Pyrimidine Deoxyribonucleotide Metabolism in Members of the Class Mollicutes

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Cell extracts from six Acholeplasma species, six Mycoplasma species, and Spiroplasma floricola 23-6T (T = type strain) were examined for enzyme activities of pyrimidine deoxyribonucleotide metabolism. All of these organisms had thymidine kinase and thymidine phosphorylase activities, and all lacked deoxyctydine triphosphatase activity. The 12 members of the Mollicutes were separated into three groups by the presence or absence of the following four enzyme activities: (i) the adenosine triphosphate-insensitive deoxyuridine triphosphate-specific hydrolyzing deoxyuridine triphosphatase, (ii) a deoxyuridine monophosphate phosphatase, (iii) deoxyctydine deaminase, and (iv) deoxyctydine monophosphate deaminase. Five of the six Acholeplasma species (all Acholeplasma species except Acholeplasma florum L1T) had all four enzymatic activities. The six Mycoplasma species only had the deoxyctydine and deoxyctydine monophosphate deaminase activities. The only two plant isolates studied, A. florum L1T and S. floricola 23-6T, lacked all four enzymatic activities.

The class Mollicutes contains two orders. The order Mycoplasmatales is composed of two families, the Mycoplasmataceae, which has two genera (Mycoplasma and Ureaplasma), and the Spiroplasmataceae, which has one genus (Spiroplasma) (4). The second order, Acholeplasmatales, contains one family, the Acholeplasmataceae, with one genus, Acholeplasma (5). A number of biochemical, nutritional, and morphological characteristics have been used to distinguish these genera and families. They include sterol requirement for growth, genome sizes, ability to hydrolyze urea, localization of reduced nicotinamide adenine dinucleotide oxidase, and presence of helical forms during growth (4, 5). However, no one has distinguished genera or families of the Mollicutes by the presence or absence of enzymes involved in pyrimidine deoxyribonucleotide metabolism.

Recently, we characterized the adenosine triphosphate (ATP)-insensitive highly specific deoxyuridine triphosphatase (dUTP)-hydrolyzing deoxyuridine triphosphate nucleotidohydrolase (dUTPase; EC 3.6.1.23) from Acholeplasma laidlawii B-PG9 (17). In the present study, we examined five other Acholeplasma species, six Mycoplasma species, and one Spiroplasma species for dUTPase and other enzyme activities involved in pyrimidine deoxyribonucleotide metabolism. Our data suggest that it may be possible, with one exception, to distinguish these genera based upon the presence or absence of the ATP-insensitive dUTP-specific dUTPase (17), deoxyuridine monophosphate (dUMP) phosphatase, deoxyctydine (dC) deaminase, and deoxyctydine monophosphate (dCMP) deaminase activities in cell extracts.

MATERIALS AND METHODS

Chemicals. Nonradioactive nucleotides were purchased from Sigma Chemical Co., St. Louis, Mo. [5-3H]dUTP (11 Ci/mmole) and [5-3H]dCMP (22 Ci/mmole) were purchased from Moravek Biochemicals, Inc., Brea, Calif.; [5-3H]dUMP (10 Ci/mmole), [5-3H]deoxyctydine triphosphate (dCTP) (21 Ci/mmole), [5-3H]dATP (29 Ci/mmole), and [2,14C]thymidine (56.6 Ci/mmole) were purchased from Amersham Corp., Arlington Heights, Ill. Polyethyleneimine-cellulose thin-layer chromatography plates were purchased from Analtech, Newark, Del.

Organisms. Acholeplasma florum L1T (T = type strain) and Spiroplasma floricola 23-6T were obtained from J. Tully, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Acholeplasma axanthum S743T, Acholeplasma granularum BTS-39T, Acholeplasma hippikon C1T, A. laidlawii B-PG9, Acholeplasma morum S2, Mycoplasma arginini G230T, Mycoplasma arthritidis 07, Mycoplasma gallisepticum S6, Mycoplasma hominis ATCC 14027, Mycoplasma pneumoniae FH1, Mycoplasma pulmonis ATCC 19612, and M. pulmonis JB were obtained from our stock collection.

Media and growth conditions. All Acholeplasma, Mycoplasma, and Spiroplasma species were grown in our modification of Edward medium (2). For growth of acholeplasmas, the medium was supplemented with 2.5% (vol/vol) heat-inactivated (56°C, 1 h) horse serum (control lots 268095 and 200011H; K. C. Biologicals, Lenexa, Kans.); for growth of mycoplasmas and spiroplasmas the medium was supplemented with this serum at a concentration of 4% (vol/vol). For growth of M. arginini and M. hominis, l-arginine hydrochloride (Calbiochem-Behring, La Jolla, Calif.) at a final concentration of 0.1% (wt/vol) was added to media. All incubations were at 37°C. Temperature-equilibrated media were inoculated with 1- to 4-day cultures (1 to 15%, vol/vol).

Preparation of cell extracts. Cells were harvested in mid-log growth (18 to 72 h), washed, hypotonically lysed, and centrifugally fractionated, as described previously for enzyme location studies (13, 14). Acholeplasmal extracts were not subjected to further disruptive procedures, but all spiroplasmal and mycoplasmal preparations were also exposed to 65 W of sonic oscillation with a model 350 Branson Sonifier (Heat Systems Co., New York, N.Y.) for three 5-s bursts while they were in a wet ice bath. Crude cell lysates were used without further preparation for the assay of thymidine kinase activity. Washed membrane and cytoplasmic fractions were prepared by differential centrifugation (14) and were used for all other enzyme assays. Before

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A. laidlawii
A. granularum
A. hippocastanum
A. laidlawii B-PG9
A. morum S2
A. florum L1T
S. floricola 23-6T
M. arginini G230T
M. gallisepticum S6
M. hominis ATCC 14027
M. arthritidis
M. pneumoniae
M. hominis
M. gallisepticum
M. hominis ATCC 14027
M. hominis

Assaying, washed membranes were suspended in TMG buffer [10 mM tris(hydroxymethyl)aminomethane hydrochloride (pH 7.5), 2 mM 2-mercaptoethanol, 1 mM MgCl2, 20% (vol/vol) glycerol].

Enzyme assays. All of the procedures used for measuring enzymatic activities have been described previously (18). Assays for dUTPase and deoxyctydine triphosphatase (dCTPase) activities were performed in the presence and absence of competing ATP (5 mM). By using these methods, we could distinguish between dUTPase and dCTPase activities (which are both ATP insensitive and specific only for the substrate, with or without ATP. The concentration of ATP added as a reaction competitor was 5 mM. The minimum detectable amount was 0.001 nmol of dUMP formed per min per mg of protein.

TABLE 1. dUTP-hydrolyzing activities in cytoplasmic fractions of members of the Mollicutes

<table>
<thead>
<tr>
<th>Organism</th>
<th>Enzyme activity</th>
<th>Without ATP</th>
<th>With ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. axanthum S743T</td>
<td></td>
<td>8.8 ± 2.1 (3)</td>
<td>7.6 ± 1.4 (3)</td>
</tr>
<tr>
<td>A. granularum BTS-39T</td>
<td></td>
<td>7.3 ± 0.5 (3)</td>
<td>6.2 ± 0.8 (3)</td>
</tr>
<tr>
<td>A. hippocastanum</td>
<td></td>
<td>7.6 (1)</td>
<td>8.3 (1)</td>
</tr>
<tr>
<td>A. laidlawii B-PG9</td>
<td></td>
<td>10.3 ± 3.1 (5)</td>
<td>9.6 ± 2.3 (7)</td>
</tr>
<tr>
<td>A. morum S2</td>
<td></td>
<td>6.8 ± 1.2 (3)</td>
<td>7.1 ± 0.6 (3)</td>
</tr>
<tr>
<td>A. florum L1T</td>
<td></td>
<td>19.7 ± 2.5 (3)</td>
<td>&lt;0.001 (3)</td>
</tr>
<tr>
<td>S. floricola 23-6T</td>
<td></td>
<td>2.1 ± 0.8 (3)</td>
<td>&lt;0.001 (3)</td>
</tr>
<tr>
<td>M. arginini G230T</td>
<td></td>
<td>3.4 ± 0.4 (3)</td>
<td>&lt;0.001 (3)</td>
</tr>
<tr>
<td>M. gallisepticum S6</td>
<td></td>
<td>5.2 ± 0.8 (3)</td>
<td>&lt;0.001 (3)</td>
</tr>
<tr>
<td>M. hominis ATCC 14027</td>
<td></td>
<td>0.4 ± 0.2 (3)</td>
<td>&lt;0.001 (3)</td>
</tr>
<tr>
<td>M. arthritidis O7</td>
<td></td>
<td>&lt;0.001 (2)</td>
<td>ND</td>
</tr>
<tr>
<td>M. pneumoniae FH</td>
<td></td>
<td>&lt;0.001 (2)</td>
<td>ND</td>
</tr>
<tr>
<td>M. pulmonis ATCC 19612</td>
<td></td>
<td>&lt;0.001 (2)</td>
<td>ND</td>
</tr>
<tr>
<td>M. pulmonis JB</td>
<td></td>
<td>&lt;0.001 (2)</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Reactions were performed as described previously (18) but using dUTP as the substrate, with or without ATP. The concentration of ATP added as a reaction competitor was 5 mM.

b Enzyme activity is expressed as nanomoles of dUMP formed per minute per milligram of protein (mean ± standard deviation). The numbers in parentheses are the numbers of different batches of cells examined.

c The minimum detectable amount was 0.001 nmol of dUMP formed per min per mg of protein.

d ND, Not done.

d The minimum detectable amount was 0.001 nmol of dUMP formed per min per mg of protein.

RESULTS

Members of three genera belonging to the class Mollicutes, each representing a different taxonomic family (4, 5), were examined for the ATP-insensitive dUTP-specific hydrolyzing dUTPase of A. laidlawii B-PG9 (17) (Table 1) and for other enzyme activities of pyrimidine deoxyribonucleotide metabolism (Table 2). Only cytoplasmic fractions from some Acholeplasma species hydrolyzed dUTP by what we identified as the dUTPase of A. laidlawii B-PG9 (Table 1). Although the cytoplasmic fractions from all other members of the Mollicutes tested lacked this activity, some contained a relatively small amount of another type of dUTP- and dCTP-hydrolyzing activity which was completely inhibited by ATP. We also found ATP-inhibitable dUTP- and dCTP-hydrolyzing activity in purified membrane fractions from all members of the Mollicutes examined (Table 3).

The cytoplasmic fractions of all members of the Mollicutes tested had thymidine phosphorylase activity, but none had detectable dUMP phosphatase activity or the specific dCTP-hydrolyzing dCTPase activity (Table 2). Except for A. florum and A. floricola, the cytoplasmic fractions of all members of the Mollicutes tested had dC deaminase and dCMP deaminase activities (Table 2). The crude lysate fractions (obtained immediately after hypotonic lysis, without further disruption or centrifugation) of all members of the Mollicutes tested had thymidine kinase activity (Table 2).

We also tested purified membrane fractions for dUMP phosphatase activity. We found that membrane fractions from A. laidlawii B-PG9 and A. axanthum S743T had dUMP phosphatase activity (4.66 ± 0.21 [n = 3] and 3.30 ± 0.34 [n = 3] nmol of dUMP hydrolyzed per min per mg of protein, respectively). We found no dUMP phosphatase activity in membrane fractions from A. florum L1T, S. floricola 23-6T, M. arginini G230T, and M. hominis ATCC 14027 (<0.001 nmol of dUMP hydrolyzed per min per mg of protein).

DISCUSSION

The pattern of our results suggests that it may be possible, after more species are studied, to distinguish some genera within the Mollicutes by the presence or absence of enzymes involved in the metabolism of pyrimidine deoxyribonucleotides. These enzyme activities are (i) the ATP-
insensitive to dUTP-specific hydrolyzing dUTPase (17), (ii) dC deaminase, (iii) dUMP phosphatase, and (iv) dCMP deaminase. Strains of *S. floricola* and *A. flororum*, the only two plant isolates tested, lacked all four enzyme activities. All other *Acholeplasma* species had all three enzyme activities, whereas all *Mycoplasma* species had dC and dCMP deaminase activities but no detectable dUTPase or dUMP phosphatase activities. In all members of the *Mollicutes* tested we detected thymidine kinase and thymidine phosphorylase activities. This confirms the results of previous studies by Hamet et al. (6), who demonstrated thymidine phosphorylase activity in a number of members of the *Mollicutes*.

Neale et al. (10, 11) reported the presence of cytoplasmic dUTPase and dCTPase activities in *Mycoplasma mycoides* subsp. *mycoides*. Although we have not examined this organism, none of the six *Mycoplasma* species which we did examine contained any detectable amounts of these specific enzymatic activities. The differences between our results and those of Neale et al. (10, 11) are probably due to intrinsic differences between *M. mycoides* subsp. *mycoides* and the *Mycoplasma* species that we studied. However, these differences may also reflect dissimilarities in the stringency of the assays used to detect these enzymes. A problem with measuring dUTPase and dCTPase activities, especially in crude extracts, is that dUTP and dCTP can be hydrolyzed by other enzymes that have a broad substrate specificity (1, 3, 8, 9). Such non-specific enzymes that hydrolyze dUTP and dCTP include nucleoside triphosphatase (1, 9), adenosine triphosphatase (8), and alkaline phosphatase (3). Both adenosine triphosphatase (6, 16) and an alkaline phosphatase-like activity (12) have been reported in extracts of *A. laidlawii*. Our results (Table 3) demonstrate that a membrane-associated enzyme from a variety of members of the *Mollicutes* nonspecifically hydrolyzes both dUTP and dCTP.

We believe that the ATP-inhibitable non-specific dUTP- and dCTP-hydrolytic activity which we found in cytoplasmic fractions was due to membrane contamination. The activities arose from solubilization of the membrane-associated enzymes or from membrane fragments containing these activities that were not sedimented during centrifugal preparation of our cytoplasmic fractions (16).

Our results also demonstrate that the two plant isolates, *A. flororum* L1T and *S. floricola* 23-6T, are identical with respect to the presence or absence of enzyme activities involved with pyrimidine deoxyribonucleotide metabolism. *A. flororum* L1T has other similarities to *S. floricola* 23-6T; they both have no detectable levels of adenosine kinase, adenylosuccinate synthetase, adenylosuccinate lyase, and inosine monophosphate dehydrogenase activities (V. V. Tryon and J. D. Pollack, unpublished data). In some respects, *A. flororum* is unlike other acholeplasmas; it does not synthesize lipids from acetate (15), and an unusually large percentage of its reduced nicotinamide adenine dinucleotide oxidase activity is localized in the cytoplasmic fraction (J. D. Pollack, K. D. Beaman, V. V. Tryon, and J. Robertson, Abstr. 4th Int. Cong. Int. Org. Mycoplasmol., Tokyo, Japan, 1982, F-1, p. 26). We hypothesize that the similarities between *S. floricola* ST-6T and *A. flororum* L1T may be related to their associations with plants.

<table>
<thead>
<tr>
<th>Organism</th>
<th>dUTP</th>
<th>dUTP + ATP(a)</th>
<th>dCTP</th>
<th>dCTP + ATP(b)</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. laidlawii</em> B-PG9</td>
<td>6.3 ± 2.1</td>
<td>NA</td>
<td>7.6 ± 1.3</td>
<td>NA</td>
<td>10.7 ± 2.3</td>
</tr>
<tr>
<td><em>A. axanthum</em> S743T</td>
<td>5.4 ± 1.1</td>
<td>NA</td>
<td>5.8 ± 1.9</td>
<td>NA</td>
<td>8.4 ± 2.1</td>
</tr>
<tr>
<td><em>A. flororum</em> L1T</td>
<td>7.6 ± 2.7</td>
<td>NA</td>
<td>8.4 ± 1.2</td>
<td>NA</td>
<td>12.5 ± 2.2</td>
</tr>
<tr>
<td><em>S. floricola</em> 23-6T</td>
<td>8.8 ± 1.2</td>
<td>NA</td>
<td>9.3 ± 1.4</td>
<td>NA</td>
<td>14.1 ± 1.5</td>
</tr>
<tr>
<td><em>M. arginini</em> G230T</td>
<td>3.3 ± 1.5</td>
<td>NA</td>
<td>4.1 ± 1.3</td>
<td>NA</td>
<td>5.6 ± 1.4</td>
</tr>
<tr>
<td><em>M. hominis</em> ATCC 14027</td>
<td>8.2 ± 1.1</td>
<td>NA</td>
<td>7.6 ± 2.3</td>
<td>NA</td>
<td>15 ± 2.3</td>
</tr>
</tbody>
</table>

\(a\) Assays for nucleotide hydrolysis were performed by using the reaction conditions described previously for the dUTPase assay (18). Values are means ± standard deviations from three cell batches (in nanomoles of nucleotide hydrolyzed per minute per milligram of protein).

\(b\) The concentration of ATP added as a reaction competitor was 5 mM.

\(c\) NA. No activity detected (the minimum detectable amount was 0.001 nmol of nucleotide hydrolyzed per min per mg of protein).

**LITERATURE CITED**


