Synthesis of Deoxyribonucleotides in Mollicutes: Dependence on Deoxyribose-1-Phosphate and PPi

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Cell extracts of Acholeplasma laidlawii B-PG9, Acholeplasma morum S2, Mycoplasma capricolum 14, and Mycoplasma gallisepticum S6 were examined for 37 cytoplasmic enzyme activities involved in the salvage and biosynthesis of purines. All of these organisms had adenine phosphoribosyltransferase activity (EC 2.4.2.7) and hypoxanthine phosphoribosyltransferase activity (EC 2.4.2.8). All of these organisms had purine-nucleoside phosphorylase activity (EC 2.4.2.1) in the synthetic direction using ribose-1-phosphate (R-1-P) or deoxyribose-1-phosphate (dR-1-P); this activity generated ribonucleosides or deoxyribonucleotides, respectively. The pyrimidine nucleobase uracil could also be ribosylated by using either R-1-P or dR-1-P as a donor. The synthesis of deoxyribonucleotides from nucleobases and dR-1-P has been reported from only one other procaryote, Escherichia coli (L. A. Mason and J. O. Lampen, J. Biol. Chem. 193:539–547, 1951). The reverse of this phosphorylase reaction is more widely known, and we found such activity in all mollicutes. Some Acholeplasma species but not the Mycoplasma species can phosphorylate deoxyribonucleotides to deoxyribonucleosides by a PPi-dependent deoxyribonucleoside kinase activity, which was first reported in this group for the ribose analogs (V. V. Tryon and J. D. Pollack, Int. J. Syst. Bacteriol. 35:497–501, 1985). This is the first report of PPi-dependent purine deoxyribonucleoside kinase activity. An ATP-dependent purine deoxyribonucleoside kinase activity is known only in salmon milt extracts (H. L. A. Tarr, Can. J. Biochem. 42:1535–1545, 1964). Deoxyribonucleotidase activity was also found in cytoplasmic extracts of these mollicutes. This is the first report of deoxyribonucleotidase activity.

Wall-less procaryotes of the class Mollicutes have an absolute nutritional requirement for the purine nucleobases or nucleotides (4, 10, 15, 22, 27, 28, 32, 33, 38). We have described the pathways involved in purine salvage and purine interconversions for a number of mollicutes (40). Similar reactions for Mycoplasma mycoides subsp. mycoides and Ureaplasma urealyticum have also been described (5, 22, 23). Pathways involved in pyrimidine deoxyribonucleotide synthesis in mollicutes are also known (24, 41). The synthesis of mononucleotides proceeds from nucleobases either with phosphoribosylpyrophosphate (PRPP) in a one-step, essentially irreversible reaction mediated by phosphoribosyltransferase (I) or in a two-step reversible reaction via the nucleoside. In the latter case, the first step is catalyzed by a phosphoribosylase (II) and requires ribose-1-phosphate (R-1-P) to form the ribonucleoside. The ribonucleoside is converted by purine nucleoside kinase activity (III), usually requiring ATP but in mollicutes requiring PPi (40), to the mononucleotide. The reverse of the latter reaction is mediated by a 5'-nucleotidase (IV) (Fig. 1).

Using crude extracts of mollicutes, we found that purine and uracil nucleobases can be ribosylated with deoxyribose-1-phosphate (dR-1-P) to form the corresponding purine and pyrimidine deoxyribonucleotides. This unusual synthesis of deoxyribonucleotides has been reported in only one procaryote, Escherichia coli (17), and in the eucaryotic cells of mammalian liver (6, 16), monkey brain (31), and fish muscle (34, 36). The reverse reaction towards nucleoside and dR-1-P has been more widely reported (1, 6, 7, 14, 16, 30, 34, 42).

Earlier we reported that ribonucleosides can be phosphorylated by a unique PPi-dependent activity known only in extracts of some mollicutes (39). In this study, we found that the same mollicutes can phosphorylate deoxyribonucleotides by the PPi-dependent nucleoside kinase activity to form deoxyribonucleotides. The phosphorylation of deoxyribonucleotides has been reported to occur only in salmon milt extracts (35). However, in that report, the synthesis of deoxyribonucleotides was ATP dependent and PPi was not tested. Our activity is different. In the mollicutes extracts we prepared, PPi was necessary to demonstrate deoxyribonucleoside kinase activity. This is the first report of PPi-dependent deoxyribonucleoside kinase activity.

In the Acholeplasma and Mycoplasma species, we detected deoxyribonucleotidase activity. Acholeplasma laidlawii is the only member of the class Mollicutes that has phosphorylase activity toward all the purine deoxyribonucleotides: dGMP, dIMP, and dAMP. This is the first report of deoxyribonucleotidase activity.

MATERIALS AND METHODS

Chemicals. The following radioactively labeled compounds were purchased from Moravek Biochemicals (Brea, Calif.): [8-3H]guanine ([8-3H]GUA), 10 Ci/mmol; [8-14C]hypoxanthine ([8-14C]HPX), 56 mCi/mmol; [8-14C]inosine ([8-14C]INO), 56 mCi/mmol; [2,8-3H]deoxyadenosine ([2,8-3H]dADO), 28 Ci/mmol; [8-14H]deoxyguanosine ([8-14H]dGUO), 16 Ci/mmol; [2,8-3H]deoxyadenosine 5'-monophosphate ([2,8-3H]dAMP), 17 Ci/mmol; [2,8-3H]adenosine 5'-monophosphate ([2,8-3H]AMP), 17 Ci/mmol; [8-14H]uridine ([8-14H]URD), 22 Ci/mmol. The following radioactively labeled compounds were purchased from Research Products International Corp. (Mt. Prospect, Ill.): [8-14C]adenine ([8-14C]ADE), 50 Ci/mmol; [8-14C]adenosine ([8-14C]ADO), 47 Ci/mmol; [8-14C]guanosine ([8-14C]GUA), 42.8 Ci/mmol. We purchased [8-14C]inosine 5'-monophosphate ([8-14C]IMP), 59 Ci/mmol, and [U-14C]deoxyguanosine 5'-monophosphate ([U-14C]DIMP) from New England Nuclear (Boston, Mass.), 50 Ci/mmol. 

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dGMP), 512 mCi/mmol, from Amersham Corp. (Arlington Heights, Ill.). [8-14C]AMP, 58 mCi/mmol; [8-3H]Juracil ([8-3H]URA), 40 Ci/mmol; and [8-3H]deoxyuridine ([8-3H]dURD). 23 Ci/mmol, were purchased from ICN Pharmaceuticals Inc. (Irvine, Calif.). [8-14C]G2-deoxyinosine ([8-14C]dINO), [8-3HI]guanosine 5'-monophosphate ([8-3H]GMP), and [8-3H]inosine 5'-monophosphate ([8-3H]dIMP) were not available, and therefore they were synthesized as described below. We calculated that their specific activities are: [8-14C]dINO, 43.5 mCi/mmol; [8-3H]GMP, 7.2 Ci/mmol; and [8-14C]dIMP, 43.5 mCi/mmol. All enzymes and most chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Organisms. A. laidlawii B-P9, Acholeplasma morum S2, Mycoplasma capricolum 14, and Mycoplasma gallisepticum S6 were obtained from our stock collection.

Media and growth conditions. All organisms were grown in our modification of Edward medium (2). Media were supplemented with heat-inactivated horse serum (lot 200031; KC Biologicals, Lenexa, Kans.) at 0% (vol/vol) for A. laidlawii B-P9, 2% (vol/vol) for A. morum S2, or 5% (vol/vol) for M. capricolum 14 and M. gallisepticum S6. Organisms were incubated statically at 37°C until the mid-log phase of growth. Cytoplasmic extracts were prepared essentially as we have described earlier (25). Cells were harvested by centrifugation, washed three to five times, and lysed by hypotonic shock. The crude cytoplasmic fraction was centrifuged at 250,000 × g for 90 min. The supernatant was dialyzed immediately and used for all assays (39). In some cases in which enzyme activity was proven to be stable to freezing, the dialyzed supernatant was stored at −40°C until assayed.

Assay. Using cytoplasmic fractions, we studied 37 enzyme activities involved in the salvage of nucleobases, nucleosides, deoxynucleosides, mononucleotides, and deoxyribonucleotides. Specific radioactive assays for the reaction in two directions were done as generally described by Tryon and Pollack (39). Briefly 10 to 20 μmol of radioactive substrates were mixed with cofactors at pH 7.4. Dialyzed cell extract containing 20 to 40 μg of protein was added to start the reaction. Protein concentration was determined by the Bio-Rad Laboratories microassay (Richmond, Calif.). The final volume of all reaction mixtures was 0.1 ml. Reaction mixtures were incubated at 37°C with shaking for 4 to 16 min. Reactions were terminated by heating at 90°C for 2 min. Samples (20 μl) of the heat-stopped mixtures were spotted onto polyethyleneimine (PEI)-cellulose plates (Analtech, Inc., Newark, Del.) along with nonradioactive standards. Radioactive substrate and product were separated by using solvents of 1 M LiCl or 1 M LiCl-0.1 M boric acid. Resolved substrate and products were visualized by UV light, scraped into counting fluid, and assayed for radioactivity by scintillation counting (LSC 7000; Beckman Instruments, Inc., Fullerton, Calif.). In all cases, greater than 80% of the applied label was recovered in the substrate and product spots. Radioisotope data were corrected for quenching, calculated as disintegrations per minute per milligram of protein, and then converted to nanomoles of product synthesized per minute per milligram of protein by calculation using the specific activity of the labeled substrate. The following enzyme assays were done by using various radioactive substrates.

Assay i. ADE phosphoribosyltransferase (AMP·PP, phosphoribosyltransferase; EC 2.4.2.7) and HPX-GUA phosphoribosyltransferase (1MP·PP, phosphoribosyltransferase; EC 2.4.2.8) were assayed as described previously (39). Reaction mixtures contained 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) (pH 7.4), 5 mM MgCl2, 4 mM PRPP, and [8-14C]ADE, [8-3H]GUA, or [8-3H]HPX. Incubation time was 8 min. Product mononucleotide was chromatographically separated from substrate nucleobase in 1 M LiCl.

Assay ii. Purine-nucleoside phosphorylase (purine nucleo-

Assay iii. AKD kinase (ATP-deoxinosine 5'-phosphotrans-

Assay iv. 5'-Nucleotidase (5'-ribonucleotide phosphory-
lase; EC 3.1.3.5) was assayed essentially as previously described (39). Reaction mixtures contained 50 mM HEPES (pH 7.4), 2 mM MgCl₂, and [8-14C]AMP, [2,8-14C]dAMP, [8-14C]GMP, [U-14C]dGMP, [8-14C]IMP, or [8-14C]dIMP. Radioactive dIMP was not commercially available. The [8-14C]dIMP reaction mixture contained [8-14C]HPX, dR-1-P, and PP, with cytoplasmic extract of A. laidlawii. The reaction mixture was mixed with nonradioactive standards and was spotted onto silica gel-H plates (Analtech, Inc.). The nucleotide was separated from the nucleoside and nucleobase in a solvent containing acetonitrile-water (90:10–0.05 M acetic acid). [8-14C]dIMP was eluted with water. [8-14C]GMP was isolated from PEI-cellulose and eluted from the matrix with methanol-water (1:1). The [8-14C]dIMP and [8-14C]GMP eluates from this step were treated and reassayed, as was [8-14C]dINO (as in assay iii).

As previously mentioned, ribo- and deoxyribonucleosides could be chromatographically separated from each other in 1 M LiCl·0.1 M boric acid. However, we could not chromatographically resolve ribo- and deoxyribonucleotides. To support our contention that we had enzymatically synthesized ribo- or deoxyribonucleosides, we further tested the mononucleotide products of A. laidlawii PP₆-nucleoside kinase reactions by a modification of the preferential-degradation method of Garrett and Santi (8). The putative ribo- and deoxyribonucleotides were extracted from PEI-cellulose and then treated by the procedure of Garrett and Santi. For controls, we used [8-14H]AMP and [8-14H]dAMP. Each treated sample was mixed with nonradioactive standards and chromatographed on PEI-cellulose plates in 1 M LiCl. UV-absorbing spots were removed and assayed for radioactivity. In the preferential-degradation procedure ribonucleosides, but not deoxyribonucleotides, are destroyed.

In most of the radioactive experiments, we used a relatively low concentration of 10 to 20 µmol of radioactive substrate. To reduce the strong possibility that our inability to detect enzyme activity was caused by limiting substrate, we increased the concentration of radioactive substrate to 100 µmol. These 10-fold assays (10-fold enhancement assays) were done for the reassessment of our negative findings of PP₆-dependent GUO and dGUO kinase and IMP and dGMP 5'-nucleotase activities. We restested extracts of A. laidlawii, A. morum, and M. gallisepticum.

We (S. P. Heron and J. D. Pollack, unpublished data) also developed a nonradioactive assay by using a fast-pressure liquid chromatography technique, particularly for the assessment of nucleoside kinase and nucleotidase activities. The technique was adapted from a high-pressure liquid chromatography procedure (9). We used this procedure for the reassessment of GMP and dIMP 5'-nucleotidase activities with extracts of A. laidlawii and M. gallisepticum. The reaction mixtures for these activities were identical to those above (as in assay iv), except that the concentration of the reactant GMP or dIMP was 1 mM and it was nonradioactive. For these assays, after the reaction mixture was heated, it was centrifuged (18,000 × g, 30 min). The supernatant was filtered through a 0.22-µm-pore-size filter membrane (Millipore Corp., Bedford, Mass.). A 100-µl sample of the filtrate was injected directly into a Mono-Q column (HR5/5) attached to the fast-pressure liquid chromatography apparatus (Pharmacia). The operating conditions were: flow rate, 2.0 ml/min; eluent A, 7 mM KH₂PO₄, 7 mM KCl, pH 4.0; eluent B, 125 mM KH₂PO₄, 250 mM KCl, pH 5.0; gradient, 0 to 20% B in 10.0 min, 20 to 40% B in 5.0 min, 40 to 60% B in 1.6 min, 60 to 100% B in 3.4 min, 100% B for 1 min; temperature, 22°C. By this procedure, we separated nucleobases and nucleotides from mononucleotides.

**RESULTS**

The cytoplasmic preparations from four mollicutes were examined for activities associated with the salvage of bases, deoxyribonucleosides, ribonucleosides, deoxyribonucleotides, and ribonucleotides. Three to nine batches of each organism were tested. The results of these assays are shown in Table 1.

A. morum and A. capricolum, like other mollicutes previously studied (40), had purine phosphoribosyltransferase activity. That is, these organisms can synthesize AMP, IMP, or GMP directly from their respective nucleobases (ADE, HPX, GUO) and PRPP (Table 1, reactions 1 to 3).

The extracts from all four mollicutes had phosphorylase activity in the synthetic direction using either R-1-P or dR-1-P. In other words, all four preparations can synthesize adenosine, deoxyadenosine, inosine, deoxyinosine, guanosine, deoxyguanosine, uridine, or deoxyuridine from their respective nucleobases (adenine, hypoxanthine, guanine, and uracil) with either R-1-P or dR-1-P (Table 1, reactions 4 to 11). Furthermore, the respective nucleobase can be formed in the reverse reaction from these nucleobases (Table 1, reactions 12 to 19).

As reported by others (11, 19, 21), we also found uridine phosphorylase activity, i.e., the interconversion of uracil and R-1-P to uridine (Table 1, reactions 7 and 15), in all mollicutes tested (Table 1). We also found that all the mollicutes could convert uracil and dR-1-P to deoxyuridine (Table 1, reactions 11 and 19).

A. laidlawii and A. morum extracts have PP₆-dependent adenosine, deoxyadenosine, inosine, and deoxyinosine kinase activities (Table 1, reactions 20, 23, 21, and 24). A. laidlawii has PP₆-dependent guanosine and deoxyguanosine kinase activity (Table 1, reactions 22 and 25), which we did not detect in A. morum extracts. M. gallisepticum and M. capricolum extracts had none of these kinase activities. The ribonucleoside kinase activities had been previously reported (40) for A. laidlawii and M. gallisepticum. In A. laidlawii, adenosine, deoxyadenosine, inosine, deoxyinosine, guanosine, and deoxyguanosine were not converted to their respective deoxyribonucleotides with ATP, ADP, AMP, dAMP, CMP, dCMP, GMP, dGMP, IMP, dIMP, UMP, or dUMP. A. morum, M. capricolum, and M. gallisepticum were examined only by using PP₆ and ATP.

A. laidlawii and A. morum extracts demonstrate AMP, dAMP, IMP, and dIMP nucleotidase activities (Table 1, reactions 26, 29, 27, and 30). A. laidlawii has GMP and dGMP nucleotidase activities (Table 1, reactions 28 and 31), which A. morum lacks. M. gallisepticum and M. capricolum demonstrate AMP and dAMP nucleotidase activities (Table 1, reactions 26 and 29). M. capricolum demonstrates IMP and dIMP nucleotidase activities (Table 1, reactions 27 and 30), which were not detected in M. gallisepticum. The nucleotidase activities for A. laidlawii and M. gallisepticum were previously reported (40).

We did not detect 5'-nucleotidase and PP₆-dependent nucleoside kinase activities in some extracts by our standard thin-layer chromatographic procedures. The absence of these activities has a significant impact on our understanding...
of the pathways of nucleic acid synthesis. The results of the alternate 10-fold enhancement assay are shown in Table 2. We confirmed our negative findings by the alternate 10-fold enhancement assays and fast-pressure liquid chromatography procedures (data not shown) and substantiated the absence of both 5'-nucleotidase and nucleoside kinase activities.

The results of the Garrett and Santi procedure for the selective degradation of ribonucleotides by extracts of *A. laidlawii* B-P9 are shown in Table 3. Radioactive standards and putative samples of ribonucleotides were degraded to nucleosides and unidentified nonmigrating materials; i.e., less than 5% of the ribonucleoside radioactivity in the reaction mixture comigrated with nonradioactive ribonucleoside standards after degradation. On the other hand, the radioactive standards and putative samples of deoxyribonucleosides were unaltered; i.e., more than 92% of the recovered radioactivity comigrated with nonradioactive deoxyribonucleoside standards. The data show, as we expected and in accord with theory, that our samples and standards of dAMP and dGMP were not degraded and that our samples and standards of AMP and GMP were degraded. Therefore, the PPn-purine nucleoside kinase activity (or activities) of *A. laidlawii* mediates a reaction of deoxyribonucleosides and PPn which results in the synthesis of deoxyribonucleotides.

**DISCUSSION**

It is generally accepted that the synthesis of the deoxy forms of the ribonucleotides, the nucleic acid precursors of DNA, can be mediated only by ribonucleotide reductases (29, 37). These enzymes reduce the ribose moiety of the nucleic acid to the 2'-deoxy form. Our study shows that the synthesis of some deoxyribonucleic acids by molluscous extracts occurs at the nucleobase level. Nucleobases that are ribosylated in vitro with dR-1-P directly form the deoxyribonucleotides and in turn be phosphorylated by a unique PPn-dependent purine deoxyribonucleoside kinase to produce deoxyribonucleotides. Although we have found these activities in vitro, we do not yet know whether they occur in the whole cell.
Since *A. laidlawii* can synthesize dAMP, dIMP, dGMP (Table 1; Fig. 2), we speculate that its ribonucleotide reductase activity may be diminished or nonexistent. All organisms are thought to have ribonucleotide reductase activity (25, 37). *A. morum* can synthesize dAMP and dIMP, but not dGMP, and *M. capricolum* and *M. gallisepticum* cannot synthesize any deoxyribonucleotide. Therefore, the latter organisms, deficient in their ability to synthesize deoxyribonucleosides, may possess some ribonucleotide reductase activity or successfully scavenge for these deoxyribonucleic acids (or both). Although we have not studied ribonucleotide reductase activity in these organisms, Neale et al. (24) have reported that *M. mycoides* subsp. *mycoides* possesses this activity.

Our data are schematically presented in Fig. 2. All the mollicutes we studied are capable of synthesizing ribonucleotides directly from the nucleoside and PRPP (Fig. 2, reactions 1 to 3). There is no known analogous one-step reaction leading to the deoxyribonucleotides. The mollicutes had nucleoside phosphorylase activity in both directions (Fig. 2, reactions 4 to 19). That is, they were able to synthesize ribonucleosides and deoxyribonucleosides from nucleobases with R-1-P or Dr-1-P, respectively (Fig. 2, reactions 4 to 11), and were also able to catabolize these nucleic acids (Fig. 2, reactions 11 to 19). In studying the catabolism of these nucleosides (Fig. 2, reactions 11 to 19), we omitted additional phosphate, and our reaction mixtures were not assayed for phosphate levels. Therefore, we are not certain whether the catabolism of these nucleosides is mediated by a phosphorylase or a hydrolase. The nucleoside hydrolase reported by Heppel and Hilmo (13) does not require P1. Some of our experiments suggest that we are dealing with a P1-requiring phosphorylase (unpublished data). *E. coli* (17) and now the mollicutes are the only procaryotes reported to synthesize deoxyribonucleosides from purine and pyrimidine nucleobases with Dr-1-P. Our data suggest that Dr-1-P may be used as a substitute for R-1-P in adenine-adenosine phosphorylase reactions which have been developed to test for mollicute contamination of tissue culture (3, 12, 18).

We found that *A. laidlawii* was capable of synthesizing the deoxyribonucleotides dAMP, dIMP, and dGMP directly from their respective deoxyribonucleosides with PP1, but not ATP (Fig. 2, reactions 23 to 25). These data coupled with our earlier studies (40) permit us to conclude that *A. laidlawii* has a unique PP1-dependent nucleoside kinase activity which is capable of phosphorylating all six of the purine deoxy- and ribonucleosides to their respective mono- and dinucleotides (Fig. 2, reactions 20 to 25). *A. morum* can similarly phosphorylate the deoxyriboadenylates and deoxyriboinosinates (Fig. 2, reactions 20, 21, 23, and 24). Neither of the *Mycoplasma* species was capable of synthesizing any detectable mononucleotide by this nucleoside kinase reaction.

The four mollicutes each had nucleotidase activity, and all were able to dephosphorylate AMP and dAMP to ADP and dADP, respectively (Fig. 2, reactions 26 and 29). The two acholoplasmas and *M. capricolum* were able to dephosphorylate IMP and dIMP to INO and dINO, respectively (Fig. 2, reactions 27 and 30). Only *A. laidlawii* was able to dephosphorylate GMP and dGMP (Fig. 2, reactions 28 and 31). Nucleotidase activity against ribonucleotides by extracts of *A. laidlawii* and *M. gallisepticum* was previously reported (40).

Our inability to detect enzyme activity does not mean that these enzyme activities are absent in the intact organism. During preparation of our cell extracts, the enzymes may be inactivated, or the activities may require more than one subcellular fraction, e.g., membranes, which are eliminated in our preparatory steps. We have reported enzyme activities as rates; such values may be in error, since all of our assays were done on crude extracts and no attempt to inhibit competing reactions was made. However, as we always recovered <80% of our starting radioactivity (unpublished data), we believe that unrecognized competing reactions do not have a significant contributory effect upon our conclusions.

In parallel with earlier studies (40), we find that where ribonucleoside phosphorylase, ribonucleoside kinase, or ribonucleotidase activity is present, deoxyribonucleoside phosphorylase, deoxyribonucleoside kinase, or deoxy-

**Table 2.** Reassessment of PP1-nucleoside kinase and 5′-nucleotidase activities by 10-fold enhancement assays

<table>
<thead>
<tr>
<th>Activity</th>
<th><em>A. laidlawii</em></th>
<th><em>A. morum</em></th>
<th><em>M. gallisepticum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>PP1-nucleoside kinase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-U + PP1 → GMP</td>
<td>41.31 (7.02)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>dG-U + PP1 → dGMP</td>
<td>37.63 (6.10)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5′-Nucleotidases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMP → INO</td>
<td>12.10 (2.10)</td>
<td>8.01 (1.9)</td>
<td>NA</td>
</tr>
<tr>
<td>dGMP → dG-U</td>
<td>8.91 (1.06)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*PP1-nucleoside kinase activity was measured as described in Materials and Methods by using 100 µmol of radioactive substrate rather than 10 µmol. Three batches of cells were tested.

*Enzyme activities are reported as the average nanomoles of product synthesized minute\(^{-1}\) milligram\(^{-1}\) of protein (± standard deviation).

*NA. No activity was detected (<0.005 nmol min\(^{-1}\) mg\(^{-1}\) of protein). *
ribononucleotidase activity is also present. Hence, until the enzymes are isolated, we speculate that the phosphor- 
ylase, PP-nucleoside kinase, and nucleotidase activities are not specific for either the deoxyribose or ribose form but can mediate the reaction involving either. We do not yet know how many different enzymes mediate these reactions.

From our studies, we conclude that in general the mollicutes can interconvert purine nucleobases and nucleo- 
sides by using either R-1-P or dR-1-P. This permits greater flexibility in synthesizing nucleic acid intermediates. Also, mollicutes can transfer the ribosylphosphate group from PRPP to nucleobases, and we speculate that this is the major route of RNA synthesis. The metabolic constraint seems to occur at the nucleoside kinase locus. Of the five species of Acholeplasma we have described here and elsewhere (40), only A. laidlawii has nucleoside kinase activity for all the 
purine nucleobases. A. morum and Acholeplasma axanthum have nucleoside kinase activity only with adenosine and 
guanosine, and Acholeplasma granulatum has nucleoside kinase activity only with adenosine. Acholeplasma floridicola, 
the three Mycoplasma species, and one species of Spiro- 
plasma we have reported here and earlier (40) have no 
detectable nucleoside kinase activity. Therefore, most of 
these mollicutes can degrade purine mononucleotides via 
nucleotidases, but they cannot all synthesize such mono- 
nucleotides, except via the phosphoribosyltransferase and 
PRPP. Hence, there may be some directional flow to purine 
salvage, especially with the Mycoplasma species, which 
proceeds "clockwise" from the nucleoside, to the 
nucleobase, then to the mononucleotide, and then either to 
oligonucleotides and presumably DNA/RNA or, if 5'- 
nucleotidase activity is present, back to the nucleoside. The 
source of the nucleobases, which are known to be transport-

FIG. 2. Schematic diagram of the proposed nucleic acid salvage pathways of four mollicutes on the basis of enzymatic studies of their cytoplasmic extracts. The enzyme assays and abbreviations are described in Materials and Methods. Numbers identify the enzyme reactions described in Table 1 in the column headed "Enzyme activity number."

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LITERATURE CITED


