Amplification of bleomycin-induced DNA cleavage by pyrrole triamide

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ABSTRACT
We investigated the amplification of bleomycin-induced DNA cleavage by synthetic pyrrole triamide (PyPyPy) using 32P-labeled DNA fragments obtained from human genes. Peplomycin, a kind of bleomycins, plus Fe(II) caused DNA cleavage at the 5'-GC-3' and 5'-GT-3' sequences (damaged bases are underlined). The addition of PyPyPy enhanced the cleavage at cytosine and thymine residues 3' to consecutive guanines, particularly at the 5'-GGGGC-3' and 5'-GGGGT-3' sequences. These results suggest that PyPyPy binds to DNA to induce its conformational change, resulting in alteration of the site specificity and amplification of DNA cleavage. The present study on amplifiers of antitumor drugs would show a novel approach to the establishment of more effective chemotherapy.

INTRODUCTION
Bleomycins are antineoplastic antibiotics, which are activated by the interaction with Fe(II) and O2 to form the bleomycin-iron-oxygen complex capable of causing DNA cleavage through hydrogen abstraction from deoxyribose1,2. Bleomycins recognize and cleave the DNA molecule preferably at the sequences of 5'-GC-3' and 5'-GT-3'2-4. The carbohydrate moiety of bleomycin is important for DNA recognition5.

It has been reported that DNA-binding molecules, such as distamycin A6 and actinomycin D5, change the site specificity of bleomycin-induced DNA cleavage. Distamycin A and actinomycin D have been reported to alter the site specificity of DNA cleavage by duocarmycin A7 and neocarzinostatin8, respectively. Polyamides containing N-methylpyrrole and N-methylimidazole rings are also DNA-binding molecules, which bind to the minor groove of DNA in a site-specific manner9. Binding of polyamides to the DNA helix may affect the site specificity of DNA cleavage by antitumor drugs and amplify their antitumor effects. In this study, we examined the effect of synthetic pyrrole triamide (PyPyPy) on site specificity of DNA cleavage induced by peplomycin, a kind of bleomycins, using 32P-5-end-labeled DNA fragments obtained from the human c-Ha-ras-1 proto-oncogene and the p53 tumor suppressor gene. The chemical structures of bleomycin (peplomycin) and PyPyPy are shown in Fig. 1.

Fig. 1. Chemical structures of bleomycin and PyPyPy.
RESULTS AND DISCUSSION

The addition of PyPyPy enhanced the cleavage of $^{32}$P-labeled DNA fragments by peplomycin plus Fe(II) and changed its site specificity. We performed a modified method of Maxam-Gilbert.\textsuperscript{10} Peplomycin plus Fe(II) induced DNA cleavage at thymine and cytosine residues at the 3'-side of guanine residues, whereas PyPyPy enhanced DNA cleavage at cytosine and thymine residues 3' to consecutive guanines, particularly at the 5'-GGGGC-3' and 5'-GGGGT-3' sequences (Fig. 2, damaged bases are underlined). PyPyPy inhibited DNA cleavage at thymine residues of the 5'-IA-3' sequence.

It has been reported that polyamides containing pyrrole and imidazole rings bind to the DNA helix in a site specific manner.\textsuperscript{7} A certain covalently-linked polyamide subunits containing PyPyPy recognizes the minor groove of DNA and specifically binds to 5'-TGXXX-3' sequences (X = A or T) with much higher affinity than unlinked PyPyPy.\textsuperscript{7} A pairing of imidazole opposite pyrrole recognizes a G•C base pair,\textsuperscript{11,12} while a Py/Py pair recognizes A•T and T•A base pairs.\textsuperscript{7}

Our results suggest that the DNA conformational change by binding of PyPyPy allows peplomycin to interact with DNA at specific sites and to cleave DNA strand at GC-rich sequences, resulting in alteration in the site specificity and enhancement of DNA cleavage. This mechanism may be explained by assuming that PyPyPy binds to the sequences of mixed G•C and A•T composition, including 5'-TGXXX-3' sequences, leading to the DNA conformational change. It has been reported that certain DNA-binding molecules enhance DNA cleavage by antitumor drugs and change the site specificity.\textsuperscript{5-8} Distamycin A enhanced DNA cleavage by bleomycin at the 5'-GGGGC-3' sequence,\textsuperscript{6} whereas actinomycin D amplified the cleavage at the 5'-GA-3' and 5'-AT-3' sequences.\textsuperscript{5} Distamycin A enhanced DNA cleavage by duocarmycin A in GC-rich regions.\textsuperscript{7} Actinomycin D enhanced double-stranded DNA cleavage by neocarzinostatin at the 5'-TCT-3'•3'-AGA-5', 5'-TGT-3'•3'-ACA-5' and 5'-ACT-3'•3'-TGA-5' sequences.\textsuperscript{8} Further investigation of enhancing effects of DNA-binding molecules on DNA cleavage by antitumor drugs would lead to reduction in drug dose and side effects and the establishment of more effective chemotherapy.

REFERENCES