6′-N-ACETYLATION OF 3′,4′-DIDEOXYKANAMYCIN B BY AN ENZYME IN A RESISTANT STRAIN OF *PSEUDOMONAS AERUGINOSA*

Sir:

As described in a previous paper[1], most of strains of *Pseudomonas aeruginosa* are sensitive to 3′,4′-dideoxykanamycin B (DKB). However, MITSUHASHI, Department of Bacteriology, School of Medicine, Gunma University, found that a strain of *Pseudomonas* (*P. aeruginosa* GN 315) isolated from a patient was resistant. This strain was not inhibited by DKB at 100 μg/ml. In this communication, we report the isolation and structure of inactivated DKB from reaction with an enzyme from this resistant strain of *Pseudomonas*.

The enzyme solution was prepared from logarithmically growing cells of *P. aeruginosa* GN315 in a nutrient broth containing 5 μg/ml of DKB. The cells were harvested by centrifugation and washed twice with buffer A: 20 mM potassium phosphate buffer (pH 7.8) containing 6 mM 2-mercaptoethanol. The washed cells were suspended in an equal volume of buffer A and disrupted by passage through a French pressure cell (1,200 kg/cm²). The ruptured cell suspension was centrifuged at 105,000 g for 90 minutes, and the supernatant was dialyzed overnight against buffer A. The dialyzed enzyme solution was diluted with buffer A to 20 mg/ml protein determined by the FOLIN method. The diluted enzyme solution was designated as S-100.

The inactivation was carried out at 37°C for 3 hours in the following reaction mixture: 90 mg (0.2 mmoles) DKB in 25 ml of distilled water, 4,842 mg (8.0 mmoles) disodium ATP in 100 ml of 0.8% $NaHCO_3$, 50 mg (0.06 mmoles) trisodium CoA in 50 ml of distilled water, 25 ml of S-100, 25 ml of 100 mM $Mg(CH_3COO)_2$–60 mM 2-mercaptoethanol and 25 ml of 1 M potassium phosphate buffer (pH 7.8). After 3-hour incubation 94% of DKB was inactivated as shown by the disc plate method using *Bacillus subtilis* PCI 219.

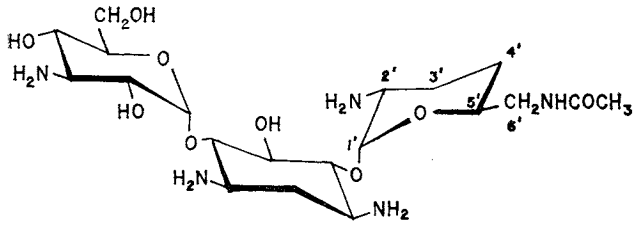
The reaction mixture was diluted with 250 ml of distilled water and kept in a boiling water bath for 10 minutes. It was filtered and the filtrate was passed through a column of Amberlite CG-50 (NH_4^+ form, 15 ml). After washed with 2,000 ml of distilled water, the inactivated DKB was eluted with 0.1 N NH_4OH. The eluate which was positive in ninhydrin and RYDON-SMITH[2] reactions was concentrated to dryness, yielding 96 mg of a brownish powder. The powder was rechromatographed on Amberlite CG-50, yielding 88 mg of a yellowish powder. It was further subjected to the chromatography of CM-Sephadex C-25 equillibrated with 0.1 M ammonium formate and the inactivated DKB was eluted with 0.6 M ammonium formate. After chromatography on Amberlite CG-50 to remove formic acid, evaporation *in vacuo* of the 0.1 N NH_4OH eluate yielded 69 mg of purified inactivated DKB as a white powder.

The inactivated DKB melted at 146~149°C. Anal. Calcd. for $C_{20}H_{39}N_5O_9 \cdot H_2O$: C 46.95, H 8.08, N 13.69, O 31.28. Found: C 47.37, H 8.02, N 13.54, O 31.06. It gave positive ninhydrin and RYDON-SMITH reactions. On high-voltage paper electrophoresis at 3,500 volts for 15 minutes, using formic acid – acetic acid – water (25 : 75 : 900 in volume), the inactivated DKB moved 11.2 cm toward the cathode, while DKB moved 13.5 cm. The UV spectrum of the inactivated DKB showed no characteristic absorption. The infrared spectrum of the inactivated DKB showed amide bands I and II (1650 and 1570 cm^{-1}).

The bioactivity of the inactivated DKB against *Bacillus subtilis* PCI 219 was about 1~1.5% of that of DKB in the usual cylinder plate assay. Hydrolysis of the inactivated DKB with 2 N NaOH regenerated the DKB as shown by the bioactivity and the high-voltage paper electrophoresis.

From the foregoing results, it was shown that the inactivated DKB is a mono-N-acetyl derivative of DKB.

The pmr spectrum of DKB has been studied in detail.[3] In the pmr spectrum of the inactivated DKB in D_2O (20 mg/0.3 ml) using tetramethylsilane as the external reference, one N-acetyl signal was seen at $\delta = 2.43$. Application of double resonance technique indicated that the signal of the



C-6′ methylene protons at $\delta = 3.15$ in DKB shifted to $\delta = 3.7$ in the inactivated DKB. Thus, the structure of the inactivated DKB was determined to be 6′-N-acetyl-3′, 4′-dideoxykanamycin B. This structure was also consistent with its mass spectrum: a peak of a mono-N-acetyl DKB at *m/e* 494 (M+1) and an intense peak at *m/e* 171 attributed to the N-acetyl-3′,4′-dideoxyneosamine C unit[4].

Acknowledgement

We express our deep thanks to Prof. S. MITSUHASHI, Medical School of Gunma University, for his supply of the valuable strain, *P. aeruginosa* GN 315.

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(Received May 30, 1972)

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