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Phosphorylation of Streptomycin by an Intracellular Enzyme of *Streptomyces griseus*

Sir:

We already reported production, isolation and chemical structure of a phosphorylated streptomycin (L)^{1~3)} which had a phosphate group at C-6 of streptidine moiety and was presumed to be a natural precursor in streptomycin biosynthesis. This substance was excreted extensively in the culture medium instead of streptomycin when the pH reaction was kept during fermentation below neutral by increase of glucose concentration in the culture medium. It was also excreted when young mycelium was suspended in glucose solution under shaking.^{4,5)}

However, there remained a question that L might be a secondary product formed by phosphorylation of streptomycin which had been excreted outside the cell. If it is the case, L is not a precursor of streptomycin. Phosphorylation of exogenous streptomycin was attempted by addition of streptomycin sulfate to culture broth or to mycelium suspension of *Streptomyces griseus* HUT 6037. But no significant phosphorylation was observed.

On the other hand, it was found that the cell-free extract of mycelium in the late stage of growth could phosphorylate considerably large amount of streptomycin. Addition of

ATP to this reaction mixture strongly stimulated the phosphorylation. A partially purified preparation of streptomycin-phosphorylating enzyme was obtained from the cell-free extract of 72 to 96 hr mycelium with column chromatography on DEAE-Sephadex A-25 which had been equilibrated previously at pH 8.0; the enzyme being eluted from the column with 2% sodium chloride in 0.03 M Tris-HCl buffer (pH 8.0). This enzyme solution was employed for preparation of the phosphorylated streptomycin. The reaction mixture consisted of enzyme solution (8 ml), streptomycin (20 μ M), ATP (60 μ M) and Mg^{2+} (0.2 mM) in 0.1 M Tris-maleate buffer at pH 7.0 (final volume 20 ml).

The phosphorylated streptomycin (I) thus produced was adsorbed on Amberlite CG-50 and eluted with 2% aqueous sodium chloride solution. This substance contained one mole of phosphoric acid and one mole of streptomycin. The phosphate group was quantitatively released with an alkaline phosphatase which was specific to L.⁶⁾ After hydrolysis of I with 1 N sulfuric acid for 48 hr according to the procedure of Candy *et al.*,⁷⁾ the hydrolysate was neutralized with sodium hydroxide, precipitating needle crystals. The crystal gave positive Sakaguchi reaction, positive phosphate reaction⁸⁾ and no maltol one. Thus, the crystal was identified to be a phospho-

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FIG. 1. IR Spectra of the Two Phosphorylated Streptidines in KBr.

— Phosphorylated streptidine obtained from L.
 ----- Phosphorylated streptidine obtained from I.

phorylated streptidine. Those facts indicate that I contains a phosphate group in the streptidine moiety as was in L. It is also considered that the streptomycin-phosphorylating enzyme of *Streptomyces griseus* HUT 6037 is not the same as the streptomycin-inactivating enzyme of *Escherichia coli* described by Ozanne *et al.*,⁹⁾ but is essentially the same as the streptidine- and streptomycin-phosphorylating enzyme reported by Miller *et al.*¹⁰⁾

The two phosphorylated streptidine which were obtained from L and I were compared in high voltage paper electrophoresis which was performed in 0.2M acetate buffer (pH 5.0) at 70 v/cm for 22 min. No difference was observed in mobility of the two substances. Both substances were subjected to periodate oxidation according to the Fleury-Lange method.¹¹⁾ One mole periodate was consumed per mole of each of them at pH 3.6 in 0.2M acetate buffer at 23°C for 54 hr. This means

that the phosphate group of I is combined to C-6 of the streptidine moiety as was discussed in L.³⁾ Also the IR spectra of the two phosphorylated streptidines gave the same pattern (Fig. 1). Judging from these results, the phosphorylated streptomycin (I) formed with an intracellular enzyme of *Streptomyces griseus* HUT 6037 was considered to be identical with L excreted in the culture broth of the same strain.

It is now under investigation if, or how, the streptomycin-phosphorylating enzyme indicated above is responsible for formation of L *in vivo*.

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