INHIBITION OF RIBOSEPHOSPHATE PYROPHOSPHOKINASE ACTIVITY BY DECOYININE, AN ADENINE NUCLEOSIDE

Alexander Bloch and Charles A. Nichol

Dept. of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, N.Y.

Received May 27, 1964

The antibiotics decoyinine (9-β-D-(5, 6-psicofuranoseenyl)-6-aminopurine) and psicofuranine (9-β-psicofuranosyl-6-aminopurine) are both produced by Streptomyces hygroscopicus (Hoeksema et al., 1964; Eble et al., 1959; Schroeder and Hoeksema, 1959). These antibiotics are structural analogs of adenosine, their modifications being in the ribofuranose moiety. While the antibacterial and antitumor activity of psicofuranine has been examined in detail, little definitive information concerning the comparative potency or mode of action of decoyinine is as yet available (Lewis et al., 1959; Evans and Gray, 1959; Tanaka et al., 1960; Tanaka, 1963).

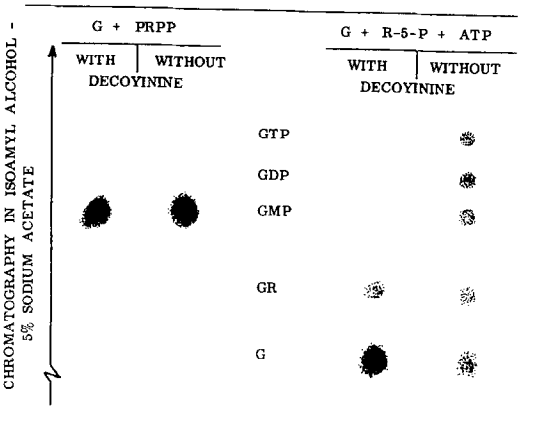
When grown in a medium lacking purines and pyrimidines (Flynn et al., 1951) 50% inhibition of the growth of Streptococcus faecalis (ATCC 8043) occurred at 5×10^{-6} M decoyinine or 6×10^{-7} M psicofuranine. Various metabolites were studied with respect to their ability to prevent the inhibitory effect of these antibiotics. Glucose, 18 amino acids, 8 vitamins, either separately or in combination, in concentrations ranging from 10^{-3} to 10^{-6} M were not able to prevent the inhibition caused by these antibiotics. Only guanine, guanosine and deoxyguanosine showed a pronounced ability to reverse the inhibition, and they did so competitively (Bloch and Nichol, 1964). The other purine and pyrimidine bases were inactive in this respect and the corresponding ribo- and deoxyribonucleosides were able to reverse the inhibition only slightly. The same competitive reversal, specifically by guanine and its nucleosides, was observed with psicofuranine.

These antibiotics were not subject to deamination by a purified preparation of

adenosine deaminase (calf intestine), nor did they interfere with the activity of this enzyme. In cell-free extracts of S. faecalis, phosphorolysis of these analogs did not occur, and no interference with adenosine phosphorylase activity was observed. Both psicofuranine and decoyinine inhibited the conversion of XMP to GMP in cell-free extracts of S. faecalis in a manner analogous to the action of psicofuranine in E. coli preparations (Schlechta, 1960; Hanka, 1960).

FIGURE 1

RADIOAUTOGRAM OF PRODUCTS FOLLOWING INCUBATION OF CELL-FREE EXTRACTS OF S. FAECALIS WITH GUANINE-8-C^{14} (G) AND PRPP OR RIBOSE-5-P + ATP



Decoyinine, and to a lesser extent psicofuranine, interfered with the formation of 5-phosphoribosyl-1-pyrophosphate (PRPP). When a cell-free extract of S. faecalis was incubated with R-5-P, ATP and radioactive guanine (or adenine), the corresponding ribonucleotides were readily formed (Fig. 1). In the presence of decoyinine, their formation was inhibited. However, when PRPP was added to the extract containing the radioactive base, then the nucleoside monophosphate was formed readily both in

the presence and absence of the antibiotic (Fig. 1). In the cell-free extracts used, nucleoside kinase activity as measured with labelled adenosine or guanosine was not detectable, and there was no chromatographic evidence for the conversion of decoyinine to its nucleotides. It is unlikely, therefore, that the conversion of the labelled bases to the nucleotides proceeded via the nucleoside phosphorylase and kinase pathway. Guanine or its nucleosides prevented the inhibitory effect of decoyinine and psicofuranine on the growth of S. faecalis in a medium free of exogenous pyrimidines. Thus, under these conditions, the biosynthesis of orotidylate is not critically limited by these antibiotics. Details of the experimental procedures and discussion of the pattern of reversal will be published elsewhere.

Both decoyinine and psicofuranine inhibit the conversion of XMP to GMP and interfere with pyrophosphokinase activity. The reaction mechanism in both cases involves the cleavage of ATP to AMP and PPi, suggesting the possibility that the mode of inhibition is the same in each instance. The binding of psicofuranine to XMP-aminase appears to be a two-step process (Udaka and Moyed, 1963), the first step consisting of a pyrophosphate-dependent reaction between psicofuranine and the enzyme, the second one requiring XMP. When all three components are present at levels sufficient for complete inhibition, they are bound in equimolar amounts (Fukuyama and Moyed, 1964). Thus, decoyinine and psicofuranine may act by occupying the ATP-site in some reactions involving pyrophosphate cleavage from ATP.

Acknowledgment. This investigation was supported in part by a research grant (CA-02906) from the National Cancer Institute of the United States Public Health Service. Decoyinine and psicofuranine were generously provided by Dr. C. G. Smith of the Upjohn Company.

REFERENCES

Bloch, A. and Nichol, C. A., Federation Proceedings 23, 324 (1964).
Eble, T. E., Hoeksema, H., Borack, E. A., and Savage, G. H., Antibiot. Chemother. 9, 419 (1959).
Evans, J. S. and Gray, J. E., Antibiot. Chemother. 9, 675 (1959).
Flynn, L. N., Williams, V. B., O'Dell, B. L., and Hogan, A. G., Anal. Chem. 23, 211 (1951).
Fukuyama, T. T. and Moyed, H. S., Federation Proceedings 23, 277 (1964).
Hanka, L. J., J. Bact. 80, 30 (1960).
Hoeksema, H., Slomp, G., and van Tamelen, E. E., submitted for publication.
Lewis, C., Reames, H. R., and Rhuland, L. E., Antibiot. Chemother. 9, 421 (1959).
Schlechta, L., Biochem. Biophys. Res. Comm. 3, 596 (1960).
Schroeder, W. and Hoeksema, H., J. Am. Chem. Soc. 81, 1767 (1959).
Tanaka, N., J. Antibiot. (A)XVI, 163 (1963).
Tanaka, N., Miyairi, N., and Umezawa, H., J. Antibiot. (A) XIII, 265 (1960).
Udaka, S. and Moyed, H. S., J. Biol. Chem. 238, 2797 (1963).