Substrate Binding Specificity and Properties of Inosine Monophosphate:Pyrophosphate Phosphoribosyltransferase (EC 2.4.2.8) from Brewers Yeast*

Richard L. Miller† and Allan L. Bieber

ABSTRACT: The substrate binding specificity of inosine monophosphate:pyrophosphate phosphoribosyltransferase from brewers yeast has been studied with 46 purine analogs.

It was found that the following structural features are required for purine binding: (1) an intact purine ring, (2) an exocyclic double bond in the 6 position of the purine ring, (3) a single bond between ring carbon 2 and substituents on this site, and (4) N-7 must be the most basic nitrogen in the purine ring. Guanine, hypoxanthine, 6-mercaptopurine, 8-azaguanine, and 2-amino-6-mercaptopurine were the only compounds found to be either substrates or inhibitors of guanosine monophosphate or inosine monophosphate synthesis. All substrates except 8-azaguanine were shown to be competitive inhibitors of guanosine monophosphate and inosine monophosphate synthesis. Michaelis constants and inhibition constants with respect to both guanosine monophosphate and inosine monophosphate synthesis were determined for these compounds. Arrhenius plots exhibit a biphasic character for guanine, 2-amino-6-mercaptopurine, and 8-azaguanine with transition temperatures of 19, 30, and 38°, respectively. Hypoxanthine does not show this biphasic character. In all cases except that of hypoxanthine the higher activation energies were found at the higher temperatures. Evidence is presented which supports the concept of a single enzyme catalyzing both inosine monophosphate and guanosine monophosphate synthesis and a temperature-dependent conformational change in the enzyme.

The enzyme inosine monophosphate:pyrophosphate phosphoribosyltransferase, first observed by Kornberg *et al.* (1955), catalyzes the condensation of hypoxanthine or guanine with PRPP[1] to form IMP or GMP. It has been partially purified and studied from a number of different sources: beef liver (Korn *et al.*, 1955), erythrocytes (Preiss and Handler, 1957; Kelley *et al.*, 1967; Henderson *et al.*, 1968), Ehrlich ascites tumor cells (Atkinson and Murray, 1965), and brewers yeast (Kornberg *et al.*, 1955; Miller and Bieber, 1968a). Partial separation of the IMP synthesis from the GMP synthesis by electrophoresis has been reported in the above-mentioned tumor system. Adye and Gots (1966), using enzyme preparations from two strains of *Salmonella typhimurium*, suggest the possibility of two separate proteins being responsible for the IMP and GMP pyrophosphorylase activities in this organism. However, evidence obtained with the enzymes from human erythrocytes (Henderson *et al.*, 1968) and brewers yeast (Miller and Bieber, 1968a) support a single enzyme catalyzing both IMP and GMP synthesis. In a recent paper concerning the mechanism of action of this enzyme from human erythrocytes Henderson *et al.* (1968) have reported that the synthesis of IMP and GMP occurs by an ordered sequence of substrate addition with the formation of ternary enzyme complexes.

In this article we describe the activity of brewers yeast inosine monophosphate:pyrophosphate phosphoribosyltransferase with 46 purines and related compounds. From these studies it has been possible to determine the structural properties required for the binding of the purine substrate or inhibitor to the active site. These data also suggest that a single enzyme catalyzes the synthesis of both IMP and GMP. A preliminary report of this work has been published (Miller and Bieber, 1968b).

Materials

Guanine-8-^{14}C and hypoxanthine-8-^{14}C were purchased from Schwarz BioResearch. 2,5-Diphenyloxazole and 1,4-bis[2-(5-phenyloxazolyl)]benzene were obtained from New England Nuclear Corp. Microcrystalline cellulose was obtained from the American Viscose Co. The sources of all other materials are as previously reported (Miller and Bieber, 1968a).

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† Present address: The Wellcome Research Laboratories, Burroughs Wellcome and Co., Inc., Tuckahoe, N. Y. 10707.

[1] The abbreviation used in this paper that is not defined in *Biochemistry 5*, 1445 (1966), is PRPP, 5-phosphorylribose 1-pyrophosphate.

Method

Inosine monophosphate:pyrophosphate phosphoribosyltransferase was prepared as described by Miller and Bieber (1968a). All studies reported in this paper were carried out with fraction IV which contained 10^{-3} M EDTA. The concentration of EDTA in the reaction mixtures was 10^{-5} M. Magnesium PRPP was converted into the sodium salt by passing it through a Dowex 50 (Na^+) column.

Enzymatic Assay

Spectrophotometric Methods. GMP SYNTHESIS. The synthesis of GMP was followed as previously described (Miller and Bieber, 1968a).

ANALOG ACTIVITIES. PRPP was added to the test cell containing $MgCl_2$ (1.0 μmole), Tris (50 μmoles, adjusted to pH 6.0, 7.4, or 8.5 with HCl), enzyme, and analog in a total volume of 1.0 ml. These test solutions were read against: (1) an enzyme blank containing $MgCl_2$ (1.0 μmole), Tris (50 μmoles), and enzyme, and (2) an analog blank containing $MgCl_2$ (1.0 μmole), Tris (50 μmoles), and analog (0.1–0.5 μmole) in a Beckman DK-2 recording spectrophotometer at 22–25° at time intervals up to 1 hr. It was assumed that if no absorbance change between 225 and 340 mμ was observed within 1 hr the analog was not a substrate. For one to observe a 10% conversion of analog into nucleotide with an absorbance change of 0.025 would necessitate a molar absorbancy difference of 1×10^3.

As shown in Figure 1, the addition of the enzyme to a reaction mixture containing 2-amino-6-mercaptopurine resulted in a progressive change in the absorption spectrum of the solution with time. The maximum absorbance change was found at 255 mμ at pH 8.5. Very little absorbance change was observed when the reaction was run at pH 7.4. With 8-azaguanine the maximum absorbance change was found at 259 mμ at pH 6.0, with no change in the absorbance of the pH 7.4 sample.

Radioactive Methods. Incubation mixtures contained Tris-HCl buffer (5 μmoles, pH 7.4), $MgCl_2$ (0.1 μmole), PRPP, and radioactive purine base in a total volume of 100 μl as described by Henderson *et al.* (1968). After incubation for 5 min at 25° the reaction was terminated by addition of 20 μl of 4 N formic acid. A 100-μl aliquot of the stopped reaction mixture was placed on Whatman No. 3MM paper with 0.1 μmole of the appropriate carrier ribonucleotide. The reaction product was separated from the substrate by ascending chromatography in 1 M ammonium acetate (pH 7.5)–95% ethanol (3:7). The reaction products were visualized with ultraviolet light, the spots were cut out, and the paper disks were counted in a Nuclear-Chicago Model 722 ambient liquid scintillation counter using a toluene scintillation solution containing 2,5-diphenyloxazole (4.0 g/l.) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (0.05 g/l.). Initial velocity measurements were used in all experiments reported in this paper. Purity of all compounds tested as substrates or inhibitors was checked by thin-layer chromatography on microcrystalline cellulose in the following solvent systems: (1) saturated $(NH_4)_2SO_4$–1 M sodium acetate–isopropyl alcohol (80:18:2), (2) *t*-amyl alcohol–90% formic acid–water (3:2:1), and (3) *n*-butyl alchol–acetone–glacial acetic acid–5% NH_4OH–water (7:5:3:3:2).

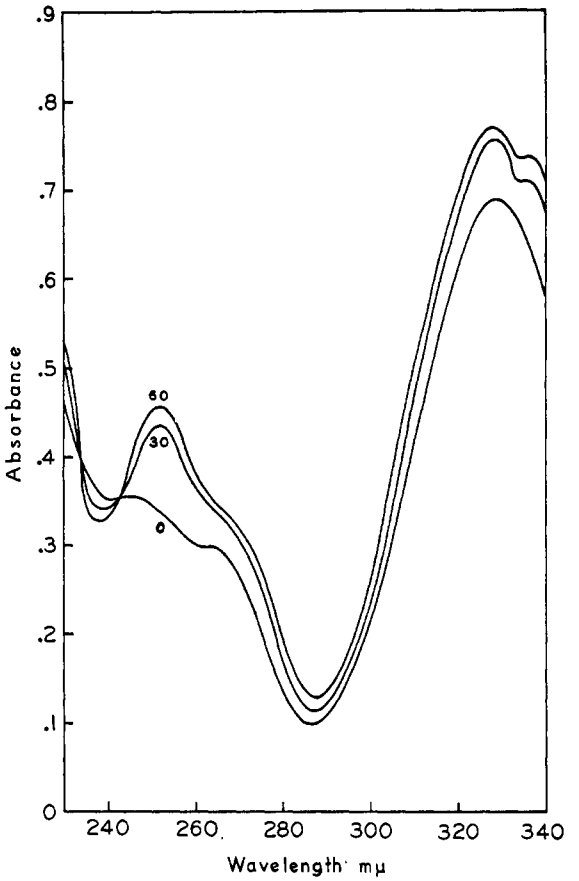

FIGURE 1: Effect of IMP pyrophosphorylase on 2-amino-6-mercaptopurine. At zero time PRPP (0.6 μmole) was added to a solution containing 10^{-3} M $MgCl_2$ and 4.6×10^{-5} M 2-amino-6-mercaptopurine in 0.05 M Tris buffer (pH 8.5) at 23°. The absorption spectrum was recorded against a buffer plus enzyme blank at the times (in minutes) indicated on the figure.

Results

Michaelis Constants for Substrates. The Michaelis constants for the purines found to be substrates are listed in Table I. In the case of 6-mercaptopurine, which has been shown to be a substrate for inosine monophosphate: pyrophosphate phosphoribosyltransferase from a number of different sources, the reaction was too slow to allow the determination of a reliable Michaelis constant by the method of Carter (1959). The Michaelis constants for 2-amino-6-mercaptopurine and 8-azaguanine were determined at pH 8.5 and 6.0, respectively, since these are the pH values at which they were found to have their maximal reaction rates as determined by the spectral assay. Note that in Figure 2 the velocity of the enzyme reaction is in terms of absorbancy change per minute instead of moles of product per minute. This is due to the fact that the difference between the molar absorptivity of substrate and product is not known for these reactions. Also listed in Table I are the K_m values for

TABLE I: K_m of Substrate and K_i of Inhibitors at 25° and pH 7.4.

Compound	K_m	K_i (Guanine)[a]	K_i (Hypoxanthine)[b]
Guanine	7.2×10^{-6}[c]		6.8×10^{-6}
Hypoxanthine	1.8×10^{-5}[c]	1.8×10^{-5}	
6-Mercaptopurine	*d*	2.4×10^{-5}	2.5×10^{-5}
2-Amino-6-mercaptopurine	1.8×10^{-5}[e]	1.8×10^{-5}	6.8×10^{-7}
8-Azaguanine	8.7×10^{-5}[f]		
PRPP	4.2×10^{-5}[g]		

[a] K_i with respect to guanine determined by the spectrophotometric assay. [b] K_i with respect to hypoxanthine determined by the radioactive assay. [c] Miller and Bieber (1968a). [d] Reacts too slowly to measure by the method of Carter (1959). [e] pH 8.5. [f] pH 6.0. [g] For either GMP or IMP synthesis.

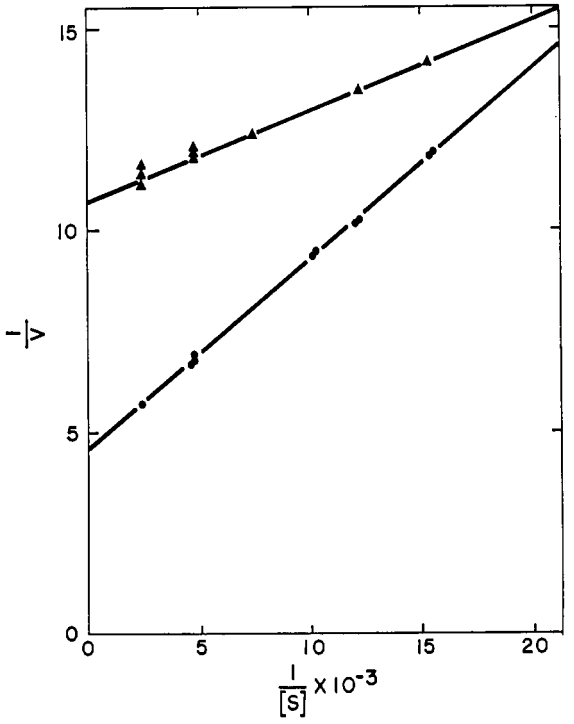

FIGURE 2: Nucleotide synthesis as a function of 8-azaguanine and 2-amino-6-mercaptopurine concentration at 25°, 10^{-3} M $MgCl_2$, and saturating PRPP concentration (0.6 μmole). Reactions were run at pH 6.0 for 8-azaguanine and pH 8.5 for 2-amino-6-mercaptopurine. The substrate concentrations are measured in moles per liter. Velocities are expressed as change in optical density per minute. (●–●–●) 8-Azaguanine and (▲–▲–▲) 2-amino-6-mercaptopurine.

PRPP determined by the radioactive assay procedure. Note that for both GMP and IMP synthesis the K_m values are identical, 4.2×10^{-5} M.

Inhibition of GMP and IMP Synthesis by Purine Analogs. Table I also contains a list of the inhibition constants found for various purines. Note that 8-azaguanine was a substrate at pH 6.0 but had no effect on GMP synthesis or IMP synthesis at pH 7.4. A possible explanation of the 8-azaguanine behavior is that it has a pK_a of 6.4 whereas the corresponding pK_a values for guanine and hypoxanthine are 9.2 and 8.9, respectively. If IMP–GMP pyrophosphorylase reacts only with the neutral species of the purine base and not the monoanion, then one would expect that 8-azaguanine would be a very poor substrate at pH 7.4. It should be noted that when either hypoxanthine or guanine was the substrate, the inhibition constants and the Michaelis constants for each of the various purine substrates were essentially identical except for 2-amino-6-mercaptopurine. In each case the inhibition was found to be competitive, as can be seen in Figures 3 and 4.

Since evidence previously reported (Miller and Bieber, 1968a) and evidence reported in this paper support the existence of one enzyme catalyzing both IMP and GMP

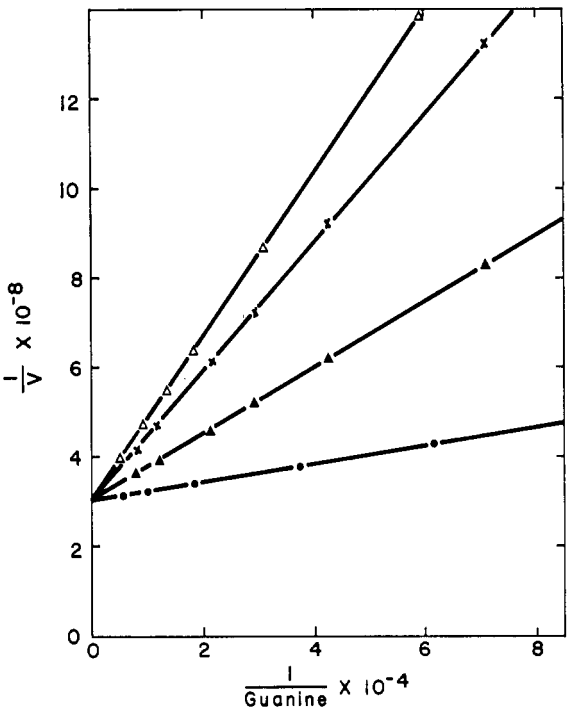

FIGURE 3: Effect of purines on GMP synthesis at pH 7.4, 25°, and saturating PRPP concentration (0.6 μmole). Concentrations were 1.3×10^{-5} M 2-amino-6-mercaptopurine, 9.8×10^{-5} M hypoxanthine, and 6×10^{-5} M 6-mercaptopurine. The substrate concentrations are measured in moles per liter. Velocities are expressed as moles of GMP synthesized per minute. (●–●–●) No inhibitors, (△–△–△) 2-amino-6-mercaptopurine, (X–X–X) hypoxanthine, and (▲–▲–▲) 6-mercaptopurine.

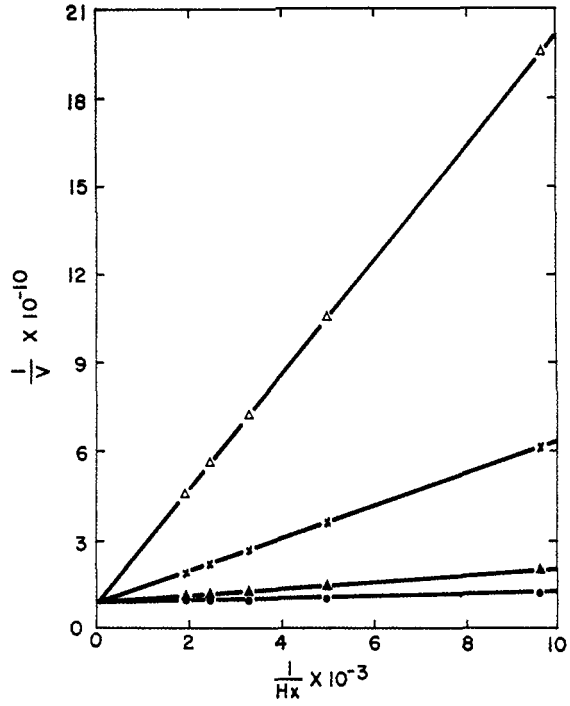

FIGURE 4: Effect of purines on IMP synthesis at pH 7.4, 25°, and saturating PRPP concentration. Concentrations were 9.7×10^{-6} M 2-amino-6-mercaptopurine, 1.5×10^{-4} M guanine, and 2.8×10^{-4} M 6-mercaptopurine. The substrate concentrations are measured in moles per liter. Velocities are expressed as moles of IMP synthesized per minute. (●-●-●) No inhibitor, (△-△-△) 2-amino-6-mercaptopurine, (X-X-X) guanine, and (▲-▲-▲) 6-mercaptopurine.

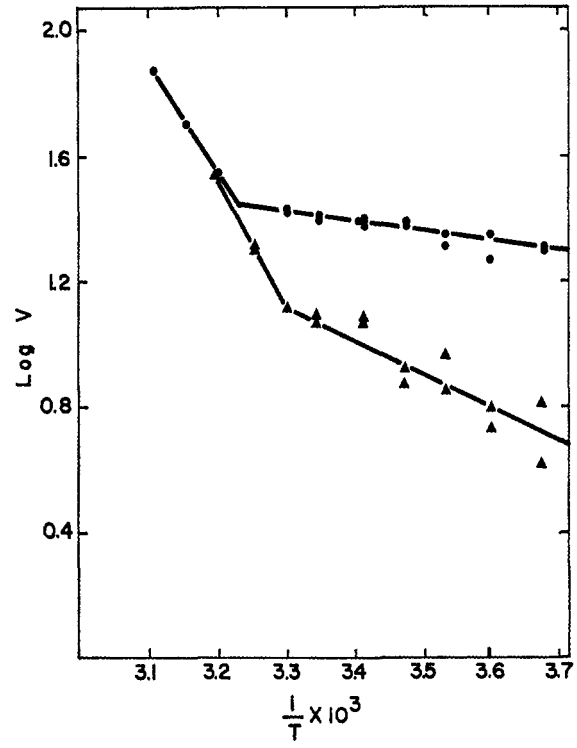

FIGURE 5: Effect of temperature on 8-azaguanine ribonucleotide and 2-amino-6-mercaptopurine ribonucleotide synthesis. Reactions for 8-azaguanine were carried out at pH 6.0. Reactions for 2-amino-6-mercaptopurine were carried out at pH 8.5. Enzyme assays were conducted at temperatures between 0 and 50°. (●-●-●) 8-Azaguanine ribonucleotide synthesis and (▲-▲-▲) 2-amino-6-mercaptopurine ribonucleotide synthesis.

synthesis, only those purine analogs which were shown to have an effect on the GMP synthesis reaction were checked for inhibition of IMP synthesis. A complete listing of the purines utilized and the sources of these analogs can be found in Miller (1968).

Effect of Temperature on Enzyme Activity. The effect of temperature on the enzyme activity with the various substrates is illustrated in Figures 5 and 6. Figure 5 shows that when either 8-azaguanine or 2-amino-6-mercaptopurine is the substrate, the curve is biphasic with transitions occurring at 38 and 30°, respectively. The calculated activation energies for 8-azaguanine are 1600 cal/mole below the transition temperature and 16,000 cal/mole above the transition temperature; for 2-amino-6-mercaptopurine they are 5000 cal/mole below and 16,000 cal/mole above the transition temperature. With guanine as substrate at pH 7.4 and p 8.5 the curve is biphasic with a transition temperature at 19°, as illustrated in Figure 6. At pH 7.4 the activation energies are 5700 cal/mole below the transition temperature and 11,600 cal/mole above the transition temperature. At pH 8.5 the values are 5400 cal/mole below and 8800 cal/mole above the transition temperature.

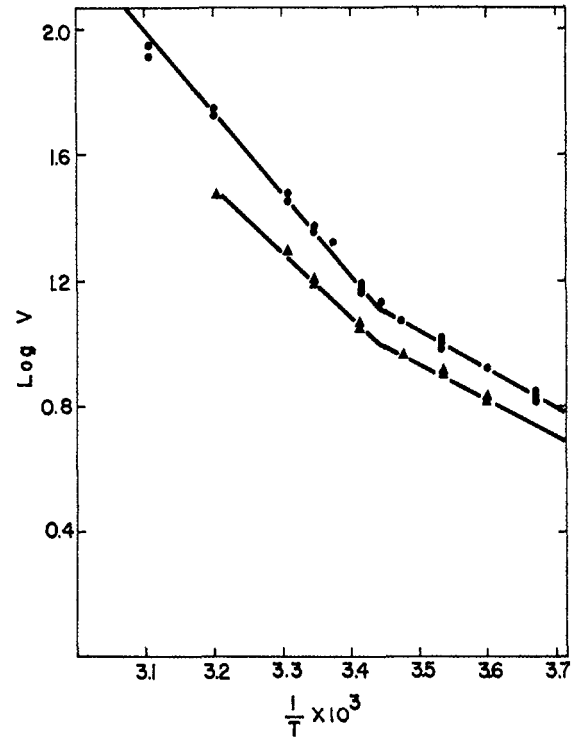

FIGURE 6: Effect of temperature and pH on GMP synthesis. Enzyme assays were conducted at temperatures between 0 and 50°. (●-●-●) GMP synthesis at pH 7.4 and (▲-▲-▲) GMP synthesis at pH 8.5.

Discussion

Evidence for the existence of a single enzyme in brewers yeast catalyzing GMP synthesis and IMP synthesis by the condensation of guanine or hypoxanthine with PRPP has been reported (Miller and Bieber, 1968a). Additional

evidence for the existence of a single enzyme and its substrate binding specificity is reported in this paper.

From the data in Table I and from the analog studies it is possible to determine the structural properties required for substrate binding to the active site. The substrate must have an intact purine ring since pteridines, pyrimidines, pyrazolo[3,4-*d*]pyrimidines, and imidazole compounds were neither substrates nor inhibitors. It has been reported (Flaks *et al.*, 1957) that 4-amino-5-imidazolecarboxamide was a substrate and a competitive inhibitor of adenosine monophosphate:pyrophosphate phosphoribosyltransferase. A possible explanation for 4-amino-5-imidazolecarboxamide showing no effect on GMP synthesis can be seen by examination of space-filling models. Comparison of models shows that it is possible for the amino function of the carboxamide group which would correspond to the 6-amino group of adenine, to lie in the same plane as the imidazole ring similar to the situation found in the planar structure of adenine. However, steric hinderence between the 4-amino group of the imidazole compound and the amino function of the carboxamide prevents the carbonyl function of the carboxamide group, which would correspond to the 6-oxo function of the purine substrates, from residing in the plane of the imidazole ring as is found in hypoxanthine and guanine. Imidazole-4-carboxylic acid also failed to inhibit GMP synthesis. Although the carbonyl function of the carboxylic acid group can lie in the same plane as the imidazole ring the negative charge of the ionized carboxylic acid group which would be present at the pH of the reaction might explain why imidazole-4-carboxylic acid is neither a substrate nor inhibitor of GMP synthesis. Attempts to synthesize imidazole-4-carboxamide by the method of Weidenhagen and Wegner (1937) have failed. Examination of a space-filling model of this compound demonstrates that the amino function and the carbonyl function of the carboxamide group can lie in the same plane as the imidazole ring and thus one might predict that this compound would be a substrate or inhibitor for GMP synthesis.

All compounds found to be substrates or inhibitors possess an exocyclic double bond in the 6 position of the purine ring and a single-bonded group on C-2 of the purine ring. Compounds which have either a substituted oxo or mercapto group or an amino group in in the 6 position such as 6-methoxypurine, 6-methylmercaptopurine, or 2,6-diaminopurine or which have no substitutent in the 6 position such as purine or 2-aminopurine cannot act as substrates or inhibitors. The importance of the single-bonded substitutent is demonstrated by the fact that 2-hydroxypurine, xanthine, and 2-thioxanthine did not function as substrates or inhibitors. In each of these compounds either an oxygen or sulfur group in which a double bond is most probable, is found in the 2 position of the purine. If this is the reason that these compounds do not bind to the active site the importance of the 2,3 region of the ring system could be verified by imidazole-4-carboximide. Nucleotide inhibition studies are in agreement with the structural properties required for purine binding (R. L. Miller and A. L. Bieber, 1968, unpublished data, manuscript in preparation).

From the studies of methylated guanines and hypoxanthines it became apparent that some of the nitrogens of the purine ring must be involved in binding. The following compounds were completely inactive as substrates or inhibitors of GMP synthesis: 1-methyl-, N^2-dimethyl-, 3-methyl-, 7-methyl-, or 9-methylguanine, 1-methyl-, 3-methyl-, 7-methyl-, or 9-methylhypoxanthine. Since neither guanine-*N*-hydroxide nor 4-hydroxypyrazolo-[3,4-*d*]pyrimidine affected GMP synthesis, the importance of N-7 is apparent. Table II contains a list of some of the purines studied, both substrates and nonsubstrates, and the most basic nitrogen in the purine ring. It is interesting to note that in all of the compounds which act as substrates or inhibitors the N-7 is the most basic. In all of the compounds which do not act as substrate or inhibitor, the N-7 is not the most basic. The exceptions to this are xanthine and 2-hydroxypurine. It should be noted that in both exceptions a double-bond exocyclic to the purine ring is found on C-2 thus explaining their lack of activity as substrate or inhibitor.

TABLE II: Ring Nitrogen Basicity *vs.* Activity.

Compound	Active	Most Basic Nitrogen[a]
Guanine	+	7
Hypoxanthine	+	7
6-Mercaptopurine	+	7
2-Amino-6-mercaptopurine	+	7
8-Azaguanine	+	7
Adenine	−	1
Purine	−	1
2,6-Diaminopurine	−	1
6-Methylpurine	−	1
2-Aminopurine	−	1
2-Hydroxypurine	−	7
2-Hydroxy-6-aminopurine	−	1
Xanthine	−	7
2-Amino-4-hydroxypyrimidine[b]	−	1
Pyrimidine[b]	−	1 = 3
Pyrazolo[3,4-*d*]pyrimidines[b]	−	3

[a] From Lister (1966), Pullman and Pullman (1963), Katritzky and Lagowski (1963), Albert (1963), and Ronca and Zucchelli (1968). [b] Numbered as if purines.

Table I lists the Michaelis constants and the inhibition constants found for the various purines. It should be noted that in all cases except that of 2-amino-6-mercaptopurine the Michaelis constants are essentially identical with the inhibitor constants. It can be seen that the addition of an amino group to the 2 position of the purine ring decreases the K_m by a factor of approximately 3. The replacement of the number 6-oxo group with a mercapto group decreases the K_m by a factor of 1.3–2.5.

The data presented in Figure 5 raises the question whether the temperature at which the break occurs in the Arrhenius plot is due to the pH at which the reactions are run or whether it is characteristic of the sub-

strate with which the enzyme is reacting. Figure 6 shows that when guanine is substrate at either pH 7.4 or 8.5, the break in the Arrhenius plot occurs at 19°. From the data in Figures 5 and 6 it can be concluded that the pH affects the activation energies and the substrate present determines the temperature at which the break in the Arrhenius plot occurs. One explanation of these data is the possibility of a conformational change in the enzyme with the conformations above and below the transition temperature having different energies of activation. This is somewhat analogous to the situation observed by Massey *et al.* (1966) for D-amino acid oxidase. It is interesting to note that the only substrate which does not have a biphasic Arrhenius plot over the temperature range of 0–50° is hypoxanthine (Miller and Bieber, 1968a). Hypoxanthine is also the only substrate which lacks an amino group in the 2 position of the purine ring causing one to suspect that the presence of the 2-amino group might be necessary for the stabilization of the enzyme in the low-activation energy form.

A comparison of the K_i values for 2-amino-6-mercaptopurine with guanine as substrate and with hypoxanthine as substrate is interesting (Table I). A possible explanation of these data is that in the presence of guanine and 2-amino-6-mercaptopurine the enzyme is held in the high-activation energy form by the presence of guanine at 25°. In the presence of hypoxanthine and 2-amino-6-mercaptopurine the conformation of the enzyme may be dictated by the 2-amino-6-mercaptopurine. Thus the low-activation energy conformation would be present at 25°. If the low-activation energy form of the enzyme reacts with the hypoxanthine more slowly or not at all a significant lowering of the K_i might be expected as is shown in Table I. Studies of the variation of the K_m and K_i for the various substrates at different temperatures are currently in progress in this laboratory. Preliminary evidence which shows that the K_m values on each side of the temperature break in the Arrhenius plot differ by a significant amount is presented in Table III. These data support the idea that the temperature break in the Arrhenius plot might be attributed to a conformational change in the enzyme such that the two conformations have different K_m values. The effect of temperature on the binding constants of the various purine substrates should help clarify the problem concerning the existence of two or more conformations of the enzyme.

TABLE III: Effect of Temperature on K_m.

Compound	Temp (°C)	K_m (M)
Guanine	10	3.8×10^{-6}
	25	7.2×10^{-6}
	37	6.8×10^{-6}
2-Amino-6-mercaptopurine	25	1.8×10^{-5}
	37	3.8×10^{-6}

Added in Proof

Information, which has been submitted for publication by U. Wölcke and G. B. Brown of the Sloan Kettering Institute and kindly made available to us as a private communication, shows that guanine is oxidized to the 3-*N*-oxide and not to the 7-*N*-oxide as previously reported. Thus it is probable that the commercial preparation of guanine 7-*N*-oxide used in the work reported in this paper is, in fact, guanine 3-*N*-oxide.

Acknowledgments

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References

Adye, J. E., and Gots, J. S. (1966), *Biochim. Biophys. Acta 118*, 344.

Albert, A. (1963), *in* Physical Methods in Heterocyclic Chemistry, Vol. 1, Katritzky, A. R., New York, N. Y., Academic, p 1.

Atkinson, M. R., and Murray, A. W. (1965), *Biochem. J. 94*, 64.

Carter, C. E. (1959), *Biochem. Pharmacol. 2*, 105.

Flaks, J. G., Erwin, M. J., and Buchanan, J. M. (1957), *J. Biol. Chem. 228*, 201.

Henderson, J. F., Brox, L. W., Kelley, W. N., Rosenbloom, F. M., and Seegmiller, J. E. (1968), *J. Biol. Chem. 243*, 2514.

Katritzky, A. R., and Lagowski, J. M. (1963), *Advan. Heterocyclic Chem. 2*, 27.

Kelley, W. N., Rosenbloom, F. M., Henderson, J. F., and Seegmiller, J. E. (1967), *Biochem. Biophys. Res. Commun. 28*, 340.

Korn, E. D., Remy, C. N., Wasilejko, H. C., and Buchanan, J. M. (1955), *J. Biol. Chem. 217*, 875.

Kornberg, A., Lieberman, A., and Simms, E. S. (1955), *J. Biol. Chem. 215*, 417.

Lister, J. H. (1966), *Advan. Heterocyclic Chem. 6*, 1.

Massey, V., Curti, B., and Ganther, H. (1966), *J. Biol. Chem. 241*, 2347.

Miller, R. L. (1968), Ph.D. Dissertation, Arizona State University, Tempe, Ariz.

Miller, R. L., and Bieber, A. L. (1968a), *Biochemistry 7*, 1420.

Miller, R. L., and Bieber, A. L. (1968b), *Abstr., Pacific Slope Biochem. Conf. Santa Barbara, Calif., Sept*, 30.

Preiss, J., and Handler, P. (1957), *J. Biol. Chem. 225*, 759.

Pullman, B., and Pullman, A. (1963), Quantum Biochemistry, New York, N. Y., Interscience, p 230.

Ronca, G., and Zucchelli, G. (1968), *Biochim. Biophys. Acta 159*, 203.

Weidenhagen, R., and Wegner, H. (1937), *Ber. 70*, 2309.