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Acetate Kinase Activity in Mycoplasmas

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Acetate kinase activity was assayed in 13 mycoplasmas. Nine species exhibited the enzymic activity in the direction of either synthesis of acetylphosphate or adenosine triphosphate. On the other hand *Mycoplasma orale*, *Mycoplasma arthritidis*, *Ureaplasma urealyticum* (10 serotypes), and two strains of *Anaeroplasma* species exhibited only minimal levels of the enzymic activity. In these four species, the enzyme does not seem to play a key role in adenosine triphosphate formation.

The established role of acetate kinase (EC 2.7.2.1) as a supplier of ATP to some anaerobic eubacteria (3, 15) led to the investigation of acetate kinase in ATP generation in mycoplasmas as well. Acetate kinase activity has been reported in the cytoplasm of both fermentative and nonfermentative mycoplasmas (5, 8, 14, 16). The presence of acetate kinase in mycoplasmas and kinetic studies of the reactions (7) indicate that under physiological conditions the enzyme is used to supplement ATP synthesis. Detailed chemical studies on the enzyme purified from *Acholeplasma laidlawii* supported the role of acetate kinase in ATP formation (7). This knowledge, particularly with respect to mycoplasmas whose energy metabolism is largely unknown, e.g., *Ureaplasma urealyticum*, would provide a greater understanding of the mechanism of generation of ATP.

Organisms and growth conditions. The following were grown in a modified Edward medium (10) with a starting pH of 7.8: *Acholeplasma axanthum* 743, *A. axanthum* 410, *A. granularum*, *Mycoplasma arginini*, *M. gallinarum*, *M. salivarium*, *M. orale*, and *M. arthritidis* 07. *A. laidlawii* and *M. gallisepticum* were grown in the same medium with a starting pH of 8.5, and *M. hominis* was grown with a pH of 6.5. *M. pneumoniae* was grown as described by Banai et al. (2). *M. salivarium* and *M. orale* were grown anaerobically under the previously described conditions. The *Anaeroplasma* species strains 61 and 7LA were grown anaerobically in a medium described by Robinson (11). Nine serotype strains of *U. urealyticum* (13) and a newly identified serotype, Western, (J. A. Robertson and G. W. Stemke, unpublished data) were grown in bromothymol blue broth (12) with a starting pH of 6.0. The growth medium for *M. arginini*, *M. hominis*, and *M. arthritidis* 07 was supplemented with 0.2 M L-arginine. The organisms were grown at 37°C for 18 to 40 h.

Separation of the soluble fraction. The organisms were harvested by centrifugation and washed twice in 0.25 M NaCl (10). The cells were disintegrated by sonication in a Heat Systems Ultrasonicator model 30. The sonication was done by using a fine probe and an output of about 100 W for 1 min. Several organisms were also osmotically lysed (10). In both cases, the soluble fraction was separated from the membranes by centrifugation (10).

Enzyme assays and analytical procedures. Protein was determined by the Folin phenol reagent (9) or by the Bradford method (4). Acetate kinase-ATP:acetate phosphotransferase, (EC 2.7.2.1) was assayed by either the formation of acetyl phosphate from acetate and ATP or the opposite reaction, formation of ATP from ADP and acetylphosphate (8). Acetylphosphate production was determined by the hydroxylamine method as described by Skarstedt and Silverstein (15); specific activity was expressed as micromoles of acetyl hydroxamate produced per minute per milligram of protein. ATP formation was determined by the method of Bowman et al. (3), using a coupled system consisting of hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49). Specific activity was expressed as micromoles of NADPH formed (equivalent to micromoles of ATP formed) per minute per milligram of protein. Phosphate acetyl transferase (EC 2.3.1.8) was determined as described by Allen et al. (1).

NADH oxidase and ATPase were assayed as previously described by Kahane et al. (6).

The results of acetate kinase activity in the various mycoplasmas are reported in Table 1. The organisms tested seemed to fall into two distinct categories: (i) organisms exhibiting acetylphosphate formation and ATP synthesis, and (ii) organisms exhibiting minimal levels of enzyme activity in both directions of the reaction. The organisms exhibiting minimal activity were those that grew to low titers, and, therefore, the possibility that the soluble fraction obtained from the harvested material contained only nonmycoplasma media components had to be ruled out. However, the harvested material was found to include viable organisms, and the soluble fraction obtained from them exhibited NADH oxidase or ATPase activity or both.

M. salivarium, *M. orale*, and all 10 serotypes of the ureaplasmas tested showed minimal but finite ATP synthesis, whereas acetyl phosphate production was nil. This may be accounted for by the insensitivity of the hydroxylamine assay. On the other hand, the low ATP formation value may be due to cross-reactivity of the enzymes catalyzing ATP synthesis, using phosphorylated substrates analogous to acetyl phosphate. This point should be further clarified by the isolation and purification of acetate kinase from these organisms.

Finally, the possibility of enzyme denaturation by the sonication procedure was ruled out by a comparison of acetate kinase activity in preparations obtained by osmotic lysis and by sonication of *U. urealyticum* Vancouver. ATP synthesis was about the same in osmotically lysed cells (specific activity, 0.173) and in sonicated cells (specific activity, 0.110). In both cases, there was no acetyl phosphate formation.

TABLE 1. *Acetate kinase activity in the soluble fraction of mycoplasmas*

Soluble fraction obtained from:	Acetate kinase sp act (μmol/min per mg of protein)	
	Acetyl phosphate formation	ATP formation
A. laidlawii	4.46	10.20
A. axanthum 743	0.27	0.20
A. axanthum 410	0.55	0.15
A. granularum	0.40	0.20
M. gallisepticum	5.53	12.50
M. arginini	1.85	0.38
M. gallinarum	1.15	0.50
M. hominis (NIH)	1.82	0.62
M. pneumoniae	9.09	3.50
M. salivarium	0	0.30
M. orale	0	0.03
M. arthritidis 07	0	0
Anaeroplasma species 61	0	0
Anaeroplasma species 7LA	0	
U. urealyticum serotypes 1 to 10	0	0.02–0.11

In summary, not all mycoplasmas exhibit acetate kinase activity. Furthermore, the enzyme does not seem to play a key role in the ATP metabolism of the ureaplasmas and anaeroplasmas. The rest of the mycoplasmas show varying levels of acetate kinase activity; therefore, a distinction between acholeplasmas and mycoplasmas is not discernable.

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