

## Characterization of Guanine and Hypoxanthine Phosphoribosyltransferases in *Methanococcus voltae*

TIMOTHY L. BOWEN,<sup>†</sup> WINSTON C. LIN, AND WILLIAM B. WHITMAN\*

*Department of Microbiology, University of Georgia, Athens, Georgia 30602-2605*

Received 4 December 1995/Accepted 21 February 1996

Phosphoribosyltransferase (PRTase) and nucleoside phosphorylase (NPase) activities were detected by radiometric methods in extracts of *Methanococcus voltae*. Guanine PRTase activity was present at 2.7 nmol min<sup>-1</sup> mg of protein<sup>-1</sup> and had an apparent  $K_m$  for guanine of 0.2 mM and a pH optimum of 9. The activity was inhibited 50% by 0.3 mM GMP. IMP and AMP were not inhibitory at concentrations up to 0.6 mM. Hypoxanthine inhibited by 50% at 0.16 mM, and adenine and xanthine were not inhibitory at concentrations up to 0.5 mM. Guanosine NPase activity was present at 0.01 nmol min<sup>-1</sup> mg of protein<sup>-1</sup>. Hypoxanthine PRTase activity was present at 0.85 nmol min<sup>-1</sup> mg of protein<sup>-1</sup> with an apparent  $K_m$  for hypoxanthine of 0.015 mM and a pH optimum of 9. Activity was stimulated at least twofold by 0.05 mM GMP and 0.2 mM IMP but was unaffected by AMP. Guanine inhibited by 50% at 0.06 mM, but adenine and xanthine were not inhibitory. Inosine NPase activity was present at 0.04 nmol min<sup>-1</sup> mg of protein<sup>-1</sup>. PRTase activities were not sensitive to any base analogs examined, with the exception of 8-azaguanine, 8-azahypoxanthine, and 2-thioxanthine. Fractionation of cell extracts by ion-exchange chromatography resolved three peaks of activity, each of which contained both guanine and hypoxanthine PRTase activities. The specific activities of the PRTases were not affected by growth in medium containing the nucleobases. Mutants of *M. voltae* resistant to base analogs lacked PRTase activity. Two mutants resistant to both 8-azaguanine and 8-azahypoxanthine lacked activity for both guanine and hypoxanthine PRTase. These results suggest that analog resistance was acquired by the loss of PRTase activity.

De novo synthesis of purine nucleotides requires a minimum of 10 enzymes for the production of IMP, the major intermediate of purine metabolism (6). The salvage of nucleobases and nucleosides provides an energy-saving alternative. Salvage pathways have been identified in eubacteria, eucaryotes, and archaeobacteria and comprise a collection of enzymes capable of converting preformed nucleobases and nucleosides to AMP, GMP, IMP, and XMP (5, 7, 10, 13, 17, 19, 21, 23, 29). These enzymes enable organisms to use exogenous bases liberated from lysed cells or recycle those produced by the breakdown of unstable RNA. A key enzyme in the pathway is phosphoribosyltransferase (PRTase). This enzyme is responsible for the conversion of bases to nucleotide 5'-monophosphates by use of 5-phosphorylribose-1-pyrophosphate (PRPP) as the phosphopentose donor. Bases can also be salvaged by nucleoside phosphorylases by use of ribose-1-phosphate as the phosphopentose donor. However, in *Escherichia coli* and *Salmonella typhimurium*, this mechanism is a minor source of nucleotides (20).

Mutants resistant to nucleobase analogs have been isolated from several archaeobacteria (2, 12, 16, 29). As with eubacteria and eucaryotes, these mutants have lost the ability to incorporate certain exogenous bases, and this loss is almost always accompanied by a loss of the corresponding PRTase activity. Thus, resistance appears to result from the inability to convert the analog to a toxic nucleotide, an activity mediated by PRTases. In this report, we characterize the hypoxanthine PRTase (EC 2.4.2.8) and guanine PRTase (EC 2.4.2.22) activ-

ities in wild-type and analog-resistant mutants of the archaeobacterium *Methanococcus voltae*.

### MATERIALS AND METHODS

**Chemicals.** Natural bases and base analogs were obtained from Sigma Chemical Co. (St. Louis, Mo.). [8-<sup>14</sup>C]guanine and [8-<sup>14</sup>C]hypoxanthine (50 Ci/mol) were obtained from NEN Research Products (Boston, Mass.). [8-<sup>14</sup>C]xanthine (50 Ci/mol) was obtained from Moravak Biochemicals, Inc. (Brea, Calif.). All other biochemicals were obtained from Sigma unless stated otherwise.

**Culture conditions.** *M. voltae* PS and the analog-resistant mutants were grown in the complex medium described by Bowen and Whitman and Whitman et al. (2, 27) with 0.2% vitamin-free Casamino Acids (Difco Laboratories, Detroit, Mich.) substituting for yeast extract. The cultures were incubated under an atmosphere of 275 kPa of H<sub>2</sub>-CO<sub>2</sub> (80:20, vol/vol) at a temperature of 37°C with shaking.

**Preparation of cell extracts.** Cultures of *M. voltae* were grown anaerobically in complex medium to an  $A_{600}$  of approximately 1. Thereafter, all manipulations were performed aerobically. After centrifugation at 5,000 × g, the cell pellet was frozen at -20°C. After 2 h, the pellet was thawed and resuspended in 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid [pH 7.4], 1 ml/g [wet weight] of cells) with DNase (50 µg/g [wet weight]). The cells were lysed under these conditions, and the extract was dialyzed twice against 6,000 volumes of 25 mM HEPES-2 mM MgCl<sub>2</sub>-7 mM 2-mercaptoethanol and stored at -20°C. Protein concentrations were determined by a modified Bradford protein assay (Bio-Rad, Richmond, Calif.).

**Enzyme assays.** Nucleoside phosphorylase and PRTase activities were determined by measuring the formation of radioactive product (26). Reaction mixtures were composed of 50 mM HEPES (pH 7.6), 5 mM MgCl<sub>2</sub>, <sup>14</sup>C-labeled base, and cell extract in a final volume of 0.050 ml. Unless noted otherwise, the concentrations of hypoxanthine and guanine were 0.01 mM and 0.1 mM, respectively. The reactions were initiated by the addition of 0.1 M ribose-1-phosphate or disodium PRPP to a final concentration of 4 mM. Because of the instability of PRPP, stock solutions were stored in aliquots at -70°C. Stock solutions of radiolabeled bases were made in 0.1 M NaOH and stored at -20°C. After termination of the reaction by the addition of 5 µl of 0.5 M disodium EDTA (pH 8.0), the radiolabeled products were separated from the substrate on plastic-backed cellulose chromatography sheets containing fluorescent indicator (no. 6065; Eastman Kodak Company, Rochester, N.Y.). The following solvents were used: 1 M ammonium acetate for guanine and 1 part of 93.6% butanol and 1 part of 44.8% propionic acid for hypoxanthine (9). The zones containing the bases

\* Corresponding author. Phone: (706) 542-4219. Fax: (706) 542-2674. Electronic mail address: whitman@uga.cc.uga.edu.

<sup>†</sup> Present address: New England Biolabs, Inc., Beverly, MA 01915.

and derivatives were visualized under UV illumination and cut out, and the radioactivity was determined with a model LS3801 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.) with ScintiVerse BD cocktail (Fisher Scientific, Pittsburgh, Pa.). A typical assay volume of 50  $\mu$ l contained 30  $\mu$ g of protein and was incubated at 37°C for 20 min. One milliunit of activity was equal to 1 nmol of product formed per min. Enzyme kinetics were measured under conditions in which no more than 10% of the substrate was converted to product. For the pH studies, the buffers used were 50 mM MES (morpholine-ethanesulfonic acid [pH 5.5 to 6.5]), HEPES (pH 7.0 to 7.5), Tris (pH 8.0 to 9.0), CHES (2-[N-cyclohexylamino]ethanesulfonate [pH 9.5 to 10.0]), and CAPS (3-[cyclohexylamino]-1-propanesulfonate [pH 10.5 to 11.5]). The specific activities at pH 9 were 4.0 and 2.0 nmol min<sup>-1</sup> mg of protein<sup>-1</sup> for hypoxanthine and guanine PRTase, respectively.

**Chromatography.** Cell extracts were fractionated at room temperature with a Pharmacia Biotech (Piscataway, N.J.) fast protein liquid chromatography system, resins, and columns. For Superose 6 chromatography, 3 ml of cell extract was applied to a 24-ml column equilibrated with 50 mM HEPES buffer (pH 7.4), 5 mM Mg<sub>2</sub>Cl, and 1 mM dithiothreitol (buffer A) under anaerobic conditions. The flow rate was 0.5 ml/min. For Resource Q chromatography, 1.5 ml of the active fractions from the Superose 6 column was applied to a 1-ml column equilibrated with 20 mM Tris chloride (pH 7.4) at a flow rate of 0.5 ml/min under aerobic conditions. The column was washed with 3 ml of buffer before beginning a 25-ml linear gradient from 0 to 0.5 M NaCl in the same buffer. At the conclusion of the gradient, the column was washed with 5 ml of 0.5 M NaCl in buffer. For phenyl-Sepharose chromatography, 4.5 ml of cell extract was applied to a 10-ml column equilibrated with buffer A plus 1.5 M KCl at a flow rate of 1 ml/min under anaerobic conditions. The column was washed with 5 ml of buffer A plus 1.5 M KCl, a 45-ml linearly decreasing gradient was run to 0 M KCl in buffer A, the column was washed with 15 ml of buffer A, and a 15-ml linear gradient was run to 50% (vol/vol) ethylene glycol in buffer A.

## RESULTS AND DISCUSSION

**Presence of salvage pathway enzymes.** The presence of the guanine and hypoxanthine PRTase activities in extracts of *M. voltae* clearly indicated the presence of a purine salvage pathway. PRTase assays were linear between 5 and 20 min and from 8 to 38  $\mu$ g of protein. Similar results were obtained when the assays were performed under aerobic or anaerobic conditions, and the PRTase activities did not appear to be affected by oxygen. Extracts of *M. voltae* had a mean specific activity for guanine PRTase of 2.7 mU mg of protein<sup>-1</sup>, with a standard deviation and range of 1.7 and 0.9 to 7.4 mU mg of protein<sup>-1</sup>, respectively, depending on the particular enzyme preparation ( $n = 30$ ). However, activity could not be measured at substrate saturation because of the limited solubility of guanine at neutral pH (see below). The activity for the hypoxanthine PRTase was comparable to that of guanine PRTase, with a mean, standard deviation, and range of 0.85, 0.56, and 0.28 to 2.4 mU mg of protein<sup>-1</sup>, respectively ( $n = 31$ ). These specific activities were obtained at 0.010 mM hypoxanthine, which was below the apparent  $K_m$  for hypoxanthine, and do not represent maximal activities. Nevertheless, they are at least an order of magnitude lower than the specific activities reported for extracts of *S. typhimurium*, *E. coli*, and *Lactobacillus casei*, which were 28, 73, and 64 mU mg of protein<sup>-1</sup>, respectively (7, 13). However, the methanococcal activities were comparable to those found in extracts of *Methanobacterium thermoautotrophicum* and members of the class *Mollicutes* (15, 29).

The guanine and hypoxanthine PRTase activities were not inducible. Growth of *M. voltae* in the presence of exogenous purines, either singly or in combination, did not change the specific activities (3). Similarly, there is no evidence for the induction of PRTase activities in other organisms by exogenous purines in the growth medium. However, amethopterin, which inhibits de novo purine biosynthesis, increases the specific activity of PRTase in *E. coli*. The effects of amethopterin in methanogens have not been well characterized.

Extracts of *M. voltae* also contained low activities for guanosine and inosine nucleoside phosphorylases. These activities were linear with time for up to 6 h and were present at 0.01 and 0.04 mU mg of protein<sup>-1</sup>, respectively. These levels were ap-

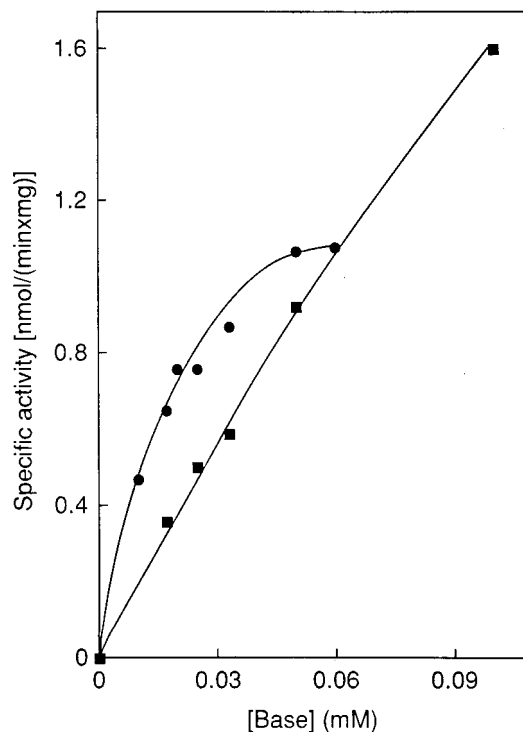


FIG. 1. Specific activities of the guanine PRTase (■) and hypoxanthine PRTase (●) with increasing guanine and hypoxanthine concentrations, respectively.

proximately 2 orders of magnitude lower than those found in the members of *Mollicutes*, organisms that have an absolute requirement for nucleobases for growth (15). Therefore, it is unlikely that these enzymes are a major route of nucleobase incorporation in *M. voltae*.

**Properties of the guanine and hypoxanthine PRTase activities.** The analysis of salvage pathway enzymes in archaebacteria has been limited. Although a number of enzymes involved in purine salvage in *M. thermoautotrophicum* have been identified, very little has been done to characterize them (29). However, PRTases in members of both the eubacteria and the eucaryotes have been well studied (5, 7, 10, 13, 17, 19, 21, 23). The guanine and hypoxanthine PRTase activities from *M. voltae* had certain similarities to those from other organisms.

The affinity of the two PRTase activities for guanine and hypoxanthine varied by nearly 10-fold. Because of the limited solubility of the substrate at neutral pH, the guanine PRTase activity could not be saturated (Fig. 1). The solubility of guanine in the reaction buffer was determined empirically to be 0.1 mM, which was somewhat higher than its solubility of 0.027 mM in distilled water (25, 28). However, even at 0.1 mM, the enzyme activity had not reached a maximum, and the apparent  $K_m$  calculated from double reciprocal plots was 0.2 mM. In contrast, saturation for hypoxanthine PRTase activity was observed at a concentration near 0.05 mM, and the apparent  $K_m$  was 0.015 mM (Fig. 1).

Both activities had a single pH optimum at approximately 9 (data not shown). Half-maximal activities were at pH 7.5 and 10 and pH 7.5 and 9.5 for the guanine and hypoxanthine activities, respectively. The guanine-xanthine PRTase of *E. coli* has a slightly lower pH optimum of about 8 (8). The enzymes from eucaryotes have somewhat higher pH optima. The hypoxanthine-guanine PRTase of *Schizosaccharomyces pombe*

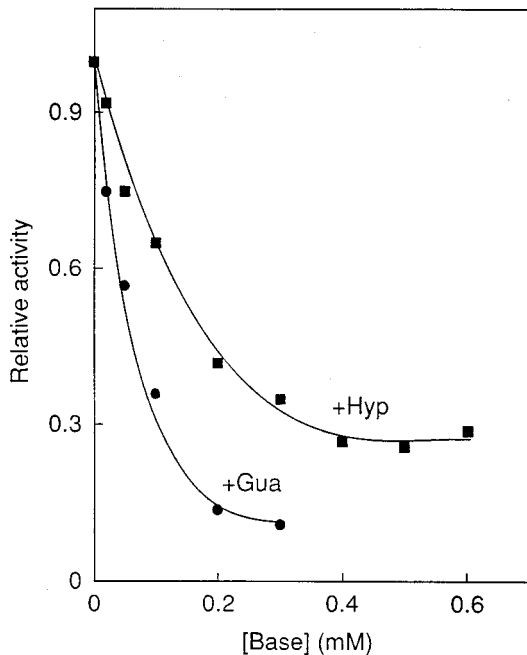


FIG. 2. Inhibition of the guanine (■) and hypoxanthine (●) PRTase activities by hypoxanthine (Hyp) and guanine (Gua), respectively. The specific activities at 100% were 4.5 and 1.2  $\text{nmol min}^{-1} \text{mg of protein}^{-1}$  for guanine and hypoxanthine PRTase activities, respectively.

has a biphasic pH response, with one optimum at pH 8.0 and another near pH 9.5 (17). Maximal activity for the same enzyme from *Plasmodium lophurae* plateaus between pH 7 and 10 (22, 24). Therefore, the pH dependence of the methanococcal PRTase activities differed from that of both the eubacterial and eucaryotic enzymes.

To assess the substrate specificity of the PRTase activities, the effects of other nucleobases and nucleoside and nucleoside analogs were determined. The rationale for these experiments was that alternative substrates would be inhibitory even though not all inhibitory compounds must be substrates. Low concentrations of hypoxanthine and guanine inhibited the guanine and hypoxanthine PRTase activities, respectively (Fig. 2). For the guanine PRTase, the concentration of hypoxanthine required for 50% inhibition was 0.16 mM. This concentration was close to the apparent  $K_m$  for guanine and 10-fold higher than the apparent  $K_m$  for the hypoxanthine PRTase activity. Likewise, the concentration of guanine required for 50% inhibition of the hypoxanthine PRTase was 0.06 mM, and this concentration was close to the apparent  $K_m$  for the hypoxanthine PRTase but threefold lower than the apparent  $K_m$  for the guanine PRTase. This pattern of inhibition is inconsistent with a simple kinetic model in which both activities are due to a single enzyme with dual substrate specificity (4). However, these results would not eliminate more complex models in which guanine and hypoxanthine are also effectors or two or more enzymes are present with overlapping substrate specificities.

For both PRTase activities, no significant inhibition was observed in the presence of 0.5 mM adenine or xanthine. The inability of adenine to inhibit is consistent with what is known about the eubacterial and eucaryotic salvage pathways, which have distinct PRTases for adenine (17, 18). In contrast, the absence of inhibition by xanthine suggests that a guanine-xanthine PRTase similar to that found in *E. coli* is not present.

Although *M. voltae* assimilates xanthine, its incorporation is only 21  $\mu\text{mol g}$  (dry weight) of cells $^{-1}$  or six- to eightfold lower than that of guanine and hypoxanthine (1). Thus, xanthine does not appear to be a major substrate for the methanococcal salvage pathway.

Both PRTase activities were also inhibited by the analogs 8-azaguanine and 8-azahypoxanthine. These analogs are bacteriocidal to *M. voltae* (2). Because the usual mechanism of toxicity requires conversion to the nucleotide (14), these analogs were expected to be alternative substrates for the PRTases. When guanine and hypoxanthine were added at concentrations less than their apparent  $K_m$ s, the responses of both PRTase activities to these analogs were almost identical, with 8-azaguanine being more inhibitory at low concentrations than 8-azahypoxanthine (Fig. 3). While this result is consistent with the presence of a single enzyme with dual substrate specificity, it could also be obtained if two PRTase enzymes with similar analog sensitivities were present.

A number of other base or nucleoside analogs that inhibit growth were also investigated (1). For the guanine PRTase, no inhibition was found with 1 mM 8-aza-2,6-diaminopurine, 1 mM 6-mercaptopyrimidine, and saturated solutions of 8-azaadenine, 5-azacytidine, 6-azauracil, 2-mercaptopyrimidine, 2-thioxanthine, 2-thiouracil, 2-amino-6-methylmercaptopyrimidine, 4-hydroxy-6-methyl-2-thiopyrimidine, 5-hydroxymethyluracil, 1-methylthymine, 6-methyluracil, 6-azauradine, 6-mercaptoguanosine, 8-aza-2,6-diaminopurine, and 6-mercaptopyrimidine. Similarly, with one exception, these compounds were also not inhibitory to the hypoxanthine PRTase activity when assayed at 0.015 mM hypoxanthine, which was the value of the apparent  $K_m$ . The exception was 2-thioxanthine, which inhibited the hypoxanthine but not the guanine PRTase by 50%. Interest-

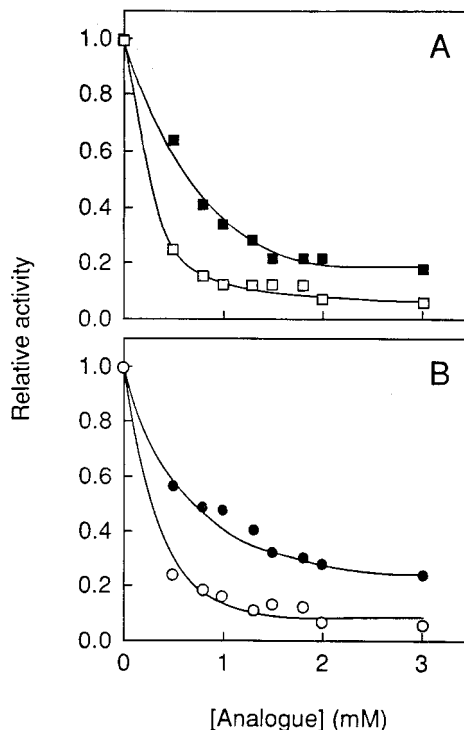


FIG. 3. Inhibition of guanine (A) and hypoxanthine (B) PRTases by azaguanine (□ and ○) and azahypoxanthine (■ and ●). The specific activities at 100% were 4.0 and 1.0  $\text{nmol min}^{-1} \text{mg of protein}^{-1}$  for the guanine and hypoxanthine PRTase activities, respectively.

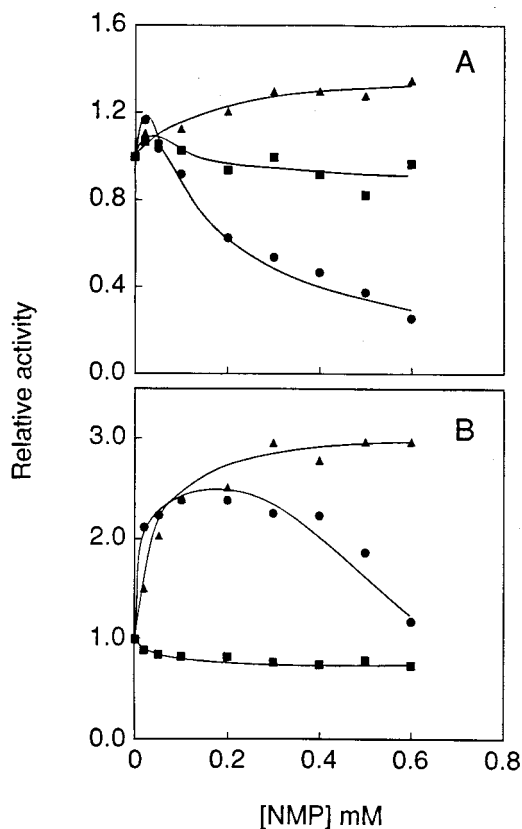


FIG. 4. Effect of IMP (▲), GMP (●), and AMP (■) on guanine PRTase (A) and hypoxanthine PRTase (B) activities. The specific activities at 100% were 2.5, 2.3, and 2.0 nmol min<sup>-1</sup> mg of protein<sup>-1</sup> for AMP, GMP, and IMP, respectively, for the guanine PRTase and 1.4, 0.9, and 0.7 nmol min<sup>-1</sup> mg of protein<sup>-1</sup> for AMP, GMP, and IMP, respectively, for the hypoxanthine PRTase.

ingly, 2-thioxanthine is not inhibitory to growth (2). With the possible exception of this latter compound, these analogs do not appear to be substrates for the guanine and hypoxanthine PRTase activities. For the most part, this result was expected from the chemical nature of the analogs and the specificity of analog-resistant mutants (2). The exception was 8-aza-2,6-diaminopurine, which was predicted to be a guanine analog (2).

**Potential effectors.** The PRTase activities were sensitive to several nucleotide effectors (Fig. 4). Guanine PRTase activity was inhibited 50% by approximately 0.4 mM GMP. IMP was slightly stimulatory, and AMP had no observable effect. Other nucleotides which had no effect at concentrations of 0.1 to 1.0 mM were ADP, ATP, GDP, GTP, IDP, ITP, XMP, and cyclic AMP. Hypoxanthine PRTase activity was stimulated two- to threefold by IMP and GMP, although this effect decreased at higher concentrations of GMP (Fig. 4). Nucleotides which had little effect on hypoxanthine PRTase activity at concentrations of 0.1 to 1.0 mM were AMP, ADP, ATP, GDP, GTP, IDP, ITP, XMP, and cyclic AMP. These data clearly indicate that guanine and hypoxanthine PRTase activities had distinct effector profiles. Guanine PRTase was significantly inhibited by GMP, whereas hypoxanthine PRTase was stimulated. In addition, stimulation by IMP was significantly greater for the hypoxanthine PRTase activity. Regulation of PRTase activity in the eubacteria and eucaryotes has been studied in some detail. Hochstadt (8) found that the guanine specific activity of the guanine-xanthine PRTase from *E. coli* was significantly inhibited by GMP and GDP. Approximately 5% activity remained

at a concentration of 1 mM. ITP, IMP, and XMP were also inhibitory but to a lesser extent. Likewise, the guanine PRTase activity in *Schizosaccharomyces pombe* was significantly inhibited by GMP, GDP, GTP, and IMP (17). Minor inhibition was also found with IDP and XMP. In contrast, guanine PRTase from *M. voltae* was inhibited only by GMP, and this regulatory pattern appears to be unique. Possible effectors of hypoxanthine PRTases in the eubacteria and eucaryotes have not been examined.

**Analog-resistant mutants.** The specific activities of the guanine and hypoxanthine PRTases in wild-type extracts were sufficient to account for the levels of guanine and hypoxanthine incorporation observed in vivo (2). In support of this role, extracts of TB4 and TB5, analog-resistant mutants of *M. voltae* which do not incorporate guanine and hypoxanthine, had much lower specific activities for these substrates (Table 1). These mutants incorporated adenine at levels equal to those of wild-type cells, confirming the conclusion that adenine is not a substrate for these PRTase activities. As a control for this experiment, extracts of the azauracil-resistant mutant TB7, which incorporates wild-type levels of purines, contained normal levels of guanine and hypoxanthine PRTase activities. This result confirmed the reliance of *M. voltae* on PRTase activities for the incorporation of exogenous purines and provided a rationale for the mechanism of analog resistance. Thus, elimination of the PRTase activities would prevent formation of the nucleotide derivatives of the analogs and their bacteriocidal effects.

For one analog-resistant mutant, TB3, a mixed phenotype was observed. This mutant was obtained after selection for 8-aza-2,6-diaminopurine resistance and had low levels of azaguanine and azahypoxanthine resistance (2). Although hypoxanthine was incorporated normally, guanine incorporation was reduced 30-fold (2), and extracts contained low levels of both PRTase activities (Table 1). Presumably, the guanine PRTase activity was sufficiently reduced to prevent significant guanine incorporation due to the low affinity for this base. Two alternatives may explain these observations. One, because 8-aza-2,6-diaminopurine was not inhibitory to the guanine and hypoxanthine PRTase activities in vitro, the conversion of this analog to a toxic nucleotide may have been mediated by another PRTase activity. The mutation in TB3 would then be pleiotropic for the guanine and hypoxanthine PRTase activities. Two, the analog may be such a poor substrate that inhibition of the PRTases was not detected in vitro, but the nucleotide product may be extremely toxic. In this case, formation of small amounts of the nucleotide by wild-type levels of the guanine and hypoxanthine PRTase activities would be sufficient to inhibit growth.

TABLE 1. Guanine and hypoxanthine PRTase activities in extracts of analog-resistant strains of *M. voltae*<sup>a</sup>

Strain	Analog used for isolation <sup>b</sup>	Bases incorporated <sup>c</sup>	% Of wild-type activity	
			Guanine PRTase	Hypoxanthine PRTase
TB3	zn <sub>2</sub> Pur	ade, hyp, ura	13	25
TB4	zGua	ade, ura	<15	<2
TB5	zHyp	ade, ura	<14	<2
TB7	zUra	ade, gua, hyp	111	109

<sup>a</sup> Strains were spontaneous mutants selected on medium containing saturating concentrations of the indicated analog (2).

<sup>b</sup> Abbreviations for analogs: zn<sub>2</sub>Pur, 8-aza-2,6-diaminopurine; zGua, 8-azaguanine; zHyp, 8-azahypoxanthine; zUra, 6-azauracil.

<sup>c</sup> The wild-type strain incorporated adenine (ade), guanine (gua), hypoxanthine (hyp), and uracil (ura) (2).

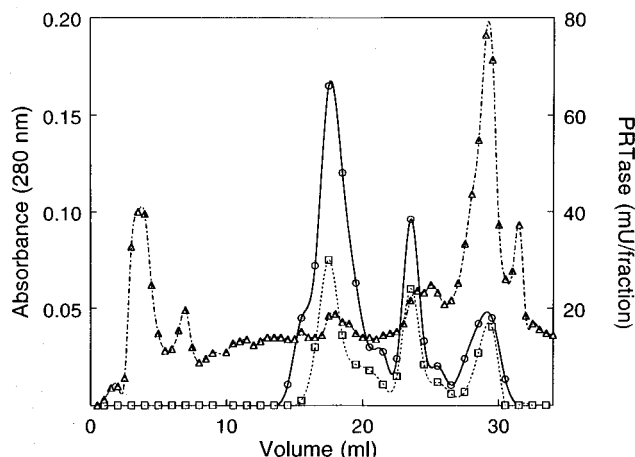


FIG. 5. Separation of PRTases by ion-exchange chromatography. The fraction volume was 0.5 ml, and the enzyme assays were performed for 90 min. Symbols: ○, hypoxanthine PRTase; □, guanine PRTase; △, absorbance.

**Separation of PRTase activities.** To determine whether more than one guanine or hypoxanthine PRTase activity was present, cell extracts were fractionated. Initially, extracts were chromatographed on phenyl-Sepharose CL-4B columns (data not shown). While multiple peaks of activity were resolved, the activity was very unstable and could not be studied further. During molecular sieve chromatography on Superose 6, a single peak containing both activities eluted at an  $M_r$  of 90,000. This activity was stable for at least 1 month at  $-20^\circ\text{C}$ . Rechromatography of the active fractions on a Resource Q ion-exchange column resolved three peaks of activity (Fig. 5). While the ratio varied somewhat, each peak contained both guanine and hypoxanthine PRTase activity. These activities were unstable, and 85% of the activity was lost in 5 days at  $-20^\circ\text{C}$ . The stability of the PRTase activities was not affected by the presence of air, and performing the chromatography anaerobically did not improve the stability. Although extracts appeared to contain multiple PRTase activities for both guanine and hypoxanthine, this conclusion could not be confirmed by comparison of the kinetics or effector responses because of the instability of the activities.

**Conclusions.** The characteristics of guanine and hypoxanthine incorporation in bacteria and eucaryotes differ greatly. These bases are incorporated by a single enzyme in eucaryotes and some eubacteria (5, 13, 17, 19, 23) or by two distinct enzymes in the enteric eubacteria, each of which has a different ability to use xanthine as a substrate (7, 10, 21). In *M. voltae*, it was not possible to fully resolve this issue, although the balance of evidence appears to suggest that more than one enzyme with overlapping substrate specificities is present. One, the kinetics of inhibition of the guanine PRTase by hypoxanthine and the inhibition of the hypoxanthine PRTase by guanine were inconsistent with both activities being catalyzed by a single enzyme. However, these results would be expected if more than one enzyme, each of which utilized guanine and hypoxanthine to various degrees, was present. Two, the effector profiles for both activities were very different, suggesting the presence of more than one enzyme. However, the stimulation of both activities by IMP and inhibition by GMP are consistent with two enzymes with overlapping specificities. Three, chromatography of cell extracts resolves at least three peaks containing both PRTase activities. Finally, while resistance to either azaguanine or azahypoxanthine was accompanied by loss of both

PRTase activities and suggested that both activities were present on a single enzyme, mutations in *S. typhimurium*, a bacterium with two distinct enzymes, can also produce this phenotype (11, 21). Moreover, this phenotype would be expected if the analogs were incorporated by more than one enzyme. Thus, only mutations which eliminated all of the guanine and hypoxanthine PRTase activities would produce the resistant phenotype. These mutations would also be expected at reasonable frequencies if the structural genes for the enzymes were genetically linked. In contrast, Worrell and Nagle (29) proposed the existence of a single enzyme for both activities in *M. thermoautotrophicum*. However, confirmation of the nature of the PRTases in both methanogens will require a more detailed biochemical and genetic analysis.

#### ACKNOWLEDGMENT

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