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COMPARISON OF THE PROPERTIES OF PHOSPHORIBOSYLPYROPHOSPHATE SYNTHETASE IN NORMAL AND LEUKEMIC HUMAN WHITE BLOOD CELLS*

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Abstract—Phosphoribosylpyrophosphate synthetase (PRPP synthetase) from normal human lymphocytes and granulocytes was compared with phytohemagglutinin (PHA)-stimulated lymphocytes and leukemic white blood cells of several types with respect to enzyme activity and kinetic constants. PRPP synthetase activity was determined by measuring the production of $^{14}CO_2$ in a coupled reaction with [^{14}C]orotic acid in the presence of orotidylate pyrophosphorylase and orotidylate decarboxylase. Enzyme activity was expressed both as nmoles/mg of protein/30 min and as nmoles/10^3 cells/30 min. In more than 50 per cent of the leukemic patients, activity was above normal values when compared on a per mg protein basis. However, when activity was compared on a per cell basis, only the acute myelocytic leukemic patients showed a change from the normal value, and in this case activity was inversely related to the percentage of blast cells in the peripheral circulation. Michaelis constants for ATP (K_m^{ATP}) and ribose-5-phosphate (K_m^{R5P}) were found to be 17.6 ± 1.8 and 51.3 ± 2.4 μM, respectively, in normal lymphocytes, and 55.9 ± 8.0 and 82.5 ± 1.1 μM in normal granulocytes. The K_m^{ATP} was found to decrease in all leukemic cell types, while the K_m^{R5P} showed deviation from normal values depending on the type of leukemia. The inhibition constants (K_i^{ATP} and K_i^{R5P}) were also compared in all leukemic cell types and showed deviations from normal which were cell type dependent. These results suggest that sufficient alteration of the properties of PRPP synthetase from leukemic cells exists to warrant further investigation into the therapeutic implications of alterations of the properties of this enzyme.

PRPP synthetase† catalyzes the formation of PRPP from ATP and R5P, and the PRPP formed in this reaction then serves as an essential substrate in purine, pyrimidine and pyridine synthesis [1]. PRPP is also necessary for the formation of the 5′-nucleotides of the purine antimetabolites such as 6-mercaptopurine and thioguanine which are used in cancer chemotherapy [2]. Therefore, a change in the activity or properties of PRPP synthetase could be involved in metabolic disorders and effectiveness of some chemotherapy regimens.

The enzyme has been studied extensively in *Salmonella typhimurium*, human red blood cells, and hepatoma cells with respect to both enzyme characteristics [3–7] and reaction mechanism [8–11]. Studies of the enzyme from human white blood cells, however, are less complete. Increased PRPP synthetase activity has been found in lymphocytes of patients with Lesch–Nyhan syndrome by Reem [12] and in lymphocytes of some patients with increased uric acid production by Becker *et al.* [13,14]. The present study provides evidence that enzyme activity is also altered in several types of leukemic white blood cells. In addition to these studies on enzyme activity, it has recently been reported by Yip *et al.* [10] that the form of the enzyme present in human lymphocytes is of a large molecular weight type which is not dissociated into subunits as is seen in other tissues such as human red blood cells. Garcia *et al.* [15] have found that several nucleotides play a role in the regulation of lymphocyte PRPP synthetase.

Because PRPP synthetase is necessary for the formation of PRPP and because this compound is essential to nucleic acid synthesis and activation of some antileukemic agents, a change in the enzyme may be involved in the leukemic process itself or may be important as a basis for effectiveness of treatment and for development of resistance to a particular drug regimen. It is the purpose of this paper, therefore, to compare enzyme activity and kinetic constants for ATP and R5P of normal lymphocytes and granulocytes with PHA-stimulated lymphocytes and white blood cells isolated from the peripheral circulation of patients with several types of leukemia and to determine differences between the enzymes in these cells. These differences might then be used as a basis for chemotherapy or to provide a possible explanation for the development of resistance to antileukemic drugs.

MATERIALS AND METHODS

Disodium D-ribose-5-phosphate, orotidine 5′-phosphate pyrophosphorylase and orotidine 5′-phosphate decarboxylase from yeast, bovine serum albumin, and disodium EDTA were purchased from the Sigma Chemical Co., St. Louis, MO. [^{14}C-carboxyl]Orotic

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† 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, chronic granulocytic leukemia; ALL, acute lymphocytic leukemia; AML, acute myelocytic leukemia, acute granulocytic leukemia; AMML, acute myelomonocytic leukemia; and PHA, phytohemagglutinin.

acid hydrate (41.25 and 42.4 mCi/m-mole) was purchased from New England Nuclear, Boston, MA. NCS Solubilizer was purchased from Amersham Searle, Arlington Heights, IL. Tris ultra pure was purchased from Schwarz-Mann, Orangeburg, NY. Ficoll-paque and Sephadex G200 were purchased from Pharmacia Chemical, Piscataway, NJ. Dithiothreitol was purchased from CalBiochem, San Diego, CA. The sodium salt of ATP was purchased from P–L Biochemicals, Milwaukee, WI; and 6% Dextran 70 in normal saline was purchased 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 purchased from Burroughs–Wellcome, Research Triangle Park, NC. All other chemicals were purchased from the Sigma Chemical Co.

Enzyme preparation. Peripheral blood samples were collected from normal volunteers and leukemic patients, and were drawn in lithium heparin vacuum tubes. These procedures have the approval of the Institutional Review Board for the Protection of Human Subjects at the University of Nebraska. Normal blood samples were diluted 1:1 with 0.9% sodium chloride, and the lymphocytes were separated using a Ficoll-paque gradient at 400 *g* for 40 min [16] in an International CRU 5000 centrifuge at 4°. Blood samples from lymphocytic leukemic patients were diluted 1:4 with 0.9% sodium chloride and centrifuged in a similar manner. Granulocytes were separated by the method of Boyum [16]. Five parts of whole blood were diluted with one part dextran solution and allowed to settle for 25 min at 37°. The plasma layer was removed and layered over Ficoll-paque and centrifuged as described above for lymphocytes. The pellet present after centrifugation contained the granulocytes with some red cell contamination. Red blood cells were removed from both the lymphocyte and granulocyte preparations by lysis with hypotonic sodium chloride [17]. The pellet containing either the lymphocyte or granulocyte fraction was then resuspended in saline and washed two more times, centrifuging for 30 min at 400 *g* after each washing. Smears of the final preparations were examined microscopically after fixing with Wright's Stain and were found to be approximately 98 per cent pure with minimal platelet and red blood cell contamination.

The white blood cells were then resuspended in 50 mM potassium phosphate buffer (pH 7.4) at a concentration of approximately 10×10^6 cells/ml. Cell counts were done on a model Z_{B1} Coulter Counter. (It is recognized that mononuclear cells, such as monocytes, macrophages and mononuclear stem cells, are trace contaminants of the isolated lymphocyte preparation; therefore, the use of the term lymphocyte in these experiments should be understood to include other mononuclear cells.) The suspended cells were disrupted by sonication for 120 sec at 0° using a Biosonik IV sonicator at maximum setting. The solution was then centrifuged at 100,000 *g* for 60 min at 4°. Enzyme studies were carried out using the supernatant fraction.

In order to determine if there were any substances present in the crude homogenate that might inhibit or stimulate enzyme activity, the following procedures were carried out. Samples of crude extract were dialyzed against 50mM potassium phosphate buffer and activity of the dialyzed preparation was compared with the undialyzed preparation. Both preparations showed linear kinetics, and no significant difference in activity was seen. A sample of the crude preparation was then applied to a Sephadex G200 column and eluted with 50 mM potassium phosphate buffer, pH7.4. Mixing experiments were conducted using various proportions of the active enzyme fraction which had been eluted from the column and the crude preparation. Again there was no change of specific activity from the predicted value, thereby indicating that there was no substance in the crude preparation which significantly stimulated or inhibited the activity of the PRPP synthetase. Therefore, undialyzed extract was used in subsequent procedures.

PHA transformation. The preparations which were used for PHA transformation were prepared as above and resuspended in RPMI-1640 with Hepes buffer. The cells were cultured by a method adapted from that of Hovi *et al.* [18]. The final cell suspensions were incubated in a total volume of 1 ml, each tube containing 2.5 μg PHA, 1.5×10^6 cells, 20% autologous serum, and 0.01 ml penicillin-streptomycin in RPMI-1640 with 25 mM Hepes buffer culture medium. Incubation was carried out for 72 hr at 37° in a humidified atmosphere [5% (v/v) CO_2] in air. There were approximately 70 per cent blast forms present. These cells were then resuspended in 50mM potassium phosphate buffer (pH 7.4) and treated as described above.

Enzyme storage. Enzyme preparations were stored at −20° as the cellular supernatant fraction in 50 mM potassium phosphate buffer (pH 7.4), at a concentration of approximately 10×10^6 cells/ml, 1 mg bovine serum albumin/ml and 0.1 mM dithiothreitol. Under these conditions, the enzyme retained 100 per cent activity for at least 4 months.

Enzyme activity. The assay for enzyme activity is modified from the method of Reem [12] and is based on the production of $^{14}CO_2$ from a [^{14}C]orotic acid precursor in the presence of orotate pyrophosphorylase and orotate decarboxylase (Fig. 1). The reaction itself was carried out in a glass scintillation vial. The $^{14}CO_2$ produced by the reaction was collected on a Whatman 3MM filter disk which had been saturated with NCS Solubilizer and inserted into the cap of the reaction vial. The reaction mixture contained, in a total volume of 200 μl, 150 μm ATP, 150 μm R5P, 4.0 mM $MgCl_2$, 1.0 mM EDTA, 30 mM potassium phosphate dibasic, 50 mM Tris-HCl (pH 7.4), 0.1 mM dithiothreitol, 0.125 mg/ml of bovine serum albumin, and 10–15 μg of enzyme protein. The orotate pyrophosphorylase–orotate decarboxylase-combined enzyme preparation was prepared in a solution of 50 mM potassium phosphate buffer (pH 7.4) immediately prior to use, and 0.1 units of enzyme were used in each reaction vial. The reaction was carried out at 37° in a shaker bath for 30 min. The reaction was linear for at least 45 min with 0–100 μg of protein. After 30 min, 50 μl of 60% perchloric acid was added to each reaction vial to stop the reaction and to facilitate the removal of CO_2 from the reaction mixture [19], and the vials were incubated for an additional 40 min. The filter disks were then removed from the reaction vials and the radioactivity was determined in a scintillation counter. Enzyme activity

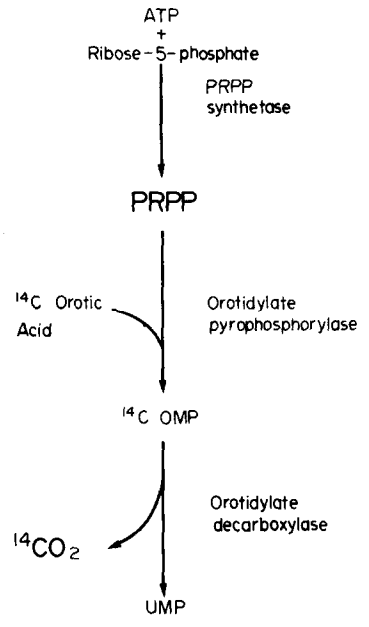

Fig. 1. Schematic diagram of PRPP synthetase assay.

is reported as nmoles of $^{14}CO_2$ produced/mg of protein/30 min and also as nmoles of $^{14}CO_2/10^3$ cells/30 min. Protein concentration was determined by the method of Lowry *et al.* [20].

Determination of kinetic constants. Nomenclature used is as defined by Cleland [21]. Given the reaction:

$$\begin{array}{ccccccc} & \text{R5P} & & \text{ATP} & & & \\ & \downarrow & & \downarrow & & \uparrow & \uparrow \\ \text{E} & & \text{E(R5P)} & & \text{E(R5P)(ATP)} \rightleftharpoons \text{E(PRPP)(AMP)} & & \text{E(PRPP)} \quad \text{E} \end{array}$$

K_m^{ATP} and K_m^{R5P} are the Michaelis constants for ATP and R5P, respectively, and express the ratio of the product of the concentrations of the transitory enzyme and substrate complexes to the ternary enzyme complex, E(R5P)(ATP), at saturating concentrations of the second substrate under steady state conditions. $K_i^{ATP} = (ATP)(E)/(E \cdot ATP)$ and $K_i^{R5P} = (R5P)(E)/(E \cdot R5P)$, and these values represent the inhibition constants. The K_i^{ATP} is of significance only under steady state conditions in which the reaction mechanism is probably random.

Double reciprocal plots of initial velocity against substrate concentration at several fixed concentrations of the second substrate were plotted using the least squares method. Kinetic constants were derived from slope and intercept replots, assuming a sequential initial velocity pattern. The substrate concentrations used permitted plotting of the data with either substrate representing the fixed substrate, facilitating calculation of both constants for each substrate. The replots were also plotted by the least squares method and K_m and K_i values were calculated from these regressions [22]. Residuals were equivalent at all concentrations and, therefore, weighted values were not used.

Statistics. Data were analyzed by a two-tailed *t*-test with significance at the 0.05 level unless otherwise indicated.

RESULTS

The optimal substrate concentration for measuring PRPP synthetase activity was determined by assaying the enzyme from normal lymphocytes and from CLL

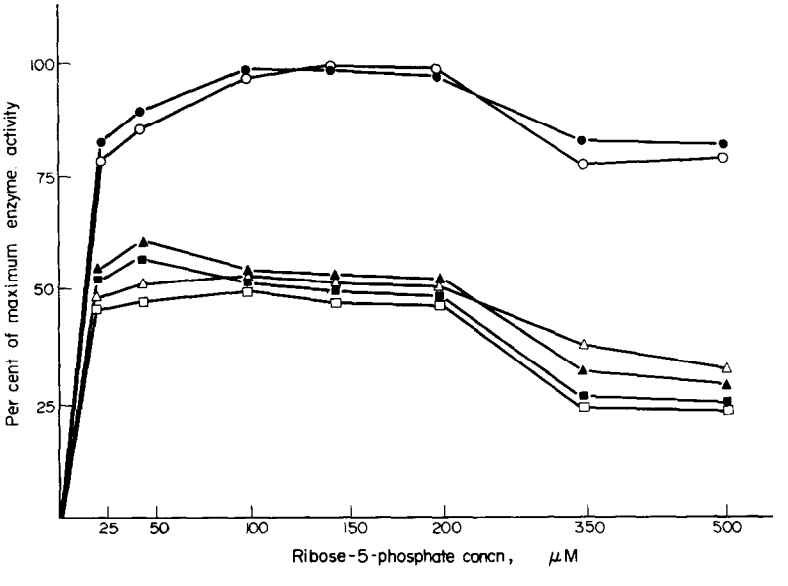

Fig. 2. Effects of substrate concentration on PRPP synthetase activity. Maximum enzyme activity is as determined under standard conditions (see Materials and Methods) at ATP and R5P concentrations of 150 μM. Values shown in this graph are calculated as percentages of the normal lymphocyte value of 36.0 nmoles/mg/30 min and a CLL value of 33.8 nmoles/mg/30 min. Open figures, CLL; closed figures, normal lymphocytes. Key: (□, ■) 30 μM ATP; (○, ●) 150 μM ATP; and (△, ▲) 500 μM ATP.

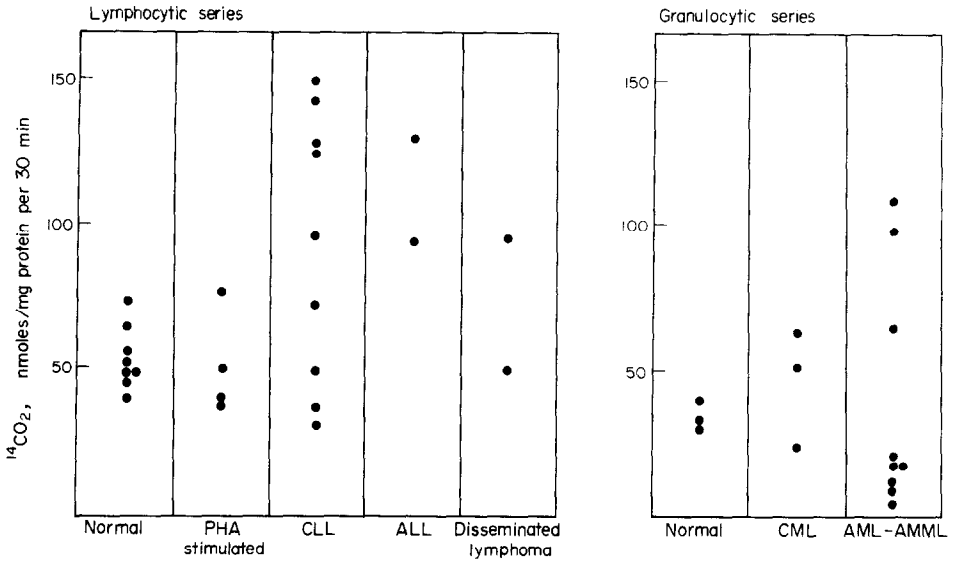

Fig. 3. Activity of PRPP synthetase in human white blood cells. Mean values for enzyme activity are expressed as nmoles/mg/30 min. Lymphocytic series: normal, 52.0 ± 3.0; PHA-stimulated, 46.1 ± 18.3; CLL, 91.8 ± 15.8; ALL, 87.0 ± 16.5; and disseminated lymphoma, 68.6 ± 13.4. Granulocytic series: normal, 38.9 ± 2.8; CML, 44.4 ± 11.5; and AML–AMML, 40.9 ± 13.4. Protein concentrations in $\mu g/10^6$ cells for the lymphocytic series were: normal, 85 ± 7; PHA-stimulated, 85 ± 9; CLL, 27 ± 4; ALL, 34 ± 6; and disseminated lymphoma, 35 ± 24. Granulocytic series: normal, 56 ± 10; CML, 56 ± 10; CML, 56 ± 15; and AML–AMML, 43 ± 7.

lymphocytes at various concentrations of ATP and R5P (Fig. 2). Similar curves were obtained with other types of leukemic cells. The maximum activity in each case was obtained when ATP and R5P were each used at a concentration of 150 μM.

Figure 3 shows the activities of PRPP synthetase in different types of normal and leukemic white blood cells. Enzyme activity from lymphocytes which had been transformed with PHA was also determined and found to be 46.1 ± 18.3 nmoles/mg/30 min; this was not significantly different from the normal value of 52.0 ± 3.0 nmoles/mg/30 min. The mean activities for the enzyme from CLL cells and ALL cells, 91.8 ± 15.8 and 87.0 ± 16.5 nmoles/mg/30 min, respectively, were significantly different from normal ($P < 0.025$). Although the mean values of the enzyme activity from ALL and CLL cells were significantly different from normal, there was such a wide range of values in each leukemic cell type that it was felt that the means did not adequately represent the data. For example, it can be seen from Fig. 3 that eight of the thirteen patients in the lymphocytic series had at least a 75 per cent increase in enzyme activity but that the remaining five patients showed no change from normal values. It was not possible to correlate enzyme activity with white blood cell count, disease stage, or type of therapy (unpublished observations).

In the granulocytic series, normal granulocytes had a mean PRPP synthetase activity of 38.9 ± 2.8 nmoles/mg/30 min, while the enzyme from CML cells had a

Table 1. Correlation of percent blast cells with PRPP synthetase activity in AML patients*

Patient	PRPP synthetase activity (nmoles/mg/30 min)	(nmoles/10^3 cells/30 min)	% Myeloblasts
R.C.	5.5	0.24	96
H.M.	1.7	0.05	93
K.B.	20.1	1.41	90
M.K.	13.1	2.44	88
V.R.	13.6	0.50	75
J.L.	4.3	0.30	52
W.W.	106.4	2.83	36
C.C.	101.2	2.50	34
J.P.	80.1	3.01	22
G.T.	63.3	4.14	3

* PRPP synthetase activity was assayed as described in Materials and Methods. Mean values ± S.E.M. for enzyme activity in patients with greater than 50 per cent blast cells are 10.8 ± nmoles/mg/30 min and 0.93 ± 0.41 nmoles/10^3 cells/30 min, and in patients with less than 50 per cent blast cells values are 95.9 ± 7.0 nmoles/mg/30 min and 2.78 + 0.1 nmoles/10^3 cells/30 min.

Table 2. Activity of PRPP synthetase in human white blood cells*

Cell type (N)	PRPP synthetase activity (nmoles/10^3 cells/30 min ± S.E.M.)
Lymphocytic series	
Normal (8)	3.6 ± 0.4
PHA-stimulated lymphocytes (3)	3.9 ± 0.7
Acute lymphocytic leukemia (2)	3.4 ± 0.3
Chronic lymphocytic leukemia (10)	3.1 ± 0.5
Disseminated lymphoma (2)	2.1 ± 1.0
Granulocytic series	
Normal (4)	2.4 ± 0.0
Acute myelocytic leukemia (4)†	3.1 ± 0.7
(6)‡	0.8 ± 0.4§
Chronic myelocytic leukemia (3)	2.2 ± 0.2

* PRPP synthetase activity was assayed as described in Materials and Methods.
† Less than 50 per cent blast cells.
‡ Greater than 50 per cent blast cells.
§ $P < 0.025$.

mean activity of 44.4 ± 11.5 nmoles/mg/30 min. AML and AMML cells had a mean activity of 40.9 ± 13.4 nmoles/mg/30 min. As with the enzyme activity patterns in the lymphocytic series, the mean values in granulocytes did not accurately reflect the data obtained. Even though the mean activities in the granulocytic leukemias did not differ from normal, six of the thirteen patients with CML, AML and AMML showed a significant increase in enzyme activity and the other seven patients showed a decrease (Fig. 3). Also, as was seen with the lymphocytic leukemias, enzyme activity could not be correlated with therapy, stage of disease, or peripheral white blood count. However, there was a correlation of activity with the percentage of blast cells in the peripheral blood (Table 1): in AML patients having over 50 per cent blast cells, enzyme activity was decreased to 9.7 ± 2.9 nmoles/mg/30 min, while in AML patients having less that 50 per cent blast cells PRPP synthetase activity was increased to 87.8 ± 9.9 nmoles/mg/30 min.

All of the above results were expressed on the basis of product formed per mg of protein. If enzyme activities were expressed on a per cell basis, a different pattern was seen, i.e. the leukemic cells were not significantly different from the normal cells (Table 2). The only exception was in the AML patients having greater than 50 per cent blast cells. In these patients enzyme activity decreased as compared to normal.

Kinetic constants. The Michaelis constants (K_m^{ATP} and K_m^{R5P}) and the inhibition constants of ATP and R5P (K_i^{ATP} and K_i^{R5P}, respectively) were calculated for each of the different types of normal and leukemic white blood cells (Table 3). The Michaelis constants were significantly different from those of normal cells in all the leukemias examined with the exception of the K_m^{R5P} of ALL and lymphoma cells. This included cells from both the lymphocytic and granulocytic series. The K_i^{ATP} and K_i^{R5P}, on the other hand, differed from the normal only in the PHA-transformed lymphocytes, CLL and lymphoma cells. The kinetic constants did not depend on previous therapy, stage of disease, or peripheral blood counts (unpublished observations), but appeared to be characteristic of a specific type of leukemia.

DISCUSSION

The activities of PRPP synthetase from lymphocytes and leukocytes obtained in the present study were comparable to the values found by Reem [12] and Yip *et al.* [10] when the differences in assay conditions were considered. The enzyme activity in isolated normal granulocytes and leukemic cells had not been deter-

Table 3. Kinetic constants for PRPP synthetase from different types of leukemic cells*

Cell type (N)	K_m^{ATP}	K_m^{R5P}	K_i^{ATP}	K_i^{R5P}
Lymphocytic series				
Normal (8)	17.6 ± 1.8	51.3 ± 2.4	28.5 ± 2.1	51.6 ± 4.5
PHA-stimulated lymphocytes (3)	10.2 ± 2.9	42.8 ± 5.6	42.3 ± 5.2†	19.5 ± 3.3‡
Acute lymphocytic leukemia (3)	3.7 ± 1.7‡	38.5 ± 17.6	21.8 ± 6.0	47.2 ± 1.8
Chronic lymphocytic leukemia (6)	4.9 ± 1.1‡	22.4 ± 6.7‡	15.3 ± 2.7	162.3 ± 13.5‡
Disseminated lymphoma (2)	8.3 ± 5.4§	69.4 ± 27.1	11.0 ± 1.5‡	165.6 ± 31.4‡
Granulocytic series				
Normal (3)	55.9 ± 8.0	82.5 ± 1.1	27.4 ± 3.5	59.9 ± 12.0
Acute granulocytic leukemia (4)	10.3 ± 5.0†	22.5 ± 11.4‡	17.6 ± 5.2	42.3 ± 2.1
Chronic granulocytic leukemia (3)	7.8 ± 2.1‡	57.5 ± 10.6†	20.2 ± 2.8	69.7 ± 6.0

* PRPP synthetase activity was assayed as described in Materials and Methods. All values are expressed as μM ± S.E.M.
† Significant difference from normal lymphocytes or granulocytes ($P < 0.025$).
‡ Significant difference from normal lymphocytes or granulocytes ($P < 0.0025$).
§ Significant difference from normal lymphocytes ($P < 0.05$).

mined previously. Also, in the present study, lymphocyte subtypes were not distinguished even though other workers have shown that some enzymes of purine metabolism are distributed primarily in either the T or B lymphocyte subtype [23].

In a majority of patients, activity of this enzyme in the lymphocytic cell series of human leukemias and in CML cells was shown to increase on a per mg of protein basis when compared to normal lymphocytes (Fig. 3). Because this increase in specific activity was not seen when results were expressed on a per cell basis (Table 2), cellular protein levels were examined. It became apparent that the increase in specific activity could be at least partially attributed to a significantly reduced ($P < 0.025$) level of intracellular protein in leukemic cells (legend of Fig. 3). These results are also consistent with an actual increase in activity of the enzyme and a concomitant decrease in the proportion of PRPP synthetase per total soluble protein. If the enzyme from leukemic cells, after purification, continues to show increased activity on a per mg basis, this would support the hypothesis that the enzyme itself is, in fact, characteristically altered in leukemic lymphocytes. This is currently under investigation.

In AML and AMML cells, however, the situation is different. Even though protein concentration per cell is not significantly different from normal cells, alterations are seen in enzyme activity both on a per cell and a per mg basis (Fig. 3 and Table 2), suggesting that the specific activity of the enzyme is actually altered. Unexpectedly, it was observed that in patients with greater than 50 per cent blast cells, the enzyme activity appeared to decrease (Table 1); however, the possibility also exists that the relative proportion of PRPP synthetase/mg of protein in the cell changes.

Extensive work has been done describing the mechanism of the reaction catalyzed by PRPP synthetase from human erythrocytes and from *S. typhimurium* [8,9]. Until recently it appeared that the reaction mechanism itself was probably an ordered bi-bi reaction with the order of substrate addition being species dependent [8,9]. Recent work indicates that R5P does bind to the free enzyme in all species examined, and ATP subsequently binds to the E·R5P complex [24]. A review of these data [8,9,24], however, suggests that the order of substrate binding is very probably random (W. W. Cleland, personal communication). We, therefore, report values of constants for ATP and R5P interaction with both the free enzyme (K_i values) and for the enzyme–substrate complexes (K_m values).

The data in Table 3 show a 2- to 5-fold decrease in K_m^{ATP} in leukemic white cells. This decrease occurred irrespective of the leukemic cell type. A less pronounced but significant decrease was seen in the product inhibition constant of ATP (K_i^{ATP}) and this appears to be somewhat more cell type specific. This decrease in the K_i and K_m for ATP could possibly be used to therapeutic advantage through selective binding of an ATP analog. Kinetic constants were not dependent on previous therapy, and several patients evaluated as much as six months apart showed no significant change in the values of kinetic constants. With respect to the R5P substrate, there is a 3-fold increase in the K_i^{R5P} seen with CLL, CML and disseminated lymphomas (Table 3) to a value which probably precludes enzyme saturation of this substance at physiologic R5P levels. This change, coupled with the previously noted ATP affinity change, could provide the basis for a synergistic therapeutic effect.

The activities and kinetic constants were also assessed in PHA-stimulated lymphocytes, and are included because they are thought to be a representative control for leukemic blast cells. In these cells, the PRPP synthetase activity and Michaelis constants for ATP and R5P were unchanged compared to normal, while the inhibition constants did show some deviation from normal values.

PRPP synthetase catalyzes the final common step in the purine and pyrimidine metabolic pathways. The activities and kinetics of this reaction, which produces a compound essential to nucleotide synthesis and formation of the active metabolites of nucleotide analogs used in chemotherapy, have been shown here to be altered in leukemic white blood cells. These alterations may provide a basis for improved chemotherapy of some leukemias.

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