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Research Article

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De Novo Purine Biosynthesis by Two Pathways in Burkitt Lymphoma Cells and in Human Spleen

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ABSTRACT This study was designed to answer the question whether human lymphocytes and spleen cells were capable of *de novo* purine biosynthesis. Experiments were carried out in cell-free extracts prepared from human spleen, and from a cell line established from Burkitt lymphoma. Burkitt lymphoma cells and human spleen cells could synthesize the first and second intermediates of the purine biosynthetic pathway. Cell-free extracts of all cell lines studied contained the enzyme systems which catalyze the synthesis of phosphoribosyl-1-amine, the first intermediate unique to the purine biosynthetic pathway and of phosphoribosyl glycinamide, the second intermediate of this pathway. Phosphoribosyl-1-amine could be synthesized in cell-free extracts from α-5-phosphoribosyl-1-pyrophosphate (PRPP) and glutamine, from PRPP and ammonia, and by an alternative pathway, directly from ribose-5-phosphate and ammonia. These findings suggest that extrahepatic tissues may be an important source for the *de novo* synthesis of purine ribonucleotide in man. They also indicate that ammonia may play an important role in purine biosynthesis. The alternative pathway for the synthesis of phosphoribosyl-1-amine from ribose-5-phosphate and ammonia was found to be subject to inhibition by the end products of the purine synthetic pathway, particularly by adenylic acid and to a lesser degree by guanylic acid. The alternative pathway for phosphoribosyl-1-amine synthesis from ribose-5-phosphate and ammonia may contribute significantly towards the regulation of the rate of *de novo* purine biosynthesis in the normal state, in metabolic disorders in which purines are excessively produced and in myeloproliferative diseases.

INTRODUCTION

The present study was undertaken to test whether human cells of extrahepatic origin could form purine ribonucleotides from small molecules and whether they were equipped with the enzyme systems which catalyze the synthesis of the first and second intermediates of the purine biosynthetic pathway. The first intermediate of this metabolic sequence is phosphoribosyl-1-amine (PRA),[1] the second, phosphoribosylglycinamide (PRG). Work by earlier investigators presented evidence that the bone marrow was dependent for its supply of purine nucleotides upon a preformed purine precursor from the liver (1). Preformed purines converted by cellular metabolism to ribonucleotides were considered the only source of purine nucleotides for these tissues. More recently, the conversion of purine bases to ribonucleotides was postulated to be involved in the regulation of purine biosynthesis (2).

In the mouse, the spleen is a hematopoietic organ. Our studies of *de novo* purine biosynthesis in cell-free extracts of spleens of leukemic mice provided evidence that these cells could synthesize purines *de novo* from small molecular precursors (3–5). We found that PRA, the first intermediate of purine synthesis, was formed by two alternative pathways:

$$\text{1. PRPP} + \text{glutamine} + H_2O \xrightarrow{Mg^{++}} \text{PRA}$$

In this reaction ammonia could replace glutamine as follows:

$$\text{PRPP} + \text{ammonia} \xrightarrow{Mg^{++}} \text{PRA}$$

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[1] *Abbreviations used in this paper:* AICA, 5-aminoimidazol-carboxamide; AICAR, 5'-phosphoribosyl-5-amine-4-imidazol carboxamide (5-amino-imidazole-4-carboxamide ribonucleotide); AMP, adenosine monophosphate; ATP, adenosine triphosphate; EDTA, ethylenediaminetetraacetate; FPRG, formylribosylglycinamide; GMP, guanosine monophosphate; IMP, inosine monophosphate; PRA, β-phosphoribosyl-1-amine; PRG, phosphoribosylglycinamide (β-glycinamide ribonucleotide); PRPP, α-5-phosphoribosyl-1-pyrophosphate; R-5-P, ribose-5-phosphate.

and by the alternative pathway:

$$\text{2. R-5-P} + \text{ammonia} + \text{ATP} \xrightarrow{Mg^{++}} \text{PRA}^2$$

In this system, glutamine could not replace ammonia.

The synthesis of PRA from R-5-P and ammonia was also studied in avian livers and was shown to be catalyzed by an enzyme system which we referred to as ribose-5-phosphate aminotransferase and which we separated from PRPP amidotransferase (EC 2.4.2.14), the enzyme which catalyzes the synthesis of PRA from PRPP and glutamine (6). PRA synthesis by this alternative pathway from R-5-P and ammonia was sensitive to inhibition by the end product of the pathway, and resembled in this respect the conventional pathway which requires PRPP and glutamine as substrates (6, 3, 4, 7).

Based on these findings, we proposed that the alternative pathway of the first step in purine biosynthesis may play an important part in the regulation of *de novo* purine biosynthesis (6). Regulation of purine synthesis may therefore be more complex than previously postulated, and may depend upon control of PRA synthesis by either of two alternative pathways rather than by a single rate-limiting reaction for the synthesis of PRA. These observations prompted this study of *de novo* purine biosynthesis in human cells.

METHODS

Materials. Purine ribonucleotides and amino acids were purchased from Calbiochem, Los Angeles, Calif., Tris-HCl ultrapure from Mann Research Labs Inc., New York, R-5-P from Sigma Chemical Co., St. Louis, Mo., PRPP as the magnesium salt from P-L Biochemicals, Inc., Milwaukee, Wis. PRPP was passed through a Na Dowex-50 column before use to convert it to the sodium salt.

Methods. Burkitt lymphoma cells[3] were maintained in tissue culture. Cells were grown in RPM1 1640 medium containing 20% fetal calf serum and supplemented with 200 μmoles *l*-glutamine, 10,000 units penicillin, 10,000 μg streptomycin, and 25 μg Fungizone per 100 ml. The medium and all ingredients were obtained from Grand Island Biological Co., Berkeley, Calif.

Preparation of cell-free extracts. Cells were harvested by centrifugation, washed three times with 0.9% NaCl, and suspended in a minimal amount of twice distilled water. Cells were broken by freeze-thawing three times, and centrifuged at 100,000 *g* for 30 min. The clear supernatant fractions so obtained were used for all enzyme assays after dialysis against 0.01 M Tris-HCl buffer pH 8.0 for 60 min.

Human spleens obtained at operation were immediately chilled and homogenized in a Waring Blendor for 3 min in 0.035 M Tris-HCl buffer pH 7.5. The homogenate was centrifuged at 100,000 *g* for 60 min and the supernatant fraction used for enzyme assays after 60 min of dialysis against 0.01 M Tris-HCl pH 8.0. All buffers contained 10^{-4} M EDTA and 10^{-3} M β-mercaptoethanol. All procedures were carried out at 4°C. One spleen was removed electively during a gastrectomy for recurrent bleeding of a peptic ulcer, and the other for diagnosis of a granulomatous disease. Studies were carried out on tissue portions that were histologically normal.

Enzyme assay. For assay and preparation of cell-free extracts of human spleen the procedures described for mouse spleen were followed (3, 4). For the determination of enzyme activity of Burkitt lymphoma cells this procedure was slightly modified. Briefly, the 100,000 *g* supernatant fractions were incubated in a total volume of 200 μl. Standard incubation mixtures contained 100 mM Tris-HCl pH 8.0, 0.5 mM ATP, 2.0 mM $MgCl_2$, 10 mM glycine, 1.0 mM Na PRPP, and 10 mM *l*-glutamine. Ammonia was substituted for glutamine as an alternative substrate for PRPP amidotransferase in the form of 80 mM NH_4Cl. At pH 8.0, 80 mM NH_4Cl is equivalent to 4.5 mM NH_3. For the assay of R-5-P aminotransferase the incubation mixture contained 2.0 mM R-5-P and 4.5 mM NH_3. Incubations were carried out in a Dubnoff Metabolic Shaker at 37°C for 15 min. For the conversion of PRA to PRG 0.04 mg of a partially purified fraction of PRG synthetase (EC 3.6.13) was added for the assay of glutamine PRPP amidotransferase (4). The 100,000 *g* supernatant fractions, however, were found to contain adequate amounts of PRG synthetase which made the addition of purified PRG synthetase optional for accurate determination of PRA. The reaction was stopped by the addition of 10 μmoles EDTA. PRG was determined after transformylation to formylribosylglycinamide (FPRG). Inosine monophosphate (IMP) serves as a formyl donor in this reaction and is converted to 5′-phosphoribosyl-5-amine-4-imidazol carboxamide (AICAR) (4). The transformylation reaction was terminated by the addition of 20 μl of 30% trichloroacetic acid in 1 N HCl and after centrifugation AICAR was determined colorimetrically by the method of Bratton and Marshall (8). Aminoimidazolcarboxamide (AICA) served as a reference standard. 10 nmoles of PRA correspond to an O.D. of 0.100. Proteins were determined by the method of Warburg and Christian (9).

RESULTS

The enzyme activities which catalyze the synthesis of PRA and PRG, the first two intermediates of the purine biosynthetic pathway, are present in cell-free extracts of Burkitt lymphoma cells grown in tissue culture. Incubation of the 100,000 *g* supernatant fractions with PRPP and glutamine, PRPP and ammonia, or R-5-P and ammonia resulted in PRA synthesis (Table I). Incubation of R-5-P and ammonia resulted in the highest specific activity from 5.6 to 12.0 nmoles PRA per min per mg protein. Glutamine could not be substituted for ammonia; incubation with 2.0 mM R-5-P and 10 mM glutamine did not result in PRA synthesis. PRA synthesis catalyzed by PRPP amidotransferase yielded the lowest specific activity when glutamine served as substrate. Values of specific enzyme activity ranged from 2.9 to 3.8 nmoles PRA per min per mg protein. Incubation of PRPP and ammonia resulted in specific activities ranging from 4.9 to 8.0 nmoles PRA per min per mg protein (Table I).

[2] To be published.

[3] We thank Dr. Jonathan Uhr for giving us his cell line.

TABLE I

PRPP Amidotransferase and R-5-P Aminotransferase Activity in Burkitt Lymphoma Cells

Experiment	Substrates: Glutamine + PRPP	NH_3 + PRPP	NH_3 + R-5-P	Glutamine + R-5-P
	*Specific activity**			
1	3.8	8.0	12.0	<0.05
2	3.8	5.7	—	—
3	2.9	4.9	5.6	<0.05
4	—	7.3	9.1	—

* Specific activity: nmoles PRA formed per minute per milligram protein of the 100,000 *g* supernatant fraction. Conditions of incubation as described in Methods.

The amount of PRA formed was dependent upon the amount of crude enzyme extract, upon the duration of incubation, and upon the nature of the substrates (Fig. 1). The amount of PRA formed was directly proportional to the time of incubation for incubations not exceeding 30 min.

These findings suggest that two enzyme systems can catalyze the synthesis of PRA in cell-free extracts of Burkitt lymphoma cells maintained in culture. This is consistent with our earlier studies of the enzyme activities purified from avian liver (6). The enzyme activity which supports PRA synthesis from R-5-P and ammonia, R-5-P aminotransferase, could be purified and separated from PRPP amidotransferase. PRPP could not be substituted for R-5-P in this reaction, nor could glutamine replace ammonia.

PRA synthesis in cell-free extracts of Burkitt lymphoma cells could be inhibited by the addition of AMP and guanosine monophosphate (GMP), the end products of the pathway. The effect of AMP and GMP on PRA synthesis is recorded in Table II. Experiments 1 and 2 show the relationship between PRPP concentration and PRA synthesis. PRA synthesis from either PRPP and glutamine or PRPP and ammonia was inhibited at all PRPP concentrations from 0.25 mmole/liter to 4.0 mmoles/liter. Both AMP and GMP were effective inhibitors and inhibited these reactions to a similar extent. The effect of AMP and GMP on PRA synthesis from R-5-P and ammonia is shown in experiment 3. While AMP inhibited this reaction significantly, GMP was a poor inhibitor of this pathway, particularly at R-5-P concentrations less than 2.0 mmoles/ liter. This is more clearly shown in Fig. 2; in this experiment R-5-P concentrations were kept at 1.0 mmole/liter. GMP, in concentrations ranging from 1.0 to 4.0 mmoles/liter, failed to inhibit PRA synthesis from R-5-P and ammonia significantly. R-5-P itself at concentrations exceeding 2.0 mmoles/liter causes partial inhibition of PRA synthesis. This is consistent with our findings in the avian enzyme (6).

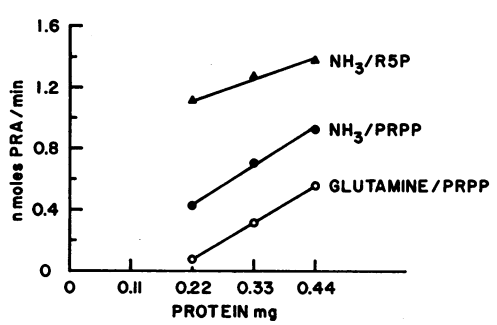


FIGURE 1 Activity of PRPP amidotransferase and R-5-P aminotransferase of Burkitt lymphoma cells as a function of protein concentration. Conditions of assay as described in Methods.

These experiments prove that PRA synthesis from PRPP and by the alternative pathway from R-5-P and ammonia are subject to end product inhibition particularly by AMP and that both pathways for the synthesis of PRA, the first intermediate, are of importance for the regulation of the entire metabolic sequence.

Investigation of the 100,000 *g* supernatant fraction obtained from human spleens showed similar results (Table III). These results indicate that purine biosynthesis in normal spleen cells and neoplastic cells are sub-

TABLE II

Inhibition of PRA Synthesis by AMP and GMP in Extracts of Burkitt Lymphoma Cells

Experiment No.	Substrates: Glutamine	Substrates: PRPP	Inhibitors: AMP 1.0 mM	AMP 2.0 mM	GMP 1.0 mM	GMP 2.0 mM
	mmoles/liter		*% inhibited*			
1	10	0.25	70	—	100	90
	10	0.5	62	76	76	83
	10	1.0	69	84	71	79
	10	2.0	82	84	84	88
	10	4.0	56	—	57	59
2	NH_4Cl	PRPP				
	80	0.25	87	—	90	96
	80	0.5	74	88	77	83
	80	1.0	59	81	58	75
	80	2.0	54	69	28	53
	80	4.0	9	—	41	28
3	NH_4Cl	R-5-P				
	80	0.25	46	64	13	16
	80	0.5	50	69	13	25
	80	1.0	50	80	2	10
	80	2.0	66	84	31	37
	80	4.0	45	44	50	24

The incubation mixtures contained in a total volume of 0.2 ml: Tris-HCl, pH 8.0, 0.1 M; $MgCl_2$, 2.0 mM; ATP, 0.5 mM; glycine, 10 mM. 0.22 mg of the 100,000 *g* supernatant fraction were incubated for experiments 1 and 2, and 0.10 mg for experiment 3. Incubations were carried out for 15 min at 37°C.

ject to regulation by end product. The specific activity of all splenic enzyme systems was considerably lower than that of Burkitt lymphoma cells maintained in tissue culture (Table III). This may be due to differences between cells grown in tissue culture and in organ systems, rather than between normal and neoplastic cells. Both pathways responded to inhibition with AMP and GMP (Table III).

DISCUSSION

The synthesis of PRA, the first intermediate of the purine pathway, is considered the rate-limiting step for the entire metabolic sequence (7, 2). Direct evidence for PRA synthesis and its regulation by AMP and GMP was presented by Caskey, Ashton, and Wyngaarden in their study of PRPP amidotransferase in avian liver (7). The present report on the early steps of *de novo* purine biosynthesis in human cells provides evidence that normal and neoplastic cells of extrahepatic origin are competent for the synthesis of PRA and PRG, the first two intermediates of the purine biosynthetic pathway. Scott studied glycine-^{14}C incorporation in the soluble nucleotide and nucleic acid purines in human lymphocytes and concluded that normal and leukemic human lymphocytes lack the enzyme systems which catalyze the first and the second steps of *de novo* purine synthesis (10). Lajtha and Vane, based on their studies of purine metabolism in rabbits, postulated that the bone marow was dependent for its supply of purine nucleotides on macromolecular precursors furnished by the liver (1).

In our earlier work we provided proof that the rate of *de novo* purine biosynthesis increased in spleens of mice infected with Friend leukemia virus (3, 4). This acceleration in the rate of purine production was linked to an increase in the specific activity of the enzymes which catalyze the synthesis of the first intermediates of the pathway.

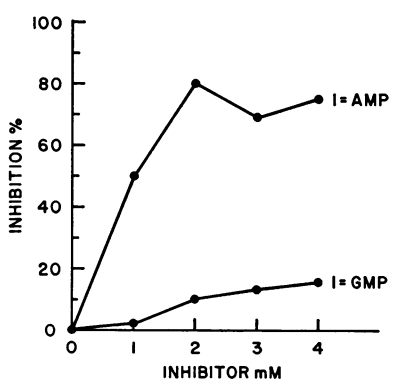


FIGURE 2 Inhibition of R-5-P aminotransferase by AMP and GMP in Burkitt lymphoma cells. Conditions of assay as described in Methods except that R-5-P concentrations were 1.0 mmole/liter for all assays.

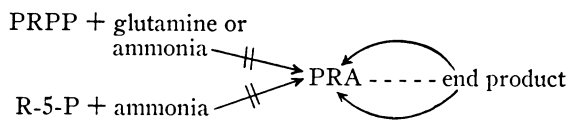
TABLE III

PRPP Amidotransferase and R-5-P Aminotransferase Activity in Human Spleen Cells

Substrates	Inhibitors: None	AMP, 2.0 mM	GMP, 2.0 mM
	*SA**	*% inhibition*	
Glutamine + PRPP	0.16	46	49
NH_3 + PRPP	0.21	69	73
NH_3 + R-5-P	0.10	42	34

* SA, specific activity: 5.7 mg of the 100,000 *g* supernatant fraction of the spleen homogenate were incubated with 75 mM Tris-HCl, pH 8.0, 0.5 mM ATP, 1.0 mM $MgCl_2$, and 10 mM glycine. Substrate concentrations were: glutamine, 10 mM; NH_4Cl, 50 mM; PRPP, 1.0 mM, R-5-P, 12.5 mM. Incubations were carried out for 10 min at 37°C.

The present study provides the first demonstration that spleen cells of a normal subject and cells from a patient with Burkitt lymphoma maintained in tissue culture possess the enzyme activities which catalyze the formation of the first two intermediates of the purine biosynthetic pathway. We found that two alternative pathways led to the synthesis of PRA in these cells and that both pathways were sensitive to regulatory control by purine ribonucleotides. Based on these findings we propose the following scheme for the regulation of PRA synthesis in human cells:



Our earlier work provided evidence that PRA synthesis was catalyzed from R-5-P and ammonia by an enzyme activity which we were able to prepare from avian liver (6). This enzyme activity was isolated and separated from PRPP amidotransferase. We found that like PRPP amidotransferase it was sensitive to inhibition of AMP and GMP, the end products of the purine pathway (6).

Our studies of the properties of PRPP amidotransferase purified from spleens of leukemic mice showed that PRPP amidotransferase catalyzed PRA synthesis from PRPP and glutamine or from PRPP and ammonia and we raised the question whether more than one PRPP amidotransferase was present in murine spleen (4, 5). This question is still unresolved. Purified PRPP amidotransferase did not support PRA synthesis from R-5-P with either glutamine or ammonia as substrate and R-5-P aminotransferase could be isolated and separated from PRPP amidotransferase (4, 6).

Work by earlier investigators led to the accepted view that PRA synthesis from PRPP and glutamine was the

first step unique to purine biosynthesis and that this step alone determined the rate of the entire metabolic sequence (7, 2). The observation that ammonia could serve as an alternative substrate for PRPP amidotransferase and that an alternative pathway for PRA synthesis from R-5-P and ammonia sensitive to feedback inhibition is operative in human cells suggests that the regulation of *de novo* purine biosynthesis is more complex than previously postulated. Two and possibly three enzyme activities catalyze PRA synthesis. The participation of ammonia for the synthesis of PRA from PRPP and from R-5-P suggests that it may have a more important role in the regulation of purine biosynthesis in man than hitherto appreciated.

Our findings support the hypothesis that the nitrogen atom 9 of the purine ring may be donated by either ammonia or glutamine since PRA contributes nitrogen atom 9 and the ribose-phosphate moiety of purine ribonucleotides. Gutman, Yu, Adler, and Javitt reported an enhanced incorporation of glycine-^{15}N into the nitrogen atoms of uric acid in gouty subjects and related it to a diminished excretion of ammonia in the urine of these patients (11). An increase in plasma glutamine and a deficiency in renal glutaminase were postulated to account for these findings. Our data are consistent with a different interpretation, namely, that an increase in the metabolic utilization of ammonia for PRA synthesis could account for the decrease in urinary ammonia excretion linked to an increased glycine-^{15}N incorporation into uric acid in these patients. Marliss, Aoki, Pozefsky, Most, and Cahill reported the daily urinary excretion of ammonia of their experimental subjects to be between 50 and 150 mmoles and they postulated that peripheral protein catabolism could serve as the ultimate source of urinary ammonia (12). It is therefore reasonable to assume that adequate concentrations of ammonia are available to the tissues for PRA synthesis.

The alternative pathway for the synthesis of PRA from R-5-P and ammonia may be of interest in disorders in which purine biosynthesis is excessive, in gout, the Lesch-Nyhan syndrome, and in myeloproliferative diseases.

The data presented in this study are consistent with the concept that extrahepatic cells are competent for the first steps of *de novo* purine biosynthesis and are not solely dependent on the liver for their supply of purines. The study of enzymes which control the rate of the early steps of purine biosynthesis in human cells may be helpful for the further investigation of the properties of these enzyme systems in cells of patients with gout, with metabolic disorders of purine biosynthesis, and in cells of patients with myeloproliferative diseases.

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