INHIBITION AND STIMULATION OF ADENINE PHOSPHORIBOSYLTRANSFERASE BY PURINE NUCLEOTIDES[1]

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Inhibition of adenine phosphoribosyltransferase (adenosine monophosphate: pyrophosphate phosphoribosyltransferase, EC 2.4.2.7) by purine nucleotides and by purine analog nucleotides has been demonstrated by Savel and Handschumacher (1965), Hori and Henderson (1966a), Berlin and Stadtman (1966), Murray (1966), Henderson et al. (1967), and Henderson and Gadd (1967). An investigation of the mechanism by which such inhibition is effected has been initiated, and this paper reports the detection of at least three types of inhibition of this enzyme by purine nucleotides. In addition, it is shown that under certain conditions some purine nucleotides cause an increase in the rate of the adenine phosphoribosyltransferase reaction.

METHODS

Purine ribo- and deoxyribonucleotides were purchased from P-L Laboratories. Sources of other materials have been listed by Hori and Henderson (1966bc).

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The initial velocity of the adenine phosphoribosyltransferase reaction was measured with a preparation partially purified from Ehrlich ascites tumor cells by Hori and Henderson (1966b). The synthesis of radioactive AMP from 5-phosphoribosyl 1-pyrophosphate (PRPP) and adenine-^{14}C was measured as described by Hori and Henderson (1966c). The concentration of Mg^{2+} was kept constant at 1 mM.

RESULTS

The adenine phosphoribosyltransferase from Ehrlich ascites tumor cells has been shown by Hori and Henderson (1966a) to be inhibited by a number of purine nucleotides. Guanylate (GMP) and deoxyadenylate (dAMP) were among the most effective inhibitors, and were chosen for more detailed study. Figure 1 shows the kinetics of inhibition of adenine phosphoribosyltransferase by GMP, dAMP, and adenylate (AMP), a product of this reaction, as

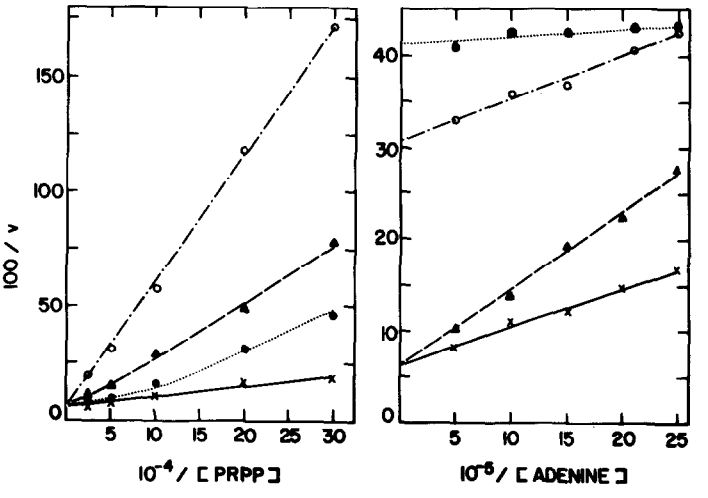

Fig. I - Inhibition of adenine phosphoribosyltransferase by purine nucleotides. Double reciprocal plots of initial velocity against: PRPP concentration at an adenine concentration of 0.01 mM; and adenine concentration at a PRPP concentration of 0.05 mM. The concentration of AMP ( ) was 0.1 mM; GMP ( ), 0.05 mM; and dAMP ( ), 0.01 mM. Control: ( ). v is expressed as μμmoles of AMP formed per minute.

functions of the concentrations of the two substrates, PRPP and adenine. (The data for AMP in Fig 1 are taken from the study of Hori and Henderson (1966c) for comparison with those for the other nucleotides.) It is apparent that each of the three purine nucleotide inhibitors produced an individual pattern of inhibition, a difference which was not apparent when only single concentrations of substrates and inhibitors were used. AMP behaved as a typical product inhibitor of this reaction, which was shown by Hori and Henderson (1966c) to be of the "ping pong" type. PRPP adds to the enzyme first to form pyrophosphate and an enzyme-bound ribose-5-phosphate intermediate, after which adenine then reacts with the latter to form AMP and free enzyme.

Double reciprocal plots of reaction rates in the presence of GMP were linear at low PRPP concentration, but curved and almost approached a common intercept on the ordinate with the control line at infinite PRPP concentration. When adenine was the variable substrate, GMP acted to lower both the apparent maximum velocity and the apparent Michaelis constant for this substrate, and the magnitude of both effects increased with increasing adenine concentration.

Double reciprocal plots of reaction rates as a function of PRPP concentration in the presence of dAMP were of similar, but not identical, shape to those with GMP under the same conditions. In contrast, dAMP was a strictly competitive inhibitor with respect to adenine, and the plot was linear.

Table I shows that at low concentrations, the same three nucleotides whose inhibitory effects have just been demonstrated, AMP, GMP, and dAMP, caused an increase in the rate of the adenine phosphoribosyltransferase reaction. Although AMP may have caused such an apparent increase in rate through isotope exchange

between adenine-^{14}C and this product nucleotide, this was not the case for GMP and dAMP.

Table I
Stimulation of adenine phosphoribosyltransferase activity by purine nucleotides

Additions	Conc. (M)	Stimulation (%)
AMP	2×10^{-5}	38
GMP	10^{-5}	33
dAMP	5×10^{-6}	30

Enzyme activity was assayed with 8×10^{-6} M adenine and 6×10^{-6} M PRPP.

DISCUSSION

Double reciprocal plots of the adenine phosphoribosyltransferase reaction are linear at reaction rates from <5 to >95 per cent of Vmax in the absence of modifers, and this enzyme may therefore be added to the small group of "regulatory" enzymes listed by Maeba and Sanwal (1966) with this characteristic. As demonstrated in Figure I, modifers such as dAMP and GMP sometimes, but not always, produce nonlinear double reciprocal plots, whereas products give linear plots.

The diversity of double reciprocal plot patterns formed when each purine nucleotide inhibitor was tested in the presence of varying concentrations of each substrate strongly suggests that GMP and dAMP do not act as simple product inhibitor analogs, and that these two nucleotides have mechanisms of inhibition which differ from each other. Whether other nucleotide inhibitors of this reaction conform to the patterns shown here, or have still different effects, remains to be determined.

Although other "regulatory" enzymes have been shown to be stimulated by modifers which at higher concentrations cause inhibition (cf. Monod et al. (1965)), such a phenomenon is uncommon among those enzymes which ordinarily give linear double reciprocal plots.

It is difficult at the present time to formulate a model for the reaction mechanism and nucleotide regulation of adenine phosphoribosyltransferase which takes into account all of these findings. Although there is no definitive evidence for the existence of subunits in this enzyme, such a possibility is being explored. Further studies on the mechanism of stimulation and inhibition of adenine phosphoribosyltransferase by purine nucleotides and nucleosides are in progress.

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