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Enzymes of Purine Metabolism in *Mycoplasma mycoides* subsp. *mycoides*

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The major pathways of ribonucleotide biosynthesis in *Mycoplasma mycoides* subsp. *mycoides* were proposed previously from studies of its usage of radioactive purines and pyrimidines. To interpret more fully the pattern of purine usage, we have assayed cell-free extracts of this organism for several enzymes associated with the salvage synthesis of purine nucleotides. *M. mycoides* possessed phosphoribosyltransferases for adenine, guanine, and hypoxanthine, purine nucleoside phosphorylase, GMP reductase, GMP kinase, adenylosuccinate synthetase, and adenylosuccinate lyase. Purine nucleoside kinase and adenosine deaminase were not detected. Examination of kinetic properties and regulation of some of the above enzymes revealed differences between *M. mycoides* and *Escherichia coli*. Most notable of these were the greater susceptibility of the enzymes from *M. mycoides* to inhibition by nucleotides and the more widespread involvement of GMP as an inhibitor. Observations on enzyme activities in vitro allow an adequate explanation of the capacity of guanine to provide *M. mycoides* with its full requirement for purine nucleotides.

Included among the growth requirements of *Mycoplasma mycoides* are the preformed bases guanine and uracil (21), which are utilized as precursors of ribonucleotides (15). By examining the incorporation of various nucleotide precursors into RNA, we have proposed pathways for nucleotide synthesis in *M. mycoides* (15) from a consideration of the conversions known to occur in the salvage synthesis of nucleotides in other bacteria (5). Whereas guanine could serve as the sole source of both guanine and adenine nucleotide, it was completely replaced as a precursor of the latter by adenine and partially so by hypoxanthine. The observation of adenine phosphoribosyltransferase in *M. mycoides* (25) would account for the utilization of adenine. This enzyme displays a sigmoidal response to phosphoribosyl pyrophosphate (P-Rib-PP) concentration and is strongly inhibited by GMP (25). Except for the demonstration of adenosine phosphorylase (EC 2.4.2.1) in many species of mycoplasma (6), there is no information on the existence of the other enzymes putatively involved in purine metabolism in *M. mycoides*. We were interested in interpreting the pattern of purine usage by this organism more fully. To this end, we have assayed cell-free extracts of *M. mycoides*, seeking to define what enzymes are available for the biosynthesis and interconversion of purine nucleotides and to determine something of the kinetic and regulatory properties of these enzymes.

MATERIALS AND METHODS

Organism and culture media. *M. mycoides* subsp. *mycoides* was kindly supplied by A. W. Rodwell, Commonwealth Scientific and Industrial Research Organisation, Division of Animal Health.

Cultures were grown in defined medium C2 of Rodwell (22) or in PPLO broth (23). By varying the purines in medium C2, cells were grown on guanine alone, guanine plus adenine, or guanine plus hypoxanthine, each at a concentration of 20 μg/ml. Cells were grown at 37°C without shaking.

Chemicals and radiochemicals. Tris(hydroxymethyl)aminomethane (Tris) 2,5-diphenyloxazole, reduced nicotinamide adenine dinucleotide phosphate (NADPH), glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and P-Rib-PP were all from Sigma Chemical Co., St. Louis, Mo. Dithiothreitol (DTT) was from Calbiochem, La Jolla, Calif. For preparing growth media, PPLO broth without crystal violet was used and was obtained from Difco Laboratories, Detroit, Mich., and yeast autolysate (Albimi) was supplied by Pfizer Diagnostics Division, New York, N.Y. All ^{14}C-labeled substrates were from the Radiochemical Centre, Amersham, England, except for [^{14}C]guanine and [^{14}C]aspartic acid, which were products of ICN Pharmaceuticals Inc., Irvine, Calif.

Preparation of extracts. Cells from 25 to 100 ml of culture were harvested, usually in late log phase, by centrifugation at 10,000 × *g* for 10 min. Cells grown in PPLO broth were washed twice, and those from defined medium were washed once, in a buffer containing 0.25 M NaCl, 0.02 M $MgCl_2$, and 0.02 M Na_2HPO_4/NaH_2PO_4 (pH 6.95). The cell pellet was suspended in 0.5 to 2.0 ml of 100 mM Tris-hydrochloride (pH 8.0) containing 1 mg of bovine serum albumin

per ml and 1 mM DTT. Cells were disrupted by sonic oscillation for 45 to 60 s in an MSE ultrasonic disintegrator. Cell debris was removed by centrifugation at 20,000 × *g* for 20 min, and the supernatant was dialyzed against Tris buffer containing DTT for 3 h at 0°C.

Estimation of protein. Protein was precipitated from extracts by treatment with 10% (wt/vol) trichloroacetic acid and the resultant pellet was washed with 2% (wt/vol) trichloroacetic acid. Protein in the pellet was estimated by the method of Lowry et al. (12), using crystalline bovine serum albumin as a standard. The protein content was corrected for bovine serum albumin in the extraction medium.

Enzyme assays. (i) Guanine and hypoxanthine phosphoribosyltransferase (EC 2.4.2.8). Unless otherwise specified, incubation mixtures contained, in a volume of 50 μl, 0.3 mM Na_4-P-Rib-PP, 0.02 mM [8-^{14}C]guanine or 0.08 mM [8-^{14}C]hypoxanthine, 5 mM $MgCl_2$, 1 mM DTT, and 0.2 mg of bovine serum albumin per ml with an appropriate amount of extract protein (usually of the order of 10 μg/ml) in 40 mM Tris-hydrochloride, pH 8.0. Addition of the radioactive precursor initiated reaction, and after incubation for 5 min at 37°C 5- to 20-μl samples were taken.

Guanine and GMP were separated by chromatography on cellulose phosphate paper (Whatman P81) as described for adenine and AMP (25). Hypoxanthine and IMP were separated as described for uracil and UMP (16) by chromatography on polyethyleneimine-cellulose thin layers (19, 20). The areas containing the products were cut out and placed in vials with 5 ml of scintillation fluid (toluene containing 5 g of 2,5-diphenyloxazole per liter) for counting in a Packard Tri-Carb liquid scintillation spectrometer.

(ii) Purine nucleoside phosphorylase (purine nucleoside:orthophosphate ribosyltransferase, EC 2.4.2.1). The reaction mixtures contained 0.5 mM [8-^{14}C]adenosine or 0.5 mM [8-^{14}C]inosine, 5 mM $MgCl_2$, and approximately 75 μg of extract protein per ml in 100 mM potassium phosphate, pH 7.2. Reaction was started by addition of the ^{14}C-labeled nucleoside to the other components after preincubation for 3 min at 37°C. At 2 min, 5-μl samples were applied to the origins of borate-treated polyethyleneimine-cellulose thin layers (24) for chromatographic separation of the nucleoside and its corresponding base as previously described (15).

The identity of the sole radioactive product of [^{14}C]adenosine degradation as adenine was confirmed by autoradiography of chromatograms of assay samples on cellulose phosphate paper, developed with water (25). In this system, R_f values for adenosine, inosine, adenine, and hypoxanthine were 0.36, 1.00, 0.14, and 0.71, respectively.

(iii) GMP reductase (NADPH:GMP oxidoreductase, EC 1.6.6.8). A mixture containing 0.27 mM NADPH, 0.67 mM glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and 0.13 mM [8-^{14}C]GMP in 15 μl of 20 mM Tris-hydrochloride (pH 8.0) was incubated for 2 min at 37°C. At zero time, the reaction was initiated by the addition of 5 μl of a solution containing extract protein to a final concentration of approximately 150 μg/ml, 0.8 mg of bovine serum albumin per ml, and 4 mM DTT in 100 mM Tris buffer.

At different times, 5-μl samples were applied to polyethyleneimine-cellulose thin layers with origins prespotted with a mixture of GMP, IMP, and guanosine as markers. The thin layers were prepared as described (16) except that the top of the layer was scraped off 6.5 cm above the origin. The chromatograms were developed twice, with intermediate drying, by ascending chromatography in 2 M formic acid. This system gave approximate R_f values of 0 for GTP and GDP (and ITP and IDP), 0.25 for IMP, 0.6 for GMP, and 1.0 for guanosine and guanine. After thorough drying of the chromatogram, markers were visualized under UV light, and the required spots were excised for liquid scintillation counting. In some experiments when we desired to separate GTP, GDP, ITP, and IDP, the samples were applied to polyethyleneimine-cellulose thin-layer sheets (20 by 20 cm) for two-dimensional chromatography. The first dimension was developed as described above. The second dimension was developed for 5 cm in 0.75 M LiCl in 1.0 M acetic acid and then for 12 cm in 1.25 M LiCl in 1.0 M acetic acid (1). This procedure effected separation of GTP, GDP, GMP, ITP, IDP, and IMP for excision and counting.

(iv) GMP kinase (ATP:nucleoside monophosphate phosphotransferase, EC 2.7.4.4). Incubation mixtures contained 10 mM ATP, 10 mM $MgSO_4$, 1 mM [^{14}C]GMP, 25 mM potassium phosphate, and extract protein in 50 mM Tris-hydrochloride, pH 8.0. Reaction was started by addition of [^{14}C]GMP after equilibration of the other components at 37°C. At various times, 5-μl samples were taken for chromatography in one dimension as described for GMP reductase.

(v) Adenylosuccinate synthetase and adenylosuccinate lyase (IMP:L-aspartate ligase (GDP-forming), EC 6.3.4.4, and adenylosuccinate AMP-lyase, E.C. 4.3.2.2). Unless otherwise specified incubation mixtures contained 1 mM IMP, 1 mM GTP, 1 mM L-[*U*-^{14}C]aspartic acid, 5 mM Mg^{2+}, 1 mM DTT, 35 mM Tris-hydrochloride (pH 8.0), extract protein (approximately 1 mg/ml), and 1.25 mM phosphoenolpyruvate with pyruvate kinase to regenerate GTP. Reaction was initiated by addition of [^{14}C]aspartate, and 5-μl samples were taken at different times and applied to polyethyleneimine-cellulose thin layers. The layers were prepared as previously described (16), except the distance from the origin to the front was 9 cm. The origins were spotted with marker solutions of fumarate and adenylosuccinate before application of the samples. Chromatograms were developed by ascending chromatography in 1.0 M formic acid. Autoradiography was used to confirm the coincidence of radioactive spots with those absorbing UV light. To measure the total reaction to adenylosuccinate, both fumarate and adenylosuccinate spots were cut out for liquid scintillation counting.

Processing of kinetic results. The data from kinetic experiments were fitted directly to the appropriate rate equation to calculate Michaelis constants, using a computer program which gives a least-squares estimation of nonlinear parameters. This program is in the University of Melbourne Computation Department Library (14). The equations used were from Cleland (3); thus, with one variable substrate,

$$v = \frac{VA}{K_a + A} \quad (1)$$

and for an ordered sequential reaction with two variable substrates,

$$v = \frac{VAB}{K_{ia}K_b + K_bA + K_aB + AB} \quad (2)$$

RESULTS

Guanine and hypoxanthine phosphoribosyltransferases. To seek an explanation of the incorporation of guanine and hypoxanthine by *M. mycoides*, cell-free extracts were assayed for the corresponding phosphoribosyltransferases. Both activities were detected. They showed pH optima at 7.8 for guanine and 8.0 for hypoxanthine, with no sharp changes in rate for pH values over the range 7.0 to 8.6. The optimum Mg^{2+} concentration was 5 mM with hypoxanthine and 10 mM with guanine, although 5 mM Mg^{2+} gave only slightly lower activity with the latter. The activities were found in the fraction precipitating from extracts between 33 and 70% saturation with ammonium sulfate. Further resolution by chromatography on a column of Sephadex G-100 (2 cm^2 by 50 cm) gave guanine and hypoxanthine phosphoribosyltransferase activities in a single peak not coincident with that of adenine phosphoribosyltransferase. In view of the detailed analysis necessary to demonstrate that guanine and hypoxanthine phosphoribosyltransferases are distinct enzymes in some other species of bacteria (4, 9), the above observation cannot be taken as evidence that one enzyme possesses both activities in *M. mycoides*.

Initial velocity studies were performed on freshly prepared, dialyzed cell-free extracts at a series of concentrations of P-Rib-PP and purine base. The data fitted equation 2 for an ordered sequential reaction to give Michaelis constants of 1.3 ± 0.4 μM for guanine and 11.2 ± 2.8 μM for P-Rib-PP with guanine phosphoribosyltransferase and 78 ± 30 μM for hypoxanthine and 66 ± 20 μM for P-Rib-PP with hypoxanthine phosphoribosyltransferase.

The activities of hypoxanthine and guanine phosphoribosyltransferases in the presence of 2 mM nucleotides are shown in Table 1. Neither activity was appreciably inhibited by pyrimidine or adenine nucleotides. For both activities, the effectiveness of inhibitors was in the order GMP > IMP > GTP. The effects of these nucleotides at more physiological concentrations are shown in Table 2.

Purine nucleoside utilization. The ability of *M. mycoides* to use purine nucleosides as precursors of nucleic acid, probably after initial degradation to the free base (15), suggests the presence of a purine nucleoside phosphorylase. Alternative possibilities for the incorporation of nucleosides could involve a kinase or, in the case of adenosine, initial deamination to inosine before phosphorolysis (or phosphorylation). Cell-free extracts catalyzed a phosphate-dependent cleavage of [^{14}C]adenosine and [^{14}C]inosine to the corresponding bases. No formation of inosine or hypoxanthine was observed from [^{14}C]adenosine. The apparent competition between adenosine, guanosine, and inosine for phosphorolysis

TABLE 1. *Effects of nucleotides on hypoxanthine and guanine phosphoribosyltransferases in M. mycoides*[a]

Nucleotide (2 mM)	Rate of reaction (% control)	
	HPRTase[b]	GPRTase[c]
Nil (control)	100	100
AMP	100	98
ATP	96	99
GMP	8	2
GTP	49	73
IMP	26	23
UMP	97	97
UTP	100	99
CMP	102	96
CTP	100	97

[a] Incubations contained 340 μM P-Rib-PP, 10 mM $MgCl_2$, 20 μM [^{14}C]guanine or 40 μM [^{14}C]hypoxanthine in 40 mM Tris-hydrochloride (pH 8.0) containing 1 mM DTT.

[b] HPRTase, Hypoxanthine phosphoribosyltransferase.

[c] GPRTase, Guanine phosphoribosyltransferase.

TABLE 2. *Effect of nucleotide concentration on hypoxanthine and guanine phosphoribosyltransferases in M. mycoides*[a]

Nucleotide and concn (mM)	Activity as % control at 200 μM P-Rib-PP			
	200 μM P-Rib-PP		20 μM P-Rib-PP	
	HPRTase	GPRTase	HPRTase	GPRTase
Nil (control)	100	100	46	70
GMP				
0.50	5	5	1	0
0.10	20	20	2	1
0.05	37	35	4	3
GTP				
2.0	23	36	3	4
1.0	47	58	7	8
0.5	63	84	12	15
IMP				
0.5	52	45	7	6
0.1	89	82	28	18

[a] Incubation mixtures as for Table 1, except containing 5 mM $MgSO_4$. Abbreviations are given in Table 1.

(Table 3) may indicate that *M. mycoides* possesses a single purine nucleoside phosphorylase displaying wide specificity, as observed in *Escherichia coli* B (11) and *Salmonella typhimurium* (10). No conditions were found under which cell-free extracts of *M. mycoides* deaminated adenosine or showed purine nucleoside kinase activity.

Metabolism of GMP by extracts of *M. mycoides*. When *M. mycoides* is supplied with guanine as the only purine, the further metabolism of the GMP derived from the phosphoribosyltransferase reaction would be critical in determining balanced rates of supply of ATP and GTP.

Using [^{14}C]GMP as substrate and a chromatographic system that separated guanosine, GMP, and IMP from each other and from the corresponding di- and triphosphates, we found that an extract of *M. mycoides* catalyzed reactions of GMP involving degradation to guanosine, phosphorylation by Mg-ATP, and reduction to IMP when the appropriate substrates for GMP kinase and GMP reductase were available (Table 4). Approximately 80% of the total radioactivity was present as [^{14}C]GTP and 13% as [^{14}C]GDP after 5 min of incubation (results not shown). The degradation to guanosine was found to be strongly inhibited by phosphate ion, and 25 mM potassium phosphate was therefore included in incubation mixtures to investigate kinetic properties of GMP reductase.

GMP reductase. GMP reductase of *M. mycoides* specifically required NADPH as substrate. The pH optimum for this enzyme was about 8.3, with little change in rate occurring in the range 8.1 to 8.6. The effect of GMP concentration on the rate of reaction was determined with NADPH at a fixed, saturating concentration of 0.5 mM, after preliminary experiments had indicated that the Michaelis constant for NADPH was less than 10 μM. Fitting the data to equation 1 gave a Michaelis constant of 9.4 $\pm$ 3.5 μM for GMP.

The effect of nucleotides on GMP reductase of *M. mycoides* is shown in Table 5. ATP without Mg^{2+} was not inhibitory toward the enzyme from *M. mycoides*, in contrast to reports that ATP is the most effective inhibitor of GMP reductase in enterobacteria (2, 13). Under these conditions of assay, only AMP and IMP caused significant inhibition of GMP reductase. Preliminary studies suggested that Mg-ATP does not inhibit GMP reductase when the GMP concentration is high (10 to 100 μM) but inhibits strongly at lower concentrations of GMP.

GMP kinase. GMP kinase of *M. mycoides* had a pH optimum of 8.0, and changes in pH from 7.0 to 8.6 caused only slight changes in the rate of reaction. The enzyme required Mg^{2+}, and maximal activity was obtained with equimolar concentrations of Mg^{2+} and ATP. Excess Mg^{2+} caused inhibition, suggesting that the true substrate was a Mg-ATP complex. Potassium ion at a concentration of 5 mM, as either the phosphate or the chloride, activated GMP kinase about twofold. Higher concentrations of K^+ did not increase the activation. The initial velocity of reaction was measured at a series of concentrations of Mg-ATP and GMP. Incubation mixtures contained an excess of 1 mM Na_4-ATP over the Mg^{2+} added to prevent any inhibition of Mg^{2+}. This concentration of ATP had previously been shown not to be inhibitory. The results fitted to equation 2 to give Michaelis constants of 6.1 to 9.0 mM for Mg-ATP and 0.40 to 0.51 mM for GMP.

We found no significant inhibition of GMP kinase by nucleotides, with the possible exceptions of GDP and GTP, the effects of which were difficult to determine owing to their con-

TABLE 3. *Competition between purine nucleosides for phosphorolysis by cell-free extracts of M. mycoides*

^{14}C-labeled nucleoside (0.5 mM)	Unlabeled nucleoside and concn (mM)	Rate of reaction (% of control)[a]
Adenosine	No addition	100
Adenosine	Inosine, 0.25	60
Adenosine	Inosine, 0.50	47
Adenosine	Guanosine, 0.25	54
Adenosine	Guanosine, 0.50	34
Inosine	No addition	100
Inosine	Adenosine, 0.25	65
Inosine	Adenosine, 0.50	44
Inosine	Guanosine, 0.25	65
Inosine	Guanosine, 0.50	51

[a] Control activity corresponded to 730 nmol/min per mg of protein for adenosine phosphorylase and 415 nmol/min per mg of protein for inosine phosphorylase.

TABLE 4. *Fate of [^{14}C]GMP incubated with M. mycoides extract*

Incubation	nmol/mg of protein		
	GDP + GTP + IDP + ITP	IMP	Guanosine
(i) 1.9 mM Mg^{2+}[a]			
2 min	0	6.6	5.5
5 min	0	15.7	16.1
10 min	0	28.5	32.8
(ii) 1.9 mM Mg-ATP[a]			
2 min	296	6.8	7.1
5 min	375	9.2	7.3
10 min	371	10.5	5.7

[a] Other components of the incubation mixture were as described for GMP reductase.

TABLE 5. *Effect of nucleotides on GMP reductase from M. mycoides*[a]

Nucleotide	Concn (mM)	Rate of reaction (% of control at 80 μM GMP)	
		80 μM GMP	20 μM GMP
Nil		100	78
AMP	2.0	44	13
	1.0	77	24
	0.5	89	39
IMP[b]	1.0	40	30
ATP	2.0	100	
	1.0	100	
CMP	1.0	82	
CTP	1.0	100	
UMP	1.0	102	
UTP	1.0	100	

[a] Incubation mixtures as described in Materials and Methods also contained 25 mM potassium phosphate.
[b] The IMP used contained approximately 2% GMP. These values have been corrected for the resulting increased concentration and decreased specific activity of substrate GMP.

tamination by GMP, which would cause changes in specific activity and concentration of the labeled GMP substrate.

Conversion of IMP to AMP. The formation of AMP from IMP, GTP, and aspartate is a two-stage reaction proceeding via the intermediate adenylosuccinate under the sequential actions of adenylosuccinate synthetase and adenylosuccinate lyase. No attempt was made to separate the two activities in cell-free extracts of *M. mycoides*. Under the conditions of assay described, the total rate of formation of adenylosuccinate was linear for incubation periods of at least 20 min. Initial velocity studies at various concentrations of one substrate (IMP, aspartate, or GTP) with fixed concentrations of the other two substrates gave apparent Michaelis constants of 30.7 ± 1.7 μM for GTP, 45.0 ± 26.2 μM for IMP, and 255 ± 27 μM for aspartate.

The effects of various nucleotides on the conversion of IMP to AMP are presented in Table 6. Under in vitro conditions, this conversion was weakly inhibited by AMP, with no other nucleotide being significantly inhibitory.

Relative activities of enzymes in cell-free extracts of *M. mycoides*. To assist in assessing the significance of the various enzymes to purine metabolism in *M. mycoides*, their specific activities in cell-free extracts were determined under optimal conditions. Table 7 lists the values for cells grown in either PPLO broth or defined medium C2 with differing purine supplements. For cells grown under these conditions, nucleic acid synthesis required incorporation of purines at a rate of 0.5 to 1 nmol/min per mg of cellular protein each for adenine and guanine nucleotide (15). The values tabulated represent rates well in excess of this and thus have scope for a regulated decrease to match the cellular requirements.

The results in Table 7 suggest that *M. mycoides* is capable of regulating enzyme synthesis to some extent in response to differing growth conditions. The variation in the ratio of guanine-to-hypoxanthine phosphoribosyltransferase activities is consistent with their being properties of different proteins. The possible regulation of enzyme levels is particularly interesting in relation to GMP reductase and GMP kinase in that the ratio of the former to the latter is severalfold greater in cells grown on medium C2 with guanine as sole purine source than in cells grown on medium containing adenine and guanine. Cells

TABLE 6. *Effect of nucleotides on the conversion of IMP to adenylosuccinate*

Nucleotide	Concn (mM)	Activity (% of control)
Nil		100[a]
AMP	1.10	38[a]
	0.83	60[a]
	0.55	73[a]
	0.28	86[a]
ATP	2.00	71
	1.10	80[a]
	0.55	90[a]
	0.28	100[a]
GMP	1.00	70
	0.50	77
	0.10	84

[a] Incubation mixtures contained 1.43 mM GTP, 1.43 mM IMP, 0.46 mM [^{14}C]aspartate, 3.6 mM Mg^{2+}, and 1.43 mM phosphoenolpyruvate.

TABLE 7. *Specific activities of various enzymes in extracts of M. mycoides*

Enzyme	Sp act (nmol/min per mg of protein)		
	PPLO broth[a]	C2 (guanine)[a]	C2 (guanine + adenine)[a]
Adenine PRTase[b]	199	84[c]	145[c]
Guanine PRTase	199	64	79
Hypoxanthine PRTase	256	195	308
Purine nucleoside phosphorylase			
With adenosine	730	—[d]	—
With inosine	415	—	—
GMP reductase	3.0	7.6	3.7
GMP kinase	150	90	145
Adenylosuccinate synthetase	1.5	—	—

[a] Growth medium for preparation of extract.
[b] PRTase, Phosphoribosyltransferase.
[c] From reference 25.
[d] —, Experiment not done.

from medium C2 with guanine and hypoxanthine showed a specific activity for GMP reductase of 5.1 nmol/min per mg of protein, intermediate between that for cells grown on guanine as the only purine and cells grown on adenine plus guanine.

DISCUSSION

M. mycoides has the enzymatic capacity to convert guanine and hypoxanthine, as well as adenine (25), to the corresponding nucleoside 5′-monophosphate by phosphoribosyltransfer from P-Rib-PP. This capacity would provide the first step for the observed incorporation (15) of these bases into RNA. For all the phosphoribosyltransferases, guanine nucleotides, particularly GMP, are the major inhibitors, with inhibition increasing at lower P-Rib-PP concentrations. *M. mycoides* also possesses GMP reductase, adenylosuccinate synthetase, and adenylosuccinate lyase, so that the sequential conversion of GMP to AMP via IMP and adenylosuccinate would be available to it.

These results account for the ability of *M. mycoides* to use guanine as the only source of purine nucleotide and are consistent with the observations on the use of other preformed bases as precursors of purine nucleotide in this organism (15).

The rapid rate of phosphorolysis of the purine nucleosides both by cell-free extracts and in cultures (15) would allow them to be utilized as the corresponding bases and suggests that the activity of purine nucleoside phosphorylase is determined largely by the availability of substrates, as has been reported for *E. coli* and *S. typhimurium* (10). Thus, the phosphoribosyltransferases would catalyze the rate-limiting step in the formation of nucleotides from either bases or nucleosides.

When guanine alone meets the full requirements of *M. mycoides* for purines, the conversion of GMP to AMP via IMP and adenylosuccinate would be of major importance. Under these conditions of growth, the intracellular utilization of GMP must be balanced between provision of adenine and guanine nucleotides, a balance that depends on the properties of GMP reductase and GMP kinase. The activity of GMP kinase would probably change in almost direct proportion to changes in concentration of GMP and ATP owing to the high Michaelis constants for both substrates. These responses would act to maintain an adequate supply of GMP for the formation of ATP, provided ATP was low. Free Mg^{2+} released from chelation might also contribute to inhibition when ATP is lowered. GMP reductase has a low Michaelis constant for GMP, and so the rate of conversion of GMP to adenine nucleotides should be relatively insensitive to changes in the level of GMP.

Studies on the incorporation of various purine bases into RNA (15) showed that the presence of adenine and guanine in the growth medium almost completely inhibited the conversion of GMP to AMP. The present data indicate, as possible factors in this effect, feedback inhibition by AMP on its synthesis from IMP, with inhibition of GMP reductase by the resultant elevated levels of IMP. However, the measured inhibitory effects from these factors in vitro appear insufficient to account for the complete cessation of ATP synthesis from guanine in the presence of adenine, unless the changes in concentration of IMP and AMP are very large. Probably the enzymes are subject to additional controlling factors in the cell, perhaps by feedback inhibition from Mg-ATP on GMP reductase at low GMP concentrations.

Another factor opposing the conversion of guanine nucleotide to adenine nucleotide in cells grown on medium containing guanine and adenine would be the lower ratio of GMP reductase to GMP kinase found in such cells as compared with cells grown on the medium with guanine as sole purine source. This factor might also apply, but to a lesser extent, for cells grown on medium containing guanine and hypoxanthine, where the latter excludes guanine from approximately one-half of the adenine nucleotide (15).

Comparative studies on the phosphoribosyltransferases and GMP reductase of *E. coli* (unpublished data) have indicated that the corresponding enzymes in *M. mycoides* are more sensitive to inhibition by purine nucleoside monophosphates. Another aspect in which *M. mycoides* differs from *E. coli* is the importance of GMP in the regulation of purine biosynthesis. Only GMP can give rise to all the purine nucleotide in the cell, since neither AMP nor IMP can be converted to GMP. This role of GMP may reflect adaptation of *M. mycoides*, the causative agent of bovine pleuropneumonia, to growth in mammalian tissues. In mammals, the liver may act as a source of preformed purines by releasing nucleosides into the blood (17). A number of possible mechanisms tend to favor the exclusion of adenosine from the purine nucleotides available for release. Mammalian, avian, and amphibian livers have a 5′-nucleotidase which is considerably more active toward IMP, GMP, and dGMP than toward AMP (8). Mammalian tissues also possess adenosine kinase and active deaminases for AMP and adenosine. The availability of free adenine would be limited by the lack of activity of purine nucleoside phosphorylase for adenosine or deoxyadenosine (18). Consistent with these mechanisms directing the

breakdown of adenosine nucleotides towards hypoxanthine and limiting the availability of adenine, hypoxanthine is the major purine component in the blood, which also contains appreciable levels of xanthine (17). The growth of *M. mycoides* on xanthine as the only purine precursor has not been tested, but xanthine phosphoribosyltransferase activity has been detected in cell extracts (unpublished observation).

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