Singh, L. H., and Lane, B. G. (1964b), *Can. J. Biochem. 42*, 1011.
Smith, J. D., and Dunn, D. B. (1959), *Biochim. Biophys. Acta 31*, 573.
Tong, G. L., Lee, W. W., and Goodman, L. (1967), *J. Org. Chem. 32*, 1984.
Townsend, L. B., Robins, R. K., Loeppky, R. N., and Leonard, N. J. (1964), *J. Am. Chem. Soc. 86*, 5320.
Wolfenden, R., Rammler, D. H., and Lipmann, F. (1964), *Biochemistry 3*, 329.

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

Richard L. Miller and Allan L. Bieber

ABSTRACT: Inosine monophosphate (IMP):pyrophosphate phosphoribosyltransferase from brewers yeast has been purified 234-fold over the original high-speed supernatant by heat denaturation, ammonium sulfate fractionation, DEAE-cellulose chromatography, and hydroxylapatite chromatography. IMP synthesis parallels guanosine monophosphate (GMP) synthesis over the entire purification range. Considerable denaturation of the enzyme occurs below pH 5.2 and above pH 10.

A broad pH optimum for activity with guanine between pH 7.0 and 8.0 and for hypoxanthine activity between pH 8.0 and 9.0 was observed. Both activities require the presence of a divalent metal ion. Optimal Mg^{2+} concentration for both activities is 1×10^{-3} M. The K_m values at pH 7.4 and 25° are 7.7×10^{-6} M for guanine, 1.8×10^{-5} M for hypoxanthine, 2.4×10^{-5} M for 5-phosphorylribose 1-pyrophosphate (PRPP) in the presence of guanine, and 4.2×10^{-5} M for PRPP in the presence of hypoxanthine. The apparent activation energies, determined by an Arrhenius plot, are 11,600 cal/mole for hypoxanthine as substrate and 5700 cal/mole below 19° and 11,300 cal/mole above 19° for guanine as substrate. Evidence is presented which supports the concept of a single enzyme catalyzing both IMP and GMP synthesis.

The direct conversion of hypoxanthine into IMP[1] was first recognized by Williams and Buchanan (1953). Pigeon liver extracts were demonstrated to be capable of forming IMP in a reaction involving hypoxanthine, ribose 5-phosphate, and ATP without the participation of inosine as an intermediate. It was later shown (Kornberg *et al.*, 1954) that ribose 5-phosphate was phosphorylated by ATP to form PRPP which reacted with hypoxanthine to form IMP. This general class of enzymes, the purine nucleotide pyrophosphorylases, was first described by Kornberg *et al.* (1955) who showed that an extract of dried brewers yeast contained purine nucleotide pyrophosphorylase activity with adenine, hypoxanthine, and guanine as substrates.

An enzyme has recently been extensively purified from Ehrlich ascites tumor cells (Hori and Henderson, 1966) which catalyzed the condensation of adenine and PRPP to form AMP. Another pyrophosphorylase system has been partially purified from beef liver (Korn *et al.*, 1955), red blood cells (Preiss and Handler, 1957), Ehrlich ascites tumor cells (Atkinson and Murray, 1965), and brewers yeast (Kornberg *et al.*, 1955). This enzyme system catalyzes a condensation reaction of hypoxanthine or guanine with PRPP to form IMP or GMP. A partial separation of the hypoxanthine activity from the guanine activity in the above-mentioned tumor system has been achieved by the use of electrophoresis. Littlefield (1964) has reported different cell lines derived from a single strain in which the ratio of IMP to GMP pyrophosphorylase activity is altered. Adye and Gots (1966) using *Salmonella typhimurium* obtained a variety of altered forms of the enzyme from mutant strains of this organism. In some cases partial loss of the hypoxanthine activity with retention of all the guanine activity was observed while in other cases partial loss of the guanine activity and retention of all of the hypoxanthine activity was reported.

The still-unresolved problem of the number of specific purine nucleotide pyrophosphorylases which exist and the related question of the specificity of purine nucleotide pyrophosphorylases with respect to purine sub-

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

strate makes it essential to purify these enzymes. Use of purified enzymes should permit the study of the specificity of these reactions in detail.

In this article we describe the extensive purification of IMP pyrophosphorylase (EC 2.4.2.8, inosine monophosphate:pyrophosphate phosphoribosyltransferase) from brewers yeast, together with some of its properties. A preliminary report of some of the data has been published (Miller and Bieber, 1967).

Materials

Dried brewers yeast was obtained from Anheuser-Busch, Inc., St. Louis, Mo. Hypoxanthine, guanine, Tris, hydroxylapatite, 5′-GMP, and EDTA were purchased from California Corp. for Biochemical Research. Xanthine oxidase was obtained from Worthington Biochemical Corp. Sephadex G-25 (coarse) and Blue Dextran 2000 were obtained from Pharmacia Fine Chemicals, Inc. PRPP was purchased from P-L Biochemicals, Inc. Bovine serum albumin was obtained from Sigma Chemical Co. All other materials used were of reagent grade.

Enzyme assays were performed on a recording spectrophotometer system consisting of a M4QIII Zeiss monochromator with Zieler sample positioner, Gilford absorbance meter, and a circulating constant-temperature bath.

Methods

Spectrophotometric Methods. GMP SYNTHESIS. The enzyme was added to blank and test cells containing $MgCl_2$ (2 μmoles), guanine (0.3 μmole), and Tris (100 μmoles, adjusted to pH 7.4 with HCl). After 2 min in the thermostatic cell holder at 25°, the reaction was started by addition of PRPP (0.5 μmole) to the test cell (final volume of cell contents, 2.0 ml). The reaction was followed by measuring the increase in absorbance at 255 mμ as described by Carter (1959). The rate of conversion of guanine into GMP at pH 7.4 was calculated by using 4.0×10^3 as the net change in molar extinction coefficient at 255 mμ. This change in extinction coefficient was obtained from the difference in absorbance of a guanine solution (1.064 μmoles/ml) and a 5′-GMP solution (1.064 μmoles/ml) at pH 7.4.

IMP SYNTHESIS. Inosine 5′-monophosphate synthesis was measured by the coupled assay of Carter (1959) using xanthine oxidase. To a solution containing $MgCl_2$ (6.0 μmoles), hypoxanthine (0.9 μmole), Tris (300 μmoles, adjusted to pH 7.4 with HCl), and enzyme, PRPP (1.5 μmoles) was added to start the reaction (final volume of solution, 6.0 ml). Aliquots (1 ml) were removed at various times and pipeted into 1.0 ml of solution containing EDTA (80 μmoles, adjusted to pH 8.0 with HCl) and sufficient xanthine oxidase to convert the hypoxanthine in the blank into uric acid in 2 min at 25°. The addition of the EDTA was found to stop the conversion of hypoxanthine into IMP instantaneously. The amount of hypoxanthine converted into IMP was assumed to be equal to the difference between the amount of uric acid produced in the test sample and a zero-time sample. The amount of uric acid produced by this method in a blank sample lacking only PRPP was constant over the time interval of the assay.

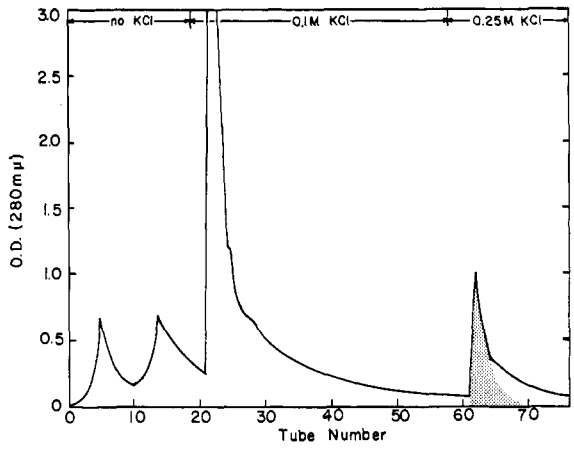

FIGURE 1: DEAE-cellulose column chromatography of GMP–IMP pyrophosphorylase. Batch elution with increasing KCl concentration in 0.01 M Tris buffer (pH 7.4) was used. Fractions of 5 ml were collected. The solid line represents the protein concentration as measured by optical density at 280 mμ. The shaded area represents enzyme activity for both IMP and GMP synthesis.

Column Assay for Either IMP or GMP Synthesis. The enzyme was added to blank and test sample containing $MgCl_2$ (2 μmoles), hypoxanthine or guanine (0.3 μmole), and Tris (100 μmoles, adjusted to pH 7.4 with HCl). After 2 min in a thermostatic bath at 25°, the reaction was started by addition of PRPP (0.5 μmole) to the test sample (final volume of blank and sample content, 2.0 ml). After an allotted amount of time the assay and blank mixtures were added to ECTEOLA-cellulose columns (1 × 3.5 cm). The free purine base was eluted with 10.0 ml of 0.001 N HCl, usually not more than 4 ml being required for complete recovery. The nucleotide was eluted with 0.1 N HCl into a 10-ml volumetric flask and the absorbance of the eluate was read at 249 mμ for IMP and at 257 mμ for GMP. To determine the amount of IMP or GMP formed, values of 11.7×10^3 and 12.2×10^3 were used for the respective extinction coefficients. This method was used only for assay of the crude fractions obtained during enzyme purification. Protein was determined by the procedure of Lowry *et al.* (1951) with crystalline bovine serum albumin as standard.

Enzyme Purification. All operations were carried out at 4° unless otherwise stated. Dried brewers yeast (100 g) was suspended in 300 ml of 0.1 M $KHCO_3$ and allowed to stand for 5–7 hr at 37°. The resulting suspension was centrifuged at 10,000*g* for 40 min. The precipitate was washed with 200 ml of 0.1 M $KHCO_3$ and centrifuged at 10,000*g* for 40 min. The original supernatant and the wash supernatant were combined and dialyzed twice against 20 l. of glass-distilled water for 24–36 hr. The dialyzed fraction was adjusted to pH 7.4 by the addition of 1.00 M potassium phosphate buffer (pH 7.4) until a final concentration of 0.033 M phosphate ion was reached (fraction I). Fraction I (460 ml) was heated to 60° in a 4-l. beaker immersed in a 74° water bath (approximately 7 min) and then placed directly into an

TABLE I: Summary of Purification of IMP Pyrophosphorylase.

Fraction	Total Protein (mg)	Total Units[e] (μmoles/min) Gu[d]	Hx[d]	Sp Act. (units/mg of protein) Gu	Hx	Yield (%) Gu	Hx	Purification Gu	Hx
I	14600	69[a,b]	458[a]	0.0047	0.031	100	100	1	1
II	5020	57[a,b]	376[a]	0.017	0.075	82	82	2.4	2.4
III	1920	45[a,b]	295[a,b]	0.023	0.139	63	64	4.9	4.5
IV[c]	204	26.3[b]	184[b]	0.128	0.90	38	40	27	29
V[c]	32.8	20.2[b]	119[b]	0.62	3.62	29	26	130	115
VI[c]	9.1	9.8[b]	60.6[b]	1.13	6.67	14	13	234	213

[a] Enzyme activity was determined by column method (see Methods section). [b] Enzyme activity was determined by appropriate spectrophotometric assay (see Methods section). [c] Fractions IV–VI are calculated on the basis of total fraction III. See text. [d] Abbreviations: Gu, GMP synthesis; Hx, IMP synthesis. [e] One enzyme unit is defined as the amount of enzyme required for the synthesis of 1 μmole of mononucleotide/min at pH 7.4 and 25°.

ice bath with intermittent stirring until the temperature dropped to 4°. This heated fraction was centrifuged at 10,000*g* for 30 min. The clear supernatant (fraction II) was adjusted to pH 6.7 with 4.4 N H_3PO_4. To each milliliter of the clear pH 6.7 supernatant 0.418 g of solid $(NH_4)_2SO_4$ was added over a period of approximately 3 hr. The suspension was then centrifuged at 37,000*g* for 30 min and the resulting precipitate was dissolved in a minimum amount of water and dialyzed against 20 l. of water overnight. Insoluble material was removed by centrifugation and discarded, leaving fraction III (~250 ml). A 10-ml aliquot of fraction III was added to a DEAE-cellulose column (10 × 200 mm) previously equilibrated with 0.01 M Tris which had been adjusted to pH 7.4 with HCl. Stepwise elution was carried out with increasing KCl concentrations buffered with 0.01 M Tris (pH 7.4) at a rate of 2 ml/min. The elution profile is presented in Figure 1. Tubes 62–64 had a 260 mμ/280 mμ absorbance ratio of less than 0.6 whereas a sharp increase in this absorbance ratio, indicating the presence of nucleic acids, was observed in subsequent fractions. The 0.25 M KCl fraction from the DEAE-cellulose column (tubes 62–64) was passed through a G-25 Sephadex column (12 × 210 mm) to remove KCl and Tris. The desalted fraction (fraction IV) was added to a hydroxylapatite column (22 × 220 mm) which had been previously equilibrated with 0.005 M potassium phosphate buffer (pH 5.4, adjusted with H_3PO_4). The column was washed with 190 ml of 0.01 M potassium phosphate buffer (pH 7.5). The enzyme was eluted with

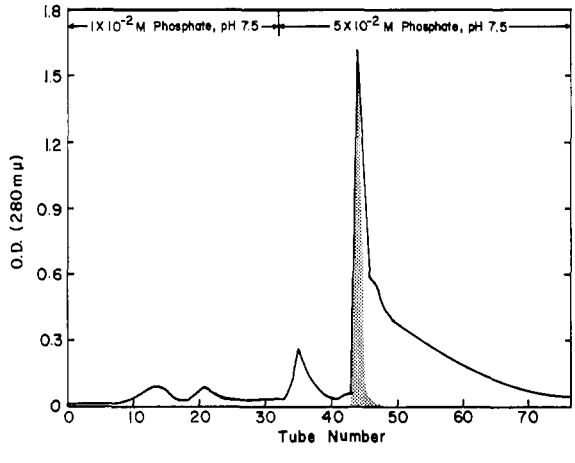

FIGURE 2: Hydroxylapatite column chromatography of GMP–IMP pyrophosphorylase. Batch elution with increasing concentrations of potassium phosphate buffer at pH 7.5 was used. Fractions of 5 ml were collected. The solid line represents protein concentration as measured by optical density at 280 mμ. The shaded area represents enzyme activity for both IMP and GMP synthesis.

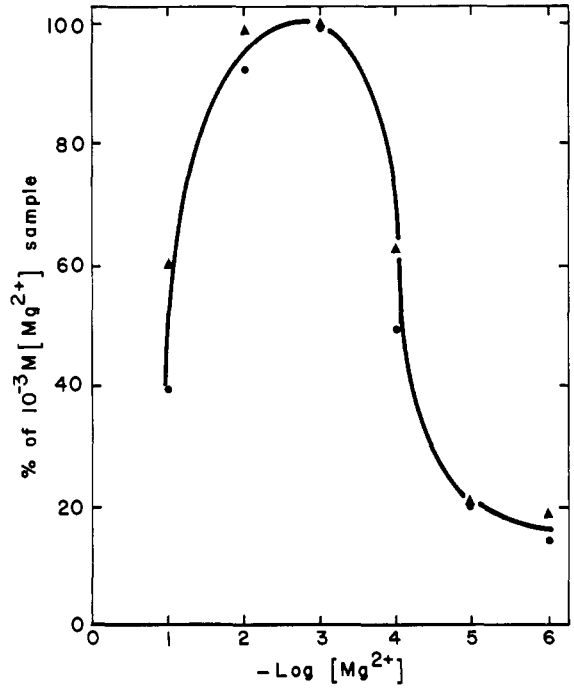

FIGURE 3: Effect of Mg^{2+} concentration on enzyme activity. The 100% value represents a synthesis of 12.3 mμmoles of GMP/min and 43.2 mμmoles of IMP/min. (●—●—●) GMP synthesis; (▲—▲—▲) IMP synthesis.

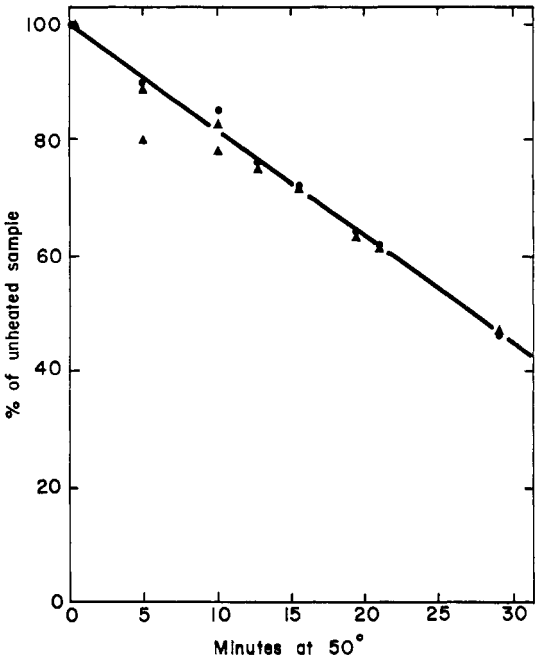

FIGURE 4: Effect of temperature on enzyme stability. Enzyme (15 ml) was heated in a 50-ml erlenmeyer flask in a constant-temperature bath at 56° until the temperature of the enzyme reached 50°. The flask containing the enzyme was then transferred to a 50° bath and 1.5-ml aliquots were withdrawn at the indicated times, cooled immediately in an ice bath, and assayed. The 100% value represents a synthesis of 13.4 mμmoles of GMP/min and 47.2 mμmoles of IMP/min. (●—●—●) GMP synthesis; (▲—▲—▲) IMP synthesis.

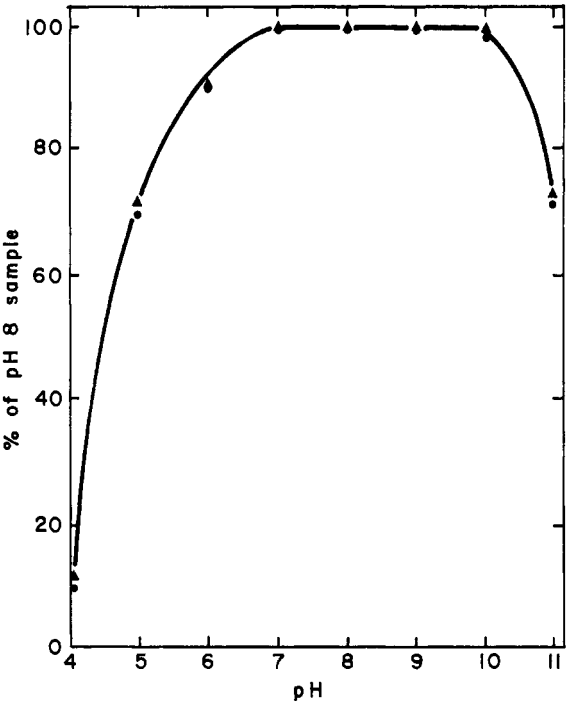

FIGURE 5: Effect of pH on enzyme stability. After a 10-min incubation at 37°, aliquots were removed and added directly to the assay mixture. The 100% value represents a synthesis of 13.2 mμmoles of GMP/min and 46.4 mμmoles of IMP/min. (●—●—●) GMP synthesis; (▲—▲—▲) IMP synthesis.

0.05 M potassium phosphate buffer (pH 7.5). The elution profile is presented in Figure 2. The fraction with the highest specific activity from the hydroxylapatite column (tube 44, fraction V, 5 ml) was dialyzed against 4 l. of 50% $(NH_4)_2SO_4$ for 6–7 hr. The suspension was centrifuged at 2000*g* for 10 min and the precipitate dissolved in 2 ml of water and desalted by passing through a G-25 Sephadex column (fraction VI).

Results

Table I summarizes the results of a typical purification using the described procedure. A 234-fold purification of GMP and IMP pyrophosphorylase activities over that found in the high-speed supernatant solution was obtained with an over-all yield of 14%. Some variation both in degree of purification and recovery of the enzyme was observed from one isolation to another.

Enzyme Storage and Stability. Fractions I and III can be stored at −20° for at least a month without appreciable loss of activity. After 3 days at −20° fraction IV loses 10% of its activity; after 5 days it loses 90% of its activity. Fractions V and VI lose 50% of their activity at −20° in 24 hr. In all cases parallel losses of IMP- and GMP-synthesizing activity were observed. Attempts to stablize fraction V to freezing with GMP, Mg^{2+}, GMP and Mg^{2+}, guanine, serum albumin, and glutathione have failed. Some evidence for a combination of PRPP and Mg^{2+} as a stabilizing agent for fraction V has been obtained but this awaits further verification. Because of the instability of the extensively purified fractions V and VI, unless otherwise indicated, fraction IV was used in all subsequent experiments.

Effect of Mg^{2+} Concentration of Enzyme Activity. Figure 3 shows the effect of Mg^{2+} concentration on IMP and GMP pyrophosphorylase activities. In the absence of Mg^{2+} neither activity was observed. The optimal Mg^{2+} concentration for both activities was shown to be 1×10^{-3} M. High concentrations of Mg^{2+} inhibited the reaction with both purine substrates.

Effect of Temperature on Enzyme Stability. Figure 4 shows both GMP and IMP pyrophosphorylase activity of fraction IV after being heated at 50° for the indicated amounts of time. It should be noted that a parallel loss in both activities occurs under these conditions.

Effect of pH on Enzyme Stability. A buffer of 0.01 M Tris–0.01 M histidine–0.01 M glycine was used to study the enzyme stability over the pH range between 4 and 11. The enzyme was incubated at 37° in the presence of buffer at the pH values indicated. After 10 min, aliquots were removed, added directly to the buffered assay mixture at pH 7.4, and assayed. No loss of activity was observed upon incubation between pH 7.0 and 10. Below pH 7.0 and above pH 10 there is rapid and parallel loss of both activities. These results are shown in Figure 5.

Effect of pH on Enzyme Activity. Figure 6 shows activities with hypoxanthine and guanine as substrates when assayed at different pH values. The GMP pyrophosphorylase activity has its pH optimum between 7 and 8 whereas the IMP pyrophosphorylase activity has a pH optimum between pH 8 and 9. It should be noted that the shapes of the IMP pyrophosphorylase

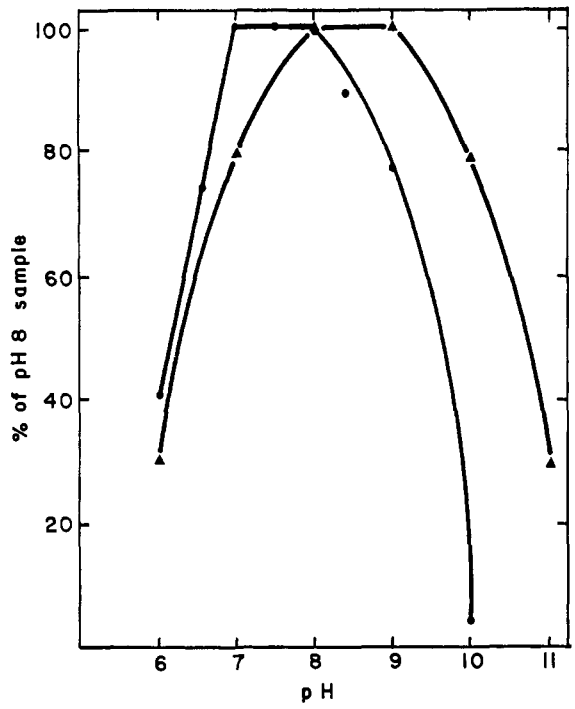

FIGURE 6: Effect of pH on enzyme activity. Enzyme assays were conducted in 0.03 M Tris–0.03 M histidine–0.03 M glycine buffers at the indicated pH. The 100% value represents a synthesis of 9.1 mμmoles of GMP/min and 32.0 mμmoles of IMP/min. (●—●—●) GMP synthesis; (▲—▲—▲) IMP synthesis.

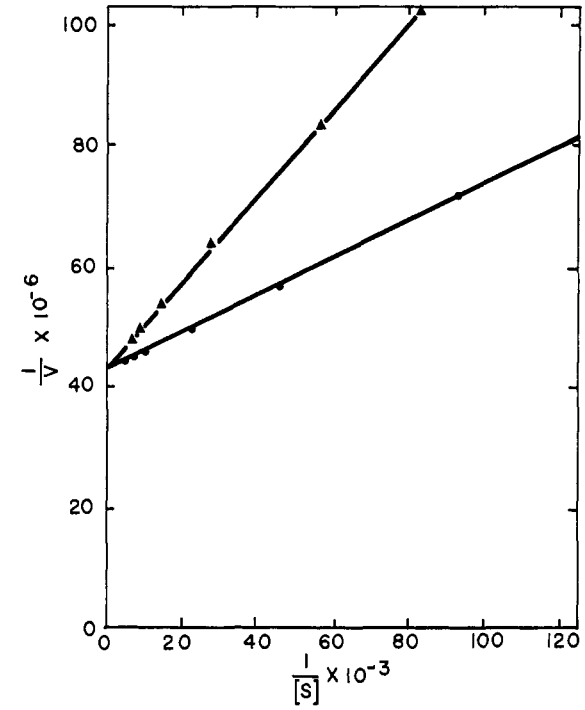

FIGURE 7: GMP synthesis as a function of substrate concentration at pH 7.4, 25°, and 10^{-3} M Mg^{2+}. The effect of each substrate concentration was measured at saturating conditions of the other substrate. The substrate concentrations were measured in moles per liter. Initial velocities were used and expressed as moles of GMP synthesized per minute. (●—●—●) Guanine; (▲—▲—▲) PRPP.

activity curve and the GMP pyrophosphorylase activity curve are very similar, the difference being that the IMP pyrophosphorylase activity curve is shifted toward a higher pH by approximately one pH unit.

Activity as a Function of Substrate Concentration. Michaelis constants for the various substrates were obtained by Lineweaver–Burk (1934) plots (Figures 7 and 8) at 25° and pH 7.4. The constants obtained for variable purine substrate at saturating PRPP concentrations are 7.7×10^{-6} M for guanine and 1.8×10^{-5} M for hypoxanthine. At variable concentrations of PRPP and saturating concentrations of purine substrates the K_m values are 2.4×10^{-5} M for PRPP with guanine and 4.2×10^{-5} M for PRPP with hypoxanthine.

Effect of Temperature on Enzyme Activity. The effect of temperature on enzyme activities with the two purine substrates is illustrated in Figure 9. The curve is biphasic, when guanine is substrate, with a sharp transition at 19°. The calculated activation energies are 5700 cal/mole below the transition temperature and 11,600 cal/mole above the transition temperature. When hypoxanthine is substrate, a linear relationship is seen over the same temperature range (0–50°). The apparent activation energy is 11,300 cal/mole for the enzyme with hypoxanthine as substrate.

Discussion

An extensive purification of IMP pyrophosphorylase from brewers yeast is reported in this paper. Kornberg *et al.* (1955) have carried out the only purification of this enzyme from Brewers yeast to our knowledge. Approximately 18-fold purification of the centrifuged lysate was reported, but yield and specific activity cannot be evaluated from the data presented. The procedure given in the present paper resulted in a specific activity of 2.25 units of guanine activity/mg of protein and 6.67 units of hypoxanthine activity/mg of protein following a 234-fold purification with a 14% yield of both activities.

The specificity of this purine nucleotide pyrophosphorylase system has not yet been determined. It is not known whether a single protein is responsible for the synthesis of IMP and GMP or whether separate enzymes catalyze each individual reaction. The data presented by Preiss and Handler (1957) on the enzymatic synthesis of nicotinamide mononucleotide in human erythrocytes suggest the possibility of separation of IMP pyrophosphorylase from GMP pyrophorylase activity by the use of calcium phosphate gel adsorption. Kelley *et al.* (1967) have recently reported the existence of an enzyme in human erythrocyte hemolysates which is capable of catalyzing the formation of xanthylic acid directly from xanthine. It is stated that genetic evidence and studies of heat inactivation suggest a single enzyme is responsible for the xanthine, hypoxanthine, and guanine phosphoribosyltransferase activity observed. Atkinson and Murray (1965), using Ehrlich ascites tumor cells as the enzyme source, have reported a partial separation of the IMP pyrophosphorylase and the GMP pyrophosphorylase activities by starch gel electro-

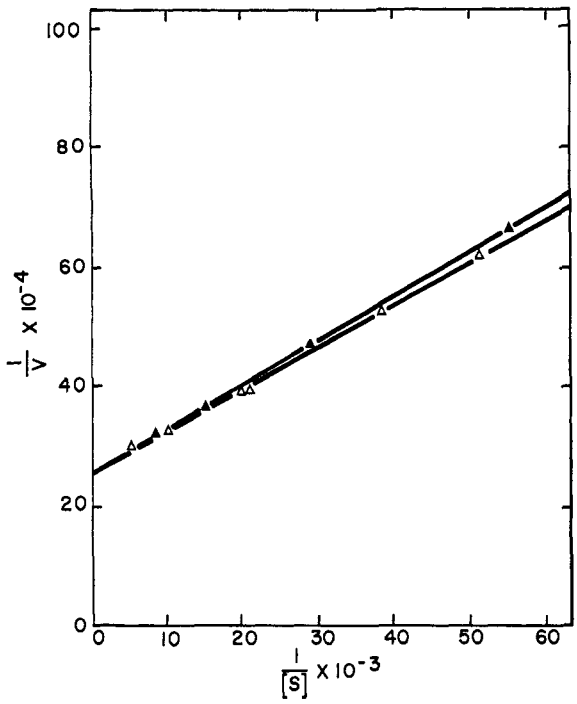

FIGURE 8: IMP synthesis as a function of substrate concentration at pH 7.4, 25°, and 10^{-3} M Mg^{2+}. The effect of each substrate concentration upon activity was measured at saturating conditions of the other substrate. The substrate concentrations were measured in moles per liter. Initial velocities were used and expressed as moles of IMP synthesized per minute. (△—△—△) Hypoxanthine; (▲—▲—▲) PRPP.

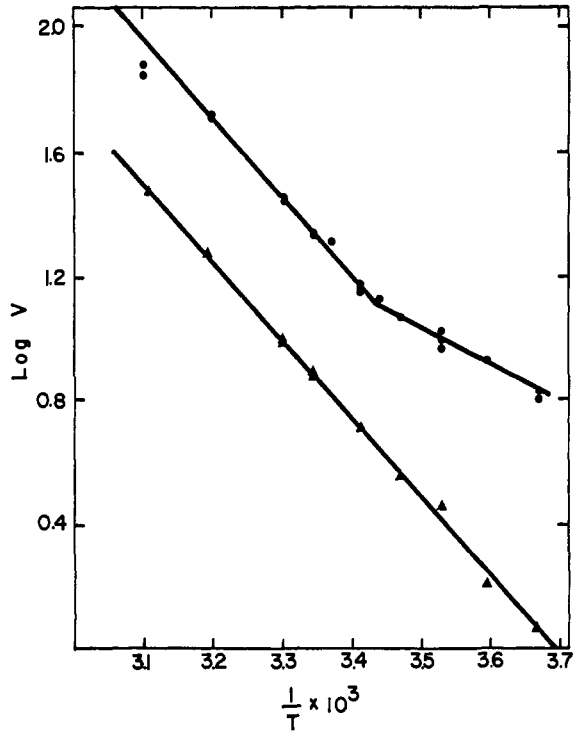

FIGURE 9: Effect of temperature on enzyme activity. Enzyme assays were conducted at temperatures between 0 and 50°. (●—●—●) GMP synthesis; (▲—▲—▲) IMP synthesis.

phoresis. Littlefield (1964) working with 8-azaguanine has shown three degrees of enzyme deficiency in mouse fibroblasts. He states that nucleotide pyrophosphorylase activity acts as if guanine and hypoxanthine were substrates for the same enzyme in mouse fibroblasts. Adye and Gots (1966) in a study of genetically altered purine nucleotide pyrophosphorylases of *S. typhimurium* reported an 8-azaguanine-resistant mutant which, although having the same K_m for guanine as the wild type, had altered K_m values for hypoxanthine and PRPP. These data suggest the possibility of two separate proteins being responsible for the IMP and GMP pyrophosphorylase activities in organisms other than the one examined here.

Michaelis constants have been reported for guanine and hypoxanthine for the IMP pyrophosphorylase from *Escherichia coli* (Carter, 1959), *S. typhimurium* (Adye and Gots, 1965), and Ehrlich ascites tumor cells (Atkinson and Murray, 1965). These K_m values were obtained under a variety of experimental conditions and with enzymes at different states of purity. Valid comparisons cannot be made until studies under identical conditions have been made.

The biphasic nature of the Arrhenius plot with guanine as substrate is of special interest. One explanation of these data is the possibility of a conformational change in the protein responsible for GMP synthesis over a narrow temperature range at 19° in the presence of guanine, with the conformations above and below 19° having different energies of activation. If one enzyme catalyzes both IMP and GMP synthesis, the single activation energy observed with hypoxanthine as substrate could be due to stabilization by hypoxanthine of one enzyme conformation over the entire temperature range studied. The same apparent activation energies at higher temperatures with guanine and hypoxanthine as substrates would support this suggestion. The conformation in the presence of hypoxanthine would be that found above 19° when guanine is substrate. This is somewhat analogous to the situation observed by Massey *et al.* (1966) for D-amino acid oxidase. Dixon and Webb (1964) have reviewed some of the enzymes which exhibit this phenomenon.

Murray (1967) has recently reported different activation energies above and below 23° for IMP pyrophosphorylase from crude extracts of Ehrlich ascites cells. Activation energies of 13,100 cal/mole above 23° and 51,000 cal/mole below 23° were reported. The former value agrees reasonably well with the value obtained for the yeast enzyme when differences in source and purity are considerd. The latter value seems inordinately high, particularly when one considers the statement by Eyring *et al.* (1954), "One of the most important and far reaching general statements that can be made is that for enzymes no activated complex structure will be important which requires a free energy much in excess of 20 kcal/mole to constitute it from the stable substances in the system." If this generalization is correct then the activation energy of 51,000 cal/mole reported

must be in error. The reaction velocities reported by Murray were determined by rate of formation of [^{14}C]-IMP from [^{14}C]hypoxanthine. The data were obtained with crude extracts and thus the presence of a phosphatase which could cleave IMP to inosine is quite probable. If such a phosphatase was present and active at temperatures below 23° and inactive or much less active at temperatures above 23°, the velocity of the pyrophosphorylase reaction at lower temperatures would be greater than the reported velocities. This could explain the extraordinarily large activation energy reported at temperatures below 23°. The data presented by Murray do not permit scrutiny of the controls which were run.

The evidence presented in this paper is consistent with a single enzyme catalyzing both IMP and GMP synthesis, namely: constant activity ratios over 234-fold purification; parallel loss of both activities in pH-stability studies and in heat-stability studies; identical requirements of both activities for Mg^{2+}; and the same activation energies above 19° with both purine substrates.

Further study of this system with more purified fractions and with substrate analogs should help clarify the problem concerning the existence of one or more purine nucleotide pyrophosphorylases. Studies of this nature are presently in progress in this laboratory.

Added in Proof

Fraction IV can be stabilized to freezing by making the enzyme solution either 10^{-3} M in EDTA or 5×10^{-4} M in Mg_2PRPP. After 13-days storage at −20°, 80% of the activity remained in the EDTA- and Mg_2PRPP-stabilized fractions whereas only 15% of the activity remained in a sample which contained no additions. Concentrations of Mg_2PRPP higher than 5×10^{-4} M resulted in decreased enzyme stability.

References

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

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

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

Dixon, M., and Webb, E. C. (1964), Enzymes, 2nd ed, New York, N. Y., Academic, p 159.

Eyring, H., Lumry, R., and Spikes, J. C. (1954), *in* Symposium on the Mechanism of Enzyme Action, McElroy, W. D., and Glass, B., Ed., Baltimore, Md., Johns Hopkins, p 131.

Hori, M., and Henderson, J. F. (1966), *J. Biol. Chem. 241*, 1406.

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. 215*, 875.

Kornberg, A., Lieberman, A., and Simms, E. S. (1954), *J. Am. Chem. Soc. 76*, 2027.

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

Lineweaver, H., and Burk, D. (1934), *J. Am. Chem. Soc. 56*, 658.

Littlefield, J. W. (1964), *Nature 203*, 1142.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

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

Miller, R. L., and Bieber, A. L. (1967), 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept, Abstract 209C.

Murray, A. W. (1967), *Biochem. J. 103*, 271.

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

Williams, W. J., and Buchanan, J. M. (1953), *J. Biol. Chem. 203*, 583.