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INOSINE 5′-MONOPHOSPHATE DEHYDROGENASE FROM SARCOMA 180 CELLS—SUBSTRATE AND INHIBITOR SPECIFICITY

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Abstract—The substrate and inhibitor specificity of IMP dehydrogenase from Sarcoma 180 ascites tumor cells has been studied with twenty purine nucleotide analogs. Several were found to be substrates with the following efficiencies (V_{max}/K_m): IMP (4000), 8-azaIMP (1360), 6-thioIMP (250), araIMP (250) and dIMP (240). While substrate activity was not detected with the 5′-phosphates of 6-methylmercaptopurine riboside or 1-ribosylallopurinol (rates less than 1/10,000th that of IMP), they were competitive inhibitors with respect to IMP (both with K_i values of 0.43 mM). Seven XMP analogs and six GMP analogs were also found to be competitive inhibitors with respect to IMP. Methods for the synthesis of the 5′-phosphates of arabinosylhypoxanthine, arabinosylxanthine, arabinosylguanine and 2′-deoxyxanthosine are described.

IMP dehydrogenase* (IMP:NAD oxidoreductase, EC 1.2.1.14) catalyzes the NAD^+-dependent oxidation of IMP to XMP. This enzymatic reaction can be considered to be the first enzymatic step unique to the biosynthetic pathway for the *de novo* synthesis of guanine nucleotides.

Many potential nucleotide substrates and inhibitors of IMP dehydrogenase are produced from pharmacologically active analogs of purine bases and nucleosides *in vivo*. Therefore, a knowledge of the substrate and inhibitor specificity of this enzyme would be useful in evaluating the mechanism of pharmacological activity of these compounds. Although studies on the substrate specificity of the enzyme from *Aerobacter aerogenes* have been reported [2–4], little is known about the substrate specificity of the mammalian enzyme. Other than IMP, only dIMP has been reported to be a substrate for this enzyme [5]. The enzyme from both mammalian [6, 7] and bacterial sources [8–10] is inhibited by XMP and GMP. It has been suggested that this inhibition is involved in the regulation of this enzyme *in vivo* [6–10]. The purpose of the present study was to extend the knowledge of the specificity of the enzyme from Sarcoma 180 ascites cells originally studied by Anderson and Sartorelli [5, 6, 11].

*Abbreviations used: IMP dehydrogenase, IMP:NAD oxidoreductase (EC 1.2.1.14); AMP deaminase, AMP aminohydrolase (EC 3.5.4.6); lactate dehydrogenase, L-lactate:NAD oxidoreductase (EC 1.1.1.27); hypoxanthine–guanine PRTase, IMP:pyrophosphate phosphoribosyltransferase (EC 2.4.2.8); hypoxanthine PRTase from *E. coli* [1]; xanthine oxidase, xanthine:O_2 oxidoreductase (EC 1.2.3.2); PP-ribose-P, 5-phosphorylribose 1-pyrophosphate; allopurinol, 4-hydroxypyrazolo(3,4-d)pyrimidine; oxipurinol, 4,6-dihydroxypyrazolo(3,4-d)pyrimidine; 1-Alo-5′-P, 1-ribosylallopurinol-5′-phosphate; 1-Oxi-5′-P, 1-ribosyloxipurinol-5′-phosphate; 7-Oxi-5′-P, 7-ribosyloxipurinol-5′-phosphate; 6-NH_2-1-Alo-5′-P, 1-ribosyl-4-hydroxy-6-aminopyrazolo(3,4-d)pyrimidine-5′-phosphate; 6-MeMPR, 6-methylmercaptopurine riboside; 6-MeMPR-5′-P, 6-methylmercaptopurine riboside-5′-phosphate; araDAP, 2,6-diaminopurine arabinoside; araDAP-5′-P, 2,6-diaminopurine arabinoside-5′-phosphate; araH, arabinosylhypoxanthine; araIMP, hypoxanthine arabinoside-5′-phosphate; araA, arabinosyladenine; araAMP, adenine arabinoside-5′-phosphate; araG, arabinosylguanine; araGMP, guanine arabinoside-5′-phosphate; araXMP, xanthine arabinoside-5′-phosphate.

MATERIALS AND METHODS

Materials. Sarcoma 180 ascites tumor cells were a gift from Dr. Alan C. Sartorelli at the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut, 06510. The sodium salt of dIMP, dGMP, TTP, the lithium salt of 8-azaGMP, the barium salt of 6-thioIMP [converted to the sodium salt on a Dowex 50 X-8 (sodium) column prior to use], NAD^+ and NADH were purchased from P-L Biochemicals; the ammonium salt of araAMP was obtained from Terra Marine Bioresearch; IMP-8-[^{14}C] and ultra-pure Tris were purchased from Schwarz Mann; and rabbit muscle AMP deaminase was purchased from Sigma Chemical Co. AraDAP-5′-P was synthesized in these laboratories by Dr. Janet Rideout according to the procedure of Yoshikawa *et al.* [12]. 6-ThioGMP, 6-thioXMP, 8-azaXMP, 1-Oxi-5′-P, 7-Oxi-5′-P, 1-Alo-5′-P and 6-NH_2-1-Alo-5′-P were synthesized and purified according to previously published procedures [13, 14]. All nucleotides were shown to be >98% chemically and/or radiochemically pure as determined by high pressure liquid chromatography [15].

Spectrophotometric enzyme assays

IMP dehydrogenase. This activity was determined by the method of Anderson and Sartorelli [6] using duplicate 250 μl reaction mixtures in 1-cm pathlength black-masked quartz micro cuvettes (Hellma Cells, Inc., Forest Hills, N.Y.) in a thermostated Guilford Model 240 spectrophotometer at 37° using a full-scale recorder deflection of 0.1 absorbance unit. Reaction mixtures contained 100 mM Tris–Cl, pH 8.0; 100 mM KCl; 0.28 mM NAD^+; IMP or analog nucleotide

Table 1. Interactions of inosinate analogs with IMP dehydrogenase

Inosinate analog	K_m* (mM)	K_i (mM)	Rel. V_{max}	V_{max}/K_m
IMP	0.025†	—	100	4000
8-AzaIMP	0.042	—	57	1360
6-ThioIMP	0.032	0.020	8	250
AraIMP	0.14	—	35	250
dIMP	0.28†	—	64	240
6-MeMPR-5′-P	—	0.43	<0.01‡	—
1-Alo-5′-P	—	0.43	<0.003**	—

* Determined using five concentrations of inosinate analog. See Materials and Methods section for concentration ranges used. When analyzed by high pressure liquid chromatography [15] under conditions where all other reaction components were clearly separated, co-chromatography of the oxidized nucleotide product with authentic compound served to verify the formation of product.

† Data confirming that previously reported [18]. The K_m value for IMP as determined by the radiochemical assay was 0.046 ± 0.005 mM (7 determinations).

‡ Lower limit of detectability observed at 18.5 mM 6-MeMPR-5′-P as analyzed by high pressure liquid chromatography.

** Lower limit of detectability observed at 3.1 mM 1-Alo-5′-P-6-[^{14}C].

substrate; and 50–200 μg of protein. Reactions were initiated by the addition of pre-warmed enzyme and the formation of NADH was monitored at 340 nm ($\Delta\epsilon$ = 6.22 mM^{-1} cm^{-1}). Reaction velocities in control reactions were typically determined from absorbance changes of 0.03 unit for the lowest IMP concentration used and 0.08 unit for the highest IMP concentration used. Due to the interfering absorbance of 6-thioIMP at 340 nm, the formation of NADH with this substrate was measured at 360 nm ($\Delta\epsilon$ = 5.27 mM^{-1} cm^{-1}). In reaction mixtures containing IMP, the formation of XMP was also monitored at 290 nm ($\Delta\epsilon$ = 5.40 mM^{-1} cm^{-1}). The initial absorbance of the reaction mixture at 290 nm was 0.8 absorbance unit for the highest concentration of IMP utilized. The following concentrations of the purine nucleoside 5′-phosphate substrates were used in determining the K_m and V_{max} values: IMP, 0.02–2.3 mM; 8-azaIMP, 0.02–0.4 mM; 6-thioIMP, 0.03–2.8 mM; araIMP, 0.16–3.5 mM; and dIMP, 0.19–9.6 mM.

NADH oxidase. To measure this activity, reaction mixtures for the IMP dehydrogenase spectrophotometric assay were used in which 0.1 mM NADH replaced the IMP and NAD^+. The oxidation of NADH was monitored as a decrease in absorbance at 340 nm ($\Delta\epsilon$ = 6.22 mM^{-1} cm^{-1}).

Radiochemical enzyme assays

IMP dehydrogenase. Reaction conditions were identical to those described for the spectrophotometric assay using 125 μl reaction volumes containing IMP-8-[^{14}C] (0.01–0.2 mM; sp. act. 2.7 Ci/mole) and 5 μg of protein. NAD^+ was omitted from control reaction mixtures. Reaction mixtures were preincubated for 3 min at 37°. Reactions were initiated by the addition of enzyme (pre-warmed to 37°). After 5 min, reactions were terminated by immersion in a boiling water bath for 3 min and were subsequently stored on ice. Aliquots (25 μl) were spotted on Whatman 3MM paper in the presence of 1 μmole of carrier IMP and XMP. The purine nucleotides were separated by high voltage electrophoresis at 2000 volts for 90 min in 50 mM potassium phosphate buffer, pH 7.5 (M_{ATP} = 100, M_{XMP} = 105, M_{IMP} = 129). IMP and XMP were visualized with u.v. light, cut out, and counted by liquid scintillation as previously described [14].

With 1-Alo-5′-P-6-[^{14}C] as substrate, reaction mixtures (100 μl) contained 100 mM Tris–Cl, pH 8.0; 100 mM KF; 1.6 mM TTP; 0.28 mM NAD^+; 3.1 mM 1-Alo-5′-P-6-[^{14}C] (3.0 Ci/mole) and 180 μg of protein. The reaction mixtures were incubated for 3 hr at 37° and the reactions were terminated by immersion in a boiling water bath for 2 min. Aliquots (25 μl) were spotted on Whatman DE-81 paper prespotted with 0.1 μmole 1-Alo-5′-P, 1-Oxi-5′-P, and allopurinol-1-ribonucleoside and chromatographed in a descending direction in 0.2 M ammonium formate, pH 5.0, for 3 hr [16] (R_f values 0.39, 0.13, 0.60 respectively). Each spot was visualized with u.v. light, cut out, and counted by liquid scintillation. Values determined by this method were subsequently verified by high pressure liquid chromatography as previously described [15].

Protein determination. Protein concentrations were determined by the procedure of Lowry *et al.* [17] using human serum albumin as the standard.

Enzyme purification. IMP dehydrogenase was purified from Sarcoma 180 ascites cells by a modification of the method of Anderson and Sartorelli [6]. The 20–40% ammonium sulfate precipitate contained appreciable NADH oxidase activity which could be removed by further ammonium sulfate fractionation at 4°. The 20–40% ammonium sulfate precipitate was dissolved in 100 mM Tris–Cl, pH 8.0, and dialyzed for 16 hr at 4° against 400 vol of 10 mM Tris–Cl, pH 8.0. After adjusting the protein concentration of the dialysate to 9 mg/ml, solid ammonium sulfate (0.1287 g/ml) was added. The resulting suspension, after 30 min, was centrifuged and the precipitate was discarded. The supernatant was dialyzed for 16 hr at 4° against 400 vol of 10 mM Tris–Cl, pH 8.0. The additional ammonium sulfate fractionation and dialysis resulted in a decrease in the NADH oxidase rate from 30% to <1% of the IMP dehydrogenase rate with a 65% recovery of the IMP dehydrogenase. This preparation had a specific activity of 1.5 milli-

units*/mg of protein which was similar to that of Anderson [18].

Preparation of nucleotides

AraIMP was synthesized by the enzymatic deamination of araAMP using rabbit muscle AMP deaminase. The reaction mixture (10 ml, 25°) contained 100 mM Tris–succinate, pH 5.9; 100 mM KCl; 5 mM β-mercaptoethanol; 69 mM araAMP and 55 units of AMP deaminase. After 3 hr, the reaction mixture was filtered (Millipore filter, 0.8 μm) and applied to a water-equilibrated polyacrylamide BioRad P-2 column (2.5 × 90 cm). After elution with water, those fractions containing araIMP as determined by t.l.c. on PEI–cellulose in 2 M formic acid–0.5 M LiCl (1:1) (R_f araIMP = 0.42; R_f araAMP = 0.86) were lyophilized, dissolved in a minimum amount of water, and reapplied to the BioRad P-2 column. Eluted fractions containing araIMP were lyophilized. The white powder (approx 200 mg) was dissolved in water, applied to preparative Silica gel t.l.c. plates and chromatographed overnight in *n*-propanol–methanol–ammonia–water (45:15:30:10) (R_f araIMP = 0.48; R_f araAMP = 0.60). The araIMP was extracted from the Silica gel with 225 ml of water. The extract volume was decreased to 10 ml by lyophilization, filtered, and applied to the BioRad P-2 column as described above. This BioRad P-2 column chromatography step was repeated. Lyophilization of the nucleotide containing fractions gave 170 mg of araIMP (71% yield) which was shown to be >99% pure by high pressure liquid chromatography [15].

AraGMP was synthesized from araDAP-5′-P by the method used for araIMP except that the reaction mixture contained 8.6 mM araDAP-5′-P in place of araAMP. After 21 hr, the nucleotides in the reaction mixture were adsorbed to charcoal and subsequently were eluted with 25 ml of 10% aqueous pyridine. The charcoal eluate was reduced to dryness at 30° under reduced pressure. The resulting powder was dissolved in 25 ml of water, filtered and lyophilized. The yellowish white powder (30 mg, 96% yield) gave a single spot on cellulose t.l.c. in 5% Na_2HPO_4–isoamylalcohol (2:1) (R_f araGMP = 0.86; R_f araDAP-5′-P = 0.55) and *n*-propanol–ammonia–water (7:3:1) (R_f araGMP = 0.34; R_f araDAP-5′-P = 0.45) and was shown to be >99% pure by high pressure liquid chromatography.

2′-DeoxyXMP was synthesized by oxidation of dIMP by the dehydrogenase fraction prior to the second ammonium sulfate fractionation (see Enzyme Purification). The reaction mixture (20 ml, 30°) contained 25 mM Tris–Cl, pH 8.0; 100 mM KCl; 0.4 mM NAD^+; 15 mM pyruvic acid; 10 mM dIMP; 15 μg lactate dehydrogenase; and 35 milliunits of IMP dehydrogenase. After 4 days, the reaction mixture was filtered and lyophilized. The lyophilized powder was dissolved in a minimum amount of water and applied to a water-equilibrated BioRad P-2 column (2.5 × 90 cm). Eluate fractions containing u.v.-absorbing material were pooled, applied to a DEAE A-25 Sephadex column and chromatographed by the method of Caldwell [19] using a pH 4.7 triethylammonium acetate buffer. Fractions containing dXMP were pooled, lyophilized, dissolved in a minimum amount of water and reapplied to the water-equilibrated BioRad P-2 column. The fractions containing dXMP were combined, the volume reduced by lyophilization and the resulting solution was applied to cellulose t.l.c. plates. The plates were developed in *n*-propanol–water (7:3), air dried, and redeveloped in the same solvent. The dXMP was extracted from the cellulose with 30 ml of cold water. The extract was filtered and lyophilized. The resulting white powder (3.3 mg, 4% yield) when dissolved in water eluted as a single symmetrical peak of >98% purity when analyzed by high pressure liquid chromatography.

AraXMP was synthesized from araIMP by the same method used for dXMP except that an additional 25 milliunits of IMP dehydrogenase were added after 24 and after 72 hr. Instead of applying the reduced volume eluate from the second BioRad P-2 column to cellulose t.l.c. plates, it was reapplied to the water-equilibrated BioRad P-2 column. Fractions containing araXMP (1% yield) were shown to be >93% pure and to contain approx 6% arabinosylxanthine† when analyzed by high pressure liquid chromatography.

8-AzaIMP was synthesized by an enzyme catalyzed condensation of 8-azahypoxanthine and PP-ribose-P. The hypoxanthine PRTase (sp. act. 0.26 units‡/mg of protein) was prepared by G-100 Sephadex chromatography [1] of an extract of *E. coli* B-96 grown as previously described [20]. The reaction mixture (100 ml, 37°) contained 50 mM potassium phosphate, pH 6.8; 5.8 mM 8-azahypoxanthine; 6 mM Mg_2PP-ribose-P; and 3.6 units of hypoxanthine PRTase. Additional Mg_2PP-ribose-P (0.15 m-moles) was added at 3, 23 and 27 hr. Additional enzyme (1.8 units) was added at 23 and 27 hr. After 48 hr the reaction mixture was concentrated to 4 ml under reduced pressure at 40°. The concentrated reaction mixture was filtered and applied to a BioRad P-2 column (2.5 × 90 cm) equilibrated with water. Eluate fractions containing the nucleotide product were lyophilized. The resulting powder was suspended in 8 ml of water and filtered. The compound was absorbed to charcoal and was eluted with 4 ml of ammonia–ethanol–water (1:5:4). The volume of the eluate was reduced, applied to a cellulose t.l.c. plate and chromatographed in *t*-amyl alcohol–formic acid–water (3:2:1) (R_f of 8-azaIMP 0.32). The 8-azaIMP was extracted from the cellulose with 10 ml of water. After lyophilization, 12 mg of 8-azaIMP (6% yield) was obtained which was shown to be >99% pure by high pressure liquid

*One unit of IMP dehydrogenase is defined as the amount of enzyme which catalyzes the formation of 1 μmole XMP/min under the conditions specified for the spectrophotometric assay.

†This compound, which was identical to the nucleoside product derived from the treatment of araXMP with 5′-nucleotidase, had the same u.v. spectra as xanthosine at both pH 1 and pH 11. Cellulose t.l.c. in 334 mM Tris–borate, pH 8.6, showed it to have an R_f (0.80) identical to that of deoxyxanthosine but different from that of xanthosine (0.92) while in *n*-propanol–water (7:3) it had the same R_f (0.31) as deoxyxanthosine and xanthosine.

‡One unit of hypoxanthine PRTase is defined as the amount of enzyme that catalyzes the formation of 1 μmole IMP/min at pH 9.25 and 37° [21].

chromatography and to have a base–ribose–phosphate ratio of 1.0:1.0:1.1.

1-Alo-5′-P-6-[^{14}C] was synthesized from allopurinol-6-[^{14}C] using the human erythrocyte hypoxanthine–guanine PRTase preparation described by Krenitsky *et al.* [22]. The nucleotide product was purified by chromatography on BioRad AG 1-X8 (chloride) with a linear gradient of 0–1 M LiCl and adsorption to and elution from charcoal with ammonia–ethanol–water (1:5:4). After removal of the ammonia and ethanol under reduced pressure, the 1-Alo-5′-P-6-[^{14}C] (3.0 Ci/mole, 43% yield) chromatographed as a single symmetrical u.v. absorbing peak and was found to be >97% radiochemically pure when analyzed by high pressure liquid chromatography.

RESULTS

Contaminating enzyme activities. In the measurement of the IMP dehydrogenase activity, the 290 nm and 340 nm spectrophotometric assays and the radioactive assay were found to agree within 5%. The agreement of the 290 nm and the 340 nm assays indicated that for each mole of IMP oxidized, one mole of NAD^+ was reduced by the final enzyme preparation. The direct NADH oxidase assay demonstrated that this activity was <1% of that of the IMP dehydrogenase. In the radiochemical assays no detectable ^{14}C-containing compounds other than [^{14}C]IMP and [^{14}C]XMP were observed after electrophoretic separation of reaction mixtures indicating that the phosphatase activity of this preparation was <2% that of the IMP dehydrogenase. Only after prolonged incubation was this phosphatase activity evident and it could be inhibited by the substitution of KF for KCl and the inclusion of TTP in these reactions. Under these conditions the IMP dehydrogenase rate was inhibited by 15% relative to the standard conditions (see Materials and Methods).

Substrate specificity. Kinetic parameters (calculated using the computer program of Cleland [23]) for the various purine and purine analog nucleotides are presented in Table 1. The K_m and V_{max} values were derived from the data obtained by the spectral assay at 340 nm (360 nm for 6-thioIMP) except for 1-Alo-5′-P which were obtained using a radiochemical assay. Of the compounds tested as substrates, the naturally occurring nucleotide IMP was found to have the highest maximal velocity (Figure 1). Replacement of the oxo group in the 6 position of IMP with a thio group caused no appreciable change in the K_m value, but did cause a decrease of >90% in the reaction rate. Oxidation of 6-MeMPR-5′-P was not detectable. Alterations in the imidazole portion of IMP did cause changes in substrate activity. Relative to IMP, the triazolo(4,5-d)pyrimidine nucleotide, 8-azaIMP, had an increased K_m value and reacted at 57% the rate of IMP (Figure 1). The corresponding pyrazolo(3,4-d)pyrimidine nucleotide, 1-Alo-5′-P, although bound to the enzyme was not oxidized at a measurable rate. Changes in the 2′-position of the ribose moiety of IMP, (i.e., dIMP and araIMP) resulted in decreased reaction velocities and increased K_m values relative to IMP.

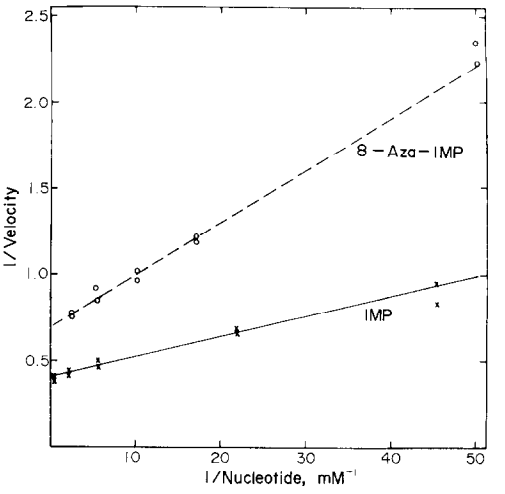

Fig. 1. Product synthesis as a function of IMP and 8-azaIMP concentration. Velocities are expressed as nmoles of product formed/min/ml enzyme.

Inhibitor specificity. The K_i values (Table 2, calculated using the computer program of Cleland [23]) were obtained using the radiochemical assay with IMP-8-[^{14}C] as the variable substrate. The XMP and GMP analogs of those compounds tested as substrates were investigated as inhibitors of IMP dehydrogenase. All of the inhibitors tested appeared to be competitive with respect to IMP. XMP and GMP had K_i values of 0.6 mM and 0.3 mM respectively. Unlike 6-thioGMP [24], 6-thioXMP exhibited non-progressive inhibition with a K_i value 3-fold lower than that of XMP. When used as an alternate-substrate inhibitor, 6-thioIMP, as previously reported [18], was also shown to be a non-progressive competitive inhibitor with a K_i value approximately equal to its K_m value. Methylation of the thio group of 6-thioIMP caused a 20-fold decrease in binding relative to 6-thioIMP (Figure 2). Although not a substrate, 1-Alo-5′-P inhibited the reaction with a K_i value of the same order of magnitude as that of XMP and GMP. This is in agreement with the recent report that 1-Alo-5′-P inhibits the human IMP dehydrogenase [7].

The pyrazolo(3,4-d)pyrimidine nucleotide analogs were bound less tightly than the corresponding purine nucleotides while the 8-azapurine nucleotide analogs were bound more tightly. DeoxyXMP was a better inhibitor than araXMP* whereas the converse was observed with the guanine nucleotides (i.e., araGMP bound more tightly than dGMP).

DISCUSSION

A number of IMP analogs were found to serve as substrates for IMP dehydrogenase. By using the value V_{max}/K_m (Table 1) as a measure of the substrate efficiency [14] of the various IMP analogs the following order of substrate activity was obtained, IMP > 8-azaIMP > araIMP = dIMP = 6-thioIMP.

*Contained 6% arabinosylxanthine (see Materials and Methods). In a separate experiment the rate of IMP oxidation (0.04 mM IMP) was unaffected by 0.2 mM arabinosylxanthine.

Table 2. Interactions of xanthylate and guanylate analogs with IMP dehydrogenase

Xanthylate analog	K_i (mM)	Guanylate analog	K_i (mM)
XMP	0.60*	GMP	0.30*
8-AzaXMP	0.17	8-AzaGMP	0.18*
6-ThioXMP	0.25	6-ThioGMP	Prog. Inhib.*†
AraXMP	3	AraGMP	0.20
dXMP	0.11	dGMP	2.5*
1-Oxi-5′-P	1.4	6-NH_2-1-Alo-5′-P	0.49
7-Oxi-5′-P	1.2		

* Data confirming that previously reported [18].

† Confirmed in an experiment in which the conditions and results were identical to those previously published by Miech *et al.* [24].

8-AzaIMP, a metabolic product reported in H.Ep. No. 2 cells treated with either 8-azainosine [25] or 8-azaadenosine [26], was found to have a substrate efficiency of 1/3 that of IMP suggesting that the *in vivo* formation of 8-azaXMP is possible. This inference is further supported by the findings of Montgomery that 8-azaIMP is metabolized to 8-azaGMP in H.Ep. No. 2 cells [27]. Although a metabolite of 8-azaguanine has been reported to cause a 70% decrease in the metabolic conversion of IMP to XMP [28], the significance of the possible inhibition of IMP dehydrogenase *in vivo* by either 8-azaXMP and/or 8-azaGMP must await the quantitative determination of the intracellular concentrations of these compounds relative to the concentration of IMP.

Low concentrations (0.001–5 μM) of 1-Alo-5′-P, 1-Oxi-5′-P, and 7-Oxi-5′-P have been reported *in vivo* in rats after high doses of allopurinol [29], a potent inhibitor of xanthine oxidase. The inability to detect any IMP dehydrogenase mediated formation of 1-Oxi-5′-P (Table 1) makes it unlikely that the 1-Oxi-5′-P found *in vivo* is formed by this route. The low concentrations of 1-Alo-5′-P, 1-Oxi-5′-P and 7-Oxi-5′-P attained *in vivo*, the reported absence of any detectable amount of 6-NH_2-1-Alo-5′-P [29], and the kinetic parameters of the enzyme (Tables 1 and 2), make it unlikely that inhibition of IMP dehydrogenase by these nucleotide products of allopurinol would be of significance.

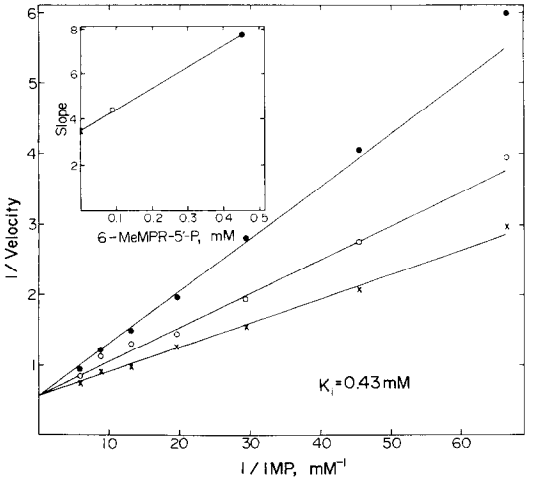

Fig. 2. 6-MeMPR-5′-P inhibition of IMP dehydrogenase-mediated oxidation of IMP. Velocities are expressed as nmoles of XMP formed/min/ml of enzyme. (×–×–×) No inhibitor, (○–○–○) 0.088 mM 6-MeMPR-5′-P, (●–●–●) 0.44 mM 6-MPR-5′-P.

Several mononucleotides are derived from 6-mercaptopurine *in vivo*: 6-thioIMP [30, 31], 6-thioXMP [15, 31, 32], 6-thioGMP [15, 31], and 6-MeMPR-5′-P [31, 33]. The observation that 6-thioXMP is formed after treatment with 6-mercaptopurine suggests that 6-thioIMP acts as a substrate for IMP dehydrogenase *in vivo*. The data in Table 1 shows that 6-thioIMP has a substrate efficiency of 1/16th that of the natural substrate, IMP. The velocity observed, 8% that of IMP, agrees with that previously reported for the enzyme from *A. aerogenes* [2]. The enzymes from *A. aerogenes* [2] and from Sarcoma 180 ascites cells [18] have been reported to be inhibited progressively by 6-thioGMP while 6-thioIMP has been shown to give normal competitive inhibition kinetics. This raises the question as to whether 6-thioXMP, which accumulates after 6-mercaptopurine administration, might also, like 6-thioGMP, be a progressive inhibitor. In the present study 6-thioXMP did inhibit IMP dehydrogenase but gave non-progressive competitive kinetics with a K_i 3-fold lower than that of XMP.

Intracellular concentrations of 6-MeMPR-5′-P as high as 2 mM have been observed *in vivo* after administration of 6-MeMPR [34]. The present studies show 6-MeMPR-5′-P to be an inhibitor of IMP dehydrogenase with a K_i value of 0.43 mM (Figure 2). In studies involving the mode of action of 6-MeMPR, Shantz *et al.* [35] have suggested that while 6-MeMPR-5′-P appears to be an inhibitor of the IMP dehydrogenase from Ehrlich ascites cells this inhibition is probably of minor significance *in vivo*. This is supported by the findings of Hill and Bennett [36] that the first enzyme of *de novo* purine biosynthesis, PP-ribose-P amidotransferase, is inhibited by 6-MeMPR-5′-P with a K_i value of 9×10^{-7} M.

Brink and LePage [37] reported that araH might be metabolized to both araA and araG nucleotides in livers of BAF_1 mice. They suggested that the araG nucleotides could be formed from araIMP by oxidation to araXMP and subsequent amination to araGMP. The present study shows that araIMP is indeed a substrate for IMP dehydrogenase with a substrate efficiency of 1/16th that of IMP.

The substrate efficiencies reported here for the oxidation of IMP analogs are useful, when considered in conjunction with the efficiency values reported for

the amination of XMP analogs [14, 38], in explaining many of the earlier metabolic investigations with analogs of hypoxanthine and inosine.

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