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Acid-accelerated DNA-cleaving activities of antitumor antibiotic varacin

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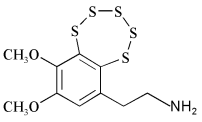
It is demonstrated in this report that the authentic molecular structure of antibiotic varacin is capable of causing DNA-cleavage with high efficiency in the presence of thiols. In addition, it is found that the DNA-cleaving activity by varacin is apparently promoted by its acidic environments.

Varacin, a metabolite isolated from the marine *ascidian Lissilinum vareau*,[1,2] is the first naturally occurring compound discovered containing benzopentathiepin as its core functionality. Subsequent biological examination revealed that this natural product exhibited potent antifungal activity against *Candida albicans* [2] as well as potent antitumor activity against human colon tumor cell line HCT 116.[2] Further preliminary biological investigation[2,3,4] using 7-methylbenzopentathiepin as a molecular model[5] suggested that implementation of the biological activity of varacin might be through its DNA damaging activity.[2,5] However, direct evidence to demonstrate the authentic structure of antibiotic varacin capable of causing efficient DNA-cleavage chemically has not yet been available.[6] With the aim of verifying the biological targets of varacin, examination of the interactions between DNA and our synthetic varacin at the molecular level were carried out recently in our laboratory. Here we report our discovery that antibiotic varacin is capable of causing DNA-cleavage with high efficiency in the presence of thiols and that the DNA-cleaving courses by this antibiotic could be accelerated by decreasing the pH values of the corresponding buffer solutions.



Varacin

The varacin used in our studies was synthesized following the synthetic procedures reported in literature[2,7] with certain modifications. The ability of varacin to cleave DNA was determined by its effectiveness in converting circular supercoiled DNA (form I) to circular relaxed (form II) DNA. For this purpose, plasmid pBR322 was accordingly incubated with varacin in phosphate buffer solutions with pH values from 5.0 to 7.5. As shown in Fig. 1, varacin caused single stranded DNA cleavage effectively at pH 5.0 (66%, Lane 2), 5.5 (81%, Lane 4) and 6.0 (54%, Lane 6) in the presence of 2-mercaptoethanol while such a DNA-cleaving activity decreased slightly as the pH of the corresponding buffer solutions increased to 7.0 (34%, Lane 10) and 7.5 (24%, Lane 12). It should be noted that the DNA-cleaving efficiency by varacin at pH 5.0 was lower than that at pH 5.5 in our studies. This happened possibly in part because the negative charges of phosphate backbones of DNA were neutralized to certain extents at such a low pH which might consequently decrease the binding affinity of the positively charged varacin to target DNA. In addition, varacin produced detectable formation of form II DNA at a concentration as low as 100 nM at pH 5.5 (Lane 3, Fig. 2) and in a concentration-dependent fashion (Fig. 2). Moreover, the DNA-cleaving course by varacin is a thiol-dependent process. As shown in Fig. 3, the amount of DNA cleavage by varacin increases with increasing the concentration of added 2-mercaptoethanol while there was no DNA-cleavage in the absence of added thiols, suggesting that a cascade of reactions of varacin activated by thiols might take place thus leading to the corresponding chemical reactions of the DNA cleavage.

In order to determine the properties of the reactive species involved in the DNA cleaving process by varacin, the inhibitory

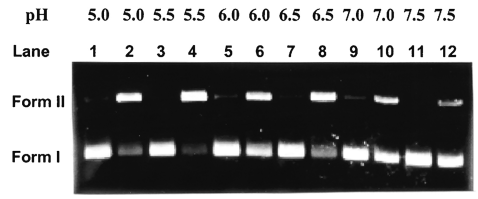


Fig. 1 DNA cleavage by varacin in phosphate buffer solutions at different pH values.† Reactions were incubated at 37 °C for 3 h in 50 mM sodium phosphate containing 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA, 10 μM varacin and in the presence or absence of added thiols. Lane 1, pH 5.0, no added thiol; lane 2, pH 5.0, 1 mM 2-mercaptoethanol; lane 3, pH 5.5, no added thiol; lane 4, pH 5.5, 1 mM 2-mercaptoethanol; lane 5, pH 6.0, no added thiol; lane 6, pH 6.0, 1 mM 2-mercaptoethanol; lane 7, pH 6.5, no added thiol; lane 8, pH 6.5, 1 mM 2-mercaptoethanol; lane 9, pH 7.0, no added thiol; lane 10, pH 7.0, 1 mM 2-mercaptoethanol; lane 11, pH 7.5, no added thiol; lane 12, pH 7.5, 1 mM 2-mercaptoethanol.

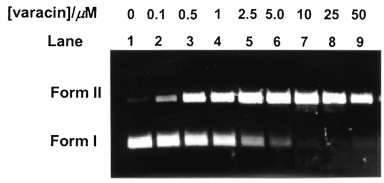


Fig. 2 DNA cleavage by various concentrations of varacin. Reactions were incubated at 37 °C for 18 h in 50 mM sodium phosphate (pH 5.5) containing 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA, varacin and 1 mM 2-mercaptoethanol. Lane 1, pBR322 alone; lane 2, 100 nM varacin; lane 3, 500 nM varacin; lane 4, 1 μM varacin; lane 5, 2.5 μM varacin; lane 6, 5.0 μM varacin; lane 7, 10 μM varacin; lane 8, 25 μM varacin; lane 9, 50 μM varacin.

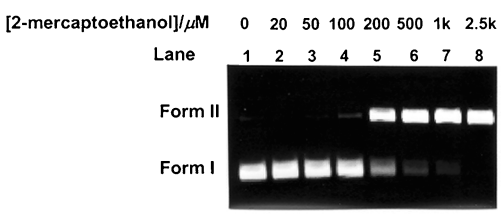


Fig. 3 DNA cleavage by varacin in the presence of varying concentrations of 2-mercaptoethanol. Reactions were incubated at 37 °C for 18 h in 50 mM sodium phosphate (pH 5.5) containing 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA, 5 μM varacin and 2-mercaptoethanol. Lane 1, no added thiol; lane 2, 20 μM 2-mercaptoethanol; lane 3, 50 μM 2-mercaptoethanol; lane 4, 100 μM 2-mercaptoethanol; lane 5, 200 μM 2-mercaptoethanol; lane 6, 500 μM 2-mercaptoethanol; lane 7, 1 mM 2-mercaptoethanol; lane 8, 2.5 mM 2-mercaptoethanol.

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effects of certain free radical scavengers were investigated. As shown in Fig. 4, the DNA cleavage course by varacin was inhibited effectively by the addition of catalase, an enzyme that reduces the concentration of hydrogen peroxide (H_2O_2) in solution (lane 3).[8,9] In addition, radical scavengers such as mannitol (lane 8), methanol (lane 6) and ethanol (lane 7) also effectively inhibit the DNA cleavage by varacin.[8,9,10] Moreover, diethylenetriamine pentaacetic acid (DETAPAC)[9,11] exhibited some inhibitory effects on the DNA-cleaving process by varacin (lane 5). Based on these observations, it can be expected that hydrogen peroxide was generated from the reaction of varacin with molecular oxygen activated by the addition of thiols, which was further decomposed into hydroxyl radicals that ultimately cause the DNA cleavage, a mechanism similar to the mode of action of antibiotic leinamycin.[5,12] Even though the addition of superoxide dismutase (an enzyme destroying superoxide in solution) to our reaction mixture did not slow down the DNA-cleaving reaction by varacin (lane 4), the involvement of superoxide radical in this DNA-cleaving process can not be ruled out. The absence of an inhibitory effect of superoxide dismutase was reported previously when superoxide dismutase was utilized to inhibit the thiol-dependent DNA-cleavage by leinamycin in which thiols functioned as a reducing agent.[9]

The cytotoxic activity of varacin was evaluated on eight different human cancer cell lines, which are expressed as the concentration of the antibiotics that inhibit 50% of cell proliferation (IC_{50}). As shown in Table 1, the IC_{50} values of varacin are all on the nanomolar scales.

In summary, our studies demonstrate that the authentic structure of varacin is capable of causing DNA cleavage chemically with high efficiency in the presence of thiols, a process that can apparently be accelerated by its acidic surroundings. Further studies on the detailed mechanisms of this DNA-cleaving process by this antibiotic are in progress.

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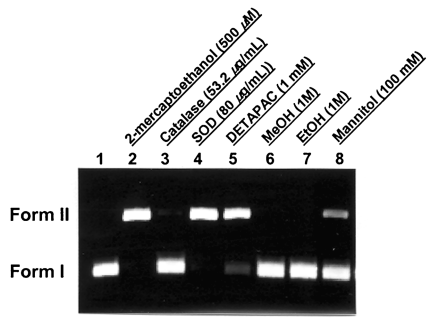

Fig. 4 DNA cleavage by varacin inhibited by the addition of radical scavengers and nucleophiles. Reactions were incubated at 37 °C for 4 h in 50 mM sodium phosphate (pH 5.5) containing 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA, 5 μM varacin, 500 μM 2-mercaptoethanol and additives. Lane 1, DNA alone; lane 2, reaction absent of additive; lane 3, 53.2 μg mL^{-1} catalase; lane 4, 80 μg mL^{-1} SOD; lane 5, 1 mM DETAPAC; lane 6, 1 M MeOH; lane 7, 1 M EtOH; lane 8, 100 mM mannitol.

Table 1 Cytotoxic property of antibiotic varacin against several cancer cell lines

Cell type	Cell line	IC_{50} varacin/nM	IC_{50} doxorubicin/nM
Colon cancer	HT-29	3.18	61.82
Prostate cancer	PC-3	0.60	49.86
Breast cancer	MDA231	9.52	1.95
Bladder cancer	UMUC3	11.41	9.24
Lung cancer	PACA2	1.8	8.79
Renal cell	A549	12.29	2.74
Human kidney carcinoma	A4982LM	48.40	52.84
Human breast carcinoma	MCF-7	1.69	1.24

Notes and references

† All DNA-cleaving reactions by varacin were analyzed by electrophoresis on 1% agarose gels in 1 M Tris-acetate buffer (100 V, 1 h). The resulting gels were subsequently photographed on a UV transilluminator after attaining with ethidium bromide. The amount of DNA in each band of ethidium-stained gels was quantified using Lumi-Imager (LumiAnalyst 3.1).

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