Communications to the editor

3''-PHOSPHORYLDIHYDROSTREPTOMYCIN PRODUCED BY THE INACTIVATING ENZYME OF *PSEUDOMONAS AERUGINOSA*

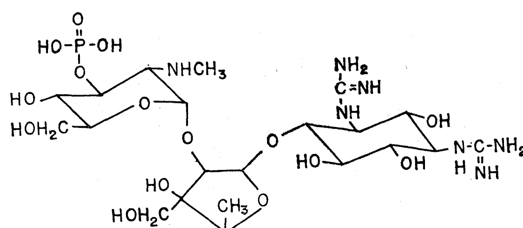
Sir :

Escherichia coli carrying R factor was reported by UMEZAWA *et al.*[1]), and TAKASAWA *et al.*[2]) to produce an enzyme adenylylating streptomycin, and *E. coli* carrying another R factor was reported by OZANNE *et al.*[3]) to produce an enzyme phosphorylating streptomycin. Though a resistant *Pseudomonas* was also observed by DOI *et al.*[4]) to produce an enzyme inactivating streptomycin in the presence of both ATP and Mg^{++}, the structure of the inactivated streptomycin produced by the enzyme of this organism was not studied. We have found[5]) *Pseudomonas aeruginosa* TI-13 (isolated from a patient) to contain a soluble enzyme fraction which inactivates both streptomycin and dihydrostreptomycin in the presence of both ATP and Mg^{++}. The inactivated product was purified by CM-Sephadex C-25 chromatography as reported elsewhere[6]). In this paper, we wish to summarize our studies on the structure of this inactivated dihydrostreptomycin.

Treatment of the inactivated product with alkaline phosphatase gave "active" dihydrostreptomycin, but treatment with phosphodiesterase did not give the "active" dihydrostreptomycin. Elemental analysis of phosphorylated dihydrostreptomycin gave : Anal. calcd. for $C_{21}H_{40}N_7O_{12}PO(OH)_2 \cdot H_2CO_3 \cdot 3H_2O$; C 33.88, H 6.48, N 12.58, P 3.97 ; found C 33.49, H 5.99, N 12.65, P 4.24.

We conclude the inactivated product to be dihydrostreptomycin-monophosphate. In order to locate the site of attachment of PO_4 to dihydrostreptomycin, periodate oxidation was carried out at 4°C for 24 hours using a reaction mixture containing 10 μmoles of the inactivated dihydrostreptomycin and 100 μmoles of sodium periodate in 5 ml of 0.2 M acetate buffer (pH 4.0). The mixture was then hydrolyzed with 6 N HCl under reflux for half an hour. The hydrolyzate was concentrated and extracted with ethanol. Thin-layer chromatography, high-voltage paper electrophoresis and gas chromotography of the extract showed the presence of N-methylglucosamine in the hydrolyzate and suggested that the moiety of N-methylglucosamine in the inactivated dihydrostreptomycin was phosphorylated and resisted periodate oxidation.

Fig. 1



The identity of the product in the extract with N-methylglucosamine was proved by the following methods comparing with an authentic sample. In thin-layer chromatography (TLC aluminum sheet cellulose) using the following solvent systems ; methanol-ethanol-conc.HCl - water (50 : 25 : 6 : 19) and propanol - pyridine - acetic acid - water (51 : 20 : 6 : 24), Rf values of the product as well as N-methylglucosamine were 0.54 and 0.37, respectively. In high-voltage paper electrophoresis using the following solvent system : acetic acid - formic acid - water (75 : 25 : 900), both the product and N-methylglucosamine moved to the cathode by 6.1 cm at 3,500 volts for 15 minutes. Moreover, in gas chromatography at 190°C using 3 mm × 2 m column (5 % Ucon LB550X on Shimalite W), the pattern of the extract which was methylated followed by persilylation was identical with that of authentic N-methylglucosamine pretreated in the same manner.

Based on the results described above, we concluded that the hydroxyl group on C-3 of the N-methylglucosamine moiety in dihydrostreptomycin was phosphorylated by the *P. aeruginosa* TI-13 enzyme and then the structure of inactivated dihydrostreptomycin is as shown in Fig. 1. This structure

is also supported by n.m.r. studies.* The mode of inactivation by *P. aeruginosa* TI-13 is similar to that observed by OZANNE *et al.*[3] using *E. coli* carrying R factor.

Acknowledgements

We express our gratitude to Dr. H. UMEZAWA, The Institute of Microbial Chemistry, for his kind advice throughout this study, and to Dr. S. KONDO, for his devoted assistance in performing gas chromatography.

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(Received May 22, 1971)

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* NAGANAWA, H.; S. KONDO, K. MAEDA & H. UMEZAWA: Private communication.