Sequence-specific alkylation by a tandem motif of pyrrole-imidazole CBI conjugate

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ABSTRACT

We have developed various types of sequence-specific alkylation agents by conjugation of Py−Im polyamides and alkylation moieties 1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-benz[el]indole (seco-CBI) with an indole linker. In order to extend the length of target DNA sequence of the hairpin Py−Im polyamide 1, we designed and synthesized Y-shaped and tandem hairpin Py−Im polyamides 2 and 3. High-resolution denaturing polyacrylamide gel electrophoresis using 5'-Texas Red-labeled 465-bp DNA fragments revealed that conjugates 2 and 3 alkylated the adenine of target DNA sequences at nanomolar concentrations. Furthermore, evaluation in human cancer cell lines revealed that these Py−Im polyamides 2 and 3 have strong cytotoxicities.

INTRODUCTION

Minor groove-binding Py−Im polyamides precisely recognize each of the four Watson−Crick base pairs according to the binding rule of Py−Im polyamides. 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 have demonstrated that various types of indole-linked hairpin alkylation polyamides possessing seco-CBI alkylate DNA fragments sequence-specifically. The alkylation of the template strand of coding regions by these polyamides causes sequence-specific gene silencing of the green fluorescent protein gene. We also developed 10-ringed hairpin polyamides that specifically alkylate the adenine of a targeted 9-bp matching sequence.

To develop an alkylation polyamide causing selective gene silencing in an endogenous target sequence of a specific gene, a new type of hairpin motif for alkylation polyamides with sequence-specific binding-site sizes over 10 bp might be required. For this reason, we searched for alternative motifs to increase the targetable binding-site size of these hairpin motifs possessing high alkylation activity and sequence-specificity. We selected Y-shaped and tandem hairpin Py−Im polyamides as the binding moiety for the alkylation agents.

RESULTS AND DISCUSSION

We synthesized hairpin conjugate 1, Y-shaped conjugate 2 and tandem hairpin Py−Im polyamide 3 (Fig. 1). Conjugates 2 and 3 were designed so that the C-terminus of two-ring linear polyamides or six-ring hairpin polyamides are covalently tethered to the α-amino group of the γ-turn of the alkylation polyamide 1 via β-alanine, respectively. The indole linked Py−Im polyamide-CBI conjugates 1-3 were synthesized as follows. Py−Im polyamides possessing a free carboxylic acid were prepared by Fmoc solid phase synthesis using a Py-coupled oxime resin followed by treatment with NaOH. The carboxylic acids were converted to an activating ester using HCTU, 1HPr3NET, followed by coupling with Indole-CBI, which was synthesized by

![Fig. 1 Chemical structures of compound 1-3.](https://academic.oup.com/nass/article-abstract/51/1/265/1023837)
coupling seco-CBI and 5-tert-butoxyamino-1H-indole-2-carboxylic acid followed by deprotection with TFA, to produce 1-3.

Sequence-selective alkylation by compound 1-3 were investigated using 5'-Texas Red-labeled 465-bp DNA fragments, employing an automated DNA sequencer, as described in a previous paper. Sequencing analyses of the alkylated DNA fragments after heat treatment revealed that conjugates 1–3 presented distinct cleavage patterns at nanomolar concentrations. As expected, DNA alkylation by conjugate 1 occurred at three match sites with sequences 5'-AACCA A-3', 5'-AACCA A-3' and 5'-TTCC A-3' with two minor mismatch alkylation sites. DNA alkylation by conjugate 2 occurred at two match sites with sequences of 5'-AAATAACC A-3' and 5'-AAATTCG A-3'. DNA alkylation by conjugate 3 occurred mainly at one match site with a sequence of 5'-AGAATAACCA A-3' and two minor mismatch site. From the densitometric analysis of the gel electrophoresis, the sequence specificities of conjugates 1–3 at the match sites are at least threefold higher than those at the mismatch sites. In particular, conjugate 2 showed relatively high alkylating activity and sequence specificity. These results demonstrate that the Y-shaped and tandem hairpin alkylating polyanides 2 and 3 extend the recognition site size of hairpin conjugates 1 from 5 bp to 8 and 10 bp without losing the high alkylating activity and sequence specificity of 1. The cytotoxicities of compound 1-3 using cancer and normal cell lines will also be discussed.

CONCLUSION

The present results suggested that the Y-shaped and tandem motifs polyanides have great potential as antitumor drugs. Further biological examinations of these agents are in progress.

REFERENCES


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