ANTAGONISTIC MECHANISM OF SULFHYDRYL COMPOUNDS ON CELLOCIDIN ACTIVITY

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Cellocidin is an antibacterial antibiotic discovered by SUZUKI *et al.*[1] Although the antibiotic shows an excellent preventive effect against rice bacterial leaf blight, its use is restricted because of its phytotoxicity to rice plants. OKIMOTO and MISATO[2] demonstrated cellocidin inhibited the metabolism of α-keto-glutarate to succinate in *Xanthomonas oryzae*. TANAKA *et al.*[3] also found that cellocidin inhibited the incorporation of ^{14}C-thymidine into the DNA of *Bacillus subtilis*. On the other hand, antibacterial activity of cellocidin is known to be blocked by sulfhydryl compounds such as cysteine, glutathione, *etc.*[2] This paper describes the antagonistic mechanism of sulfhydryl compounds on cellocidin activity.

Effect of cellocidin on the thiol-disulfide exchange reaction between 5,5-dithio bis (2-nitrobenzoic acid) [DTNB] and reduced glutathione was examined. The reaction mixture consisted of 2.0 ml of 0.1 M phosphate buffer pH 7.6 containing 0.3 mM ethylenediaminetetraacetate, 0.2 ml each of 4 mM DTNB and 0.5 or 1.0 mM reduced glutathione, 0.5 ml of cellocidin solution and water in a final volume of 4.0 ml. The reaction was initiated by the addition of DTNB in a cuvette of 10-mm light path. After 10-minute incubation, absorbance increase was measured at 412 nm by a Shimadzu UV-200 spectrometer. Thiol-disulfide exchange reaction between glutathione and DTNB was markedly blocked at a 10 μg/ml of cellocidin as shown in Table 1. This result suggested that cellocidin reacted to sulfhydryl compounds chemically.

The reaction of cysteine to cellocidin was made by stirring the mixture of 10 mmol cysteine-HCl, 10 mmol cellocidin and 0.01 N NaOH in a volume of 1.0 liter at 25°C for 5 hours. The reactants were chromatographed on a silica gel TLC by *n*-butanol - acetic acid - water (4 : 1 : 1, v/v), and were detected with ninhydrin reagent. Besides cysteine and cystine spots, two ninhydrin-positive spots were observed, and their Rf values were 0.64 (designated compound **I**) and 0.45 (designated compound **II**). In order to isolate the two products, the reaction mixture was concentrated and applied to an Avicel column. The column was eluted with *n*-butanol - acetic acid - water (2 : 1 : 1, v/v), and the eluate was fractionated in each 15 ml. Two ninhydrin-positive fractions other than cysteine and cystine were collected and concentrated, and each product was purified by crystallization. Compounds **I** and **II** obtained as needle crystals were water-soluble under acid or alkaline conditions. Antibacterial activities were not observed for these compounds even at a concentration of 100 μg/ml *in vitro*.

Melting points of compounds **I** and **II** were 193°C (decomp.) and 205~207°C (decomp.), respectively. The UV absorption spectra were measured in 0.01 N NaOH according to SUZUKI *et al.*[1], and the maximum absorptions were 290 nm for compound **I** and 285 nm for compound **II**. The IR spectra of both compounds showed the broad absorptions for amino acids at 3400~2400 cm^{-1}, and the specific absorptions for -NH_3^+, -COO^- and $RCONH_2$, but not the absorptions for R-SH with cysteine. These results suggested that sulfhydryl group of cysteine might be bound to cellocidin.

Elemental analytic data of compounds **I** and **II** gave the composition formulae of $C_{14}H_{24}O_9N_6S_2$ and $C_{10}H_{20}O_7N_4S_2$, respectively. Considering these elemental analyses on the basis of NMR analytic data described below, the molecular formulae were estimated as $C_7H_{11}O_4N_3S\cdot\frac{1}{2}H_2O$ for compound **I** and $C_{10}H_{18}O_6N_4S_2\cdot H_2O$ for compound **II**.

The NMR spectrum was recorded on a Nihondenshi C-60 HL spectrometer by using D_2O solution containing DCl. The NMR spectra of

Table 1. Effect of cellocidin on thiol-disulfide exchange reaction

Concentration of glutathione	Cellocidin (μg/ml)		
	0	1	10
25 μM	0.410	0.310	0.155
50	0.865	0.861	0.345

Data are optical densities at 412 nm after thiol-disulfide exchange reaction between DTNB and reduced glutathione for 10 minutes.

Fig. 1. NMR spectrum of compound **I**

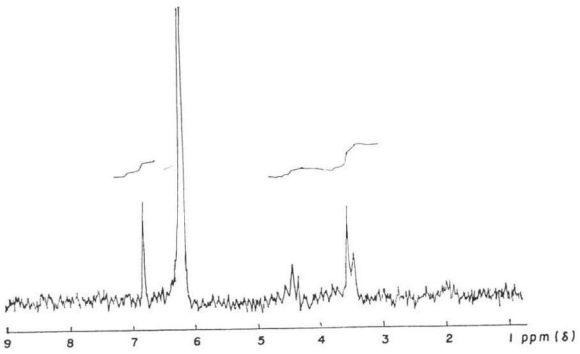


Fig. 2. NMR spectrum of compound **II**

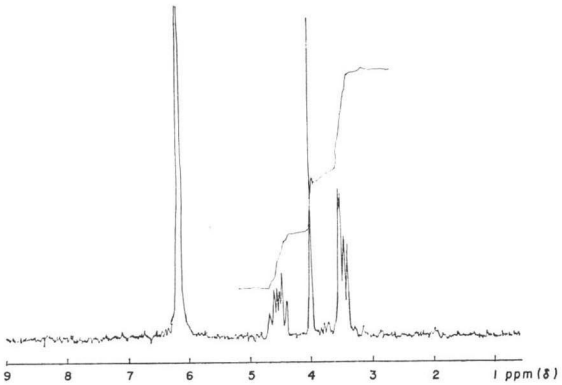
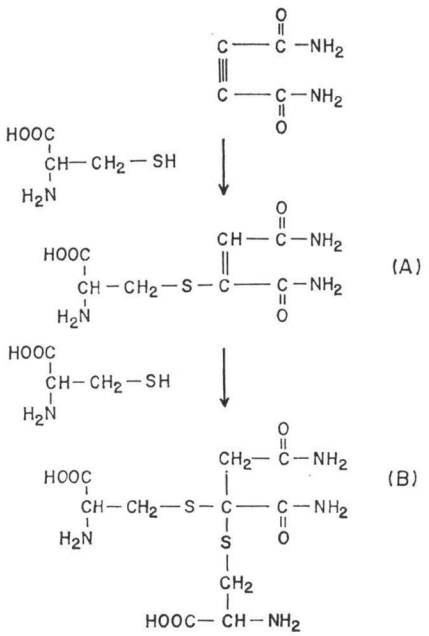


Fig. 3. Mechanism of reaction of cellocidin with cysteine



compound **I** in Fig. 1 showed the presence of a methylene proton (doublet at δ 3.57, 2 H) and a methine proton (triplet at δ 4.47, 1 H), and these peaks correspond to the protons of cysteine residue. The other singlet peak at δ 6.83 (1 H) indicated the presence of the functional group of -C=CH- in compound **I**. From NMR and elemental analytic data, the structure of compound **I** was concluded to be the adduct of one molecule of cysteine to the triple bond of cellocidin as shown in Fig. 3 (A).

The NMR spectra of compound **II** in Fig. 2 gave the multiplet peaks derived from two cysteine molecules at δ 3.56 (2 H) and δ 4.51 (1 H). Since both multiplets were slightly shifted between two molecules, the two molecules are asymmetrically bound to cellocidin. The other singlet peak at δ 4.05 (2 H) indicated the presence of the functional group of -C-CH_2- in compound **II**. From NMR and elemental analytic data of compound **II**, the structure was concluded to be the adduct of two molecules of cysteine to one triple bond carbon of cellocidin as shown in Fig. 3 (B).

The addition of cysteine to cellocidin was considered to react according to the rule of MARKOWNIKOFF. These results show that cellocidin loses its antibacterial activity by the chemical reaction with sulfhydryl compounds.

Literature

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