INHIBITION OF XANTHOSINE-5'-PHOSPHATE AMINASE BY PSICOFURANINE

Libor Slechta

Research Laboratories, The Upjohn Company,
Kalamazoo, Michigan

Received November 11, 1960

Evidence has been obtained from studies with intact E. coli B (Slechta, 1960), S. aureus (Hanka, 1960) and the Walker carcinoma of the rat (Magee and Eberts, 1960) that psicofuranine (6-amino-9-D-psicofuranosylpurine, Schroeder and Hoeksema, 1959) isolated from the culture media of Streptomyces hygroscopious var. decoyinine (Eble, Hoeksema, Boyack and Savage, 1959) inhibited the biosynthesis of guanosine-5'-phosphate from xanthosine-5'-phosphate. This finding has now been substantiated by studies at the enzyme level.

A cell-free extract from E. coli B, prepared according to Carter (1959) was found to contain the enzyme xanthosine-5'-phosphate aminase. In agreement with the conclusions from studies with whole cells of E. coli B, the amination of xanthosine-5'-phosphate to guanosine-5'-phosphate by this extract was inhibited by psicofuranine, as shown in Fig. 1.

Carter (1959) described the enzyme 5-phosphoribosylpyrophosphorylase from E. coli B, which converted hypoxanthine, guanine and 6-mercaptopurine to the respective 5'-nucleotides. The crude bacterial extract used in this study showed also 5-phosphoribosylpyrophosphorylase activity with xanthine as substrate. Therefore, it was possible to couple this reaction, using C^{14}-labeled xanthine, with the subsequent amination of labeled xanthosine-5'-phosphate to labeled guanosine-5'-phosphate and to observe the inhibitory effect of psicofuranine by measuring the radioactivity of xanthosine-5'-phosphate and guanosine-5'-phosphate. Aliquots of the reaction mixture, described in the legend to

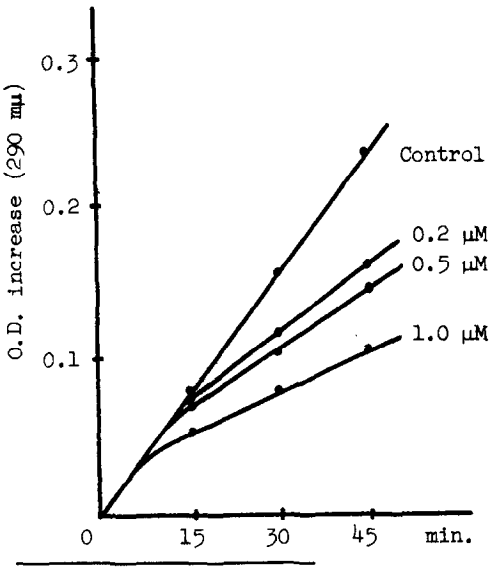

Fig. 1. Inhibition of xanthosine-5'-phosphate aminase by psicofuranine. Each test tube contained: 0.25 ml of bacterial extract; 100 μM of Tris buffer, pH 8.5; 150 μM of $(NH_4)_2SO_4$; 10 μM of $MgCl_2 \cdot 6H_2O$; 2 μM of adenosine-triphosphate; 1.2 μM of xanthosine-5'-phosphate; water to make a total volume of 0.5 ml. Incubation was at room temperature. At the depicted time intervals, the reaction mixture was stopped by the addition of 5.5 ml of 3.5% $HClO_4$ (v/v) and the precipitated proteins were removed by centrifugation. Concentration of guanosine-5'-phosphate in the supernatant was estimated by ultraviolet light absorption at 290 mμ (Moyed, 1957). Amounts of psicofuranine (in μM) added to the reaction mixture are given in the figure.

Table I, were analyzed after deproteinization by paper chromatography with saturated ammonium sulphate/n-butanol/water as the solvent system (Bergkvist, 1958). In this system, xanthine (R_f = 0.19) was separated from xanthosine-5'-phosphate and guanosine-5'-phosphate (both R_f = 0.46). Spots of the combined nucleotides were quantitatively eluted with 10 ml of 1 N HCl and the eluates heated for 60 minutes in a boiling water bath to hydrolyze the nucleotides to free purine bases. The hydrolyzates were evaporated *in vacuo* over NaOH and the dry residues dissolved in 0.5 ml of water. Xanthine and guanine in aliquots of these solutions (50 μl) were separated by paper chromatography in isoamylalcohol/Na_2HOP_4 solvent system (Carter, 1950). To locate the spots by ultraviolet light absorption, inactive carriers (5 μg of each) were applied onto the paper chromatograms prior to the analyzed radioactive solutions. Spots corresponding to xanthine and guanine were eluted with 5 ml of water directly into the counting vials and after evaporation of the solvent the radioactivity of the residues was measured in a Packard Tri-Carb Scintillation Spectrometer with the mixture of toluene and absolute ethanol (7:3) with 0.4% 2,5-diphenyloxazole and 0.01% 1,4-di-[2-(5-phenyloxazolyl)] benzene as the phosphors.

TABLE I

Inhibitory Effect of Psicofuranine on the Conversion of C^{14}-Labeled Xanthosine-5'-Phosphate to Guanosine-5'-Phosphate.

Incubation Time	Without Psicofuranine		With Psicofuranine	
	Total Radioactivity from:			
	Xanthosine-5'-Phosphate	Guanosine-5'-Phosphate	Xanthosine-5'-Phosphate	Guanosine-5'-Phosphate
0 min	trace	0	trace	0
30 min	1.57×10^4 cpm	3.44×10^4 cpm	3.98×10^4 cpm	0.83×10^4 cpm

Each of two incubation vessels contained: 1 ml of the bacterial extract, 400 μM of Tris buffer, pH 8.5; 400 μM of $(NH_4)_2SO_4$, 40 μM of $MgCl_2 \cdot 6H_2O$; 8 μM of adenosinetriphosphate, 10 μM of 5-phosphoribosyl-pyrophosphate; 2 μM of inactive xanthine and 0.5 μc of xanthine-8-C^{14} (1.6 μc/mg); water to final volume of 2 ml. One vessel contained 5 μM of psicofuranine. Incubation was at room temperature. Aliquots of the reaction mixtures (0.6 ml) were removed at specified time periods and added to 0.6 ml of 10% $HClO_4$ (v/v). Precipitated proteins were removed by centrifugation, the pH of the supernatant adjusted to 7 with 5 N KOH and $KClO_4$ centrifuged off. Aliquots of the neutral supernatant (100 μM) were analyzed by paper chromatography as described in the text.

The radioactivities of xanthosine-5'-phosphate and guanosine-5'-phosphate found in the absence and in the presence of psicofuranine, as shown in Table I, confirmed the validity of the results obtained by the spectrophotometric determination. Recently Moyed (1960) has confirmed the inhibitory action of psicofuranine on xanthosine-5'-phosphate aminase using a highly purified preparation isolated from a mutant of Aerobacter aerogenes.

References

Bergkvist, R., Acta Chem. Scand., 12, 1544 (1958).

Carter, C. E., J. Am. Chem. Soc., 72, 1466 (1950).

Carter, C. E., Biochem. Pharmacology, 2, 105 (1959).

Eble, T. E., Hoeksema, H., Boyack, G. A. and Savage, G. M., Antibiotics and Chemotherapy, 9, 419 (1959).

Hanka, L. J., J. Bact., 80, 30 (1960).

Magee, W. E. and Eberts, F. S., submitted for publication.

Moyed, H. S. and Magasanik, B., J. Biol. Chem., 226, 351 (1957).

Moyed, H. S., (1960), personal communication.

Schroeder, W. and Hoeksema, H., J. Am. Chem. Soc., 81, 1767 (1959).

Slechta, L., Fed. Proc., 19, 308 (1960).

Slechta, L., Biochem. Pharmacology, (1960), in press.