A NEW ANTISTREPTOLYSIN, M-9337, FROM ACTINOMYCETES

Sir:

It is known that bacterial toxins have a significant role in the pathogenesis of bacterial infections and antitoxic sera have marked prophylactic and therapeutic value for certain diseases. Streptolysin, a type of hemolysin, is considered to be a bacterial toxin; it exerts several types of damaging effects on various mammalian cells. A substance which exhibits inhibitory activity against streptolysin has been purified from the culture broth of *Streptomyces* strain M-9337, an organism isolated from local soil. The antistreptolysin substance has been designated M-9337.

Antistreptolysin activity was tested as follows: a mixture consisting of 1.0 ml of streptolysin O buffer containing different quantities of a test material and 0.5 ml of streptolysin O reagent (Eiken) in the same buffer, was incubated at 37°C for 15 minutes. Then 0.5 ml of defibrinated rabbit red cells (5%) was added and incubation was continued for an additional 45 minutes at 37°C. The end point was considered to be the last tube showing no hemolysis of the supernatant fluid and the result was expressed in Todd units.

M-9337 was produced by *Streptomyces* M-9337, when the organism was inoculated to a medium consisting of 2% glucose, 2% soybean meal, 0.2% meat extract, 0.2% yeast extract, 0.2% peptone, 0.2% NaNO₃, 0.1% K₂HPO₄, 0.05% KCl, 0.05% MgSO₄·7H₂O, 0.001% FeSO₄·7H₂O (pH 6.8 ~ 7.0). The culture was grown on a shaker at 30°C for 96 hours.

The organisms were removed from the medium by centrifugation at 8,000 rpm for 20 minutes. The supernatant fluid was adjusted to pH 3.0 with 1 N HCl and the precipitate was collected. The precipitate was washed with cold acetone and ethyl ether after which the active substance was eluted with a mixture of chloroform and methanol (2:1). The eluate was evaporated under reduced pressure and the powder thus obtained was dissolved in 85% ethanol and purified by DEAE-Sephadex A-50 column chromatography with a solvent of chloroform - methanol - 0.8 M sodium acetate (30: 60: 8). The active fraction was concentrated *in vacuo* to dryness and the powder was dissolved in methanol. It was further purified by thin-layer chromatography on silica gel 60 (Merck) developed with chloroform - methanol - water (65: 25: 4). The active spot was scraped and was extracted with methanol. The extract was evaporated to yield a yellowish-white powder.

Purified M-9337 melts at 170 ~ 175°C with decomposition. The analytical data indicate the composition to be: C 52 ~ 53%, H 7 ~ 8%; no sulfur or nitrogen was present. The purified material dissolved in methanol has no absorption below 220 nm; the infrared absorption spectrum is shown in Fig. 1. The purified substance is soluble in dimethyl sulfoxide and in a mixture of chloroform and methanol, is sparingly soluble in a water and in methanol, and is insoluble in ethyl acetate and in chloroform. It gave positive reactions to anthrone, ammonium molybdate-perchloric and anisaldehyde tests, and gave negative reactions to ninhydrin and Dragendorff tests. It was stable in aqueous solution (pH 3 ~ 9) at 100°C for 5 minutes.

Purified M-9337 inhibited the activity of streptolysin O (Eiken) at 40 Todd units/mg. It showed no antibacterial and no antifungal activity at 1 mg/ml, and the median lethal dose for mice was 290 mg/kg intraperitoneally.

Furthermore, the protective effect of M-9337 against experimental infection in mice was examined. Mice were injected intraperitoneally with 0.5 ml of an overnight Todd-Hewitt broth culture of *Streptococcus haemolyticus* O-78 classified group A and type 12, and 3 hours later they were injected subcutaneously with 0.2 ml of M-9337 (20 mg/ml). As a result, it was observed that all of 10 untreated mice succumbed within 1 to 3 days while 4 of 10 mice of the treated group survived at least one week.

*Streptomyces* M-9337 which produced M-9337 was found during our screening program for new antibiotics. The substance is not antibiotic since it shows no growth inhibition of microorganisms. Recently UMEZAWA²,³ has reported many enzyme inhibitors produced by *Streptomyces* and designated these products as "microbial secondary metabolites". According to his classification,
M-9337 belongs in this group. However, from the viewpoint of clinical usefulness, a microbial antihemolysin such as M-9337 has a special significance. Further investigations will be expected not only because of the intrinsic biological interest of this compound but also for clinical application of the antitoxic substances as well as antitoxic sera.

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References
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