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Synthesis of Deoxyribomononucleotides in Mollicutes: Dependence on Deoxyribose-1-Phosphate and PP_i

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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 deoxyribonucleosides, 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 deoxyribonucleosides 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 studied. Some *Acholeplasma* species but not the *Mycoplasma* species can phosphorylate deoxyribonucleosides to deoxyribomononucleotides by a PP_i-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 PP_i-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). Deoxyribomononucleotidase activity was also found in cytoplasmic extracts of these mollicutes. This is the first report of deoxyribomononucleotidase activity.

Wall-less procaryotes of the class *Mollicutes* have an absolute nutritional requirement for the purine nucleobases or nucleosides (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 phosphorylase (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 PP_i (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 deoxyribonucleosides. This unusual synthesis of deoxyribonucleosides 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 nucleobase 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 PP_i-dependent activity known only in extracts of some mollicutes (39). In this study, we found that the same mollicutes can phosphorylate deoxyribonucleosides by the PP_i-dependent nucleoside kinase activity to form deoxyribomononucleotides. The phosphorylation of deoxyribonucleosides has been reported to occur only in salmon milt extracts (35). However, in that report, the synthesis of deoxyribomononucleotides was ATP dependent and PP_i was not tested. Our activity is different. In the mollicutes extracts we prepared, PP_i was necessary to demonstrate deoxyribonucleoside kinase activity. This is the first report of PP_i-dependent deoxyribonucleoside kinase activity.

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

MATERIALS AND METHODS

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

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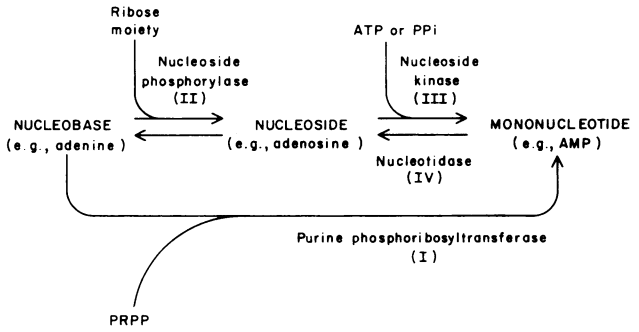

FIG. 1. Schematic diagram of the pathways of purine mononucleotide synthesis.

dGMP), 512 mCi/mmol, from Amersham Corp. (Arlington Heights, Ill.). [8-^{14}C]AMP, 58 mCi/mmol; [8-^{3}H]uracil ([8-^{3}H]URA), 40 Ci/mmol; and [8-^{3}H]deoxyuridine ([8-^{3}H]dURD), 23 Ci/mmol, were purchased from ICN Pharmaceuticals Inc. (Irvine, Calif.). [8-^{14}C]2′-deoxyinosine ([8-^{14}C]dINO), [8-^{3}H]guanosine 5′-monophosphate ([8-^{3}H]GMP), and [8-^{14}C]inosine 5′-monophosphate ([8-^{14}C]dIMP) were not available, and therefore they were synthesized as described below. We calculated that their specific activities are: [8-^{14}C]dINO, 43.5 mCi/mmol; [8-^{3}H]GMP, 7.2 Ci/mmol; and [8-^{14}C]dIMP, 43.5 mCi/mmol. All enzymes and most chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Organisms. *A. laidlawii* B-PG9, *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-PG9, 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 260,000 × *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 deoxymononucleotides. 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 by 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$_i$ phosphoribosyltransferase; EC 2.4.2.7) and HPX-GUA phosphoribosyltransferase (IMP-PP$_i$ phosphoribosyltransferase, EC 2.4.2.8) were assayed as previously described (39). Reaction mixtures contained 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.4), 5 mM $MgCl_2$, 4 mM PRPP, and [8-^{14}C]ADE, [8-^{3}H]GUA, or [8-^{14}C]HPX. Incubation time was 8 min. Product mononucleotide was chromatographically separated from substrate nucleobase in 1 M LiCl.

Assay ii. Purine-nucleoside phosphorylase (purine nucleoside-P$_i$ ribosyltransferase; EC 2.4.2.1) activity was assayed essentially as previously described (39). Reaction mixtures for the base-to-nucleoside conversion contained 50 mM HEPES (pH 7.4), 2 mM $MgCl_2$, 4 mM R-1-P, or 4 mM dR-1-P, and [8-^{14}C]ADE, [8-^{3}H]GUA, or [8-^{14}C]HPX. For the nucleoside-to-base conversion, the reactions were the same except that [8-^{14}C]ADO or [2,8-^{3}H]dADO replaced [8-^{14}C]ADE; [8-^{14}C]GUO or [8-^{3}H]dGUO replaced [8-^{3}H]GUA; and [8-^{14}C]INO or [8-^{14}C]dINO replaced [8-^{14}C]HPX. Incubation time was 4 min. Nucleosides were chromatographically separated from nucleobases on PEI-cellulose in 1 M LiCl. Using PEI-cellulose matrix, we could chromatographically separate ribonucleosides from deoxyribonucleosides with a solvent containing 1 M LiCl–0.1 M boric acid.

In reactions essentially identical to the purine nucleoside phosphorylase assays, we tested for uridine phosphorylase activity (uridine:P$_i$ ribosyltransferase, EC 2.4.2.3). In these experiments, we substituted [8-^{3}H]URA for the radioactive purine nucleobase, or [8-^{3}H]URD or [8-^{3}H]dURD for the purine nucleoside. The reaction mixtures were processed exactly as for the purine nucleoside phosphorylase assay.

Assay iii. ADO kinase (ATP-adenosine 5′-phosphotransferase; EC 2.7.1.20) and nucleoside kinase (ATP-inosine 5′-monophosphotranferase; EC 2.7.1.73) activities utilizing PP$_i$ was assayed essentially as previously described (39). Reaction mixtures contained 50 mM HEPES (pH 7.4), 2 mM $MgCl_2$, 4 mM sodium PP$_i$, and [8-^{14}C]ADO, [2,8-^{3}H]dADO, [8-^{14}C]GUO, [8-^{3}H]dGUO, [8-^{14}C]INO, or [8-^{14}C]dINO. ATP, ADP, AMP, dAMP, CMP, dCMP, GMP, dGMP, IMP, dIMP, UMP, and dUMP were substituted for PP$_i$ with *A. laidlawii*. Radioactive dINO was not commercially available. This product was synthesized by reacting [8-^{14}C]HPX and dR-1-P with *A. laidlawii* extract (as in assay ii). Incubation time was 6 min. Product nucleotide was separated from substrate nucleoside by chromatography on PEI-cellulose in 1 M LiCl. The [8-^{14}C]dINO was extracted from the PEI-cellulose with methanol-water (1:1). The extract was reduced to dryness at 45°C under N_2. The residue was dissolved in water or buffer for storage at −20°C. Before use, the solution was rechromatographed with standards to assess purity and confirm the identity of the solute.

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 $MgCl_2$, and [8-^{14}C]AMP, [2,8-^{3}H]dAMP, [8-^{3}H]GMP, [U-C^{14}]dGMP, [8-^{14}C]IMP, or [8-^{14}C]dIMP. Radioactive dIMP was not commercially available. The [8-^{14}C]dIMP reaction mixture contained [8-^{14}C]HPX, dR-1-P, and PP_i 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-^{14}C]dIMP was eluted with water. [8-^{3}H]GMP was made by reacting [8-^{3}H]GUA and PRPP via the GUA phosphoribosyltransferase reaction with *A. laidlawii* cytoplasmic extract (as in assay i). [8-^{3}H]GMP was isolated on PEI-cellulose and eluted from the matrix with methanol-water (1:1). The [8-^{14}C]dIMP and [8-^{3}H]GMP eluates from this step were treated and reassayed, as was [8-^{14}C]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 deoxyribomononucleotides. To support our contention that we had enzymatically synthesized ribo- or deoxyribomononucleotides, we further tested the mononucleotide products of *A. laidlawii* PP_i-nucleoside kinase reactions by a modification of the preferential-degradation method of Garrett and Santi (8). The putative ribo- and deoxyribomononucleotides were extracted from PEI-cellulose and then treated by the procedure of Garrett and Santi. For controls, we used [8-H^{3}]AMP and [8-H^{3}] 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 ribomononucleotides, but not deoxyribomononucleotides, 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 PP_i-dependent GUO and dGUO kinase and IMP and dGMP 5′-nucleotase activities. We retested 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 heat-stopped, 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 KH_2PO_4, 7 mM KCl, pH 4.0; eluent B, 125 mM KH_2PO_4, 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 nucleosides from mononucleotides.

RESULTS

The cytoplasmic preparations from four mollicutes were examined for activities associated with the salvage of bases, deoxyribonucleosides, ribonucleosides, deoxyribomononucleotides, and ribomononucleotides. 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, GUA) 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 nucleosides (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 PP_i-dependent adenosine, deoxyadenosine, inosine, and deoxyinosine kinase activities (Table 1, reactions 20, 23, 21, and 24).

A. laidlawii has PP_i-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 deoxyribomononucleotides 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 PP_i 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 PP_i-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

TABLE 1. Enzyme activities[a]

Enzyme activity no.[b]	Enzyme and reaction[c]	Species			
		A. laidlawii	*A. morum*	*M. capricolum*	*M. gallisepticum*
	Phosphoribosyltransferases				
1	ADE + PRPP → AMP + PP_i	5.21 (1.06)[d]	3.96 (0.97)	3.46 (0.91)	1.36 (0.61)[d]
2	HPX + PRPP → IMP + PP_i	3.42 (0.96)[d]	5.16 (0.53)	3.41 (0.87)	6.41 (1.21)[d]
3	GUA + PRPP → GMP + PP_i	2.89 (0.99)[d]	1.37 (0.10)	10.26 (1.39)	15.21 (2.36)[d]
	Nucleoside phosphorylases				
4	ADE + R-1-P → ADO	7.54 (1.39)[d]	6.59 (1.21)	13.61 (2.01)	14.62 (3.11)[d]
5	HPX + R-1-P → INO	7.39 (1.46)[d]	5.32 (1.01)	1.36 (0.09)	2.64 (0.36)[d]
6	GUA + R-1-P → GUO	10.16 (2.10)[d]	7.61 (1.13)	9.36 (1.02)	12.39 (2.11)[d]
7	URA + R-1-P → URD	3.06 (0.90)	2.20 (0.92)	3.67 (1.01)	3.34 (0.97)
8	ADE + dR-1-P → dADO	8.30 (1.21)	6.35 (0.83)	8.43 (1.10)	13.90 (2.17)
9	HPX + dR-1-P → dINO	8.01 (1.16)	6.21 (0.73)	4.10 (0.09)	3.10 (1.00)
10	GUA + dR-1-P → dGUO	9.36 (1.34)	8.11 (1.31)	6.91 (0.91)	10.21 (1.97)
11	URA + dR-1-P → dURD	2.90 (0.89)	4.06 (1.53)	2.99 (0.67)	4.78 (0.97)
12	ADO → ADE + R-1-P	2.32 (0.15)[d]	1.37 (0.10)	1.03 (0.13)	1.31 (0.24)[d]
13	INO → HPX + R-1-P	2.11 (0.63)[d]	1.89 (0.21)	0.91 (0.05)	0.73 (0.11)[d]
14	GUO → GUA + R-1-P	2.06 (0.41)[d]	2.13 (0.36)	0.38 (0.05)	0.98 (0.17)[d]
15	URD → URA + R-1-P	1.01 (0.09)	2.01 (0.31)	1.07 (0.21)	2.81 (0.19)
16	dADO → ADE + dR-1-P	2.01 (0.67)	1.97 (0.37)	0.93 (0.09)	1.97 (0.19)
17	dINO → HPX + dR-1-P	1.91 (0.49)	1.01 (0.10)	0.98 (0.07)	0.62 (0.50)
18	dGUO → GUA + dR-1-P	2.21 (0.89)	1.88 (0.26)	1.01 (0.07)	0.71 (0.10)
19	dURD → URA + dR-1-P	2.36 (0.39)	1.91 (0.29)	0.97 (0.09)	0.89 (0.21)
	PP_i-nucleoside kinases				
20	ADO + PP_i → AMP	11.91 (2.11)[d]	9.03 (1.37)	NA[e]	NA[d]
21	INO + PP_i → IMP	7.32 (1.62)[d]	4.61 (0.93)	NA	NA[d]
22	GUO + PP_i → GMP	9.10 (1.81)[d,f]	NA[f]	NA	NA[d,f]
23	dADO + PP_i → dAMP	12.31 (2.06)	6.13 (1.05)	NA	NA
24	dINO + PP_i → dIMP	6.46 (1.71)	4.13 (0.97)	NA	NA
25	dGUO + PP_i → dGMP	8.91 (1.09)[f]	NA[f]	NA	NA[f]
	5′-Nucleotidases				
26	AMP → ADO	11.31 (1.76)[d]	4.02 (0.93)	1.93 (0.17)	0.097 (0.05)[d]
27	IMP → INO	2.09 (0.19)[d,f]	1.06 (0.07)[f]	1.07 (0.09)	NA[d,f]
28	GMP → GUO	1.09 (0.13)[d,g]	NA[g]	NA	NA[d,g]
29	dAMP → dADO	10.61 (2.01)	3.97 (0.79)	1.33 (0.21)	1.02 (0.10)
30	dIMP → dINO	1.11 (0.31)[g]	2.01 (0.63)	0.93 (0.05)	NA[g]
31	dGMP → dGUO	2.41 (0.63)[f]	NA[f]	NA	NA[f]

[a] Enzyme activities are expressed as the average nanomoles of product synthesized $minute^{-1}$ $milligram^{-1}$ of protein (± standard deviation). The number of different batches of cells tested was 3 to 9.
[b] As reported in Results and Discussion.
[c] Abbreviations and reaction conditions are described in Materials and Methods.
[d] Similar data were previously reported from our laboratory (40).
[e] NA, No activity was detected (<0.005 nmol of product synthesized min^{-1} mg of $protein^{-1}$).
[f] This result was reassessed by the 10-fold enhancement assay procedure reported in Table 2.
[g] This result was reassessed by fast-pressure liquid chromatography.

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-PG9 are shown in Table 3. Radioactive standards and putative samples of ribomononucleotides were degraded to nucleosides and unidentified nonmigrating materials; i.e., less than 5% of the ribomononucleotide radioactivity in the reaction mixture cochromatographed with nonradioactive ribomononucleotide standards after degradation. On the other hand, the radioactive standards and putative samples of deoxyribomononucleotides were unaltered; i.e., more than 92% of the recovered radioactivity cochromatographed with nonradioactive deoxyribomononucleotide 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 PP_i-purine nucleoside kinase activity (or activities) of *A. laidlawii* mediates a reaction of deoxyribonucleosides and PP_i which results in the synthesis of deoxyribomononucleotides.

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 mollicutes extracts occurs at the nucleobase level. Nucleobases that are ribosylated in vitro with dR-1-P directly to form the deoxyribonucleosides can in turn be phosphorylated by a unique PP_i-dependent purine deoxyribonucleoside kinase to produce deoxyribomononucleotides. 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 (29, 37). *A. morum* can synthesize dAMP and dIMP, but not dGMP, and *M. capricolum* and *M. gallisepticum* cannot synthesize any deoxyribomononucleotide. 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 ribomononucleotides directly from the nucleobase and PRPP (Fig. 2, reactions 1 to 3). There is no known analogous one-step reaction leading to the deoxyribomononucleotides. 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 Hilmoe (13) does not require P_i. Some of our experiments suggest that we are dealing with a P_i-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 mollicutes contamination of tissue culture (3, 12, 18).

We found that *A. laidlawii* was capable of synthesizing the deoxyribomononucleotides dAMP, dIMP, and dGMP directly from their respective deoxyribonucleosides with PP_i 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 PP_i-dependent nucleoside kinase activity which is capable of phosphorylating all six of the purine deoxy- and ribonucleosides to their respective mononucleotides (Fig. 2, reactions 20 to 25). *A. morum* can similarily 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.

TABLE 2. Reassessment of PP_i-nucleoside kinase and 5'-nucleotidase activities by 10-fold enhancement assays[a]

Activity	Amt of product synthesized[b]		
	A. laidlawii	*A. morum*	*M. gallisepticum*
PP_i-nucleoside kinase			
GUO + PP_i → GMP	41.31 (7.02)	NA[c]	NA
dGUO + PP_i → dGMP	37.63 (6.10)	NA	NA
5'-Nucleotidases			
IMP → INO	12.10 (2.10)	8.01 (1.9)	NA
dGMP → dGUO	8.91 (1.06)	NA	NA

[a] Assays were done as described in Materials and Methods by using 100 μmol of radioactive substrate rather than 10 μmol. Three batches of cells were tested.

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

[c] NA, No activity was detected (<0.005 nmol min^{-1} mg^{-1} of protein).

TABLE 3. Selective degradation of putative deoxy- and ribomononucleotide samples isolated from PP_i-dependent nucleoside kinase reactions with *A. laidlawii* B-PG9 extracts[a]

Sample degraded	Avg % total radioactivity recovered after PEI-thin-layer chromatography of the degradation reaction mixture[b]		
	Mononucleotide	Nucleoside	Origin
Standard			
[^{3}H]dAMP	97.9 (5.8)	1.6 (0.4)	0.6 (0.1)
[^{3}H]AMP	1.5 (0.2)	78.3 (4.9)	20.2 (3.1)
Putative			
dAMP	93.3 (7.2)	5.0 (1.0)	1.7 (0.8)
dGMP	92.4 (10.2)	5.3 (2.1)	1.4 (0.7)
AMP	3.4 (0.4)	70.8 (9.1)	25.7 (4.9)
GMP	2.5 (0.9)	75.7 (5.1)	21.6 (2.6)

[a] We isolated the deoxy- and ribomononucleotides from PP_i-nucleoside kinase reactions by using either radioactive deoxyribonucleosides or ribomonucleotides as reactants and *A. laidlawii* extracts as described in Materials and Methods. These reaction mixtures were then chromatographed on PEI-cellulose plates with 1 M LiCl–0.1 M boric acid to separate the deoxy- and ribomononucleotides. The isolated radioactive mononucleotides were eluted with methanol-water (1:1). To further establish that these isolated radioactive mononucleotides were either ribo- or deoxyribomononucleotides, the samples and radioactive dAMP and AMP standards were separately subjected to the selective degradation procedure of Garrett and Santi (8). After the periodate and methylamine treatment steps, the reaction mixtures were rechromatographed on PEI-cellulose plates in LiCl-boric acid along with nonradioactive nucleic acid standards. Resolved UV-absorbing spots and the origin area were removed and assayed for radioactivity. By this procedure, deoxyribomononucleotides are not degraded and are reisolatable as deoxyribomononucleotides, whereas ribomononucleotides are degraded to nucleosides and unidentified products.

[b] Data are recorded as the average percentage of the total radioactivity recovered (± standard deviation); $n = 3$.

The four mollicutes each had nucleotidase activity, and all were able to dephosphorylate AMP and dAMP to ADO and dADO, respectively (Fig. 2, reactions 26 and 29). The two acholeplasmas 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 ribomononucleotides 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 ribomononucleotidase activity is present, deoxyribonucleoside phosphorylase, deoxyribonucleoside kinase, or deoxy-

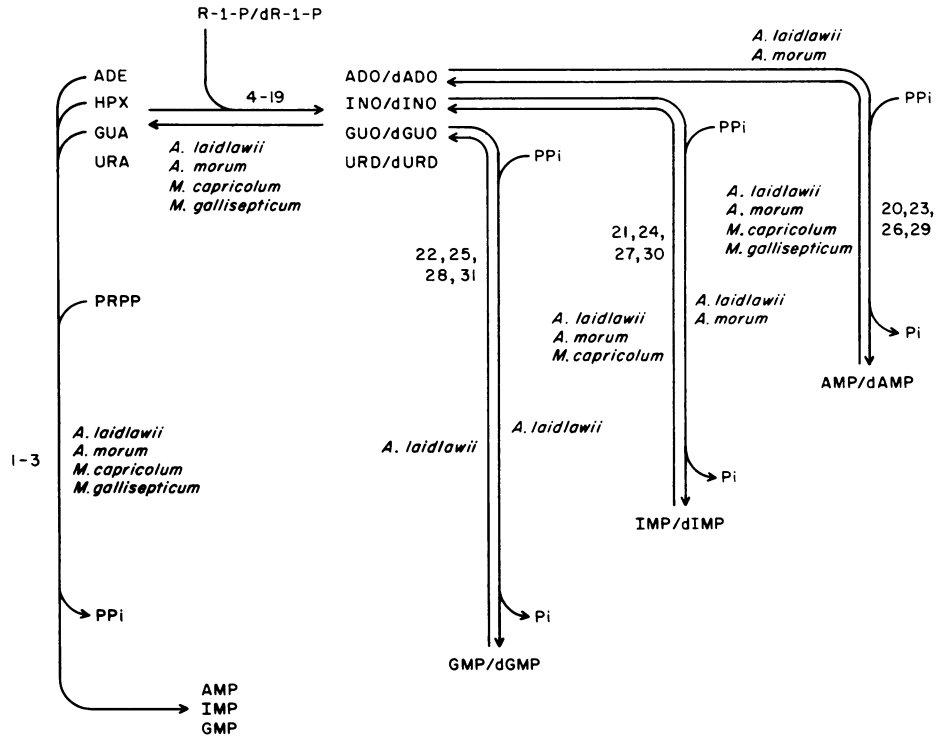

FIG. 2. Schematic diagram of the proposed nucleic acid salvage pathways of four mollicutes on the basis of enzymatic studies of their cytoplasmic extracts. The enzyme assays and abbreviations are described in Materials and Methods. Numbers identify the enzyme reactions described in Table 1 in the column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

ribomononucleotidase activity is also present. Hence, until the enzymes are isolated, we speculate that the phosphorylase, PP_i-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 nucleosides 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 nucleosides. *A. morum* and *Acholeplasma axanthum* have nucleoside kinase activity only with adenosine and guanosine, and *Acholeplasma granularum* has nucleoside kinase activity only with adenosine. *Acholeplasma floricola*, the three *Mycoplasma* species, and one species of *Spiroplasma* 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 mononucleotides, 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 nucleosides, which are known to be transportable as are nucleobases (20), may be the nucleic acids, oligonucleotides, or DNA added to the medium, or the source may be the homologous DNA which is degraded by endogenous nucleases (26).

The relative isolation and metabolic limitations of the guanylate locus in the mollicutes, which were recognized before (40), is further accentuated by this work. This metabolic constraint, e.g., the paucity of guanosine kinase activities, may be related to the low percent G + C content in the DNA of these organisms (43), but proof of any cause-and-effect relationship is not presently available.

Although not emphasized, the probable ability of *A. laidlawii* extracts to synthesize new, rare, or unusual nucleic acid intermediates may be valuable. Our success in the relatively simple synthesis of small quantities of radioactive dINO, GMP, and dIMP suggests that this method may be useful and have other applications. In particular, we believe that the synthesis of nucleoside or mononucleotide intermediates of other pentoses, as arabinose, xylose, or lyxose or their deoxy forms is possible.

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