Im hairpin polyamides precisely recognize each of the four Watson–Crick base pairs according to the binding rule of Py–Im polyamides.1 Antiparallel pairing of Im opposite Py (Im/Py) recognizes a G–C base pair, whereas a Py/Py pair recognizes A–T or T–A base pairs.

We demonstrated that Py–Im hairpin polyamides possessing vinyl linker selectively alkylate target sequences according to the recognition rule of Py–Im polyamides.2–3 We showed that alkylation by Py–Im polyamides that recognize specific sites on the template strand of the coding region in the green fluorescent protein (GFP) gene effectively inhibits transcription in an in vitro translation system.4 We also demonstratedenantioselective DNA alkylation by conjugates of Py/Im polyamide and (S)-1,2,9,9a-tetrahydrocyclopenta[1,2-c]benzo[1,2-e]indol-4-one (S-CBI), which can be synthesized from commercially available 1,3-naphtalenediol.5

However, it is difficult for us to synthesize sufficient quantities of Py–Im CBI conjugates, because the vinyl linker is unstable under basic and acidic conditions. To overcome this problem, we searched for an alternative linker moiety that was equivalent to the vinyl linker. We selected 5-amino-indole-2-carboxylic acid, because it gives approximately the same distance and geometry as the Py-vinyl linker. The introduction of indole linker greatly facilitated the syntheses of sequence-specific alkylation Py–Im polyamides, which made it possible for us to perform large-scale syntheses for biological studies and extension of the sequence recognition of the alkylation polyamides.

RESULTS AND DISCUSSION

The indole linked Py–Im polyamide–CBI conjugates 1 and 3–7 were synthesized as follows. Py–Im polyamides possessing a free carboxylic acid were prepared by Fmoc solid phase synthesis using oxime resin and coupled with 5-amino-indole-2-carboxylic acid. Finally, the synthesis of conjugates was accomplished by coupling with seco-CBI using NaHCO3. After purification using HPLC, the hairpin polyamide–CBI conjugates were used in DNA alkylation experiments. It was found that the CBI conjugates with indole linker were significantly more stable under basic and acidic conditions.

Sequence-selective alkylation by compound 1 was investigated using high-resolution denaturing gel electrophoresis, 5′-Texas Red-labeled 500-bp DNA

Figure 1 Chemical structures of compound 1-7.
fragments, employing an automated DNA sequencer, as described in a previous paper. Sequencing analyses of the alkylated DNA fragments after heat treatment revealed that alkylation by the Py–Im hairpin polyamide–CBI conjugates occurred predominantly at the A of the matched nine base pair sequence at nanomolar concentrations. The results clearly indicated that the selectivity and the efficiency of DNA alkylation by conjugate possessing indole linker were comparable with those of the conjugate with vinyl linker.

We compared the cytotoxicities of compound 2 and the five indole-linked Py–Im polyamide–CBI conjugates 3–7, which recognize different sequences, using 10 different cell lines. The results clearly demonstrated that the growth inhibitory effects of compound 2 are altered dramatically by conjugation with Py–Im polyamides. These results also suggested that DNA-sequence specificities might contribute to the cytotoxic potency of alkylating agents.

Furthermore, we investigated the gene silencing activities of polyamides 4, 5 and 7 that selectively alkylate specific sequences in the promoter region, coding strand, and noncoding strand of the GFP gene. The GFP vector was transfected into human colon carcinoma cells and the cells were treated with 100 μM of the polyamides for 24 h. Fluorescence detection and real-time PCR analysis indicated that the Py–Im polyamide that target the coding strand offer a novel approach for sequence-specific gene silencing.

CONCLUSION

The present results suggested that indole-linked polyamides have great potential as antitumor drugs. Further biological examinations of these agents are in progress.

REFERENCES