Chinese Hamster Hypoxanthine-Guanine Phosphoribosyltransferase

PURIFICATION, STRUCTURAL, AND CATALYTIC PROPERTIES*

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SUMMARY

Hypoxanthine-guanine phosphoribosyltransferase from Chinese hamster brain, liver, and V79 tissue culture cells appears to have identical structural and catalytic properties. The enzyme has been purified 540-fold to apparent homogeneity from Chinese hamster brain. The native molecular weight is 78,000 to 85,000 determined by Sephadex G-100 column chromatography and acrylamide gel electrophoresis. The enzyme appears to consist of three subunits of molecular weight 25,000 determined by sodium dodecyl sulfate acrylamide gel electrophoresis. Electrophoresis and acrylamide gel electrophoresis demonstrate the presence of at least three isozymes. The enzyme is remarkably stable at 85° if first incubated in 1 mM 5-phosphoribosyl 1-pyrophosphate. The enzyme is active from pH 5.5 to 11 with maximum activity at pH 10. The enzyme displays Michaelis-Menten kinetics with apparent Michaelis constants for hypoxanthine, guanine, and phosphoribosylpyrophosphate of 0.52, 1.1, and 5.3 μM, respectively.

Purine nucleotides are formed either by a multistep de novo synthetic pathway, or by salvage enzymes which enable the utilization of preformed purine bases. Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) is a salvage enzyme responsible for the conversion of the purine bases hypoxanthine or guanine to the corresponding 5'-ribonucleotides IMP or GMP.

Hypoxanthine or

Guamine + PRPP + Mg²⁺ → IMP or + PP₁ GMP

This reaction provides purine ribonucleotides at a much lower energy expenditure than the de novo synthetic pathway. Although tissue culture cells can survive on either de novo or salvage pathways alone, whole animals apparently require both. Even in Lesch-Nyhan syndrome patients who show marked deficiency of hypoxanthine-guanine phosphoribosyltransferase, low enzyme activity remains (1-3). Total deletion of this salvage enzyme would probably be lethal since some cells are incapable of de novo purine synthesis (4-6).

The dependence upon salvage pathways of leukocytes forms the rationale for chemotherapeutic treatment of leukemia patients (7). Purine base analogs are administered to kill preferentially actively dividing cells that have an absolute requirement for preformed purines. Treatment is often initially successful, but after a time the leukemic cells often appear resistant to the drug(s). It would be valuable to know whether altered forms of hypoxanthine-guanine phosphoribosyltransferase could account for this resistance. Also, a better understanding of the mechanisms of purine regulation might suggest improved methods of cancer chemotherapy.

Since it is difficult to study human patients, we have chosen to investigate mechanisms of drug resistance and purine regulation in tissue culture cells. Chinese hamster cells offer numerous advantages over human cells for genetic and regulatory studies (8). Methods for selecting drug-resistant variants are well documented, and we have produced phenotypically different variants in many different ways. Hypoxanthine-guanine phosphoribosyltransferase activity in drug-resistant variants ranges from total absence to normal levels. An analysis of these variants requires knowledge of the properties and characteristics of the enzyme. In this paper, we present the purification procedure for a homogeneous enzyme from Chinese hamster brain and a comparison of the physical and biochemical properties of the brain enzyme with the enzyme prepared from liver and tissue culture cells.

EXPERIMENTAL PROCEDURE

Enzyme Assay

The formation of IMP was measured in a radioisotope assay. A standard assay mixture contained 50 mM Tris-HCl (pH 7.8), 5 to 9 mM MgCl₂, 2 mM dithiothreitol, 1 mM PRPP (tetrasodium salt, Sigma), 0.06 mM hypoxanthine, 8-1H-hypoxanthine (Schwarz, 25.3 Ci per mmole) to give 30,000 to 300,000 cpm per assay, and enzyme sample in a volume of 100 μl. In cases where indicated, bovine serum albumin at final concentrations of 0.1 or 1.0 mg per ml was included in the assay to improve the stability of the more purified enzyme fractions. The reaction mixtures were incubated for 15 min at 37° and the incubation was terminated by adding 150
μl of cold 50 mM Tris-HCl, pH 8, and placing the mixture on ice. A 200-μl aliquot was removed and added to a Pasteur pipette column (3.5 × 0.5 cm) of DEAE-cellulose (Bio-Rad Cellex D), capacity 0.6 meq per g) equilibrated with 50 mM Tris-HCl, pH 8, and washed with 5 ml of the same buffer to remove unretracted hypoxanthine. The nucleotide was eluted with 1.5 ml of 1 M KCl and counted in 10 ml of scintillation fluid (0.1 g of New England Nuclear Omnifluor in 2000 ml of toluene and 1170 ml Triton X-100) in a Packard Tri-Carb with an efficiency of 30%. A low background was achieved by removing from the labeled hypoxanthine contaminants which bind to DEAE-cellulose. The [3H]hypoxanthine was added to a Cellex D column and eluted with buffer as described above. About 80% of the radioisotope was recovered. The solution was quickly cooled, then centrifuged for 30 min. The solution was quickly cooled, then centrifuged for 30 min. The supernatant was brought to 40% saturation in ammonium sulfate by the addition of a 0.2 volume of 1 M sodium acetate, pH 4.5. After 20 min, the precipitate was removed by centrifugation and the supernatant was brought to 70% saturation by the addition of 1 ml of cold 50 mM Tris-HCl and placing the mixture on ice. After 20 min, the precipitate was removed by centrifugation at 20,000 × g for 15 min, and the supernatant was adjusted to pH 7.8 by the addition of a 0.2 volume of 2 M Tris-HCl, pH 8.0.

65°C Supernatant—The pH 5.4 supernatant was added. 0.01 volume of 100 mM TRP, the solution was heated at 65°C for 10 min, quickly chilled, and centrifuged at 20,000 × g for 15 min.

40 to 70% Ammonium Sulfate Fraction—The 65°C supernatant was brought to 30% saturation in ammonium sulfate and the addition of a 0.67 volume of saturated ammonium sulfate solution. After 20 min, the precipitate was removed by centrifugation and the supernatant was brought to 70% saturation by the addition of 1 volume of saturated ammonium sulfate. After 20 min, the suspension was centrifuged and the supernatant was discarded. The precipitate was dissolved in a small volume of enzyme buffer (20 mM sodium acetate, pH 4.5, 20 mM KCl, 0.1 mM EDTA, and 2 mM dithiothreitol) containing 0.1 mM PRPP. DEAE-Sepharose Fraction—The ammonium sulfate fraction was dialyzed against enzyme buffer and applied to a column (0.0 × 20 cm) filled with DEAE-Sepharose A-50 equilibrated with enzyme buffer. The column was washed with 40 ml of enzyme buffer and the enzyme was eluted with a KCl gradient (20 to 200 mM) in enzyme buffer. Fractions of 2 ml were collected and assayed for enzyme activity. Peak fractions (Numbers 43 to 50) were pooled and concentrated in an Amicon ultrafiltration cell with a PM-10 membrane. To the concentrated enzyme was added 0.01 volume of 100 mM TRP.

85°C Supernatant—PRPP was added to the DEAE-fraction to a concentration of 1 mM and the enzyme was heated at 85°C for 10 min. The solution was quickly cooled, then centrifuged for 50 min at 50,000 × g to remove denatured contaminants.

Purification from Chinese Hamster Liver

An alternate procedure was employed for the purification of the enzyme from liver.

Crude Extract—Livers (19.8 g) from 12 adult male Chinese hamsters were homogenized as described above except that 20 ml of buffer were added each time, and no PRPP was added.

High Speed Supernatant—The crude extract was centrifuged for 90 min at 237,000 × g and the supernatant was removed.

30 to 70% Ammonium Sulfate Fraction—This was the same procedure as that described for the brain enzyme, but the fractionation was from 30 to 70% saturation in ammonium sulfate.

DEAE-Sepharose Fraction—The 30 to 70% ammonium sulfate fraction (10 ml) was applied to a column (5 × 100 cm) filled with DEAE-Sepharose equilibrated with enzyme buffer. The column was washed with 85 ml of enzyme buffer, and the enzyme was eluted with 100 ml of enzyme buffer and fractions (15 ml) were assayed for enzyme activity. The peak fractions were pooled and concentrated in an Amicon ultrafiltration cell with a PM-10 membrane.

DEAE-Sepharose Fraction—This was the same procedure as that used for the brain enzyme except that the column was larger (2.5 × 25 cm). After application of the enzyme, the column was washed with 85 ml of enzyme buffer, and the enzyme was eluted with a 100 ml KCl gradient. The active fractions were pooled and concentrated but no PRPP was added.

pH 4.5 Supernatant—The procedure was the same as that for the brain enzyme, except that a 0.05 volume of 1 M sodium acetate and a 0.05 volume of 2 M Tris-HCl were added.

Sodium Dodecyl Sulfate Gel Electrophoresis

The gel and buffer system of Laemmli (12) was used with minor modifications. A separating gel of 12.5% acrylamide and a stacking gel of 4.5% acrylamide were used. The gels were run at room temperature at 100 volts until the dye band entered the separating gel, and at 200 volts until the dye band reached the bottom of the gel.

Dodecyl Sulfate Gel Electrophoresis

A modification of the procedure of Davis (13) was used to prepare separating gels of 4 to 15% acrylamide and stacking gels of 3% acrylamide. The electrode buffer (pH 8.5) contained 10 mM Tris-base, 75 mM glycine, and 2 mM 2-mercaptoethanol. Gels were run at 5-100 volts at 200 volts until the dye band entered the separating gel, and at 200 volts until the dye band reached the bottom of the gel. The sections of the gel to be assayed were cut into 2.5-mm slices, placed into tubes, and 100 μl of the standard assay mixture were added. These were then incubated at 37°C for 15 min, and the products were separated as described above.

Isoelectric Focusing

Electrofocusing was performed at 4°C using an LKB model 8101 Ampholine column. A 110 ml, 0 to 40% sucrose gradient with 1% ampholyte, pH 5 to 7, and 2 mM dithiothreitol was prepared manually following the procedure in the LKB manual. The enzyme sample (1 to 3 ml) was introduced into the gradient by substituting it for an equal volume of the "light" solution near the middle of the gradient. The column was run for 65 to 70 hours. During this time, the voltage was increased from 300 to 1000 volts. At the end of the run, 1-ml fractions were collected from the bottom of the column and their pH was measured at 4°C. The fractions were then assayed for enzyme activity.

RESULTS

The quantity of IMP produced in 15 min in our standard assay is a direct measure of the rate of the reaction. The formation of IMP from hypoxanthine and PRPP mediated by brain crude extracts is a linear function of time for at least 2
Table I

Purification of Chinese hamster brain hypoxanthine-guanine phosphoribosyltransferase

| Fraction                  | Volume | Total protein | Specific activity | Cumulative recovery | Purification |
|---------------------------|--------|---------------|------------------|--------------------|--------------|
| Crude extract             | 24.7   | 361           | 0.017 / mgl        | 100.0              | 1.0          |
| pH 4.5 Supernatant        | 34.2   | 140           | 0.023             | 52.7               | 1.4          |
| 65°C Supernatant          | 30.2   | 30.2          | 0.054             | 26.7               | 3.2          |
| 40 to 70% Ammonium sulfate |       |               |                   |                    |              |
| DEAE-Sephadex A-50        | 3.6    | 12.6          | 0.232             | 48.3               | 14           |
| 85°C Supernatant          | 3.3    | 0.7           | 1.23             | 14.8               | 75           |

* One unit is defined as the amount of enzyme which catalyzes the formation of 1 mmole of IMP per min at 37°C.

* Assay included 1 mg per ml bovine serum albumin.

* Protein concentration determined from intensity of enzyme band on sodium dodecyl sulfate acrylamide gel.

Fig. 1. DEAE-Sephadex chromatography of brain hypoxanthine-guanine phosphoribosyltransferase. Enzyme activity (O-O) and protein concentration (O---O) are determined as described under "Experimental Procedure." The dashed line indicates the KCl gradient.

Fig. 2. Sodium dodecyl sulfate gel electrophoresis of hypoxanthine-guanine phosphoribosyltransferase. Electrophoresis is performed as described under "Experimental Procedure." The samples are: A, carbonic anhydrase, 2 µg; B, brain DEAE-fraction, 4 µg; C, brain 85°C supernatant fraction, 0.6 µg; D, same as C, but enzyme heated for an additional 10 min at 85°C; E, chymotrypsinogen, 2 µg.

50% loss in enzyme activity. We have tested for the removal of essential factors by combining various fractions but have not been able to restore or to enhance enzyme activity. The final step in the purification scheme depends on the high heat stability of enzyme first incubated in PRPP. After heating DEAE-fraction enzyme for 10 min at 85°C, 80% of enzyme activity remains and other proteins are denatured and precipitate. The complete purification procedure can be conducted in 2 days and results in a 540-fold increase in enzyme specific activity. The purified enzyme retains at least 60% of its activity after 4 months of storage in a liquid nitrogen freezer. Enzyme fractions through the DEAE step are not inactivated by repeated freezing and thawing.

We have purified hypoxanthine-guanine phosphoribosyltransferase from brain because the specific activity of brain extracts (17 units per g) is about 4 times that of liver (4.0 units per g) or cell extracts (4.2 units per g). An alternate procedure is described for the purification of enzyme from liver, and the purification procedure for enzyme from tissue culture cell extracts will be described in a subsequent paper.

Sodium dodecyl sulfate acrylamide gel electrophoresis demonstrates that incubation of the DEAE-fraction for 10 min at 85°C produces a pure enzyme (Fig. 2). The sodium dodecyl sulfate gel of the DEAE-fraction displays three major and five minor bands. Only a single protein band remains after the 85°C heat step, and this band migrates with a mobility slightly greater than a chymotrypsinogen marker. The intensity of the corresponding band in the DEAE-fraction is consistent with a 7-fold purification by the 85°C heat step. A single band in sodium dodecyl sulfate gels implies that the enzyme consists of subunits of identical molecular weight. The subunit molecular...
and relative distribution between brain and liver enzymes reveal whether the observed differences in isoelectric pH values peaks containing 8, 22, and 70 kDa of enzyme activity with isoelectric pH values of 6.33, 6.49, and 6.70, respectively. It is not known whether the observed differences in isoelectric pH values peaks containing 22, 28, and 50 kDa of enzyme activity (Fig. 7).

Experiments with liver DEAE-fraction also give three major peaks with isoelectric pH values of 6.24, 6.43, and 6.55, respectively. The native molecular weight of the enzyme determined by column chromatography on Sephades G-100 (Fig. 4) is 78,000. An error of one fraction on either side of the assigned peak would give a molecular weight range of 73,000 to 83,000.

When run on non-denaturing acrylamide gels, the purified enzyme appears to consist of three or four bands (Fig. 5). Assay of gel slices indicates a broad peak of activity covering the area of these bands (Fig. 5). The slices were too wide to detect the possible existence of separate peaks of activity. The native molecular weight was determined from the mobility of the enzyme in acrylamide gels of varying acrylamide concentration as described by Hedrick and Smith (19). The log of the mobility was plotted as a function of gel concentration for protein standards and for the enzyme bands on gels of 4.5, 6, 7.5, and 9% acrylamide. The three enzyme bands which we could measure give a family of parallel lines (Fig. 6A) which implies that they have the same molecular weight but different net charges. The slopes of the plot of the log of the mobility versus gel concentrations are proportional to the molecular weight as shown in the standard curve in Fig. 6B. The enzyme native molecular weight determined from the standard curve is 89,000.

Electrofocusing of the brain DEAE-fraction gives three major peaks containing 29, 28, and 50% of enzyme activity (Fig. 7) with isoelectric pH values of 6.24, 6.43, and 6.55, respectively. Experiments with liver DEAE-fraction also give three major peaks containing 8, 22, and 70% of enzyme activity with isoelectric pH values of 6.33, 6.49, and 6.70, respectively. It is not clear whether the observed differences in isoelectric pH values and relative distribution between brain and liver enzymes represent real variation, or reflect differences in the methods of preparing the two enzymes.

In the presence of the substrate PRPP, hypoxanthine-guanine phosphoribosyltransferase is extremely heat-stable. Incubating the enzyme with PRPP increases the activity 2.5-fold and protects the enzyme against heat inactivation, as shown in Table II. The protected enzyme retains 96 and 80% activity after 10 min at 65 and 85°C, respectively, while the unprotected enzyme retains only 66 and 19% activity at these two temperatures. Measurements of IMP formation as a function of time (not shown) indicate that the increased enzyme activity observed after incubation with PRPP is due to an increase in the rate of IMP synthesis.

Chinese hamster hypoxanthine-guanine phosphoribosyltransferase is active over a broad pH range, with optimum activity at pH 10 to 10.5. As shown in Fig. 8, the activity increases about 2.5-fold as the pH changes from pH 5.6 to 7, is fairly constant from pH 7 to 8, and then increases 8-fold as the pH rises from 8 to 10.5. Beyond pH 10.5, the activity falls off sharply. The effect of pH on enzyme activity may reflect the ionic state of the substrates. PRPP has pK values of 5.9 and 6.7 (20), and hypoxanthine has a pK of 8.9.
Hypoxanthine-guanine phosphoribosyltransferase displays Michaelis-Menten kinetics when one of the substrates is in limiting amounts and the other is in excess. Lineweaver-Burk plots of $v$ versus $s$ for PRPP, hypoxanthine and guanine are shown in Fig. 9. The velocities are expressed as $V_{max}/V$ so that the data for the enzyme from liver, brain, and cells can be plotted on the same scale. The enzyme from these three sources has identical $K_m$ values for a given substrate. The apparent $K_m$ values for hypoxanthine and guanine are 0.52 and 1.1 $\mu$M, respectively. The $K_m$ for PRPP depends on the concentration of MgCl$_2$ (Fig. 9A). At 5.6 mM MgCl$_2$ the $K_m$ is 5.3 $\mu$M, while higher $K_m$ values of 13.2 and 20 $\mu$M are observed at 10-fold lower (0.5 mM) or 10-fold higher (50 mM) MgCl$_2$, respectively.

![Image](http://www.jbc.org/)

**Table II**

Heat inactivation of hypoxanthine-guanine phosphoribosyltransferase

| Temperature | Enzyme activity |
|-------------|-----------------|
|             | Without PRPP    | With PRPP     |
| 0$^\circ$   | 1.65            | 4.09          |
| 65$^\circ$  | 1.08            | 3.91          |
| 85$^\circ$  | 0.31            | 3.29          |

**Fig. 7.** Electrofocusing of hypoxanthine-guanine phosphoribosyltransferase. Electrofocusing is performed as described under "Experimental Procedure" with brain DEAE-fraction. Enzyme activity (○—○) is measured as described under "Experimental Procedure."

**Fig. 8.** pH activity profile. Liver DEAE-fraction (0.6 $\mu$g) is used in each assay. Enzyme activity is measured as described under "Experimental Procedure," except that the Tris-HCl buffer is replaced either by a combination of 50 mM sodium glycine, 50 mM histidine, 50 mM Tris-HCl (○—○), or by 50 mM cyclohexylaminopropane sulfonic acid (Δ—Δ) at the pH indicated.
Table III

Effect of purine nucleotides on hypoxanthine-guanine phosphoribosyltransferase activity

Lever DEAE-fraction (14 μg) was assayed as described under "Experimental Procedure" except that nucleotides at the concentrations indicated were included in the reaction mixtures. The values given represent per cent of control activity (0.24 milliunits per mg).

| Nucleotide | Activity |
|------------|----------|
|            | 0.01 mm  | 0.1 mm  | 1 mm    | 5 mm    |
| IMP        | 97       | 73      | 34      | 18      |
| GMP        | 85       | 69      | 42      | 24      |
| AMP        | 100      | 100     | 100     | 100     |

The activity of hypoxanthine-guanine phosphoribosyltransferase is subject to inhibition by its product nucleotides IMP or GMP, but not by AMP, as shown in Table III. The inhibition by IMP is neither strictly competitive with PRPP, nor strictly noncompetitive. At 1 mM IMP, the K_m for PRPP is increased to 28 μM, and the V_max is decreased to one-third of normal (data not shown). No inhibition is seen with ribose-1-P as high as 5 mM. The nucleotide inosine is slightly inhibitory (81% of control activity at 5 mM inosine).

Discussion

Hypoxanthine-guanine phosphoribosyltransferase from Chinese hamster brain, liver, and cultured cells appears identical in native molecular weight, subunit molecular weight, and kinetic properties. Based on the specific activities of the crude extracts, the enzyme represents 0.3% of the soluble protein in brain, and 0.04% of the soluble protein in liver and in cells. An extract from 10^6 cultured cells contains approximately 1 mg of soluble protein. Based on a native molecular weight of 80,000, each cell contains approximately 3 x 10^9 molecules of hypoxanthine-guanine phosphoribosyltransferase. Using this figure, one can calculate the potential contribution of the enzyme to the synthesis of purine nucleotides in cells. Under optimal conditions the enzyme in each cell could synthesize 0.2 pmol of IMP per generation time of 12 hours. The average DNA plus RNA content of an animal cell in culture is 40 pg (21), or about 0.06 pmol of purine mononucleotides. Thus, a cell could synthesize by hypoxanthine and guanine salvage alone, about 3 times the minimum amount of purine nucleotides needed to duplicate its DNA and RNA in one generation. The capability of cultured Chinese hamster cells to supply their purine nucleotide needs by salvage pathways can be investigated by blocking de novo purine synthesis with the drug aminopterin. Under these conditions, cells show only a minor increase in generation time implying that hypoxanthine-guanine phosphoribosyltransferase must be operating at close to maximal efficiency. From these data we conclude that the concentration of PRPP and hypoxanthine in cells must approach or exceed the K_m values for the enzyme. Hypoxanthine is present in excess (30 μM) in F12 tissue culture medium. We estimate that the intracellular concentration of PRPP is 0.1 mM based on the rate of IMP formation by crude cell extracts in the absence of added PRPP.

Table IV presents a comparison of hypoxanthine-guanine phosphoribosyltransferase isolated from Chinese hamsters, human red blood cells, rat brain, and brewers' yeast. Although the specific activities of all of the enzymes are approximately the same, the K_m values observed for PRPP, hypoxanthine, and guanine for the Chinese hamster enzyme are all considerably lower than those reported for the enzyme from other sources. The most striking differences occur in the molecular weight determinations. The human enzyme has a native molecular weight of 800,000, whereas the Chinese hamster enzyme has a native molecular weight of 180,000.
We have characterized hypoxanthine-guanine phosphoribosyltransferase from Chinese hamster brain, liver, and tissue culture cells. The enzymes from all three sources appear identical in structural and catalytic properties. Thus, we can be reasonably confident that any differences we observe in drug-resistant cells result from post-translational modifications. An interesting aspect of our results is the similarity in molecular weight and subunit composition of hypoxanthine-guanine phosphoribosyltransferase from Chinese hamster brain, liver, and tissue culture cells. The enzymes from all three sources appear identical in structural and catalytic properties. Thus, we can be reasonably confident that any differences we observe in drug-resistant cells result from post-translational modifications. A Chinese hamster hypoxanthine-guanine phosphoribosyltransferase appears to be quite different.

The native molecular weight of Chinese hamster hypoxanthine-guanine phosphoribosyltransferase is 78,000 to 85,000. Sodium dodecyl sulfate gel electrophoresis gives a subunit molecular weight of 25,000. The simplest interpretation of these data is that Chinese hamster hypoxanthine-guanine phosphoribosyltransferase consists of three identical subunits of molecular weight 25,000 for a combined native molecular weight of 75,000. However, since the native molecular weight may be as high as 85,000, we cannot eliminate the possibility that there are additional subunits of combined molecular weight less than 10,000. Our current techniques are not sufficiently sensitive to detect a small amount of low molecular weight protein.

At least three apparent isozymes of hypoxanthine-guanine phosphoribosyltransferase are present in our preparations and can be distinguished either by polyacrylamide gel electrophoresis or by electrofocusing. The irregular peak we observe on elution of the brain enzyme from DEAE-Sephadex may also indicate some separation of isozymes. Similar isozymes have been described by Arnold and Kelley (24) for the human enzyme, although their isoelectric points are somewhat lower. All evidence indicates that there is only a single gene locus for hypoxanthine-guanine phosphoribosyltransferase. Experiments in this laboratory indicate that Chinese hamster purine nucleoside phosphorylase from calf spleen appears to be a trimer with a subunit molecular weight of 25,000 and a native molecular weight of 84,000 (27). The trimeric structure of human purine nucleoside phosphorylase is also suggested by the demonstration of three binding sites for hypoxanthine per enzyme molecular weight of 81,000 (28), and by in vitro hybridization of mouse and human enzymes (29). Davies and Dean (30) found three or four coincident peaks of hypoxanthine-guanine phosphoribosyltransferase and purine nucleoside phosphorylase activity on electrofocusing of human erythrocyte lysates, suggesting that the activity of the two enzymes may reside in the same protein.

We have examined the relationship between hypoxanthine-guanine phosphoribosyltransferase and purine nucleoside phosphorylase from Chinese hamsters. Purified hypoxanthine-guanine phosphoribosyltransferase fails to convert hypoxanthine to inosine in the presence of ribose-1-P. Furthermore, the substrate and product of purine nucleoside phosphorylase, ribose-1-P and inosine, have a negligible effect on the activity of hypoxanthine-guanine phosphoribosyltransferase. Experiments in this laboratory indicate that Chinese hamster purine nucleoside phosphorylase is much more heat-labile than hypoxanthine-guanine phosphoribosyltransferase. Similar differences in heat stability have been reported for human purine nucleoside phosphorylase and hypoxanthine-guanine phosphoribosyltransferase (1). Yet, it is still possible that the two enzymes share common subunits. We are presently investigating this possibility.

| Specific activity (units/mg) | Chinese hamster brain | Human erythrocytes | Rat brain* | Brewers' yeastb |
|----------------------------|-----------------------|-------------------|-----------|----------------|
| Crude extract              | 0.017                 | 0.00135           | 0.004     | 0.031          |
| Purified enzyme            | 9                     | 3.8e              | 2.8       | 6.67           |
| $K_m$ ($\mu M$)            |                       |                   |           |                |
| PRPP                       | 5.3                   | 250e              | 200       | 42             |
| Hypoxanthine               | 0.52                  | 17e               | 18        |                |
| Guanine                    | 1.1                   | 5e                | 2.7       |                |
| Isozymes                   |                       |                   |           |                |
| Number observed            | 3                     | 3c                | 3         |                |
| pH                         | 6.24, 6.43, 6.55       | 5.64, 5.82, 5.97c |           |                |
| Native molecular weight    | 78-85,000             | 68,000c           |           |                |
| Subunit molecular weight   | 25,000                | 34,000c           |           |                |
| Number of subunits         | 3                     | 2                 |           |                |

*a Enzyme purified to about 50% purity (22).
*b Activity determined at 25°C; enzyme purified 213-fold (23).
*c Enzyme purified to apparent homogeneity (24).
*d Purified enzyme appeared as single band on gel electrophoresis (25).
*e Determined for erythrocyte lysates (26).
are not artifacts. We are now comparing the enzyme isolated from normal tissue culture cells with those found in drug-resistant phenotypes.

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