THE EFFECT OF 6-MERCAPTOPURINE AND ITS DERIVATIVES ON MAMMALIAN RNA-DEPENDENT RNA SYNTHESIS[1]

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Received November 12, 1973

Summary - The effect of 6-mercaptopurine (6-MP) and its nucleoside and nucleotide derivatives on the rate of RNA synthesis with reticulocyte RNA-dependent RNA polymerase has been examined. Of the 6-MP derivatives tested only 6-mercaptopurine ribo-5'-monophosphate (6-MPR-P) significantly stimulates the rate of RNA synthesis with hemoglobin mRNA as a template while 6-MP and 6-MPR have little or no effect. Kinetic studies have demonstrated that 6-MPR-P activates the RNA-dependent RNA polymerase by increasing the Vmax of the enzyme rather than by decreasing the Km values for the substrates. It is suggested that the stimulation of RNA-dependent RNA synthesis by 6-MPR-P may explain the transient development of megaloblastic anemia following 6-MP therapy.

The purification of an RNA-dependent RNA polymerase from rabbit reticulocytes has recently been described (1). This enzyme is unable to utilize either native or denatured DNA but preferentially utilizes messenger RNA as a template for RNA synthesis. The presence of this activity in the cytoplasm of a mammalian cell has very broad biological implications, since it would provide a mechanism for the synthesis of mRNA without concomitant DNA transcription. Furthermore, the RNA-dependent synthesis of RNA in the cytoplasm may be under different regulatory mechanisms than the transcription of nuclear genes.

Many drugs which interfere with nucleic acid metabolism are effective anti-tumor agents. Purine analogs and their nucleotide derivatives interfere with nucleic acid metabolism at many sites, although the precise basis of their chemotherapeutic effect is not known (2,3). The purine analog

1. This research was supported by grants from the National Institute of Health (NIH AM 09001 and NIH-5-T01-AM-5472-03), American Heart Association (72-967) and Milton N. Weir, Jr. Cancer Research Fund.
2. Established Investigator of the American Heart Association.

6-mercaptopurine (6-MP) and its nucleotide derivatives are known to inhibit the synthesis of purine nucleotides and to be incorporated into nucleic acids, both DNA and RNA (4). In this communication we wish to report the effects of 6-MP and its derivatives on the RNA-dependent synthesis of RNA.

MATERIALS AND METHODS

[^{3}H]UTP was purchased either from International Chemical and Nuclear Corp. or Schwarz-Mann; unlabeled ribonucleoside triphosphates, purine analogs and cytosine β-D arabinofuranoside 5'-monophosphate (Ara-CMP) were obtained from Sigma Chemical Co.; 5-fluorodeoxyuridine 5'-monophosphate (5-FdUMP) and 5-bromouridine 5'-monophosphate (5 BUMP) were purchased from Markson Science Co., Del Mar, California.

RNA-dependent RNA polymerase was prepared from rabbit reticulocytes as previously described (1). RNA was prepared from reticulocyte polysomes by the method of Oda and Jolick (5) and fractionated by sucrose density gradient sedimentation (6).

Assay for RNA-dependent RNA Polymerase

The reaction mixture contained in a final volume of 0.25 ml: 80 mM Tris, pH 7.8; 1.6 mM $MnCl_2$; 1.0 mM EDTA; 1.0 mM dithiothreitol; 80 mM ammonium sulfate; 0.16 mM each ATP, GTP and CTP; 8.0 μM [^{3}H]UTP, 250 μCi/μmole; 5 μg mRNA and 10-15 μg of enzyme. After incubation at 37°C, the reaction was stopped by the addition of 2 ml of cold 5% trichloroacetic acid and the precipitate was collected on a glass fiber filter (Whatman GF/C). The filter was washed with 30 ml of 5% trichloroacetic acid and 10 ml of 95% ethanol, dried, and counted in a liquid scintillation spectrometer.

RESULTS

The Effect of 6-MP and its Nucleoside and Nucleotide Derivatives on the Rate of RNA Synthesis

The effects of 6-MP, 6-MPR and 6-MPR-P on the rate of RNA synthesis are shown in Table I.

Of the 6-MP derivatives tested the nucleotide 6-MPR-P significantly

Table I

Effects of 6-Mercaptopurine and its Derivatives on the Rate of RNA Synthesis

Addition	[^{3}H]UMP Incorporated (cpm)	% Stimulation (above control)
none	3,140	0
6-MP	3,540	12.0
6-MPR	3,510	11.8
6-MPR-P	16,450	424.0

The reaction mixtures were as described in Materials and Methods except for the indicated additions. The final concentration of each purine analog was 0.8 mM.

stimulates the rate of RNA synthesis with hemoglobin mRNA as a template while 6-MP and 6-MPR have little or no effect. To test the specificity of this effect, several purine and pyrimidine nucleotides and sulfhydryl containing reagents were tested for stimulatory activity. These results are shown in Table II.

With the exception of 6-MPR-P, all of the nucleotides tested have little or no effect on the rate of synthesis of RNA. 5'-AMP, 5'-IMP, and 5-BUMP all stimulate about 30% while 3'-AMP, 5'-GMP, Ara-CMP and 5-FdUMP each stimulate about 10-15%. The sulfhydryl reagent dithiothreitol also has no effect on the rate of RNA synthesis. It should be emphasized that the RNA-dependent RNA polymerase requires 1.0 mM dithiothreitol for optimal activity and the additional dithiothreitol was supplementary to the sulfhydryl reagent already present in the mixture.

The Effect of 6-MPR-P on the Rate of RNA Synthesis

The effect of increasing concentrations of 6-MPR-P on the rate of RNA synthesis is shown in Figure 1. Increasing concentrations of 6-MPR-P increase

Table II

Effects of Various Nucleotides on the Rate of DNA Synthesis

Addition	[3H]UMP Incorporated (cpm)	% Stimulation (above control)
none	3,140	0
6-MPR-P	16,450	424
5'-AMP	4,150	32.3
3'-AMP	3,490	11.3
5'-GMP	4,119	31.2
5'-IMP	4,230	34.7
5'-UMP	4,169	32.8
5-FdUMP	3,570	13.7
5-BUMP	4,230	34.7
Ara-CMP	3,610	15.0
dithiothreitol	3,260	3.9

The assay conditions were as described in Materials and Methods except for the addition of nucleotides as indicated to a final concentration of 0.8 mM. The 0.8 mM dithiothreitol added was supplementary to the 1.0 mM dithiothreitol already present in the reaction mixture.

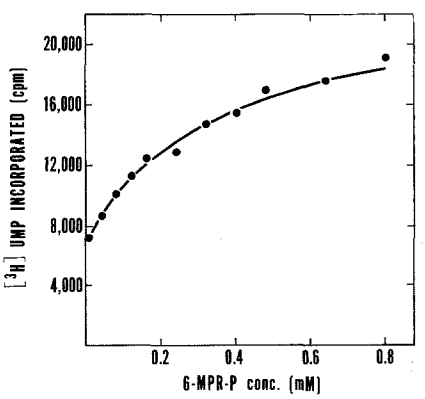

Figure 1. The Effect of 6-MPR-P on the Rate of RNA Synthesis. Assay conditions were as described in Materials and Methods except for the addition of 6-MPR-P as indicated.

the rate of RNA synthesis hyperbolically and at 0.8 mM 6-MPR-P the rate of RNA synthesis is stimulated about 2.5-fold.

The stimulatory effect of 6-MPR-P could be the result of either increasing the maximal velocity of the enzyme or decreasing the Km values for the substrates. The effects of 6-MPR-P on the kinetic constants are shown in Figure

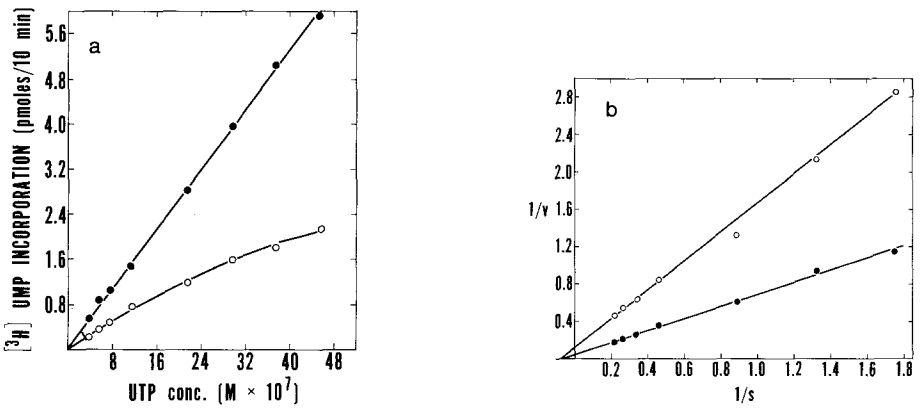

Figure 2. The Effect of Substrate Concentration on the Rate of RNA Synthesis. A: The concentration of ATP, GTP and CTP were held constant at 0.16 mM and the concentration of [^{3}H]UTP was varied from 0.3 μM to 4.5 μM in the presence (●—●) and absence (○—○) of 6-MPR-P. B: Double-reciprocal plot of the data in Figure 2A.

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2. In this case the concentrations of ATP, GTP, and CTP were held constant at 0.16 mM and the concentration of [^{3}H]UTP was varied either in the presence or absence of 6-MPR-P. The apparent Km for UTP was found to be 5×10^{-6} M either in the presence or absence of 6-MPR-P. However, the Vmax in the presence of 6-MPR-P was 268 pmoles/ml/10 min as compared to 68 pmoles/ml/10 min in the absence of 6-MPR-P. Thus, 6-MPR-P has no effect on the apparent Km for UTP but increases the maximal velocity approximately 3-fold.

DISCUSSION

The presence of an RNA-dependent RNA polymerase in the cytoplasm of a mammalian cell which is able to synthesize RNA with mRNA as a template pro-

vides a new site for control of gene expression in higher organisms as well as a new pathway for synthesis of RNA without concomitant DNA transcription (1). The widespread occurrance of this enzyme in mammalian cells might explain apparent paradoxes such as the increased rate of protein synthesis in the presence of Actinomycin D, an inhibitor of DNA-dependent RNA synthesis (7,8,9), since this antibiotic has no effect on the RNA-dependent synthesis of RNA. Furthermore the existence of this enzyme also calls for a careful re-evaluation of data on the half-life of mRNA in eukaryocytes that are based on the use of Actinomycin D to prevent RNA synthesis. It is possible that other inhibitors of DNA-dependent RNA synthesis may also have quite different effects on RNA-dependent RNA synthesis.

The purine analog 6-MP is an effective anti-tumor agent which interferes with nucleic acid metabolism. The nucleotide derivative 6-MPR-P is thought to be the active antimetabolite since it has been shown to inhibit purine nucleotide synthesis. However, it has not been established that the inhibition of purine nucleotide synthesis is responsible for the anti-tumor activity of this drug.

In this communication we have shown that 6-MPR-P stimulates the rate of RNA synthesis with the RNA-dependent RNA polymerase of reticulocytes. This stimulation is not due to 6-MPR-P acting as a sulfhydryl reagent since 6-MP and 6-MPR as well as higher concentrations of dithiothreitol have little or no effect on the rate of RNA synthesis. We have also considered the possibility that the effect of 6-MPR-P is due to its ability to complex metal ions. However the optimal concentration of $MnCl_2$ remains unchanged either in the presence or absence of this purine nucleotide analog (data not shown), thus ruling out this possibility. Kinetic studies have demonstrated that 6-MPR-P activates the RNA polymerase by increasing the Vmax of the enzyme rather than decreasing the Km values for the substrate.

The stimulation of the rate of RNA synthesis by 6-MPR-P may explain some of the effects of *in vivo* administration of 6-MP. It has been observed that

6-MP therapy results in the transient development of megaloblastic anemia (10), which is thought to be the result of selective impairment of DNA synthesis without impairment of RNA synthesis. It is possible that the stimulation of RNA-dependent RNA polymerase by 6-MPR-P is responsible for this phenomenon.

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