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HUMAN KIDNEY THIOPURINE METHYLTRANSFERASE
PURIFICATION AND BIOCHEMICAL PROPERTIES*

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Abstract—Thiopurine methyltransferase (TPMT) catalyzes the *S*-methylation of thiopurines and thiopyrimidines. Human kidney TPMT was purified over 300-fold and its biochemical properties were determined. TPMT was "soluble" and had a molecular weight of approximately 36,000 daltons as estimated by gel filtration chromatography. The pH optimum of the purified TPMT was 6.7. "True" K_m values for 6-mercaptopurine (6-MP) and *S*-adenosyl-L-methionine (SAM), the two cosubstrates for the reaction, were 0.30 mM and 2.7 μM respectively. "Apparent" K_m values for 6-thioguanine and 2-thiouracil, two other methyl acceptor substrates, were 0.55 and 2.0 mM respectively. Aliphatic thiol compounds were either poor substrates for TPMT or were not methylated. *S*-Adenosyl-L-homocysteine was a competitive inhibitor of TPMT when the varied substrate was SAM, and 6-methylmercaptopurine was a noncompetitive inhibitor with respect to 6-MP. Purified TPMT was neither activated nor inhibited by 1 mM Ca^{2+} or Mg^{2+}, but exposure to reagents such as *N*-ethylmaleimide and ethacrynic acid that interact with sulfhydryl groups inactivated the enzyme. Tropolone inhibited TPMT with a K_i of approximately 0.85 mM. Finally, human kidney TPMT activity could be distinguished from human kidney thiol methyltransferase (EC 2.1.1.9) activity on the basis of subcellular distribution, substrate specificity, kinetic characteristics and differential sensitivity to inhibitors.

Thiopurines, such as 6-mercaptopurine, 6-thioguanine and azathioprine, are used in the treatment of patients with neoplastic and autoimmune disease as well as patients undergoing organ transplantation [1, 2]. The major catabolic pathways for thiopurines are oxidation catalyzed by xanthine oxidase and *S*-methylation catalyzed by thiopurine methyltransferase (TPMT) [1, 2]. *S*-Methylated ribonucleotides of thiopurines are "active" metabolites that are capable of inhibiting purine biosynthesis [3]. TPMT activity can be measured in human erythrocytes [4], and wide individual variations in levels of human red blood cell (RBC) TPMT activity are due primarily to the effects of inheritance [5]. Genetically regulated variations in human RBC TPMT activity reflect individual variations in levels of the enzyme activity in the kidney [6, 7] and the lymphocyte [8, 9], two potential "target" tissues when thiopurine drugs are used clinically. It has been suggested that "pharmacogenetic" variations in TPMT activity might represent one factor responsible for individual differences in the therapeutic or toxic effects of thiopurine drugs [5, 10].

The properties of highly purified human TPMT have not been described previously. A thorough understanding of the biochemical properties of this drug-metabolizing enzyme will be important in future studies of the regulation and functional role of TPMT. This report describes the purification and biochemical properties of human kidney TPMT. Kidney was used as a source for the enzyme because studies in rodents had shown that the kidney had the highest level of TPMT activity of any organ tested [11, 12] and because experiments with human tissue had shown that kidney TPMT activity was much greater than that in the erythrocyte [6, 7]. In addition, thiopurine drugs are used clinically to treat both recipients of transplanted kidneys and patients with renal disease such as glomerulonephritis [1, 2].

Finally, there has occasionally been confusion with regard to the nomenclature for TPMT. At times the enzyme has been referred to as "thiol methyltransferase" (TMT, EC 2.1.1.9) even though the original description of TPMT [11] clearly differentiated this "cytoplasmic" activity capable of catalyzing the *S*-methylation of thiopurines and thiopyrimidines from the membrane-bound TMT activity that had been reported previously to catalyze the *S*-methylation of "non-physiologic" aliphatic sulfhydryl compounds [13]. Therefore, one additional goal of these experiments was to compare the properties of human kidney TPMT with those of human kidney TMT activity.

MATERIALS AND METHODS

Source of tissue. Renal tissue was obtained from patients who underwent nephrectomy for removal of tumor. Resected tissue that was free of tumor was placed immediately on dry ice and was stored at −85°. Renal TPMT was stable under these conditions. All renal tissue was obtained under guidelines established by the Mayo Clinic Human Studies Committee.

TPMT assay. TPMT activity was measured by the

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method of Weinshilboum *et al.* [4]. The assay is based on the conversion of 6-mercaptopurine (6-MP) to radioactively labeled 6-methylmercaptopurine (6-MMP) with [^{14}C]methyl-*S*-adenosyl-L-methionine (SAM) as the methyl donor. Substrate concentrations used in the assay were 3.7 mM for 6-MP and 25 μM for SAM. The reaction pH was 6.7. The reaction product was isolated by organic solvent extraction, and its radioactivity was measured in a liquid scintillation counter. The organic solvent used was 20% isoamyl alcohol in toluene in all assays except those in which 2-thiouracil was tested as a substrate. Water-saturated isoamyl alcohol was used to perform the organic solvent extraction for those assays. Blank samples contained all reagents except 6-MP. One unit of TPMT activity represented the formation of 1 nmole 6-MMP/hr of incubation at 37°.

TMT assay. TMT activity was measured by a modification of the method of Weinshilboum *et al.* [14]. The assay is based on the conversion of 2-mercaptoethanol (2-ME) to 2-methylmercaptoethanol with [^{14}C]methyl-*S*-adenosyl-L-methionine as the methyl donor. Unless otherwise stated, the final concentration of 2-ME in these experiments was 150 mM. One unit of enzyme activity represented the formation of 1 nmole 2-methylmercaptoethanol/hr of incubation at 37°.

Catechol-O-methyltransferase assay. Catechol-*O*-methyltransferase activity was measured by the method of Raymond and Weinshilboum [15].

Protein assay. Protein concentrations were measured by the dye-binding method of Bradford [16] with bovine serum albumin as a standard.

Subcellular distribution studies. TPMT and TMT activities were measured after differential centrifugation of pooled renal cortical homogenates. Three kidney samples were homogenized separately in 9 vol. of 5 mM potassium phosphate buffer, pH 7.5, with a Polytron homogenizer. Each homogenate was filtered through two layers of gauze and 2.5-ml aliquots were taken from each of the filtrates and were combined to make the pooled sample. The pooled sample was centrifuged at 1000 *g* for 10 min, the supernatant fraction was removed, the pellet was resuspended in buffer, and the centrifugation step was repeated. Supernatant fractions from the two centrifugation steps were combined and were centrifuged at 10,000 *g* for 20 min to yield a "mitochondrial" pellet. This pellet was "washed" three times by resuspension followed by centrifugation. The supernatant fraction from the initial 10,000 *g* centrifugation step and the supernatant fraction from the first wash of the mitochondrial preparation were combined and were centrifuged at 100,000 *g* for 60 min to yield a "microsomal" fraction. The pellets from each centrifugation step were resuspended in adequate buffer to result in volumes identical to those present prior to centrifugation.

Before TPMT activity was assayed, 100 μl of each fraction was mixed with 800 μl of 5 mM potassium phosphate buffer, pH 7.5, that contained 2.5 mg bovine serum albumin/ml and 100 μl of Chelex-100 chelating resin in a 1:1 suspension in water. The chelating resin was included to remove magnesium and thus to inhibit hypoxanthine guanine phosphoribosyltransferase activity [4]. These diluted preparations were mixed gently by rotation at 12 rpm for 1 hr at 4°. They were then centrifuged at 2500 *g* for 10 min, the supernatant fractions were removed, and 100μl aliquots were used for the measurement of TPMT activity. Prior to the assay of TMT activity, an aliquot of each fraction was diluted 1:20 with 5 mM Tris–HCl buffer, pH 7.8, and 100-μl aliquots of the diluted preparation were used for the measurement of TMT activity.

Enzyme purification. Human kidney TPMT was partially purified by a modification of the method of Woodson *et al.* [17]. Thirty-four grams of human renal tissue was homogenized in 5 vol. of 5 mM potassium phosphate buffer, pH 7.5, with a Polytron homogenizer. The homogenate was centrifuged at 10,000 *g* for 10 min, and the supernatant fraction was centrifuged at 100,000 *g* for 60 min. Solid ammonium sulfate was added to the supernatant fraction to a final concentration of 50% of saturation. During addition of the ammonium sulfate, Trizma base was added to the solution to adjust the pH to 7.5. The resulting suspension was centrifuged at 10,000 *g* for 10 min, and the supernatant fraction was brought to 75% of saturation with additional ammonium sulfate. The pellet formed after centrifugation of this suspension at 10,000 *g* for 10 min was dissolved in a minimal volume of 10 mM potassium phosphate buffer, pH 7.5. This solution was dialyzed against 100 vol. of 10 mM potassium phosphate buffer, pH 7.5, that contained 1 mM dithiothreitol (DTT) and 0.2 mM EDTA. The dialysis fluid was changed after 16 hr, and dialysis was continued for an additional 24 hr. Sixty-eight milliliters of dialysate that contained 890 mg of protein was applied to a 2.5 × 15 cm column that contained 75 ml of DEAE Sephadex A-25 that had been equilibrated with 20 mM Tris–HCl buffer, pH 7.5, containing 1 mM DTT and 0.2 mM EDTA. Buffer (100 ml) containing DTT and EDTA was passed through the column, and TPMT was then eluted with a linear gradient varying from 20 to 150 mM Tris–HCl, pH 7.5, that contained 1 mM DTT and 0.2 mM EDTA (Fig. 1A). TPMT activity and protein concentrations were measured in each fraction, and the fractions with the highest specific activities were pooled and were concentrated in an Amicon pressure dialysis chamber with a PM-10 membrane. The pooled concentrate containing 11.2 mg protein was applied to a 2.5 × 90 cm column of Sephadex G-100 Superfine that was equilibrated with 20 mM Tris–HCl buffer, pH 7.5, that contained 1 mM DTT and 0.2 mM EDTA. The column was eluted with the same buffer (Fig. 1B), and the fractions with the highest TPMT specific activities were pooled and were concentrated. The purified enzyme was stored in 250-μl aliquots at −85°. The protein concentration of these aliquots was approximately 70 μg/ml. TPMT activity was stable when stored under these conditions.

Molecular weight determination. The molecular weight of purified human kidney TPMT was estimated by gel filtration chromatography. A 1.5 × 90 cm column of Sephadex G-100 Superfine was equilibrated with 80 mM potassium phosphate buffer, pH 7.5, that contained 1 mM DTT and 0.2 mM EDTA. The void volume of the column was determined with Blue Dextran 2000. Ribonuclease

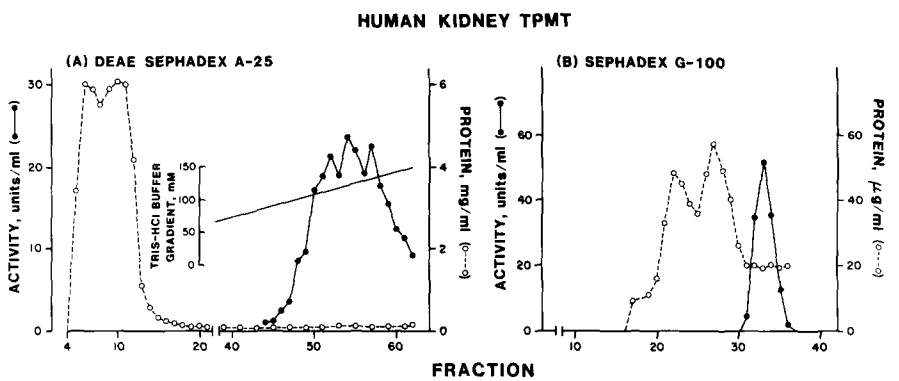

Fig. 1. Elution patterns for human kidney TPMT from columns of (A) DEAE Sephadex A-25 and (B) Sephadex G-100. Enzyme activities are indicated by the closed symbols (●) and protein concentrations are indicated by the open symbols (○).

A (molecular weight 13,700 daltons), ovalbumin (molecular weight 43,000 daltons) and bovine serum albumin (molecular weight 67,000 daltons) were used as molecular weight calibration standards. The calibration standards were applied to the column in 1.5-ml samples that contained 5.6 mg protein, and elution was monitored by measuring absorbance at 280 nm. Molecular weights were estimated as described by Whitaker [18].

Kinetic analysis. Michaelis–Menten (K_m) values were estimated by the method of Wilkinson [19] with a program written by Cleland [20]. Double-reciprocal plots were constructed using these estimates of kinetic parameters. A Hewlett–Packard 9845B computer was used to perform the calculations.

Materials. [^{14}C]Methyl-*S*-adenosyl-L-methionine (sp. act. 58 mCi/mmole) was purchased from the New England Nuclear Corp., Boston, MA. *S*-Adenosyl-L-methionine hydrochloride, *S*-adenosyl-L-homocysteine, dithiothreitol, allopurinol, 6-mercaptopurine, 2-mercaptoethanol, 6-thioguanine and 2-thiouracil were purchased from the Sigma Chemical Co., St. Louis, MO. A low molecular weight marker kit was purchased from Pharmacia Fine Chemicals, Piscataway, NJ. Tropolone was purchased from the Regis Chemical Co., Morton Grove, IL. Ethacrynic acid was obtained from Merck Sharp & Dohme, Inc. *N*-Ethylmaleimide was purchased from Eastman Kodak, Rochester, NY. Chelex-100 and Dye Reagent for the protein assay were purchased from Bio-Rad Laboratories, Richmond, CA. SKF-525A was a gift of Dr. R. Van Dyke of the Mayo Clinic.

RESULTS

Purification of human kidney TPMT. Application of ammonium sulfate precipitation, ion exchange chromatography, and gel filtration chromatography resulted in a 314-fold purification of human kidney TPMT activity as compared with the activity in a 100,000 *g* supernatant fraction of kidney homogenate (Fig. 1, Table 1). Although further purification of the enzyme could be achieved by affinity chromatography using *S*-adenosyl-L-homocysteine as the bound ligand, the TPMT in this more highly purified preparation was very unstable. During the first 24 hr after affinity chromatography, 79% of the enzyme activity was lost. Therefore, the 314-fold purified enzyme preparation obtained by use of the procedures outlined in Table 1 was used to characterize the biochemical properties of human kidney TPMT.

Effect of pH on human kidney TPMT. The effect of pH on purified human kidney TPMT was determined. pH values were measured at 20° in the presence of all components of the reaction mixture. The pH optimum of the enzyme was 6.7 when potassium phosphate was used as buffer.

Substrate kinetics. Substrate kinetic studies were performed with purified human kidney TPMT. "True" K_m values were determined for 6-MP and for SAM, the two cosubstrates used in the assay. Double-reciprocal plots of data obtained with various concentrations of these two substrates are shown in Figs 2A and 3A. Plots of the reciprocals of cosubstrate concentration versus reciprocals of apparent V_{max} values were also made (Fig. 2B and Fig. 3B).

Table 1. Purification of human kidney TPMT

	Enzyme activity (units)	Protein (mg)	Specific activity (units/mg protein)	Purification (fold)	Yield (%)
100,000 *g* Supernatant	5240	1598	3.28	1.0	100
50–75% Saturated ammonium sulfate fraction	4673	890	5.24	1.6	89.2
DEAE-A25 chromatography	1346	11.2	120	36.8	26
Sephadex G-100 chromatography	952	0.92	1030	314	18

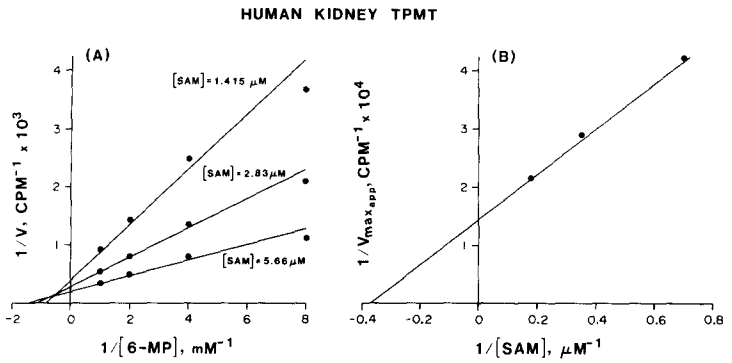

Fig. 2. TPMT substrate kinetics. (A) Plots of $1/V$ versus 1/[6-MP] with different concentrations of *S*-adenosyl-L-methionine (SAM). Each value is the mean of three determinations. (B) Plot of reciprocal apparent V_{max} values versus corresponding reciprocal SAM concentrations.

K_m and V_{max} values calculated from these data are shown in Table 2.

Another thiopurine, 6-thioguanine, and 2-thiouracil, a thiopyrimidine, were also tested as substrates. Both compounds were substrates for the purified human kidney enzyme just as they are for the rodent kidney enzyme and for human RBC TPMT [4, 11]. Apparent K_m and V_{max} values for these two compounds were calculated from kinetic data obtained by assay in the presence of various concentrations of 6-thioguanine and 2-thiouracil (Table 2).

TPMT activity has been distinguished from "thiol methyltransferase" (TMT) activity in part on the basis of differences in substrate specificity. Bremer and Greenberg [13] described TMT as capable of catalyzing the *S*-methylation of "non-physiologic" aliphatic sulfhydryl compounds such as 2-mercaptoethanol (2-ME) and dithiothreitol (DTT). Therefore, DTT and 2-ME were also tested as potential substrates for purified human kidney TPMT. When DTT at concentrations ranging from 1.5 to 150 mM was tested, no methyltransferase activity was observed. However, methyl conjugating activity was observed with high concentrations of 2-ME. The apparent K_m value for 2-ME determined from the double-reciprocal plot of these data was very high, 168 mM (Table 2).

Table 2. TPMT kinetics*

Substrate	K_m	V_{max}
S-Adenosyl-L-methionine	2.7 μM	370
6-Mercaptopurine	0.30 mM	476
6-Thioguanine	0.55 mM	545
2-Thiouracil	2.0 mM	217
2-Mercaptoethanol	168 mM	128

* V_{max} values are expressed as units per mg protein. Values for *S*-adenosyl-L-methionine were determined with 6-mercaptopurine as the cosubstrate.

End-product inhibition. Many SAM-dependent methyltransferases are inhibited by *S*-adenosyl-L-homocysteine, a product of reactions in which SAM serves as a methyl donor [21]. Inhibition of TPMT by 6-methylmercaptopurine, the methylconjugated product of the reaction when 6-MP is used as substrate, has also been reported [11]. Therefore, *S*-adenosyl-L-homocysteine and 6-methylmercaptopurine were tested as possible inhibitors of purified human kidney TPMT. Both compounds inhibited the reaction. Figure 4 shows the effect of various concentrations of *S*-adenosyl-L-homocysteine on the TPMT reaction. The pattern is compatible with competitive inhibition with respect to SAM, with a K_i value of approximately 0.75 μM. This mechanism

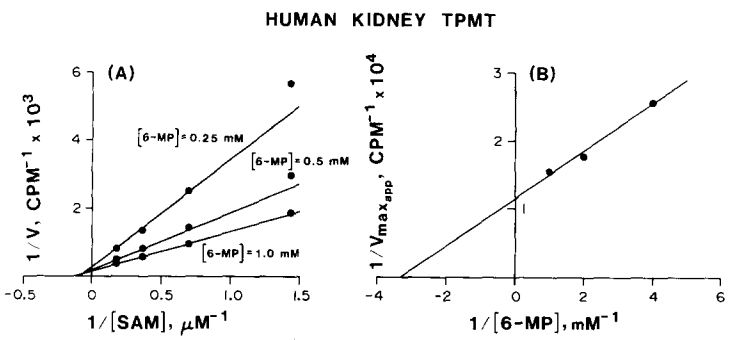

Fig. 3. TPMT substrate kinetics. (A) Plots of $1/V$ versus 1/[SAM] with different concentrations of 6-MP. Each value is the mean of three determinations. (B) Plot of reciprocal apparent V_{max} values versus corresponding reciprocal 6-MP concentrations.

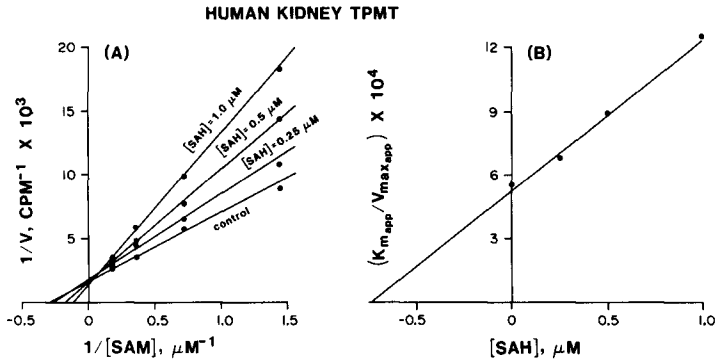

Fig. 4. TPMT inhibitor kinetics. The effect of *S*-adenosyl-L-homocysteine (SAH) on TPMT activity. (A) Plots of $1/V$ versus 1/[SAM] with different concentrations of SAH. Each value is the mean of three determinations. (B) Plot of slopes calculated from the double-reciprocal plots in Fig. 4A versus concentrations of SAH.

of inhibition by *S*-adenosyl-L-homocysteine is typical of many SAM-dependent methyltransferases [21]. 6-Methylmercaptopurine also inhibited the reaction. Inhibition by 6-methylmercaptopurine was noncompetitive with respect to 6-MP (Fig. 5). The calculated K_i value was approximately 0.56 mM.

Effects of divalent cations and enzyme inhibitors. The human kidney contains many methyltransferase enzymes other than TPMT. Included among these are catechol-*O*-methyltransferase (COMT) [22] and TMT. COMT is magnesium dependent [23] and is inhibited by calcium and by tropolone [24, 25]. Human RBC TMT activity is not affected by either calcium or magnesium at 1 mM concentrations, but it is inhibited by SKF-525A [14]. The effects of Ca^{2+}, Mg^{2+}, tropolone, and SKF-525A on purified human kidney TPMT were determined (Table 3). TPMT activity was not altered by 1 mM concentrations of either Ca^{2+} or Mg^{2+}. SKF-525A at 0.5 mM, a concentration that inhibited human RBC TMT activity by 60% [14], also failed to influence TPMT activity. In contrast, 1 mM tropolone inhibited the TPMT activity by 52%. Since inhibition by tropolone was unexpected, the purified human kidney TPMT preparation was tested to determine whether it was capable of catalyzing the COMT reaction. It contained no detectable COMT activity. An experiment was then performed to study the kinetic characteristics of the inhibition of TPMT by tropolone (Fig. 6). When 6-MP was the substrate that was varied, the kinetic pattern was consistent with noncompetitive inhibition. The K_{is} for the inhibition by tropolone was estimated to be 0.85 mM and the K_{ii} was estimated to be 1.63 mM.

Table 3. Effect of inhibitors and ions on human kidney TPMT activity*

Reagent or ion tested	% Baseline activity
Ca^{2+}, 1 mM	97 ± 2.3
Mg^{2+}, 1 mM	102 ± 2.1
Tropolone, 1 mM	48 ± 1.5
SKF-525A, 0.5 mM	105 ± 3.6
N-Ethylmaleimide, 1 mM	3 ± 0.7
Ethacrynic acid, 1 mM	23 ± 1.0

* TPMT activity was 0.19 units for experiments with Ca^{2+}, Mg^{2+}, tropolone and SKF-525A. The basal enzyme activity was 0.055 units for experiments with *N*-ethylmaleimide and ethacrynic acid. Each value is the mean ± S.E.M. of three determinations.

Finally, the effects on purified human kidney

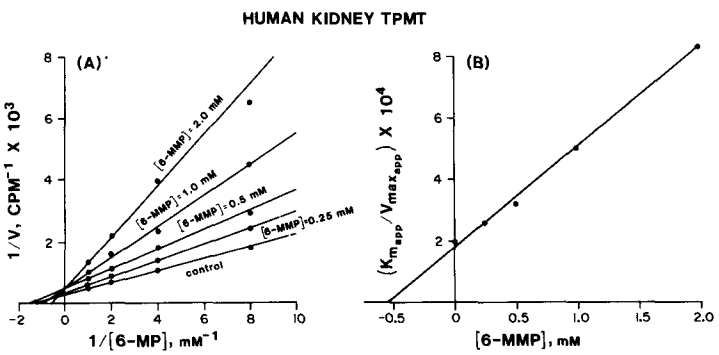

Fig. 5. TPMT inhibitor kinetics. The effect of 6-methylmercaptopurine (6-MMP) on TPMT activity. (A) Plots of $1/V$ versus 1/[6-MP] with different concentrations of 6-MMP. Each value is the mean of three determinations. (B) Plot of slopes calculated from the double-reciprocal plots in Fig. 5A versus concentrations of 6-MMP.

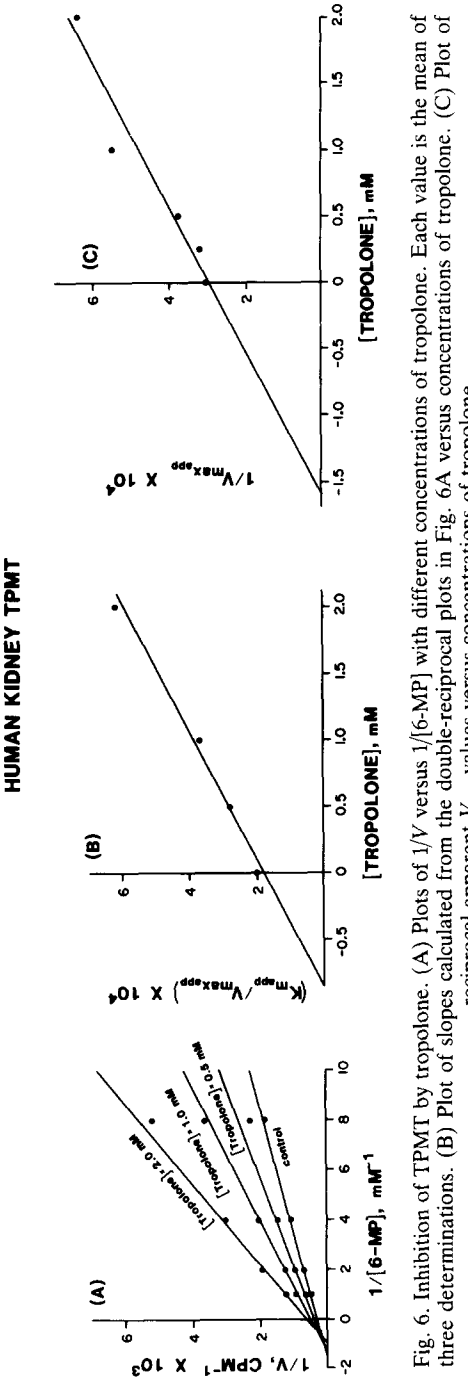

Fig. 6. Inhibition of TPMT by tropolone. (A) Plots of $1/V$ versus 1/[6-MP] with different concentrations of tropolone. Each value is the mean of three determinations. (B) Plot of slopes calculated from the double-reciprocal plots in Fig. 6A versus concentrations of tropolone. (C) Plot of reciprocal apparent V_{max} values versus concentrations of tropolone.

TPMT of ethacrynic acid and *N*-ethylmaleimide, agents that interact with sulfhydryl groups, were tested. After incubation with the enzyme, the ethacrynic acid and *N*-ethylmaleimide were removed by dialysis to prevent their interaction with the thiol substrate used in the enzyme assay. Exposure to 1 mM *N*-ethylmaleimide and to 1 mM ethacrynic acid inhibited TPMT activity by 97 and 77% respectively (Table 3).

Molecular weight estimate. The molecular weight of purified human kidney TPMT was estimated by gel filtration chromatography. The results indicated that the molecular weight of human kidney TPMT was approximately 36,000 daltons. As in all cases in which only one technique is used to estimate a molecular weight, this value should be considered only an estimate until it is verified by other methods.

Human kidney TMT activity. Although purified human kidney TPMT was unable to catalyze the methylation of DTT, one substrate that has been used to measure TMT activity [14], it was capable of catalyzing the methylation of 2-ME, another TMT substrate [13, 14]. The fact that the apparent K_m for this reaction was extremely high, 168 mM, made it unlikely that this activity was related to the membrane-associated TMT activity described by Bremer and Greenberg [13]. However, a series of experiments was performed to compare the characteristics of human kidney TMT with those of human kidney TPMT activity.

The first experiment examined the subcellular distribution of TPMT and TMT activities in homogenates of human renal tissue. When differential centrifugation was performed as described under Materials and Methods, TPMT activity was present entirely in the soluble supernatant fraction while over 87% of the total TMT activity recovered was particle associated (Table 4). These results were similar to those that have been reported for rat and mouse liver and kidney TPMT [11, 13]. Differences in subcellular distribution formed one of the criteria by which TPMT was differentiated from TMT [11].

The next series of experiments was concerned with substrate kinetics and with the effect of inhibitors on renal TMT activity. These studies were performed with microsomal preparations of human kidney homogenates prepared as described in Materials and

Table 4. Subcellular distribution of human kidney TPMT and TMT activities*

	TPMT (%)	TMT (%)
Homogenate	100	100
Nuclei	0	11.5
Mitochondria	0	36
Microsomes	0	20.6
100,000 *g* Supernatant	82.8	10
Total activity recovered	82.8	78.1

* Enzyme activities are expressed as percentages of the total activity in the homogenate. Homogenate activities were 4.02 units/mg protein for TPMT and 9.02 units/mg protein for TMT. Each value is the mean of three determinations.

Methods and "washed" one time with 5 mM potassium phosphate buffer. TMT activity in these preparations was not affected significantly by either 1 mM Ca^{2+} or 1 mM Mg^{2+}. However, TMT was inhibited 56% by 0.5 mM SKF-525A and 62% by 1.0 mM tropolone. The inhibition by SKF-525A contrasted sharply with the lack of effect of that drug on purified kidney TPMT (Table 3).

Finally, the apparent K_m value for the methylation of 2-ME catalyzed by purified human kidney TPMT was extremely high (168 mM). This value exceeded by at least an order of magnitude K_m values reported for the methylation of 2-ME catalyzed by TMT in either the human RBC [14] or rat liver [26]. Therefore, an experiment was performed to determine the apparent K_m value for 2-ME as a substrate for the membrane-associated TMT activity found in the human kidney. When this activity was measured in the presence of nine different concentrations of 2-ME ranging from 0.625 to 160 mM, the kinetic pattern on a double-inverse plot was complex and was similar to that which has been reported for TMT activity in human RBC membrane preparations [14]. Two apparent K_m values were present. The highest of these was 15.5 mM, a value very similar to that of 20 mM which was reported for the highest apparent K_m for the human RBC membrane activity [14]. This K_m value for renal tissue was an order of magnitude lower than that which we observed for the reaction catalyzed by purified human kidney TPMT.

DISCUSSION

TPMT plays an important role in the catabolism of thiopurines and thiopyrimidines [1, 2]. Although the regulation of this drug-metabolizing enzyme activity has been studied in the rat [12, 17], until recently little was known of its regulation in man. The recent description of a sensitive radiochemical enzymatic assay for TPMT [4] made it possible to measure the enzyme activity in the human RBC. Human RBC TPMT is regulated primarily by a common genetic polymorphism [5], and this "pharmacogenetic" variation in the RBC enzyme activity reflects variation of TPMT activity in other tissues and cells such as the kidney [6, 7] and the lymphocyte [8, 9]. These observations have led to suggestions that individual variations in TPMT activity may represent one factor involved in differences in the effect or toxicity of thiopurine drugs [5, 10]. All of these developments have increased the importance of knowledge of the properties of human TPMT.

This report has described the purification and biochemical properties of human kidney TPMT. The human kidney enzyme was purified over 300-fold and was found to have an approximate molecular weight of 36,000 daltons as estimated by gel filtration chromatography. The pH optimum of the enzyme was 6.7. K_m values for 6-MP and SAM, two cosubstrates for the reaction, were 0.30 mM and 2.7 μM respectively. 6-Thioguanine, another thiopurine, and 2-thiouracil, a thiopyrimidine, were also able to serve as substrates for the enzyme with apparent K_m values of 0.55 and 2.0 mM respectively. *S*-Adenosyl-L-homocysteine and 6-methylmercaptopurine were both inhibitors of the reaction catalyzed by human kidney TPMT. Neither Ca^{2+} nor Mg^{2+} at concentrations of 1 mM influenced the enzyme reaction, and it was not altered by SKF-525A at a final concentration of 0.5 mM. However, tropolone, a drug usually considered primarily an inhibitor of COMT [25], was also an effective inhibitor of partially purified TPMT even though the TPMT preparation was unable to catalyze the COMT reaction. Since no potent or specific inhibitors of TPMT are available as either research tools or, possibly, for future clinical applications, it would be of interest to study systematically other COMT inhibitors to determine whether they are capable of inhibiting TPMT.

TPMT has occasionally been confused with TMT, a membrane-associated activity reported to catalyze the *S*-methylation of "non-physiologic" aliphatic sulfhydryl compounds such as DTT and 2-ME [13]. Human kidney TPMT was found to differ from human kidney TMT on the basis of subcellular distribution, substrate specificity, kinetic behavior and response to inhibitors. All of these observations are compatible with the conclusion that TMT and TPMT represent separate enzyme activities. A final decision on the relationship of these two activities must await purification and comparison of the two enzymes.

Finally, the significance of even the partial purification of human TPMT extends beyond merely a description of the biochemical properties of the enzyme. The purification of kidney TPMT has made it possible to develop antibodies directed against human TPMT that have been used to help clarify the molecular mechanism of the genetic polymorphism regulating the enzyme activity in man. The use of these antibodies has demonstrated that subjects with inherited low levels of TPMT enzymatic activity also have low levels of immunoreactive TPMT protein in their erythrocytes [7, 27]. Results such as those and the results reported here should increase our understanding of the regulation and the functional role of this important drug-metabolizing enzyme activity.

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