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REGULATION OF PHOSPHORIBOSYLPYROPHOSPHATE SYNTHETASE BY ENDOGENOUS PURINE AND PYRIMIDINE COMPOUNDS AND SYNTHETIC ANALOGS IN NORMAL AND LEUKEMIC WHITE BLOOD CELLS

MARY K. DANKS* and ERIC M. SCHOLAR
Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68105, U.S.A.

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Abstract—Phosphoribosylpyrophosphate (PRPP) is essential for the formation of both purine and pyrimidine nucleotides as well as for the active nucleotide form of some chemotherapeutic agents. The formation of PRPP is catalyzed by the enzyme PRPP synthetase, and many different compounds are known to affect the activity of this enzyme. This report examines the effects of endogenous purine and pyrimidine nucleotides, nucleosides, and several analogs of these compounds on the activity of PRPP synthetase from different types of normal and leukemic white blood cells (i.e. normal lymphocytes, normal granulocytes, phytohemagglutinin-stimulated lymphocytes, and acute and chronic leukemic cells). Our results show that the effect varied with each individual compound, and the magnitude of the effect was dependent on the source of the enzyme. Since it appears possible to differentially affect PRPP synthetase activity from the different types of leukemic cells, this enzyme may be a potential target site in the chemotherapy of leukemia.

Phosphoribosylpyrophosphate (PRPP) is essential for the formation of purine and pyrimidine nucleotides. The formation of PRPP from ATP and ribose-5-phosphate (R5P) is catalyzed by the enzyme PRPP synthetase.† Many different types of compounds are known to influence the activity of this enzyme, including nucleotides, nucleosides and their bases [1, 2].

Inhibition of PRPP synthetase activity by purine and pyrimidine compounds has been shown in human red blood cells (RBCs) [2], Ehrlich ascites tumor cells [3], rat liver [4], and lymphocytes [5]. ADP is one of the most potent inhibitors of the activity of PRPP synthetase from RBCs [2], and the mechanism of this inhibition is apparent competition at the ATP binding site. Inhibition produced by ADP is physiologically important; its intracellular concentration is greater than the K_i for PRPP synthetase [2, 6]. GDP is also a potent inhibitor of PRPP synthetase activity. The mechanism of this inhibition is noncompetitive with respect to substrate binding [2], but the exact mechanism of interaction is unknown. There is also inhibition of PRPP synthetase by the products of the reaction, PRPP and AMP.

In contrast to the inhibition of enzyme activity, several endogenous compounds are known to increase PRPP synthetase activity; these compounds include inorganic phosphate and Mg^{2+} [1, 2, 5, 7]. However, the mechanism of activation by these cofactors and the physiological significance of the effect of phosphate ions are uncertain [7]. PRPP synthetase activity is also increased by cAMP and glucagon in cultured hepatoma cells [8]; this cAMP stimulation is antagonized by cGMP. Green and Martin [9] reported a cGMP-stimulated PRPP synthetase activity which was not associated with phosphorylation of protein in rat hepatomas. PRPP synthetase from human lymphocytes is stimulated by IMP [5].

Although PRPP synthetase is essential to nucleotide production and for the formation of the nucleotides of several purine and pyrimidine analogs used in chemotherapy, little is known about regulation of the activity of the enzyme in normal and leukemic white blood cells (WBCs). This paper compares the effect of endogenous nucleotides, nucleosides, and several analogs of these compounds on the activity of PRPP synthetase from several different types of normal and leukemic WBCs. Differences found in the regulation of PRPP synthetase by these compounds might be used as a basis for understanding underlying biochemical changes of the leukemic process, as a basis for chemotherapy, or to provide an explanation for resistance to antileukemic drugs.

Our results show that each compound included in the study affects PRPP synthetase activity to a different degree, and the magnitude of the effect is dependent on the source of the enzyme.

* Address correspondence to: Mary K. Danks, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38101, U.S.A.

† Abbreviations: PRPP synthetase, phosphoribosylpyrophosphate synthetase (EC 2.7.6.1, ATP: D-ribose-5-phosphate pyrophosphotransferase); R5P, ribose-5-phosphate; CLL, chronic lymphocytic leukemia; CML, chronic myelocytic leukemia; CGL, chronic granulocytic leukemia; ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia; PHA, phytohemagglutinin; RBC, red blood cell; WBC, white blood cell; BSA, bovine serum albumin; 6-MP, 6-mercaptopurine; 6-MP monophosphate, 6-mercaptopurine monophosphate; TG, thioguanine; ara-C, cytosine arabinoside; and FdUMP, fluorodeoxyuridine monophosphate.

MATERIALS AND METHODS

[^{14}C-carboxyl]Orotic acid hydrate (42.5 and 42.4 mCi/mmole) was purchased from the New England Nuclear Corp. (Boston, MA), NCS solubilizer from Amersham Searle (Arlington Heights, IL) and Tris ultra pure from Schwarz-Mann (Orangeburg, NY). Ficoll-paque and Sephadex G200 were obtained from Pharmacia Chemical (Piscataway, NJ) and dithiothreitol was purchased from Calbiochem, San Diego, CA. The sodium salt of ATP, adenosine, guanosine, hypoxanthine, inosine, AMP, GMP, CMP, TMP, IMP, and ADP were purchased from P-L Biochemicals (Milwaukee, WI); and 6% Dextran 70 in normal saline was obtained from McGaw Laboratories (Glendale, CA). RPMI-1640 with Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer and penicillin–streptomycin solution were purchased from Grand Island Biologicals (Grand Island, NY). PHA (HA16) was acquired from Burroughs–Wellcome (Research Triangle Park, NC), and 6-aminohexanoic acid from Eastman Kodak (Rochester, NY). Disodium D-ribose-5-phosphate, orotate phosphoribosyltransferase and orotidine 5′-monophosphate (OMP) decarboxylase from yeast, bovine serum albumin (BSA), disodium EDTA, GDP, CDP, GTP, dADP, dGDP, cAMP, cGMP, 6-MP, 6-MP monophosphate, TG, cytosine arabinoside (ara-C), fluorodeoxyuridine monophosphate (FdUMP), and all other chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO).

Enzyme preparation. Peripheral blood samples were collected from normal volunteers and leukemic patients. Blasts and lymphocytic cells were separated from whole blood by the Ficoll–Hypaque method [10] as described previously [11]. Granulocytic cells were separated from plasma by the same technique after the whole blood had been allowed to settle at 37° as described previously [11]. Contaminating RBCs were removed by hypotonic lysis. The cell preparations were then suspended in 50 mM potassium phosphate buffer (pH 7.4), sonicated, and centrifuged at 100,000 *g*. PRPP synthetase activity was determined using the supernatant fraction.

Enzyme activity. Enzyme activity was assayed by the production of $^{14}CO_2$ from a [^{14}C]orotic acid precursor, as described previously [11]. Protein concentration was determined by the method of Lowry *et al.* [12].

Partial enzyme purification. The crude enzyme preparation which had been prepared by sonication and centrifugation was then applied to a Sephadex G200 column measuring 1.5 cm i.d. × 25 cm. The PRPP synthetase was eluted from the column with 50 mM potassium phosphate buffer (pH 7.4), 0.15 mM ATP, 4 mM $MgCl_2$, and 10 mM 6-aminohexanoic acid as a nonspecific protease inhibitor. Eluted fractions were assayed for enzyme activity as described above. Specific activity of the PRPP synthetase preparation increased approximately 7-fold under these conditions. Elution profiles obtained with PRPP synthetase from all types of WBCs in this study were similar. The partially purified enzyme was used for the inhibition studies.

Inhibition studies. Uninhibited enzyme activity (control activity) was determined for each enzyme preparation and the values in the tables represent per cent of uninhibited (control) enzyme activity for that same enzyme preparation. Purine and pyrimidine compounds and analogs were initially screened at concentrations of 10^{-5}, 10^{-4}, and 10^{-3} M for effect on PRPP synthetase activity. For compounds that produced less than 50% inhibition of enzyme activity at 1 mM concentration, results are expressed as per cent of control enzyme activity. For compounds that produced greater than 50% inhibition at a 1 mM concentration, an ID_{50} value (i.e. the concentration of inhibitor required to reduce enzyme activity 50%) was also determined. This was done graphically.

Statistics. Statistics were done using Student's *t*-test, with significance at the 0.05 level.

RESULTS

The types of WBCs included in the study were normal lymphocytes and granulocytes, PHA-stimulated normal lymphocytes (which are often thought to be a control for leukemic blast cells), acute and chronic lymphocytic leukemic (ALL and CLL) cells, acute nonlymphocytic leukemic (ANLL) cells, and chronic myelocytic leukemic (CML) cells.

The purine and pyrimidine compounds and analogs studied for their effect on PRPP synthetase activity in the lymphocytic series of WBCs are listed in Tables 1 and 2. The effects of these compounds on the enzyme from the nonlymphocytic series are shown in Tables 3 and 4. Although most of these compounds produced some inhibition of enzyme activity, a few were shown to increase PRPP production. These compounds are identified by per cents greater than 100. Average PRPP synthetase activity for each normal and leukemic cell has been reported previously [11]. None of the compounds used for the inhibition studies or for elution of protein from the column had a significant effect on the assay enzymes, i.e. orotate phosphoribosyltransferase and OMP decarboxylase (data not shown).

A greater than 50% inhibition of PRPP synthetase from normal lymphocytes was seen with 1 mM AMP, GMP, ADP, GDP, CDP, and dGDP (Table 1). Each leukemic cell type had a characteristic inhibition pattern. The pattern of inhibition of the enzyme from PHA-stimulated normal lymphocytes was similar to that of ALL and of CLL. In the nonlymphocytic series (Table 3), at least a 50% decrease in activity of the enzyme from normal cells was seen with 1 mM AMP, ADP, GDP, CDP, GTP, dADP, and dGDP. The ID_{50} values of these compounds were determined and the results are shown in Tables 5 and 6. AMP and dGDP were the most potent inhibitors of PRPP synthetase activity in both the lymphocytic and nonlymphocytic cell types. Deoxycoformycin and methylmercaptopurine riboside were also tested, but the ID_{50} values were too high to be considered pharmacologically significant (data not shown).

DISCUSSION

In agreement with previous studies [1, 2, 4, 5, 7, 13, 14], this paper shows that ADP,

Table 1. Effects of purines and pyrimidines on PRPP synthetase activity in normal and leukemic lymphocytes*

Compound	Per cent of control PRPP synthetase activity Cell type, lymphocytic series (N) Normal (3)	PHA (3)	ALL (2)	CLL (3)
Adenosine	68 ± 4	89 ± 2†	55 ± 5	54 ± 5
Guanosine	90 ± 3	134 ± 13	88 ± 5	109 ± 10
Hypoxanthine	68 ± 0	83 ± 1†	14 ± 3†	33 ± 4†
Inosine	108 ± 12	102 ± 12	50 ± 2†	58 ± 17†
AMP	30 ± 2	14 ± 5†	18 ± 2†	4 ± 1†
GMP	28 ± 8	53 ± 7†	73 ± 1†	20 ± 3
CMP	63 ± 4	74 ± 1	67 ± 2	74 ± 8
TMP	71 ± 6	100 ± 1†	75 ± 3	95 ± 5†
IMP	66 ± 5	80 ± 3†	56 ± 8	98 ± 8†
ADP	42 ± 2	74 ± 4†	57 ± 5	40 ± 1
GDP	20 ± 2	45 ± 10†	17 ± 3	25 ± 3
CDP	13 ± 10	43 ± 2†	23 ± 9	53 ± 6†
GTP	70 ± 6	51 ± 1†	47 ± 8†	39 ± 2†
dADP	84 ± 3	26 ± 10†	31 ± 1†	82 ± 5
dGDP	10 ± 3	40 ± 3†	17 ± 3	39 ± 9†
cAMP	76 ± 2	95 ± 2†	79 ± 1	72 ± 7
cGMP	112 ± 12	124 ± 11	92 ± 3	124 ± 16

* All values are expressed as per cent of control enzyme activity ± S.E.M. Procedure was as described in Materials and Methods. The concentration of all compounds was 1 mM.

GDP, AMP, GMP, dADP, and dGDP are potent inhibitors of PRPP synthetase activity. But while ADP is the most potent diphosphate inhibitor of enzyme activity in RBCs [2], rat liver [4], and *Salmonella typhimurium* [14], we have shown GDP to be a more potent inhibitor than ADP with the enzyme from lymphocytes. Inhibition of PRPP synthetase activity was also shown by both purine and pyrimidine monophosphates, and this is similar to the effect on the enzyme from other mammalian and bacterial sources [2, 4, 14]. However, in contrast to results shown in this paper, Garcia *et al.* [5] reported an increase in PRPP synthetase activity by both IMP and GMP with the enzyme from human WBCs. Different methods of enzyme preparation and different assay conditions may account for the differences reported. The cGMP-stimulated PRPP synthetase activity reported by Green and Martin [9] was not evident with the enzyme from WBCs. Overall, the inhibition and stimulation patterns of PRPP synthetase activity vary with the source of the enzyme.

The analog nucleotides used in this study failed to show a greater than 50% inhibition of PRPP synthetase activity with the enzyme from any one type of leukemic cells. In another study by Yen *et al.* [15], however, a purine analog has been shown to be an effective inhibitor of PRPP synthetase activity in cultured fibroblasts, at inhibitor concentrations in the micromolar range. It appears possible, therefore, that high local concentrations of these nucleotides may yet be found within a cell, or compounds other than those in our study may be effective in inhibiting nucleic acid production at this step of synthesis.

The inhibition pattern of PRPP synthetase by

Table 2. Effects of purine and pyrimidine analogs on PRPP synthetase activity in normal and leukemic lymphocytes*

Compound	Per cent of control PRPP synthetase activity Cell type, lymphocyte series (N) Normal (3)	PHA (3)	ALL (2)	CLL (3)
6-Mercaptopurine (6-MP)	72 ± 2	72 ± 3	54 ± 11	65 ± 14
6-MP monophosphate	66 ± 3			
Thioguanine	123 ± 11	131 ± 15	108 ± 7	114 ± 10
Cytosine arabinoside	68 ± 6	84 ± 2	75 ± 3	78 ± 2
Fluorodeoxyuridine 5'-monophosphate	54 ± 6	82 ± 9†	82 ± 4†	71 ± 0†

* All values are expressed as per cent of control enzyme activity ± S.E.M. Procedure was as described in the text. The concentration of all compounds was 1 mM.

† Significantly different from enzyme activity in normal lymphocytes ($P < 0.05$).

Table 3. Effects of purines and pyrimidines on PRPP synthetase activity in normal and leukemic granulocytes*

Compound	Per cent of control PRPP synthetase activity Cell type, granulocytic series (N) Normal (3)	ANLL (4)	CML (3)
Adenosine	66 ± 4	72 ± 2	60 ± 3
Guanosine	68 ± 4	78 ± 2	76 ± 1
Hypoxanthine	75 ± 2	88 ± 4	87 ± 4
Inosine	72 ± 3	77 ± 5	85 ± 3
AMP	11 ± 1	15 ± 2	13 ± 2
GMP	76 ± 3	51 ± 2†	54 ± 2†
CMP	69 ± 1	79 ± 4	62 ± 3
TMP	79 ± 2	69 ± 3	85 ± 3
IMP	98 ± 2	99 ± 4	85 ± 6
ADP	46 ± 4	52 ± 5	40 ± 2
GDP	45 ± 1	36 ± 6	49 ± 2
CDP	33 ± 3	36 ± 3	32 ± 3
GTP	48 ± 3	55 ± 3	18 ± 2†
dADP	33 ± 4	37 ± 2	9 ± 5†
dGDP	20 ± 2	20 ± 3	1 ± 3†
cAMP	77 ± 2	80 ± 4	67 ± 5
cGMP	78 ± 3	78 ± 4	89 ± 5

* All values are expressed as per cent of control enzyme activity ± S.E.M. Procedure was as described in the text. The concentration of all compounds was 1 mM.

† Significantly different from enzyme activity in normal granulocytes ($P < 0.05$).

endogenous compounds differs between normal and leukemic WBCs. Varying inhibition patterns have also been seen by investigators working with PRPP synthetase from other neoplastic tissues. One of the ways in which inhibition patterns deviate from normal is shown by a decreased sensitivity to control of enzyme activity, reflected by a higher ID_{50} of a particular compound with the PRPP synthetase from malignant versus normal tissue. For example, different inhibition patterns of PRPP synthetase activity have been seen with the enzyme from rat hepatoma cells versus normal rat liver cells [16]. Compared to the control, the enzyme from the hepatoma cells shows a decreased sensitivity to feedback control of enzyme activity reflected by higher ID_{50} values of the inhibitors. While the PRPP synthetase from WBCs failed to show an overall decrease in sensitivity to control of enzyme activity, several individual compounds did have higher ID_{50} values for inhibition of PRPP synthetase from leukemic cells. In ALL cells, for example, GMP, ADP, and GDP had higher ID_{50} values than in normal cells; and in CLL cells, CDP and dGDP had higher ID_{50} values than in normal cells. We can conclude that the concentration of the compounds required to regulate PRPP synthetase activity is dependent upon the type of cell from which the enzyme was obtained.

On the basis of the varying patterns of inhibition seen in this study, it appears possible to differentially affect the enzyme from different types of leukemic

Table 4. Effects of purine and pyrimidine analogs of PRPP synthetase activity in granulocytes*

Compound	Per cent of control PRPP synthetase activity Cell type, granulocytic series (N) Normal (3)	ANLL (4)	CML (3)
6-Mercaptopurine (6-MP)	50 ± 0	52 ± 6	98 ± 3†
6-MP monophosphate	80 ± 4	67 ± 5	98 ± 1†
Thioguanine	102 ± 4	116 ± 4	91 ± 6
Cytosine arabinoside	67 ± 3	79 ± 5	85 ± 7
Fluorodeoxyuridine 5′-monophosphate	95 ± 3	100 ± 3	60 ± 2†

* All values are expressed as per cent of control enzyme activity ± S.E.M. Procedure was as described in the text. The concentration of all compounds was 1 mM.

† Significantly different from enzyme activity in normal granulocytes ($P < 0.05$).

Table 5. ID_{50} Values of compounds known to inhibit PRPP synthetase activity from normal and leukemic lymphocytes by greater than 50% at a 1 mM concentration of inhibitor*

Inhibitor	ID_{50} (mM) Cell type, lymphocytic series (N) Normal (3)	PHA (3)	ALL (3)	CLL (3)
Hypoxanthine	>1.00	>1.00	0.60 ± 0.02†	0.80 ± 0.14†
AMP	0.28 ± 0.04	0.62 ± 0.05†	0.36 ± 0.01	0.32 ± 0.04
GMP	0.51 ± 0.08	>1.00†	>1.00†	0.23 ± 0.20
ADP	0.72 ± 0.10	>1.00†	1.00 ± 0.01†	0.95 ± 0.21
GDP	0.30 ± 0.11	0.67 ± 0.14	0.65 ± 0.03†	0.44 ± 0.10
CDP	0.32 ± 0.04	0.96 ± 0.02†	0.35 ± 0.02	>1.00†
GTP	>1.00	>1.00	1.00 ± 0.08	0.89 ± 0.02†
dGDP	0.47 ± 0.05	0.85 ± 0.01†	0.38 ± 0.01	0.84 ± 0.06†

* Values are expressed in mM concentration ± S.E.M.

† Significantly different from normal control value ($P < 0.05$).

Table 6. ID_{50} Values of compounds known to inhibit PRPP synthetase activity from normal and leukemic granulocytes by greater than 50% at a 1 mM concentration of inhibitor*

Inhibitor	ID_{50} (mM) Cell type, granulocytic series (N) Normal (3)	ANLL (4)	CML (3)
AMP	0.42 ± 0.04	0.41 ± 0.03	0.35 ± 0.07
ADP	1.00 ± 0.21	1.00 ± 0.09	0.75 ± 0.15
GDP	0.25 ± 0.07	0.30 ± 0.13	0.38 ± 0.01
CDP	0.85 ± 0.06	0.85 ± 0.05	0.82 ± 0.12
GTP	0.95 ± 0.21	1.00 ± 0.00	0.50 ± 0.08†
dADP	0.62 ± 0.10	0.90 ± 0.02†	0.63 ± 0.12
dGDP	0.72 ± 0.08	0.44 ± 0.10†	0.10 ± 0.25†

* Values are expressed in mM concentration ± S.E.M.
† Significantly different from normal control values ($P < 0.05$).

cells, as well as leukemic versus normal cells. Despite the observation that the analogs used here were not potent and selective inhibitors of PRPP synthetase activity, it is known that PRPP is essential to nucleotide formation of some antileukemic agents and that inhibition of formation of PRPP by synthetic compounds is possible. Therefore, this metabolic step may still prove to be an important target site in the chemotherapy of leukemia.

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