THE INHIBITION OF POLYNUCLEOTIDE PHOSPHORYLASE BY 6-MERCAPTOPURINE RIBOSIDE 5'-DIPHOSPHATE

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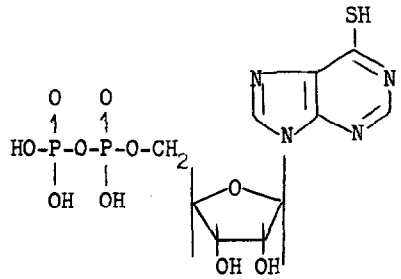
The purine antimetabolite, 6-mercaptopurine (6-MP), has been found to possess significant tumor growth inhibitory properties in animals, and has been widely tested clinically against a variety of human neoplasms (cf. Farber et al., 1956). Evidence has accumulated to indicate that 6-MP inhibits protein synthesis in several organisms, although the exact mechanism of this inhibition has not been elucidated (for leading references see Lee, 1960).

We have discovered that 6-mercaptopurine riboside 5'diphosphate (6-MPRDP) is a potent inhibitor of the enzyme, polynucleotide phosphorylase, obtained from M. lysodeikticus. This finding could have some bearing on the potent growth-inhibitory effects of 6-MP, since it is known that purine antimetabolites can be converted to various nucleotide derivatives in vivo (Way, Dahl, and Parks, 1959). The metabolic importance of polynucleotide phosphorylase has not yet been fully established, however.



6-MPRDP

6-Mercaptopurine riboside 5'-monophosphate (6-MPRMP) was synthesized as previously described (Roy et al., 1961; Montgomery and Thomas, 1961). This material was converted to the 5'-phosphoromorpholidate derivative using the excellent general method of Moffatt and Khorana (1961), and obtained as the bis-(4-morpholine N,N'-dicyclohexylcarboxamidine) salt in 62% yield [Calcd. for $C_{14}H_{20}N_5O_7PS \cdot 2(C_{17}H_{31}N_3O) \cdot H_2O$: C, 55.3; H, 8.17; N, 14.8. Found: C, 55.5: H, 7.95; N, 14.6]. Using ascending paper chromatography, this material displayed R_f 0.36 (ethanol-1M ammonium acetate, 5:2) and R_f 0.67 (isopropyl alcohol-1% ammonium sulfate, 2:1). Small spots corresponding to 6-MPRMP were also obtained in some preparations.

The 5'-phosphoromorpholidate derivative was treated with tri-n-butyl ammonium phosphate in dry pyridine in a manner analogous to that used by Moffatt and Khorana (1961) for the preparation of other nucleoside 5'-diphosphates. The resulting mixture of 6-MPRMP and 6-MPRDP was separated on a column of Dowex 2-X8 (chloride form), the monophosphate being eluted with 0.01 N HCl-0.05 N LiCl and the diphosphate with 0.05 N HCl-0.05 N LiCl. There was thus obtained a 17% recovery of 6-MPRMP and a 41% yield of 6-MPRDP as the tri-lithium salt [Anal. Found: Total P: labile P: 6-mercaptopurine = 2.03:0.82:1.00]. This material gave one spot (R_f 0.14) using ascending paper chromatography (isopropyl alcohol - 1% ammonium sulfate; 2:1). The ultraviolet absorption spectrum was identical to that previously reported for 6-mercaptopurine riboside (Fox et al., 1958).

Polynucleotide phosphorylase was prepared from acetone-dried M. lysodeikticus cells as directed by Beers (1957 and 1960). The enzyme was assayed using ADP as substrate by measuring the release of inorganic phosphate and by the increase in optical density at 257 mμ of the acid-insoluble fraction (poly A)(Beers, 1957). Preliminary experiments soon indicated that the 6-MPRDP was not a substrate of poylnucleotide phosphorylase, even at high concentrations of enzyme or in the presence of ADP. In fact, 6-MPRDP has proven to be a powerful inhibitor of this enzyme, giving measurable inhibition of poly A synthesis at concentrations as low as 1.67×10^{-5} M

(Table I). In contrast to these results, the 5'-monophosphate (6-MPRMP) was not inhibitory at 1.67 mM.

TABLE I

Polynucleotide Phosphorylase Inhibition by Various Concentrations of 6-MPRDP using 1.67 mM ADP as Substrate

6-MPRDP X 10^{-5} M	% Inhibition (5 min.)	% Inhibition (20 min.)
1.67	15.8	27.1
3.34	57.6	48.4
6.67	70.7	63.7
16.7	95.8	92.8
33.4	100.0	99.4

Reaction mixtures (3.0 ml.) contained 0.167 M KCl, 1.33 X 10^{-3} M $MgCl_2$, 16.7 X 10^{-3} Tris buffer (pH 9.0), 1.67 X 10^{-3} M ADP, 6-MPRDP or water, and enzyme. After incubation at 37° for the indicated times, aliquots were withdrawn and assayed for poly A as directed by Beers (1957).

Attempts to determine the nature of this inhibition of poly A synthesis (competitive or noncompetitive) are complicated by the fact that the kinetics of poly A synthesis by this enzyme often do not follow the Michaelis-Menten equation (Beers, 1958). However, as shown in Table II, the inhibition of poly A synthesis by 6-MPRDP is dependent upon the concentration of the substrate (ADP), indicating a competition between ADP and 6-MPRDP for the active site of the enzyme.

Although the polynucleotide phosphorylase from M. lysodeikticus is known to be sensitive to changes in magnesium ion concentration, the concentration of magnesium chloride used in these experiments is quite high (1.33 X 10^{-3} M) compared with that of the inhibitor (10^{-5} - 10^{-4} M). The effect of the inhibitor is therefore probably not due to the binding of magnesium ions.

TABLE II

Polynucleotide Phosphorylase Inhibition by 6.67×10^{-5} M 6-MPRDP

ADP, mMolar	% Inhibition (20 min.)
2.50	58.7
1.60	63.2
1.17	66.8
0.67	80

Reaction mixtures (3.0 ml.) contained 0.167 M KCl, 1.33×10^{-3} M $MgCl_2$, 16.7×10^{-3} M Tris buffer (pH 9.0), ADP, 6.67×10^{-5} M 6-MPRDP or water, and enzyme. After incubation at 37° for 20 min., aliquots were withdrawn and assayed for poly A as directed by Beers (1957).

Further studies concerning the inhibition of polynucleotide phosphorylase by 6-MPRDP are in progress and will be reported elsewhere.

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