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

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The major pathways of ribonucleotide biosynthesis in *Mycoplasma mycoides* subsp. *mycoides* have been proposed from studies on its use of radioactive purines and pyrimidines. To interpret more fully the observed pattern of pyrimidine usage, cell extracts of this organism have been assayed for several enzymes associated with the salvage synthesis of pyrimidine nucleotides. *M. mycoides* possessed uracil phosphoribosyltransferase, uridine phosphorylase, uridine (cytidine) kinase, uridine 5'-monophosphate kinase, and cytidine 5'-triphosphate synthetase. No activity for phosphorolysis of cytidine was detected, and no in vitro conditions were found to give measurable deamination of cytidine. Of the two potential pathways for incorporation of uridine, our data suggest that this precursor would largely undergo initial phosphorolysis to uracil and ribose-1-phosphate. Conversely, cytidine is phosphorylated directly to cytidine 5'-monophosphate in its major utilization, although conversion of cytidine to uracil, uridine, and uridine nucleotide has been observed in vivo, at least when uracil is provided in the growth medium. Measurements of intracellular nucleotide contents and their changes on additions of pyrimidine precursors have allowed suggestions as to the operation of regulatory mechanisms on pyrimidine nucleotide biosynthesis in *M. mycoides* in vivo. With uracil alone or uracil plus uridine as precursors of pyrimidine ribonucleotides, the regulation of uracil phosphoribosyltransferase and cytidine 5'-triphosphate synthetase is probably most important in determining the rate of pyrimidine nucleotide synthesis. When cytidine supplements uracil in the growth medium, control of cytidine kinase activity would also be important in this regard.

Mycoplasma mycoides subsp. *mycoides* requires for growth both uracil and guanine as precursors of ribonucleotides (15). From an examination of the incorporation of precursors into RNA (9) and a consideration of the known pathways of salvage nucleotide synthesis in other bacteria (14), we have outlined possible pathways of nucleotide metabolism in *M. mycoides* (9). Our results indicate that this organism lacks the reactions of de novo synthesis of nucleotides from simple precursors and displays a limited ability to interconvert nucleotides.

Of the enzymes associated with the proposed pathways, those relating to purine nucleotides have been partially defined and were found to differ from the corresponding *Escherichia coli* enzymes in some aspects of their regulation (10, 19). In relation to pyrimidine nucleotide metabolism, uridine phosphorylase (EC 2.4.2.3) has been detected in many strains of mycoplasmas during the search for methods to assess mycoplasmal contamination of mammalian cell cultures (6).

To extend this limited knowledge of the pyrimidine nucleotide metabolism in mycoplasmas, we have assayed cell extracts of *M. mycoides* for the various enzymes suggested from our previous studies and have investigated aspects of the kinetic properties and regulation of those enzymes likely to be significant in determining the rate of in vivo nucleotide synthesis. To test the effectiveness of in vitro regulatory mechanisms in controlling the synthesis of ribonucleotides in vivo, we have measured the intracellular contents of some ribonucleotides and the changes which occur in them in response to the addition of supplementary nucleosides or bases as potential precursors of pyrimidine nucleotides.

MATERIALS AND METHODS

Organism and culture medium. *M. mycoides* subsp. *mycoides* was kindly supplied by A. W. Rodwell, Commonwealth Scientific and Industrial Research Organisation, Division of Animal Health. For estimation of intracellular nucleotide contents, cells were grown in a modified medium C2 of Rodwell (16) as previously described (9). For the preparation of extracts for assay

of enzymes, cells were grown in PPLO broth (17).

Chemicals and radiochemicals. Tris, 2,5-diphenyloxazole, and tetrasodium 5-phosphoribosyl-1-diphosphate (P-Rib-PP) were all from the Sigma Chemical Co. Dithiothreitol, 2-phosphoenolpyruvate, pyruvate kinase, and crystalline bovine serum albumin were from Calbiochem. The radiochemical substrates [2-^{14}C]uracil (61 Ci/mol), [2-^{14}C]uridine (59 Ci/mol), [4-^{14}C]UMP (24 Ci/mol), and [4-^{14}C]UTP (48 Ci/mol) were all supplied by the Radiochemical Centre, England; [2-^{14}C]cytidine (43 Ci/mol) was a product of ICN Chemical and Radioisotope Division.

Preparation of extracts for enzyme assays. Extracts of *M. mycoides* were prepared by sonic oscillation of cells from late-log-phase or stationary-phase cultures as previously described (10). Unless specified, extracts were dialyzed for 4 to 6 h against 100 mM Tris-hydrochloride at 0°C. Protein content was estimated by the method of Lowry et al. (8) as previously described (10).

Enzyme assays. (i) Uracil phosphoribosyltransferase. In the uracil phosphoribosyltransferase (UMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.9) assay, unless otherwise specified, reaction mixtures, incubated at 37°C, contained 5 mM $MgSO_4$, 50 μM [^{14}C]uracil, 500 μM P-Rib-PP, bovine serum albumin (0.2 mg/ml), and extract protein (to approximately 40 μg/ml) in 40 mM Tris-hydrochloride (pH 8.0). When GTP was included, 1 mM 2-phosphoenolpyruvate and pyruvate kinase were also added. The reaction was initiated by the addition of [^{14}C]uracil to a mixture of the other components after preincubation of the mixture at 37°C. At various times, 5-μl samples were applied to the origins of polyethyleneimine-cellulose (PEI-C) thin layers for chromatographic isolation of the product for quantitation by liquid scintillation counting (11).

(ii) Uridine phosphorylase. In the uridine phosphorylase (uridine:orthophosphate ribosyltransferase) assay, reaction mixtures, incubated at 37°C, were buffered at pH 7.2 with 40 mM Tris-hydrochloride and contained 5 mM $MgSO_4$, various concentrations of the substrates [^{14}C]uridine and potassium phosphate, and extract protein (to approximately 160 μg/ml). The reaction was started by the addition of the [^{14}C]uridine to a mixture of the other components at 37°C. Samples of the incubation mixture, taken at various times, were streaked on the origins of PEI-C thin layers in the borate form (18) for chromatography and counting of products as described earlier (9).

(iii) Uridine (cytidine) kinase. In the uridine (cytidine) kinase (ATP:uridine 5′-phosphotransferase, EC 2.7.1.48) assay, reaction mixtures, incubated at 37°C, contained 5 mM Mg acetate, 1 mM ^{14}C-labeled nucleoside, 2 mM ATP, 40 mM Tris-hydrochloride (pH 7.8), and extract protein (to approximately 400 μg/ml). Sampling, chromatography, and liquid scintillation counting were as described for uracil phosphoribosyltransferase (11).

(iv) Cytidine deaminase. In the cytidine deaminase (cytidine aminohydrolase, EC 3.5.4.5) assay, to assay for the formation of [^{14}C]uridine from [^{14}C]cytidine, cell extracts of *M. mycoides* were incubated at 37°C in 40 mM Tris-hydrochloride (pH 7.2), containing 5 mM $MgSO_4$. At the required times, 5- to 20-μl samples of incubation mixture were spotted at the origins of cellulose phosphate paper strips for chromatography as described (9).

(v) UMP kinase. In the UMP kinase (ATP:UMP phosphotransferase) assay, reaction mixtures, incubated at 37°C, contained 5 mM ATP, 0.2 mM [^{14}C]-UMP, 5 mM $MgCl_2$, 40 mM Tris-hydrochloride (pH 7.8), and extract protein to approximately 50 μg/ml. Reaction was started by the addition of [^{14}C]UMP to a mixture of the other components at 37°C. At different times, 5-μl samples were applied to PEI-C thin layers (11), 10 cm long. The origins had previously been spotted with a marker solution (2 μl, containing 3.3 mM UTP, UDP, and UMP). The chromatograms were developed once with distilled water, dried, then redeveloped in 1.0 M acetic acid containing 0.75 M LiCl. The first development moved any uracil or uridine to the front, whereas the redevelopment effectively separated UMP, UDP, and UTP which were located by the UV absorbance of the marker compounds to allow their excision for liquid scintillation counting.

(vi) CTP synthetase. In the CTP synthetase (UTP:ammonia ligase [ADP], EC 6.3.4.2) assay, reaction mixtures, incubated at 37°C, contained 1 mM ATP, 0.31 mM [^{14}C]UTP, 0.5 mM GTP, 5.0 mM glutamine, and 10.0 mM Mg acetate in 37.5 mM Tris-hydrochloride (pH 7.2), and extract protein (to approximately 400 μg/ml). The reaction was begun by the addition of [^{14}C]UTP to all other components at 37°C. At various times, 5-μl samples were applied to PEI-C thin-layer strips (11), 20 cm in length. The origins had previously been spotted with a marker solution (2 μl, containing UTP, UDP, UMP, CTP, CDP, and CMP, each at a concentration of 1.6 mM). Chromatograms were developed for 9 cm in 1.0 M formic acid containing 0.4 M LiCl, and for an additional 9 cm in 1.0 M formic acid containing 1.0 M LiCl. This procedure effectively separated UTP and CTP from the corresponding nucleoside mono- and diphosphates. The six nucleotides were located by the UV absorbance of the marker compounds to allow their excision for liquid scintillation counting.

Processing of kinetic results. For the estimation of kinetic parameters as described earlier (10), the data for kinetic experiments were fitted to the rate equations describing ordered sequential, equilibrium-ordered, and ping-pong reaction mechanisms (3) for two substrate reactions. For each of the enzymes studied, best fit of the kinetic data was achieved with the rate equation for the ordered sequential reaction mechanism.

Measurement of intracellular ribonucleotide contents. [^{32}P]orthophosphate, to a specific activity of 60 to 70 Ci/mol, was added to cultures at a cell density corresponding to approximately 25 μg of cellular protein per ml of culture. Uniform labeling was ensured by allowing at least two doubling times before commencement of sampling. Extraction of cells in ice-cold $HClO_4$, and subsequent procedures for the estimation of nucleotides were performed as described by Bagnara and Finch (1). To study the effects of added nucleosides and bases on the intracellular nucleotide

content, the additions were made as a 1 mg/ml solution to give a concentration of 20 μg/ml in the culture.

RESULTS

Uracil phosphoribosyltransferase. To seek explanation of the ability of *M. mycoides* to incorporate uracil, cell extracts were assayed for uracil phosphoribosyltransferase. The reaction was found to be dependent on the presence of Mg^{2+}, and the rate was stimulated three- to sixfold by saturating concentrations of GTP, depending on the concentration of P-Rib-PP used (Fig. 1a). A double reciprocal plot of the GTP-dependent component of the rates shown in Fig. 1a gives straight lines (Fig. 1b) intersecting the absicissa at -40 mM^{-1}, suggesting that the dissociation constant (K_D) for GTP from the enzyme is approximately 25 μM.

The results of initial velocity studies at a series of concentrations of uracil and P-Rib-PP and a fixed concentration of 1 mM GTP gave K_m values of 2.6 ± 0.4 μM for uracil and 51.3 ± 6.7 μM for P-Rib-PP and a V_{max} of 120 μmol min^{-1} g^{-1} of extract protein when fitted to the model for an ordered sequential mechanism.

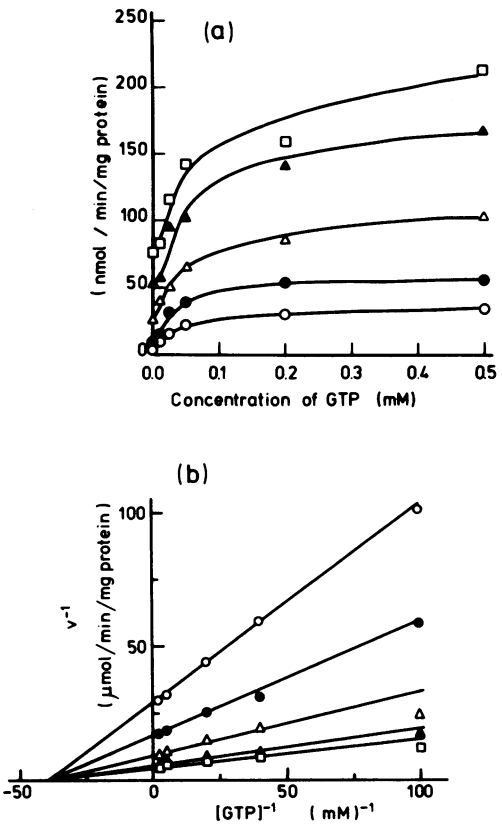

FIG. 1. *Effect of GTP on the kinetics of uracil phosphoribosyltransferase with respect to P-Rib-PP. Standard assay conditions were used, except that the concentrations of P-Rib-PP were: 5 μM (○); 10 μM (●); 25 μM (△); 100 μM (▲); and 400 μM (□). (a) Direct plot of rate of reaction versus GTP concentration. (b) Double reciprocal plot of the GTP-dependent component of the data in a.*

Table 1 shows the effects of several nucleotides, added together with 1 mM GTP, on the activity of uracil phosphoribosyltransferase. No other purine or cytidine nucleotide significantly affected the rate of reaction. The inhibition by uridine nucleotides increases with inhibitor concentration and in the order UTP, UDP, UMP (Table 2). Double reciprocal plots (Fig. 2) of data for the effect of P-Rib-PP concentration on the rate of reaction at various concentrations of UMP (Fig. 2) showed that the effect of UMP was to change the slope, but not the intercept, of the linear curve, suggesting that the inhibition by UMP was competitive with respect to P-Rib-PP. The data suggest K_i values for UMP in the range 19 to 22 μM with 0.1 mM GTP (Fig. 2a) and 30 to 50 μM with 1.0 mM GTP (Fig. 2b). Other data confirm K_i values in these ranges for UMP and suggest values of approximately 100

TABLE 1. *Effect of nucleotides added together with 1 mM GTP on uracil phosphoribosyltransferase*

Nucleoside	Activity (% control) with added nucleotide (2 mM)		
	Monophosphate	Diphosphate	Triphosphate
Adenosine	97	104	101
Inosine	94	94	103
Guanosine	82	101	100[a]
Uridine	10	25	80
Cytidine	108	101	108

[a] Final GTP concentration 2 mM—control.

TABLE 2. *Response of uracil phosphoribosyltransferase to various concentrations of uridine nucleotide added together with 1 mM GTP*

Nucleotide	Concn (mM)	Activity as % of control[a]	
		50 μM P-Rib-PP	500 μM P-Rib-PP
UMP	0.1	28	66
	0.5	9	22
	1.0	5	15
UDP	0.1	64	79
	0.5	21	51
	1.0	13	42
UTP	0.1	100	95
	0.5	71	76
	1.0	53	69

[a] Control assays contained 1 mM GTP, 50 or 500 μM P-Rib-PP as indicated, and no added uridine nucleotide.

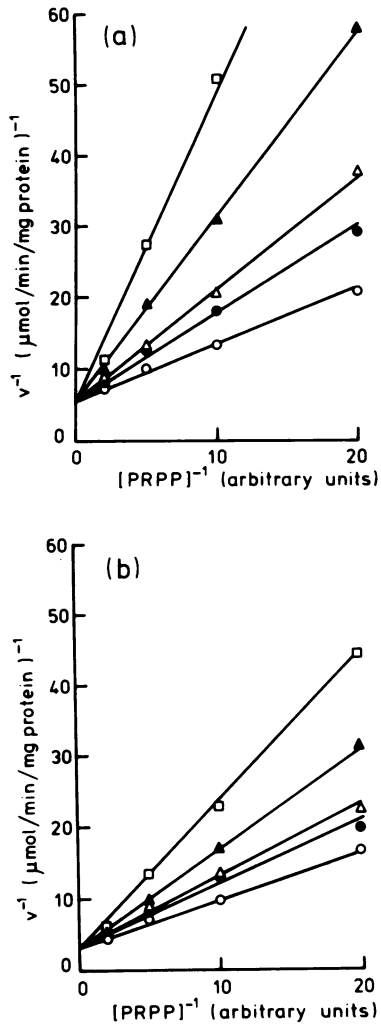

FIG. 2. *Double reciprocal plots of rate of reaction versus P-Rib-PP concentration for uracil phosphoribosyltransferase for various concentrations of UMP. Except for varied P-Rib-PP concentration, standard assay conditions were used with 0.1 mM GTP (a) or 1.0 mM GTP (b) and UMP at various concentrations: 0 μM (○); 10 μM (●); 20 μM (△); 50 μM (▲); and 100 μM (□).*

and 650 μM for UDP and UTP, respectively, with GTP at 1.0 mM.

Without GTP, the rate of reaction followed a sigmoidal curve when plotted against P-Rib-PP concentrations over the range of 70 to 150 μM, and the enzyme appeared to be saturated with uracil at concentrations below 2.5 μM. Also, without GTP, uracil phosphoribosyltransferase was somewhat more susceptible to inhibition by uridine nucleotides and was slightly activated by GDP. Since it is likely that GTP is always present in the cell, these latter two effects are probably not of physiological importance.

Uridine phosphorylase. The ability of *M. mycoides* to use uridine as a precursor of RNA, probably after initial degradation to the free base (9), is suggestive that this organism possesses uridine phosphorylase. Cell extracts of *M. mycoides* catalyzed a phosphate-dependent release of uracil from uridine as expected for uridine phosphorylase. Similar to other organisms (4, 13), *M. mycoides* displayed no phosphorolytic activity toward cytidine.

The data for initial velocity studies at a series of concentrations of phosphate and uridine yielded K_m values of 1.99 ± 0.98 mM for phosphate and 2.16 ± 0.68 mM for uridine, and a V_{max} of 860 μmol min^{-1} g^{-1} of protein when fitted to the equation for an ordered sequential reaction mechanism.

Uridine (cytidine) kinase. The effective utilization of cytidine by *M. mycoides* as a precursor of cytidine nucleotide in RNA (9) suggested the involvement of a cytidine kinase. This activity and a uridine kinase activity were demonstrated in dialyzed cell extracts of *M. mycoides*. The specific activity of uridine (cytidine) kinase in these extracts was 12.5 μmol min^{-1} g^{-1} of protein with uridine as substrate and 6.5 μmol min^{-1} g^{-1} of protein with cytidine. The enzyme required Mg^{2+} for activity, although concentrations above 10 mM were somewhat inhibitory.

ATP and to some extent GTP were substrates of uridine kinase, but cytidine kinase used only ATP. Inclusion of 1 mM cytidine in the assay of uridine kinase caused 30% inhibition of this reaction. Studies on the effects of various nucleotides on uridine (cytidine) kinase are shown in Tables 3 and 4. Both activities were inhibited by uridine and cytidine nucleotides, with cytidine kinase being more susceptible to inhibition by all effectors tested. These results contrast with those for the *E. coli* enzyme, which was found to be largely specific for GTP as phosphoryl donor and to be strongly inhibited by CTP, less strongly inhibited by CMP, and almost unaffected by uridine nucleotides (John Baird, M.Sc. thesis, University of Melbourne, Parkville, Victoria, Australia, 1973). Kinetic studies on uridine (cytidine) kinase were not undertaken, owing to the instability of cytidine kinase and the difficulty in suppressing uridine phosphorylase in assays. Extensive dialysis was only partially successful in alleviating the latter problem because phosphate in assay mixtures appeared to derive from ATP.

Cytidine deaminase. Cytidine is incorporated into the UMP isolated from RNA of *M. mycoides* (9) and, upon addition of [^{14}C]cytidine

TABLE 3. *The effect of pyrimidine nucleotides on uridine (cytidine) kinase*

Added nucleotide (2 mM)	Activity as % of control	
	Uridine kinase	Cytidine kinase
None	100	100
UTP	16	2
UDP	23	7
UMP	62	19
CTP	18	1
CMP	35	4

TABLE 4. *Effect of CTP and CMP on cytidine kinase*

Nucleotide concn (mM)	Activity with added nucleotide (% control)	
	CMP	CTP
0.0	100	100
0.1	73	67
0.5	37	15
1.0	17	1
2.0	10	1

to cultures, [^{14}C]uracil, derived via [^{14}C]uridine, accumulates extracellularly (9). These findings imply that *M. mycoides* may possess cytidine deaminase. However, no deamination of cytidine was measurable on incubation of the nucleoside with cell extracts with or without sulfhydryl reagents under conditions of varying pH or Mg^{2+} concentrations. Cytidine deaminase is inducible in *E. coli* and *Salmonella typhimurium* (5, 12), but no activity was detectable in extracts of *M. mycoides* grown in medium C2 supplemented with 2 mM cytidine.

UMP kinase. For *M. mycoides* grown on uracil as the sole precursor of pyrimidine ribonucleotides, the activity of UMP kinase may be a factor in controlling the supply of UTP and CTP to the cell. High levels of UMP kinase and significant levels of UDP kinase were measurable in cell extracts. The rate of formation of UTP was greatly decreased by lowering the amount of extract protein in assays and, under these conditions, the appearance of UDP and any UTP was taken as a measure of total UMP kinase activity. UMP kinase was activated by Mg^{2+} concentrations up to 5 mM, with slight inhibition occurring at higher concentrations.

Analysis of initial velocity data at a series of concentrations of UMP and ATP gave K_m values of 530 ± 90 μM for ATP, 60 ± 10 μM for UMP, and a V_{max} of approximately 200 μmol min^{-1} g^{-1} of extract protein. Potential inhibitory effects of UDP and UTP were not investigated because of difficulties in ascertaining the effect of contaminant UMP on the specific activity of the [^{14}C]UMP substrate.

CTP synthetase. In keeping with the observations that *M. mycoides* can use uracil as the source of both uridine and cytidine nucleotide, CTP synthetase was demonstrated in cell extracts of the organism. Autoradiography of the chromatograms obtained in the assay of CTP synthetase revealed the formation of UDP, UMP, CDP, and CMP, as well as CTP, from the radioactive UTP used as substrate. Owing to these further complications to the assay of the already complex CTP synthetase reaction, no attempt was made to study the kinetic properties of this enzyme in crude cell extracts.

The enzyme from *M. mycoides* resembled that from *E. coli* in not requiring a sulfhydryl reagent for activity (7). Although the addition of ATP and GTP was not absolutely essential to the demonstration of activity with undialyzed cell extracts, their omission decreased activity by more than 90%. Presumably, nucleotides present at low levels in the extract may have accounted for the background activity. Mg^{2+} was essential for reaction. Preliminary studies (Table 5) show that the enzyme was inhibited by CTP.

The effects of addition of supplementary precursors of pyrimidine nucleotides on the intracellular contents of some ribonucleotides. Figure 3 shows the effects of addition of uridine to cells of *M. mycoides* grown in a low-phosphate modification (9) of medium C2 of Rodwell (16) with guanine as the sole source of purine nucleotide. The addition caused a marked and rapid increase in the level of UTP and a slower, significant increase in the content of CTP. ATP was maintained at a somewhat higher steady-state level 5 min after the addition of uridine and GTP slowly decreased in concentration after an initial increase. When uridine was added to cells grown on medium in which adenine supplemented guanine to provide an alternative source of purine nucleotide (result not shown), the effects on pyrimidine nucleotides were very similar to those shown in Fig. 3. None of the observed changes is attributable to uridine providing a source of extra uracil, which is already 0.18 mM in medium C2 (16), since supplementation with additional uracil failed to elicit any effect on the intracellular ribonucleo-

TABLE 5. *Effect of CTP on CTP synthetase*

Concn of CTP (mM)	Activity (μmol min^{-1} g^{-1} of protein)
0.00	2.46
0.05	2.00
0.10	1.37
0.50	0.55

tide contents of a culture grown in this medium (result not shown).

The effects of cytidine on the ribonucleotide levels of cells grown in medium with guanine alone or guanine plus adenine are shown in Fig. 4. In each case, the addition of cytidine resulted in marked increases in the concentrations of UDP and CTP. However, the culture containing guanine plus adenine (Fig. 4a) showed the greater response, with CTP increasing steadily to a maximum of fivefold by 15 min. This increase was largely retained over 80 min. With the culture containing guanine as the only purine (Fig. 4b), both CTP and UTP doubled to a maximum by 5 min. Thereafter, UTP fell rapidly to be near its initial level by 10 min, whereas CTP remained slightly below the maximum. In the latter culture, but not the former, there was an appreciable increase in the ratio of diphosphate to triphosphate for the purine nucleotides.

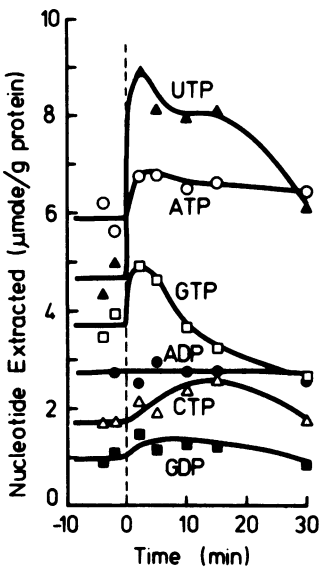

FIG. 3. *Effect of uridine on the intracellular ribonucleotides of M. mycoides grown on guanine as the sole source of purine nucleotide. Uridine was added at zero time to a concentration of 20 μg per ml of culture, and samples were taken for estimation of ribonucleotides at the indicated times. Symbols: □, GTP; ○, ATP; △, CTP; ▲, UTP; ■, GDP; ●, ADP.*

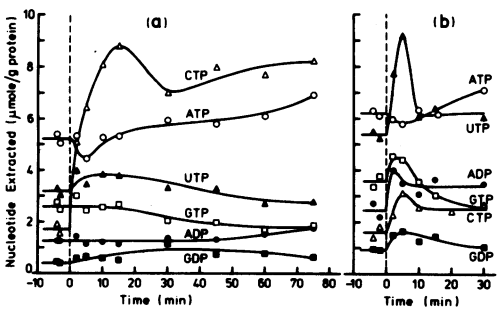

FIG. 4. *Effect of cytidine on the intracellular ribonucleotides of M. mycoides. The conditions were as described for Fig. 2, with cytidine replacing uridine. (a) Cells grown on medium containing guanine and adenine. (b) Cells grown on medium containing guanine as the only source of purine nucleotide.*

DISCUSSION

The present findings substantiate earlier proposals, based on the incorporation of precursors into RNA (9), for the reactions of pyrimidine nucleotide biosynthesis in *M. mycoides*. The organism possessed uracil phosphoribosyltransferase, UMP kinase and CTP synthetase for the provision of all pyrimidine ribonucleotides from uracil. The activation of uracil phosphoribosyltransferase from *M. mycoides* by GTP is similar to the effects observed with this enzyme from *E. coli* K-12 (11), and from *E. coli* B and *Klebsiella aerogenes* (unpublished data). The CTP synthetase also appears similar to that from *E. coli* (7) in being activated by GTP and inhibited by CTP. Uridine phosphorylase was very active in cell extracts, in keeping with the extensive degradation of uridine in cultures of *M. mycoides* (8). The high level of this enzyme would suggest that the direct phosphorylation of uridine to UMP via uridine kinase would only have a transitory significance in cultures unless uridine is supplied continuously. The observations on nucleotide contents support this suggestion. The rapid increase in concentration of UTP upon the addition of uridine is likely to arise through the provision of an alternative reaction for the synthesis of UMP via uridine kinase in addition to that catalyzed by the phosphoribosyltransferase. Before the provision of this additional pathway, the synthesis of uridine nucleotides would be regulated at the point of UMP biosynthesis by the activating effect of GTP and the inhibitory effect of uridine nucleotides on uracil phosphoribosyltransferase, perhaps also influenced by the P-Rib-PP concentration which lies in the range of 15 to 60 μM in the total cell water (unpublished data). The increase in GTP concentration (Fig. 3) could further increase the rate of UMP production through activation of uracil phosphoribosyltransferase. To limit the rate of UTP production back to the requirements of the cell, the rise in UTP would initially provide feedback on both uridine kinase and uracil phosphoribosyltransferase. Subsequently, the fall in concentration of GTP and decreased availability of uridine (9) would limit the capac-

ity for UMP synthesis such that a rate of synthesis appropriate to cellular requirements is only achieved at a lower concentration of the feedback inhibitor, UTP. The increased CTP concentration is a predictable consequence of elevated levels of ATP, GTP, and UTP. The return toward the original CTP level would occur as the inhibition of CTP synthetase by CTP became effective.

Our observations that *M. mycoides* is unable to incorporate cytosine into RNA (9), whereas it effectively uses cytidine, are consistent with the failure of cell extracts to catalyze the phosphorolysis of cytidine. The ability to use cytidine as a ready source of cytidine nucleotide (9) finds explanation in the availability of cytidine kinase. However the inability to demonstrate cytidine deaminase activity in extracts gave no support for the plausible hypothesis that this enzyme might account for our observation that cytidine serves as a precursor of a significant fraction of uridine nucleotide in RNA (9). Although it is unlikely, cytidine may appear as uridine nucleotide following deamination at the nucleotide level. Alternatively, *M. mycoides* may resemble a pyrimidine-requiring mutant of *S. typhimurium* reported to lack detectable cytidine deaminase, yet to retain the ability to convert cytidine to uridine (2). Possibly, a slow deamination to maintain a low concentration of uridine might allow this product to be preferentially used by a uridine kinase with a high affinity for it, rather than being degraded by a uridine phosphorylase with a low affinity.

With the addition of cytidine to cells growing in medium with guanine or guanine plus adenine, a pathway for the direct synthesis of cytidine nucleotides, independent of UTP, becomes available via cytidine kinase. The very large difference in the extent to which CTP concentrations increase in the two cultures may stem from the enhanced ability of cultures containing guanine as well as adenine to support the additional phosphorylations required to maintain a raised content of CTP and UTP. Feedback inhibition by the increased concentration of CTP on its further synthesis from UTP may have the effect of sparing UTP and so contributing to its increased concentration, as would activation of uracil phosphoribosyltransferase by the initially enhanced GTP content. Also, any conversion of cytidine to uridine would influence the intracellular content of uridine nucleotides by making available a pathway for their synthesis via uridine kinase. The in vivo studies on the effects of cytidine addition suggest that the concentration of CTP necessary to inhibit CTP synthetase must be considerably less than that required to inhibit cytidine kinase, since the concentrations of both CTP and UTP rise markedly. Such an effect would account for the strong preference shown for cytidine as a source of cytidine nucleotide in RNA when both uracil and cytidine are available as precursors (9).

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