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Some Properties of a Pyrimidine Phosphoribosyltransferase from Murine Leukemia Cells

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SUMMARY

A pyrimidine phosphoribosyltransferase (approximate mol wt 100,000) with a sharply defined specificity was partially purified from ascitic cells of the P388/38280 murine leukemia. This enzyme is involved in the conversion of the antineoplastic drug 5-fluorouracil (K_m = 100 μM) to pharmacologically active nucleotides. The lowest K_m value was obtained with orotic acid as substrate (K_m = 50 μM). The enzyme could also utilize 5-fluoroorotate (K_m = 85 μM) and uracil (K_m = 5 mM). Inhibition studies, using fluorouracil as substrate, indicate that this enzyme has a strong affinity for pyrimidines with a carboxyl or amino group at position 6, or a fluorine (but not a larger halogen) at position 5. A methyl group at position 5 markedly decreases affinity of the enzyme for all pyrimidines. The affinity of the enzyme for 6-carboxypyrimidines was greatly increased in the presence of dimethylsulfoxide, but the rate of the enzyme-catalyzed reaction was markedly decreased. The enzyme requires Mg^{2+} and phosphoribosyl pyrophosphate; the latter promotes stability at all temperatures. Enzyme extracted from a cell line made resistant to fluorouracil showed a decreased capacity to utilize fluorouracil as a substrate.

INTRODUCTION

Although purine phosphoribosyltransferases from mammalian sources have been studied in detail (1–7), the analogous pyrimidine-utilizing enzymes have been considered only briefly. One such enzyme (8–12) seems to be involved in the biotransformations of the antineoplastic drug 5-fluorouracil. This enzyme can apparently utilize fluorouracil, uracil, or orotic acid as substrate, requires phosphoribosyl pyrophosphate and Mg^{2+}, and catalyzes the formation of pyrimidine 5′-monophosphate.

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We have reported that this enzyme can account for nucleotide formation from fluorouracil in a murine cell line (P388/38280) which lacks uridine phosphorylase (11). In the present report we have partially characterized the phosphoribosyltransferase from this cell line. Studies were carried out to delineate the rather unusual substrate specificity of the enzyme, kinetic constants, and factors affecting enzyme activity. The phosphoribosyltransferase from a fluorouracil-resistant cell line was also examined.

MATERIALS AND METHODS

Substrates. Both 5-fluorouracil and 5-fluoroorotate, labeled with ^{14}C in position 2 of the ring, were purchased from New England Nuclear Corporation; uracil, similarly labeled, was obtained from Calbiochem. These substrates were purified by ascending

thin-layer chromatography on cellulose sheets, using 77% 1-butanol–13% water–10% concentrated formic acid. The ultraviolet-absorbing material at R_F 0.4–0.6, identified with appropriate markers, was recovered and then diluted with water to 10 mM (3–5 mCi/mmole). The sodium salt of PRPP[2] and the other pyrimidines were purchased from Sigma Chemical Company. A later assay of PRPP from this supplier indicated an approximate purity of 50%. Its further purification as described by Flaks (13) did not alter the results shown here, nor did the use of the sodium salt of PRPP prepared from dimagnesium PRPP (Calbiochem) as described by Reyes (10). The principal impurity in the Sigma PRPP preparation was said to be the 3,5-cyclic phosphate of ribose 1-pyrophosphate. We have not examined the properties of this material in the present system. 2-Ethylmercapto-5-fluoroorotic aldehyde and 6-(2-thio-4-oxy-6-pyrimidylmethylidine)-5′-oxo-2′-penyloxazoline were generously provided by Dr. J. Logan Irvin, University of North Carolina.

Cell lines. CFD_1 male mice were inoculated with 1×10^6 P388/38280 cells, and the ascitic tumor was collected 7 days later as described previously (11). Drug-resistant cells were obtained by treating tumor-bearing animals with 25 mg/kg of 5-fluorouracil from day 1 to day 10 after transplant. After 50 transplant generations, a fluorouracil-resistant line was obtained. The drug-resistant line was maintained in animals which received 25 mg/kg of fluorouracil on days 1 and 2 after transplant.

Enzyme assays. The assay for phosphoribosyltransferase activity was described previously (11); it involves the use of DEAE-impregnated cellulose discs to separate pyrimidine 5′-monophosphate from the pyrimidine substrates. The latter are readily washed from the disc in 0.01 M citric acid. This assay is suitable for use with either uracil (final level, 5–10 mM) or fluorouracil (final level, 0.5–1 mM) as substrate. Since both orotic and fluoroorotic acids are strongly bound by DEAE-cellulose, a different assay was used with these substrates. A 25-μl volume contained 1 mM PRPP, 1 mM $MgCl_2$, 0.5 mM 2-mercaptoethanol, 100 mM buffer (generally glycine at pH 9), enzyme, and 500 μM substrate. The reaction was terminated, usually after 10 min at 37°, by the addition of 5 μl of 0.5% phosphotungstic acid in 0.1 M HCl. The precipitate was removed by centrifugation, and a 15-μl portion of the supernatant fluid was applied near one end of a 2 × 20 cm strip of Whatman No. 1 chromatography paper. The strip was developed overnight in the solvent system described above. Markers were used to identify OMP (which remained at the origin) and unreacted substrate, which migrated down the strip. In both assay systems, blanks were run either by omitting enzyme or by terminating the reaction immediately after adding enzyme. Radioactivity was measured by liquid scintillation counting.

[2] The abbreviations used are: PRPP, phosphoribosyl pyrophosphate; FUMP, 5-fluorouridine 5′-monophosphate; DMSO, dimethylsulfoxide.

RESULTS

Partial enzyme purification. Ten-gram batches of tumor cells (wet weight) were suspended in 20 ml of buffer (50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 6.8, and 50 mM glycine). The suspension was frozen in a −70° bath, then thawed at 25°. This process was repeated, and the suspension was centrifuged at 100,000 × *g* for 60 min. The supernatant fluid was treated with $MnCl_2$ (final level, 50 mM) to remove nucleic acids. The precipitate was removed by centrifugation, and the solution was brought to 35% saturation with ammonium sulfate. This precipitate was discarded, and the ammonium sulfate concentration was then raised to 60%. The resulting precipitate was retained, dissolved in a minimal volume of 0.1 M glycine buffer at pH 7.5, and dialyzed against two changes of the same buffer. The addition of 1 mM PRPP promoted enzyme stability, as discussed below. The resulting preparation was stable to storage at −20°. The murine leukemia enzyme could be subjected to further steps in the procedure of Kasbekar *et al.* (8), but difficulty in obtaining large quantities of tumor cells, together with large losses in total activity during further

purification steps, limited our studies along these lines.

The methanol step involved treatment of 10 ml of the dialyzed enzyme from the ammonium sulfate procedure with 2 ml of methanol. This operation was carried out at −5°. After 10 min, the precipitate was collected by centrifugation and discarded. The supernatant fluid was mixed with 1.2 ml of 0.08 M sodium acetate buffer (pH 4.0) in 16.5% methanol. The resulting precipitate was removed by centrifugation, dissolved in 2–3 ml of 0.1 M glycine buffer, pH 7.5, and dialyzed against the same buffer containing 1 mM PRPP.

Chromatography was carried out on a 1 × 5 cm column of DEAE-cellulose equilibrated with 0.03 M glycine buffer, pH 7.6. The enzyme was eluted with a linear gradient beginning with 0.03 M glycine, pH 7.6, and ending with 0.3 M glycine, pH 7.0. Fractions containing enzyme activity were pooled and concentrated by repeated dialysis against 0.1 M glycine buffer containing 1 mM PRPP and 30% polyethylene glycol (mol wt 6000) and finally by dialysis against glycine buffer plus 1 mM PRPP.

Purification data are summarized in Table 1.

Reaction products. With fluorouracil as substrate, the reaction required PRPP and Mg^{2+}. The purified enzyme preparations contained sufficient magnesium so that the reaction was decreased to only 75% of control values by omission of $MgCl_2$. Addition of 2 mM EDTA inhibited the reaction, however. Optimal utilization of fluorouracil at saturating levels ($5 \times K_m$) occurred with the addition of 1 mM PRPP and 1 mM $MgCl_2$. The product, FUMP, was characterized as described by Reyes (10) and Dahl *et al.* (14). With orotate as substrate, the product was identified by thin-layer chromatography as OMP plus UMP. With uracil as substrate, only UMP was formed. Solvent systems are described by Berlin (4) and Reyes (10). The preparations of phosphoribosyltransferase used here were contaminated with OMP decarboxylase, as shown by catalysis of the conversion of OMP to UMP. Any OMP thus transformed was also detected in our assay for enzyme activity, since either nucleotide remains at the origin of the paper chromatographic system used, and both nucleotides are therefore measured. The magnesium requirement of the reaction has been documented (10). In the present study, we found the apparent K_m for PRPP to be 100 μM with fluorouracil as substrate.

Enzyme stability. The enzyme, purified through the ammonium sulfate step, was protected against inactivation by 1 mM PRPP. After 18 hr at 0°, optimal stability was found if the enzyme had been main-

TABLE 1

Enzyme purification

Cell extracts were treated as described in the text. One unit of activity is defined as the conversion of 1 μmole of fluorouracil to FUMP per hour.

Fraction	Total protein	Total activity	Specific activity
	mg	*units*	*units/g protein*
Cell extract	600	4.5	7.5
$MnCl_2$	580	4.5	7.7
Ammonium sulfate	180	4.0	20
Methanol	60	2.1	35
DEAE-cellulose	6	1.1	180

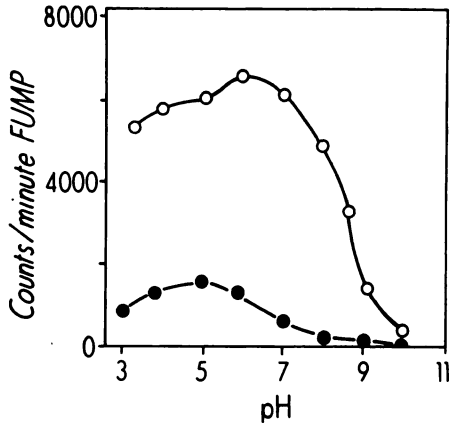

FIG. 1. *Phosphoribosyltransferase activity after storage overnight at 0° at pH 3–11*

Ordinate, FUMP formed during a standard incubation (counts per minute); abscissa, pH of storage. The assay was carried out at pH 9 in glycine buffer. ○, 2 mM PRPP present; ●, PRPP absent during storage at 0°.

tained at pH 6.8 with PRPP present. Without PRPP, considerable inactivation occurred, and the pH for optimal storage was shifted to 5.5 (Fig. 1). Addition of 1 mM PRPP strongly promoted stability at both 50° and 60° (Fig. 2). Enzyme stability was not enhanced by the addition of $MgCl_2$, orotate, uracil, fluoroorotate, or any combination of these substances, at 1–10 mM. Addition of 1 mM $MgCl_2$ and 1 mM PRPP afforded less protection at 60° than did PRPP alone. However, the combination of 1 mM PRPP and 2 mM EDTA was also less effective than was PRPP alone. Endogenous levels of magnesium ion apparently contribute to the protective effect of PRPP. These data are summarized in Table 2.

Molecular weight. A 2.5 × 45 cm column of Sephadex G-150 was used, together with markers of blue dextran, bovine γ-globulin, ovalbumin, cytochrome *c*, and tritiated water. The column was equilibrated with 0.1 M glycine buffer, pH 7.2, containing 1 mM PRPP; this mixture was used to elute the enzyme. In the absence of PRPP, no activity survived the treatment. Phosphoribosyltransferase activity (substrate, fluorouracil) was eluted in the region corresponding to a molecular weight of 100,000 (Fig. 3). This result may have been influenced by the possible aggregation of the enzyme with orotidylate decarboxylase, producing a complex of higher molecular weight than either enzyme alone (15). In addition, the presence of the substrate PRPP may have changed the enzyme configuration and thus altered the apparent molecular weight as measured on a Sephadex column.

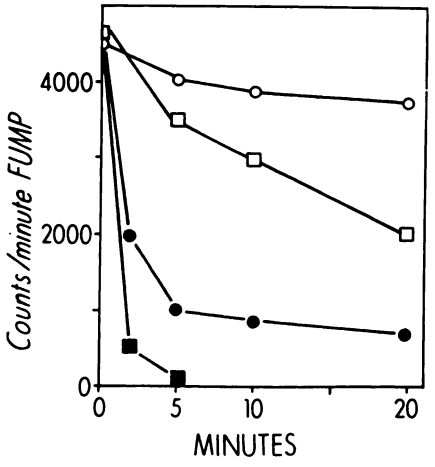

FIG. 2. *Heat inactivation of enzyme at 50° or 60°*
○, 50°, PRPP present; ●, 50°, PRPP absent; □, 60°, PRPP present; ■, 60°, PRPP absent. After heating for the specified interval, the enzyme was chilled. Activity was measured at 37° in the standard assay at pH 9 in glycine buffer.

TABLE 2
Effect of magnesium and PRPP on enzyme stability at 60°

These assays were carried out by heating the enzyme in 0.1 M glycine, pH 7, with specified levels of $MgCl_2$, EDTA, and PRPP for 10 min at 60°. The mixture was chilled and then brought to 1 mM PRPP, 1 mM $MgCl_2$, and 1 mM fluorouracil. Excess $MgCl_2$ was added to compensate for EDTA, if present. Activity was measured at pH 9 and compared with an unheated control.

Additions	Enzyme activity
	%
None	0
1 mM PRPP	75
1 mM PRPP + 1 mM $MgCl_2$	50
1 mM PRPP + 2 mM EDTA	40
2 mM EDTA	0

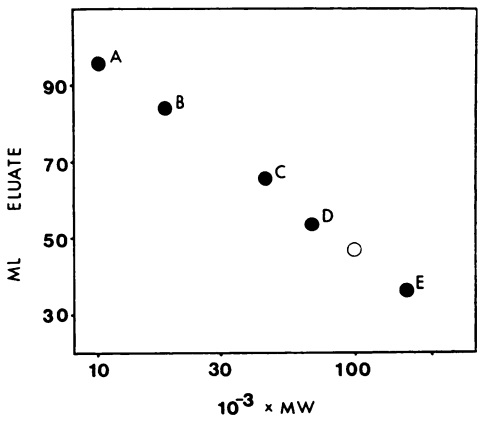

FIG. 3. *Relationship between molecular weight and elution volume from Sephadex G-150*
●, standards: *A*, cytochrome *c*; *B*, myoglobin; *C*, ovalbumin; *D*, bovine serum albumin; *E*, γ-globulin. ○, enzyme activity.

pH optima. With fluorouracil as substrate, optimal activity was found at pH 9.3 (Fig. 4). The optimum with uracil as substrate was pH 10.5 (Fig. 5). With orotic acid as sub-

strate (Fig. 6), a complex optimum was found which was dependent on the particular buffer employed. Glycine appeared to promote enzyme activity at high pH values, a finding also reported with a purine phosphoribosyltransferase (6) when the substrate was guanine. To test the entire pH range, a composite buffer composed of equimolar portions of glycine, histidine, and Tris was used.

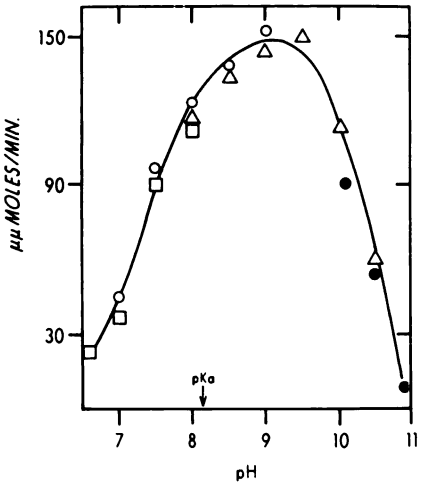

FIG. 4. *pH optimum of phosphoribosyltransferase with fluorouracil as substrate*

Ordinate, rate of formation of FUMP. Buffers: □, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; ○, Tris; △, glycine; ●, lysine. The pK_a of fluorouracil is shown.

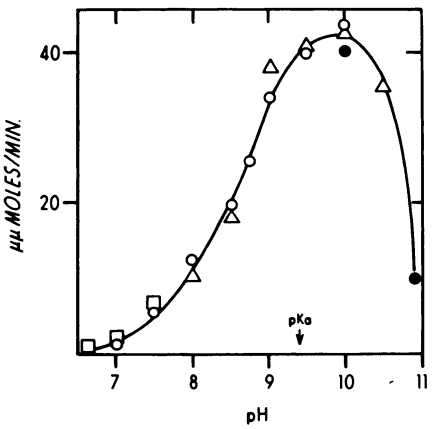

FIG. 5. *pH optimum of phosphoribosyltransferase with uracil as substrate*

See the legend to Fig. 4 for details.

Activation energy. The initial rate of the reaction with fluorouracil as substrate varied with the incubation temperature. From these data (Fig. 7) an activation energy of 11,200 cal/mole was determined.

Kinetic constants. The apparent K_m for fluorouracil at pH 9 in glycine buffer was 100 μM; in equimolar glycine–histidine–Tris buffer at pH 6.3, a K_m value of 560 μM was obtained. At pH 9 in glycine buffer, apparent

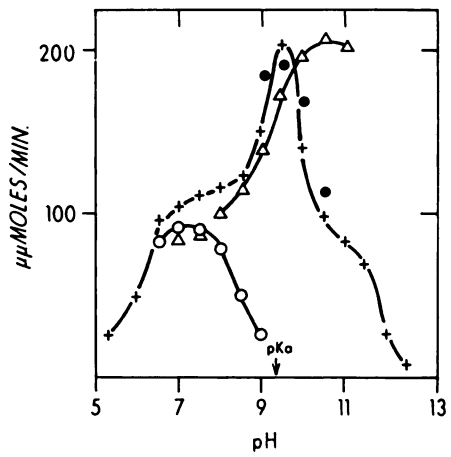

FIG. 6. *pH optimum of phosphoribosyltransferase with orotic acid as substrate*

+, equimolar glycine–histidine–Tris buffer. For other details, see the legend to Fig. 4.

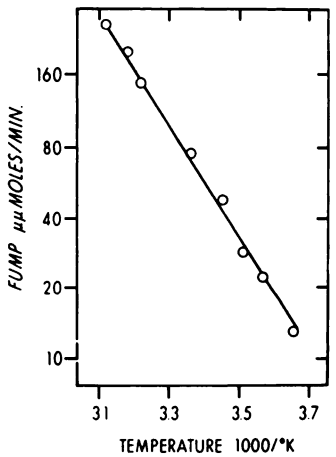

FIG. 7. *Effect of temperature of incubation on phosphoribosyltransferase activity with fluorouracil as substrate*

A value of ΔH = 11,200 cal/mole was calculated.

K_m values of 50 μM for orotic acid, 80 μM for fluoroorotate, and 5 mM for uracil were obtained. Studies on the V_{max} of the reaction at pH 9 with the four substrates yielded the following values (units are micromoles of product formed per milligram of protein per minute): fluorouracil, 300; uracil, 70; orotate, 400; fluoroorotate, 600.

Inhibition studies. A group of substituted pyrimidines were found to be competitive inhibitors of the enzymatic conversion of fluorouracil to FUMP. Plots of velocity vs. velocity/substrate concentration were used throughout. The fluorouracil concentration was varied from 10 μM to 1 mM. The most potent such inhibitor was orotic acid (K_i = 50 μM). Fluoroorotate was almost as effective (K_i = 85 mM), but uracil was much less effective (K_i = 5.0 mM). The K_i values agree closely with the apparent K_m values for these compounds. It is not known whether other substituted pyrimidines can serve as substrates for the enzyme. Of the competitive inhibitors listed in Table 3, the most effective were 6-aminouracil, 5-aminoorotate, and 5-nitroorotate. Introduction of a 5-methyl group strongly diminished inhibitory capacity, as did a large halogen at position 5. Other findings are discussed below.

TABLE 3

Competitive inhibitors of pyrimidine phosphoribosyltransferase

The substrate was fluorouracil in 0.1 M glycine buffer, pH 9. Initial rates of FUMP formation were measured at 1, 2, and 5 min. pK_a values are from refs. 16 and 17.

Competitor		K_i	pK_a
		μM	
2,4-Diketopyrimidines			
Position 5	*Position 6*		
H	COOH	50	9.45
F	COOH	85	
NO_2	COOH	300	
NH_2	COOH	1,300	
CH_3	COOH	10,000	7.93
H	NH_2	500	
F	H	100	8.15
NH_2	H	850	
NO_2	H	2,300	5.56
H	H	5,000	9.45
COOH	H	8,000	
N_2^+	H	10,000	
F_3C	H	10,000	
CH_3	H	>10,000	
Br	H	>10,000	
Other pyrimidines			
L-Dihydroorotate		800	
D-Dihydroorotate		3,300	
OMP		100	
UMP		2,500	
2-ethylmercapto-5-fluoro-orotic aldehyde		2,000	
6-(2-Thio-4-oxy-6-pyrimidylmethylidine)-5'-oxo-2'-phenyloxazoline		5,000	
2-Hydroxyprimidine		8,000	
2-Thiouracil		>10,000	
5-Azauracil		4,000	6.73
6-Azauracil		5,000	7.00

TABLE 4

Effect of DMSO on inhibition of phosphoribosyltransferase activity

These assays were carried out with 8% DMSO present as specified. The level of inhibitor required to inhibit the formation of FUMP by 50%, under standard conditions, is shown. The level of inhibitor needed to cause 50% inhibition in the absence of DMSO was also determined, and the ratio of the two values was calculated.

Inhibitor	I_{50} with DMSO present	$\frac{I_{50}\text{ (DMSO absent)}}{I_{50}\text{(DMSO present)}}$
	μM	
Orotic acid	3	17
5-Fluoroorotate	6	14
OMP	25	4
5-Aminoorotate	65	20
5-Nitroorotate	150	2
5-Fluorouracil	50	1
6-Aminouracil	500	1
5-Aminouracil	850	1
Uracil	5000	1

Effect of dimethylsulfoxide. The addition of DMSO (8%, v/v) to incubation mixtures decreased the rate of conversion of orotic acid to OMP 10-fold. With fluorouracil or uracil as substrate, the reaction rate was unaffected by this DMSO level. Increasing the DMSO concentration to 30% essentially abolished conversion of orotate to OMP by the phosphoribosyltransferase, but only

slowed the conversion of fluorouracil to FUMP by 20%. These data suggested that DMSO might also decrease the affinity of the enzyme for orotate. Orotate, however, was a much more potent inhibitor of the enzymatic conversion of fluorouracil to FUMP with DMSO present. The data (Table 4) show the concentration of 6-carboxypyrimidines required to inhibit fluorouracil conversion to FUMP by 50% in the presence and absence of DMSO.

Phosphoribosyltransferase activity in beef thymus. We carried out the partial purification of a pyrimidine phosphoribosyltransferase preparation from beef thymus as described by Kasbekar *et al.* (8). At 600-fold purification, the enzyme could utilize orotate, fluoroorotate, fluorouracil, or uracil as substrate. The respective K_m values were 50 μM, 75 μM, 80 μM, and 4 mM. These studies were carried out in 0.1 M glycine buffer, pH 9, as described above.

Phosphoribosyltransferase activity in a fluorouracil-resistant cell line. We compared the rates of conversion of orotate and fluorouracil to nucleoside 5′-monophasphates, using homogenates of P388/38280 cells and the drug-resistant subline P388/38280/FU. The drug-resistant line had somewhat impaired levels of enzyme activity toward orotate; the analogous conversion of fluorouracil was decreased 10-fold (Table 5). In other studies we found that the apparent K_m for orotate was 60 μM, using enzyme derived from the P388/38280/FU line, and the K_m for fluorouracil was 550 μM. These determinations were carried out at pH 9 in 0.1 M glycine buffer, using enzyme purified through the ammonium sulfate step.

TABLE 5

Rate of FUMP formation by P388/38280 and P388/38280/FU cells

Cell homogenates were incubated in the standard assay mixture with 1 mM radioactive fluorouracil or 0.5 mM orotate. The data represent the mean values of five determinations; the range was ±15%.

Cell line	FUMP formed	OMP formed
	μmoles/g protein/hr	
P388/38280	7.5	22.0
P388/38280/FU	0.8	12.5

DISCUSSION

This report describes some of the properties of a pyrimidine phosphoribosyltransferase partially purified from P388/38280 cells. This cell line lacks uridine phosphorylase, so that the only route for FUMP formation involves phosphoribosyltransferase activity (11). Our studies on substrate specificity initially showed that the enzyme could utilize orotate (K_m = 50 μM), fluoroorotate (K_m = 80 μM) fluorouracil (K_m = 100 μM), or uracil (K_m = 5 mM) as substrate. The low apparent K_m value found for orotic acid suggests that the enzyme under study is the orotidine 5′-monophosphate phosphoribosyltransferase (EC 2.4.2.10) described by Lieberman, Kronberg, and Simms (18). The observed K_m value for PRPP was 100 μM.

The suggestion of a rather unusual substrate specificity prompted a study of determinants of enzyme-substrate affinity. For this purpose, fluorouracil was used as substrate, and K_i values were measured for other substituted pyrimidines. The K_i values obtained with orotate, fluoroorotate, and uracil (Table 2) agree closely with the apparent K_m values obtained with these compounds as substrates. These data suggest that a single enzyme is capable of using any of these four pyrimidines as a substrate. Addition of a methyl group at position 5 markedly decreased competitive inhibition. In the case of 5-methylorotate, the decrease was more than two orders of magnitude. Substitution of the electronegative fluorine atom at position 5 strongly increased enzyme-pyrimidine affinity, but the larger bromine atom had no such effect. Furthermore, substitution at position 5 with the more strongly electronegative trifluoromethyl group, which lowers the pK_a of the pyrimidine to 5.7,[3] yielded an inactive compound. Substitution of nitro or amino groups at position 5 lowered, but did not abolish, the affinity of the enzyme for pyrimidines such as 5-nitroorotate. The hydrophobic nature of the 5-methyl group may make 5-methylpyrimidines unsuitable substrates for the

[3] pK_a values are found in refs. 19 and 20.

phosphoribosyltransferase. Steric factors could be responsible for the apparent poor affinity of 5-bromouracil and 5-trifluoromethyluracil for the enzyme.

Studies carried out in the presence of DMSO showed that the addition of this compound has two demonstrable effects. The rate of conversion of 6-carboxypyrimidines (orotate, fluoroorotate) to nucleoside 5′-monophosphates is decreased, but the ability of these compounds to inhibit enzyme is promoted. These findings suggest that substrate (or product) affinity of 6-carboxypyrimidines for the phosphoribosyltransferase is so strongly increased in the presence of DMSO that the reaction rate is decreased. The effect of DMSO on pyrimidines unsubstituted at position 6 was negligible; 5-nitroorotic acid was affected to an intermediate extent.

Two pyrimidines which inhibit the conversion of orotic acid to OMP by intact Ehrlich ascites tumor cells, 2-ethylmercapto-5-fluoroorotic aldehyde and 6-(2-thio-4-oxy-6-pyrimidylmethylidine) - 5′ - oxo - 2′ - phenyloxazoline (21), were poor inhibitors of the analogous reaction with fluorouracil. Perhaps biotransformation of these compounds by intact cells promotes their inhibitory ability. Alternatively, these compounds may be more effective as inhibitors of the conversion of orotate to OMP. Our studies on the phosphoribosyltransferase with orotate as substrate will be described later.

There is considerable variation in reports on the substrate specificity of pyrimidine phosphoribosyltransferases. In bacterial systems two enzymes have been described (22), one utilizing orotate and fluoroorotate as substrates, the other utilizing uracil and fluorouracil. The structural specificity of the former enzyme has been extensively analyzed (16). A yeast enzyme (14) utilizes orotate and fluoroorotate, but not fluorouracil; 5-nitroorotate is not an inhibitor. An enzyme from beef erythrocytes utilizes orotate or uracil (12) as substrate; the highly purified phosphoribosyltransferase from calf thymus utilizes orotate or fluorouracil as substrate (8). We have confirmed the suitability of either compound as substrate for the last-mentioned enzyme, using a 600 fold-purified preparation. An enzyme isolated from murine leukemia P1534J (10) could apparently utilize fluorouracil, uracil, or orotate as substrate. The high pH optimum of the reaction, with uracil as substrate, together with the low V_{max} and high K_m values, may be responsible for the failure of other investigators to identify a uracil-utilizing phosphoribosyltransferase in mammalian cell extracts (e.g., ref. 17).

There is no present evidence for multiple forms of pyrimidine phosphoribosyltranferases in mammalian cell extracts. Kinetic constants which we have obtained suggest that a single such enzyme is present.

Data on pH optima have suggested to other investigators that pyrimidines are utilized as substrates in their anionic form, since optimal values of pH were always above the corresponding pK_a values. The pH optimum with orotate as substrate was complex (Fig. 6), a finding also reported previously (8). There is some evidence that glycine stabilizes the enzyme at high pH.

This pyrimidine phosphoribosyltransferase was stabilized by PRPP at all temperatures. The addition of Mg^{2+} antagonized this stabilization, as did EDTA. Apparently the endogenous magnesium in enzyme preparations is sufficient to permit maximum stabilization by PRPP. PRPP was also found to protect purine phosphoribosyltransferases against inactivation by sulfhydryl reagents (2). With the aid of PRPP, we could stabilize the pyrimidine transferase sufficiently to permit determination of its molecular weight on Sephadex G-150. The value of 100,000 obtained is higher than the 60,000 (3) and 45,000 (4) reported for purine transferases or the value of 62,000 reported for a pyrimidine phosphoribosyltransferase from pea cotyledons (23). The present data may reflect the molecular weight of a transferase–orotidylate decarboxylase complex (15) or the effect of PRPP on the enzyme configuration. The activation energy of the pyrimidine transferase reported here (11,200 cal/mole) agrees closely with the value of 12,600 cal/mole reported for a purine phosphoribosyltransferase (1).

The data of Table 4, together with K_m measurements reported above, show that phosphoribosyltransferase from a fluorouracil-resistant subline of P388/38280 had a

reduced affinity for fluorouracil. Since this enzyme is presumably involved in an important metabolic process, the conversion of orotate to OMP, it would clearly be advantageous to a fluorouracil-resistant cell to diminish enzymatic activity toward fluorouracil while retaining the capacity to form OMP. The conversion of exogenous cytidine derivatives to uracil nucleotides via a deamination process could provide an additional route of pyrimidine synthesis in drug-resistant cells with a defective OMP phosphoribosyltransferase.

We conclude that the enzyme studied here is clearly capable of catalyzing the formation of FUMP from fluorouracil and has indeed been implicated as a major determinant of responsiveness to this drug (24) in murine leukemias. Considerations of structural specificity indicate that the substitution of an electronegative fluorine atom at position 5 of the uracil molecule serves to promote enzyme-pyrimidine affinity. Substitution of bulkier or more hydrophobic atoms impedes such affinity. The finding that DMSO strongly promotes enzyme affinity for 6-carboxypyrimidines, but not for fluorouracil, suggests that this enzyme may associate with the former compounds somewhat differently than with the latter. The finding that a fluorouracil-resistant cell line contains pyrimidine phosphoribosyltransferase with impaired affinity for fluorouracil but not for orotate provides additional evidence that orotate and fluorouracil may interact differently with the active site of the enzyme. Alterations in the enzyme, apparently under genetic control, can alter substrate specificity.

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