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# Purine and Glycine Metabolism by Purinolytic Clostridia

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Cell extracts of *Clostridium acidiurici*, *C. cylindrosporum*, and *C. purinolyticum* converted purine, hypoxanthine, 2-hydroxypurine, 6,8-dihydroxypurine, and uric acid into xanthine by the shortest possible route. Adenine was transformed to xanthine only by *C. purinolyticum*, whereas the other two species formed 6-amino-8-hydroxypurine, which was neither deaminated nor hydroxylated further. 8-Hydroxypurine was formed from purine by all three species. Xanthine dehydrogenase activity was constitutively expressed by *C. purinolyticum*. Due to the lability of the enzyme activity, comparative studies could not be done with a purified preparation. All enzymes reported to be involved in formiminoglycine metabolism of *C. acidiurici* and *C. cylindrosporum* were present in *C. purinolyticum*. However, glycine was reduced directly to acetate in all three species, as indicated by radiochemical data and by the detection of glycine reductase in cell extracts of *C. cylindrosporum* and *C. purinolyticum*. The expression of glycine reductase and the high ratio of glycine fermented to uric acid present points to an energetic advantage for the glycine reductase system, which is expressed when selenium compounds are added to the growth media.

Purines are important components of nucleic acids and nucleotides. However, free purine bases can be quite toxic for bacteria and eucaryotes (19). A variety of bacteria transform or degrade purines (43). The enzyme xanthine dehydrogenase, a molybdo-iron-sulfur flavoprotein, is generally responsible for the transformation of the purine ring system. Its substrate specificity and pattern of hydroxylation depend on the organism involved (11). Recently, the trace element selenium was found to be important for the expression of an active xanthine dehydrogenase in bacteria specialized in anaerobic purine degradation (13, 46). The three known species, *Clostridium acidiurici*, *C. cylindrosporum*, and *C. purinolyticum*, are phenotypically very similar, but genotypically quite distinct. The spectrum of purines utilized by each of these bacteria for growth differs slightly (13). In a study on xanthine dehydrogenase of *C. cylindrosporum* (6), a preference of the enzyme for attacking position 8 of the purine ring system was found, and uric acid was concluded to be a central intermediate in purine transformation of this organism. The purified xanthine dehydrogenase used was, however, much less active than crude extracts prepared from selenium-supplemented cells (46).

Recent studies with *C. purinolyticum* showed that in selenium-deprived cells uric acid has a central position in purine degradation (14). In contrast, selenium-supplemented cells oxidize hypoxanthine directly to xanthine (15), and not via 6,8-dihydroxypurine and uric acid as reported for *C. cylindrosporum* (6). The hydrolysis of xanthine to formiminoglycine occurs in *C. purinolyticum* via the same intermediates as those reported for *C. cylindrosporum* (15, 24). However, the metabolism of glycine seems to be different: *C. acidiurici* and *C. cylindrosporum* convert glycine via serine and pyruvate to acetate (9, 28, 31, 44), whereas *C. purinolyticum* reduces glycine directly to acetate by the action of one enzyme, glycine reductase, which requires selenium compounds (16, 36). Therefore, purine and glycine metabolism by *C. acidiurici* and *C. cylindrosporum* were reexamined. The results obtained by using selenium-supplemented cells revise the hydroxylation pattern of purine compounds and of glycine metabolism compared with previous data. Thus, the availability of selenium influences the substrate specificity and the flow of purine carbon in these organisms by the formation of more active and favorable enzymes.

## MATERIALS AND METHODS

**Organisms and growth media.** *C. purinolyticum* WA-1 (DSM 1384) was grown on adenine as described previously (13). *C. acidiurici* 9a (ATCC 7906, DSM 604) and *C. cylindrosporum* HC-1 (ATCC 7905, DSM 605) were obtained from the Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of

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Germany, and were cultured in a similar medium with adenine replaced by uric acid (10 mM). In some experiments, glycine (100 mM) was added. *C. sticklandii* (ATCC 12662, DSM 519) was kindly provided by A. C. Schwartz, Institute of Botany, University of Bonn, Federal Republic of Germany, and was cultivated according to Stadtman (36). All media were prepared under strictly anaerobic conditions (13).

**Analytical methods.** Acetate was determined by an enzymatic procedure (12), formate and glycine by colorimetric methods (22, 33). Identification and quantification of purines were performed by means of high-pressure liquid chromatography on a reversed-phase ($C_{18}$) column, using potassium phosphate buffer (100 mM, pH 2.7 to 3.7) as eluent (15). 8-Hydroxypurine eluted in advance of the unsubstituted purine.

**Tracer experiments.** Fermentation of radioactive glycine in the presence of uric acid by resting cells of *C. acidiurici* and *C. cylindrosporum* was performed in principle as already described (16). In this case, the reaction buffer had the same composition as the growth medium except that glycine, potassium bicarbonate, and yeast extract were omitted and 0.15 μM thiamine was added.

**Enzyme assays.** Cell extracts were prepared by suspending 1 g of cells (wet weight, harvested at the end of the exponential-growth phase) in 2 ml of potassium phosphate buffer (10 mM, pH 7.1); adding 1 mg of lysozyme, 0.1 mg of DNase, and 1 mM dithioerythritol; and incubating anaerobically for 1 h at 37°C. Subsequently, the suspension was passed once through a chilled French pressure cell at 1,050 kPa/cm$^2$. After centrifugation at 40,000 × *g* (30 min) and 4°C, the supernatant was kept at 0°C under an atmosphere of nitrogen in the dark and immediately used for enzymatic assays. Cells for the glycine reductase assay were stored according to Barnard and Akhtar (4). The protein content of the extracts was determined by the biuret method (5). All enzymatic tests were performed under strictly anaerobic conditions at 37°C. The following assays were used: xanthine dehydrogenase (13), carbamate kinase (34), glycine formiminotransferase (40), methenyltetrahydrofolate cyclohydrolase (23, 41), methylenetetrahydrofolate dehydrogenase (23, 42), formyltetrahydrofolate synthetase (23, 27), L-serine dehydratase (16) (activation of extract according to Carter and Sagers [8]), serine hydroxymethyltransferase (4), glycine decarboxylase (32), and glycine reductase (36). One unit of enzyme activity was defined as 1 μmol of substrate transformed or product formed per min at 37°C. About 80% of the xanthine dehydrogenase activity was lost in cell extracts within 3 days at 4°C when 50 mM potassium phosphate buffer, pH 8.0, or 50 mM Tricine-KOH, pH 8.5, was used. We have not yet found better conditions to stabilize the activity. Purification by affinity chromatography with an allopurinol derivative coupled to Sepharose (17) failed, although the enzyme was about one-third as active against allopurinol as against xanthine. Ferredoxin of *C. purinolyticum* could not be tested as a potential physiological electron acceptor of xanthine dehydrogenase (6) since it was even more unstable than that from *C. cylindrosporum* (9) by the procedures generally employed (9, 35).

**Chemicals.** 8-Hydroxypurine and 6-amino-8-hydroxypurine were synthesized according to Isay (20) and Robins (30), respectively. The identity of 8-hydroxypurine was additionally established by cochromatography with the actual substance, kindly donated by H. A. Barker, University of California, Berkeley. Attempts to prepare 3-(1*H*-pyrazolo[3,4-*d*]pyrimidine-4-ylamino)-1-propyl-6-aminohexanoate (=compound III) for affinity chromatography of xanthine dehydrogenase as described previously (17) proved to be unsuccessful. However, the substance could be prepared by changing the sequence of additions during the synthesis. It was absolutely necessary to add *N,N'*-carbonyldiimidazole in advance of compound I. Compound III synthesized in this way proved to be identical with material kindly provided by G. Blankenhorn, University of Konstanz, Federal Republic of Germany. Tetrahydrofolic acid was kindly provided by Lynnè Quandt and M. Braun, University of Göttingen, Federal Republic of Germany. Formiminoglycine-β-phenylethylester hydrochloride was synthesized according to Freter et al. (18). Polymin P used for purification of ferredoxin (35) was donated by BASF, Ludwigshafen, Federal Republic of Germany. All other chemicals used were of the highest purity commercially available.

## RESULTS

**Purine interconversion.** Cell extracts of *C. purinolyticum*, *C. acidiurici*, and *C. cylindrosporum* were allowed to act on buffered purine solutions (4 mM potassium phosphate buffer, pH 7.5) in the presence of 6 mM EDTA. This agent does not inhibit purine interconversion but prevents purine breakdown (15), which generally starts from xanthine (24). Xanthine was found to be a transformation product of all purine compounds tested except for adenine in the case of *C. acidiurici* and *C. cylindrosporum* (Table 1). Unlike *C. purinolyticum*, these organisms seemed to be unable to deaminate adenine to hypoxanthine; instead, adenine was hydroxylated to yield 6-amino-8-hydroxypurine. 6-Amino-2-hydroxypurine (isoguanine) and 6-amino-2,8-dihydroxypurine were not detected as products. The unsubstituted purine ring was hydroxylated in positions 6 and 8. However, 8-hydroxypurine was only transiently formed within the first 2 h by *C. purinolyticum*, whereas its concentration did not decrease with time in the extracts of the other two organisms. Xanthine was the only product detected after incubation of the extracts with hypoxanthine, 2-hydroxypurine, and uric acid, indicating a direct hydroxylation to yield xanthine. Uric acid was an intermediary product only with 6,8-dihydroxypurine. The lower recovery of purine compounds observed in some experiments shown in Table 1 probably reflects a cleavage of the ring system (15).

The methyl viologen-dependent xanthine dehydrogenase activity was constitutively expressed by *C. purinolyticum*; extracts of cells grown on glycine exhibited an activity of 0.85 U compared to 1.6 U per mg of protein after growth on adenine (13). The induction pattern of

TABLE 1. Purine interconversions by cell extracts of purinolytic clostridia[a]

| Substrate added (concn) | Compounds detected | *C. purinolyticum* | | | *C. acidiurici* | | | *C. cylindrosporum* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Concn (mM) | Incubation time (h) | Total recovery of purine compounds (%) | Concn (mM) | Incubation time (h) | Total recovery of purine compounds (%) | Concn (mM) | Incubation time (h) | Total recovery of purine compounds (%) |
| Purine (4 mM) | Purine | 1.40 | | | 1.66 | | | 1.49 | | |
| | 8-Hydroxypurine | 0[b] | | | 1.11 | | | 1.55 | | |
| | Hypoxanthine | 0.82 | | | 0.56 | | | 0.33 | | |
| | Xanthine | 0.83 | 23 | 76 | 0.25 | 23 | 90 | 0.45 | 23 | 96 |
| Adenine (4 mM) | Adenine | 1.73[c] | | | 3.31 | | | 3.67 | | |
| | 6-Amino-8-hydroxypurine | 0[c] | | | 0.70 | | | 0.54 | | |
| | Hypoxanthine | 0.19[c] | | | 0 | | | 0 | | |
| | Xanthine | 1.54[c] | 16 | 77 | 0 | 4 | 100 | 0 | 4 | 105 |
| Hypoxanthine (2 mM) | Hypoxanthine | 0.52[c] | | | 0.56 | | | 0.41 | | |
| | Xanthine | 0.61[c] | 6 | 56 | 0.74 | 6 | 65 | 1.00 | 4 | 71 |
| 2-Hydroxypurine (1 mM) | 2-Hydroxypurine | 0.60 | | | 0.48 | | | 0.08 | | |
| | Xanthine | 0.41 | 1 | 101 | 0.06 | 4 | 54 | 0.93 | 6 | 101 |
| 6,8-Dihydroxypurine (2 mM) | 6,8-Dihydroxypurine | 0.54 | | | 1.20 | | | 1.04 | | |
| | Uric acid | 0.54 | | | 0.45 | | | 0.36 | | |
| | Xanthine | 0.20 | 4 | 64 | 0.59 | 6 | 112 | 0.17 | 4 | 79 |
| Uric acid (2 mM) | Uric acid | 1.80 | | | 1.72 | | | 1.79 | | |
| | Xanthine | 0.02 | 6 | 91 | 0.06 | 6 | 89 | 0.02 | 6 | 91 |
| Xanthine (2 mM) | Xanthine | 1.68[c] | | | 1.46 | | | 1.18 | | |
| | Uric acid | 0[c] | 4 | 84 | 0.29 | 4 | 88 | 0.48 | 8 | 83 |

[a] Samples were taken at hourly intervals and analyzed by high-pressure liquid chromatography (15). The data shown represent a characteristic analysis. The protein content was 2 mg/ml.

[b] Initially 8-hydroxypurine accumulated but disappeared later.

[c] Data were taken for comparison from reference 15.

xanthine dehydrogenase could not be tested in *C. acidiurici* and *C. cylindrosporum* (46) since these species grow only on purines (13).

**Purine decomposition and formiminoglycine metabolism.** As has been demonstrated for *C. cylindrosporum* by Rabinowitz (24) and partly for *C. acidiurici* by Radin and Barker (28), *C. purinolyticum* degrades xanthine via 4-ureido-5-imidazolecarboxylate, 4-amino-5-imidazolecarboxylate, and 4-aminoimidazole to formiminoglycine. These substances have been detected previously by high-pressure liquid chromatography as degradation products (15). 4(5)-Imidazolone was not found as a product by the latter method due to its instability. However, extracts of *C. purinolyticum* exhibited a 4(5)-imidazolone amidohydrolase activity (Fig. 1) as reported for *C. cylindrosporum* (18). 4(5)-Imidazolone was formed first within the cuvette from formiminoglycine-β-phenylethylester by an equimolar addition of KOH (18). Although the hydrolysis of 4(5)-imidazolone, as monitored by a decrease of its extinction, was dependent on the concentration of active enzyme, the reaction ceased before it reached completion. This might be due to an inhibition by polymeric products of imidazolone (18). The enzymatic reaction was present in adenine-grown and glycine-grown cells of *C. purinolyticum*.

All enzymes known to be involved in the degradation of formiminoglycine by *C. acidiurici* and *C. cylindrosporum* (9) were detected in cell extracts of *C. purinolyticum* (Table 2). The latter organism contained rather high activities of glycine formiminotransferase and formyltetrahydrofolate synthetase. The assay system used for glycine formiminotransferase (40) additionally allowed the detection of formiminotetrahydrofolate cyclodeaminase activity in *C. purinolyticum*. Glycine was catabolized by a glycine reductase which catalyzed a direct reduction of glycine to acetate with dithiothreitol as an electron donor (36). For comparison, the specific activity of glycine reductase in cell extracts of *C.*

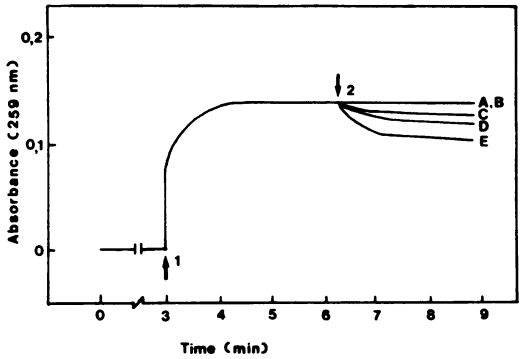


FIG. 1. Decomposition of 4(5)-imidazolone by cell-free extracts of *C. purinolyticum*. (1) Addition of KOH (10 μmol) for liberation of 4(5)-imidazolone from formiminoglycine-β-phenylethylester. (2) Start of the reaction by adding: A, water (control); B, 5 μl of extract from adenine-grown cells (about 100 μg of protein) incubated for 5 min at 100°C; C, 2.5 μl of extract from adenine-grown cells (about 50 μg of protein); D, 5 μl of extract from glycine-grown cells (about 90 μg of protein); or E, 5 μl of extract from adenine-grown cells (about 100 μg of protein).

*sticklandii* was 0.06 U/mg of protein. *C. purinolyticum* contained methenyltetrahydrofolate cyclohydrolase and glycine decarboxylase in about the same low activities as those reported for *C. acidiurici* and *C. cylindrosporum* (9). Methylenetetrahydrofolate dehydrogenase was active with NAD and NADP as coenzymes, but NADP was about ten times more effective, as reported for the enzyme of *C. cylindrosporum* in crude extracts (42). Serine hydroxymethyltransferase and L-serine dehydratase were present, but exhibited rather low activities compared with the other purine-fermenting clostridia (8, 9). A carbamate kinase activity found in *C. purinolyticum* was independent of tetrahydrofolate.

**Comparative studies with glycine as substrate.** Glycine is an intermediary product of anaerobic purine degradation to acetate (43). *C. acidiurici* and *C. cylindrosporum* cannot grow on glycine as sole substrate, but the organisms can utilize, in cell suspensions, 1.5 mol of glycine in cometabolism with 1 mol of uric acid (2). We found that the ratio of glycine to uric acid used could be increased to 10:1 by using cell suspensions grown under these conditions in the presence of $10^{-7}$ M selenite; 100 mM glycine and 10 mM uric acid were completely fermented by *C. acidiurici* and *C. cylindrosporum* to acetate, $CO_2$, and $NH_3$, but not to formate. These resting cells of both organisms were used to ferment [2-$^{14}$C]glycine in the presence of unlabeled uric acid (Table 3). The acetate produced by *C. acidiurici* and *C. cylindrosporum* was highly labeled in the methyl position. Some glycine (or acetate) was converted into cell material, because all missing radioactivity as calculated from the balances could be detected within the cells. Since the labeling data indicated the function of a glycine reductase as

TABLE 2. Specific activities of enzymes participating in formiminoglycine degradation by *C. purinolyticum*[a]

| Enzyme | Sp act (U/mg of protein) |
|---|---|
| Glycine formiminotransferase | 4.6 |
| Formiminotetrahydrofolate cyclodeaminase | Present |
| Methenyltetrahydrofolate cyclohydrolase | 0.02 |
| Formyltetrahydrofolate synthetase | 44.0 |
| Formate dehydrogenase | 1.8[b] |
| Methylenetetrahydrofolate dehydrogenase (NADP dependent) | 0.7 |
| Glycine decarboxylase | 0.008 |
| Glycine reductase | 0.05 |
| Serine hydroxymethyltransferase | 0.1 |
| L-Serine dehydratase | 0.7 |
| Carbamate kinase | 0.5 |

[a] Cells were grown on adenine as substrate.
[b] Data taken from reference 13.

TABLE 3. Distribution of radioactivity in the products of glycine fermentation in the presence of uric acid by resting cells of *C. acidurici* and *C. cylindrosporum* with [2-$^{14}$C]glycine as substrate[a]

| Organism | Specific radioactivity (dpm/mmol) | | | | $^{14}$C recovery (%) |
|---|---|---|---|---|---|
| | Added radioactivity ([2-$^{14}$C]glycine) | Products | | | |
| | | $CH_3$ group of acetate | COOH group of acetate | $CO_2$ | |
| *C. acidiurici* | 370,000 | 165,700 (78.1)[b] | 46,480 (21.9) | 84,930 | 66.8 |
| *C. cylindrosporum* | 370,000 | 266,580 (93.7) | 17,860 ( 6.3) | 142,260 | 96.1 |

[a] The reaction vessels contained 40 mM glycine and 4 mM uric acid in a total volume of 30 ml. The resting cells had an absorbance at 600 nm of 11.7 within the reaction mixture. Less than 1% of the substrates could be detected in the supernatant of the medium after 7 h of fermentation at 37°C. End products were only acetate, $CO_2$, and $NH_3$. Calculations were done according to the following fermentation equations: 1 glycine + 0.5 $H_2O$ → 0.75 acetate + 0.5$CO_2$ + 1 $NH_3$; 1 uric acid + 5.5 $H_3O$ → 0.75 acetate + 3.5$CO_2$ + 4 $NH_3$.
[b] Numbers in parentheses indicate the percentage of acetate labeled in the group specified.

observed in *C. purinolyticum* (16), the activity of this enzyme was tested in cell extracts of both organisms. *C. cylindrosporum* contained 0.03 U/mg of protein; no activity could be detected in extracts of *C. acidiurici* by the standard test (36). The enzyme activity is known to be labile (4, 16, 36), and this may account for failure to find the activity in *C. acidiurici*.

## DISCUSSION

According to this study, the hydroxylation pattern of purines and the pathway of their degradation are almost identical for the three clostridia which are specialized on purine utilization. One explanation for the differences observed among this study and previous studies of *C. acidiurici* and *C. cylindrosporum* (2, 3, 6, 21, 25, 44) is the improved growth conditions due to supplementation of the media with the trace element selenium. This guarantees that these bacteria form the active enzymes required for substrate attack and product formation, such as xanthine dehydrogenase (46), formate dehydrogenase (45), and glycine reductase (36).

All purines tested are generally transformed by cell extracts into xanthine by the shortest possible route (Fig. 2). In a former study with purified xanthine dehydrogenase of *C. cylindrosporum* (6), the oxidation of position 8 was found to be the preferred reaction, with uric acid as a central intermediate. In this study, however, uric acid was only detected with 6,8-dihydroxypurine as substrate. The scheme observed for *C. purinolyticum* is almost identical to that of *Peptococcus aerogenes* (B. S. Woolfolk and H. R. Whiteley, Bacteriol. Proc., p. 108, P28, 1962). Three differences were noted between *C. purinolyticum* and *C. acidiurici* and *C. cylindrosporum*. (i) Under the conditions used, xanthine was not converted to uric acid by extracts of *C. purinolyticum*, explaining to some degree the central position of uric acid proposed for the latter organisms (6). (ii) 8-Hydroxypurine was only temporarily formed from purine by *C. purinolyticum*, but accumulated in cell extracts of the other two species. (iii) Adenine was transformed to hypoxanthine by *C. purinolyticum* (15), but 6-amino-8-hydroxypurine accumulated in extracts of the other two organisms. Therefore, the introduction of a hydroxyl group in position 8 of purine or adenine might be a dead-end reaction. 6-Amino-8-hydroxypurine is a poor substrate for the xanthine dehydrogenase of *C. cylindrosporum* (6), and 6-amino-2,8-dihydroxypurine is a strong competitive inhibitor of xanthine dehydrogenase (37). The differences observed for the clostridial species correspond to their ability to utilize purine and adenine for growth. However, some purines such as 2-hydroxypurine and 6,8-dihydroxypurine were converted into xanthine although they do not serve as growth substrates for *C. acidiurici* and *C. cylindrosporum* (13). This inability might be due to a deficiency in induced transport for these compounds. An energy-linked transport system has been found for formiminoglycine in *C. acidiurici*, which depends on xanthine or uric acid as energy source and on sodium salts (10).

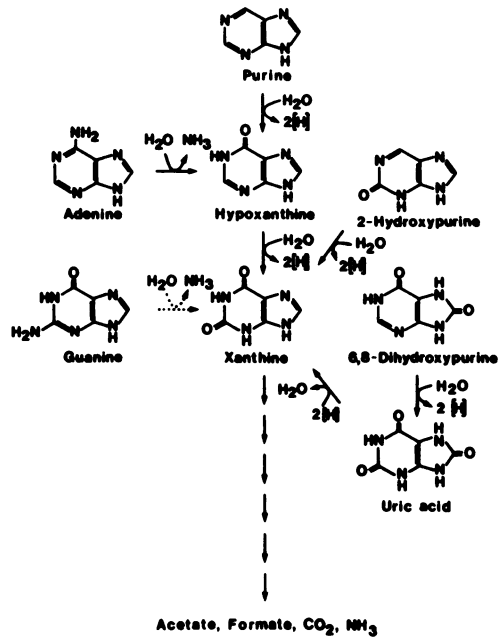


FIG. 2. Purine interconversions catalyzed by *C. purinolyticum*. The deamination of guanine to xanthine as indicated by the dotted line has been demonstrated for *C. acidiurici* (29) and *C. cylindrosporum* (26), but not for *C. purinolyticum*.

According to the present results, *C. cylindrosporum* and possibly *C. acidiurici* can use two systems for glycine metabolism as part of the purine breakdown. (i) Under selenium deficiency, the glycine-serine-pyruvate interconversion, initiated by the serine hydroxymethyltransferase reaction, is operative as indicated by labeling and enzymatic data (3, 9, 21, 25, 28, 31, 44). (ii) After selenium supplementation, the glycine reductase system is responsible for most of the acetate formed as in *C. purinolyticum*. This is indicated by the labeling data presented in this study, although all the enzymes involved in a glycine-serine-pyruvate interconversion are present in cell extracts. The specific activities of glycine reductase in extracts of *C. cylindrosporum* and *C. purinolyticum* were about the same as we obtained for *C. sticklandii*, which contains the best-characterized enzyme (36). In extracts of *C. acidiurici*, no glycine reductase

activity was found. This might be explained by greater instability (4) or by inappropriate assay conditions, such as the use of the artificial electron donor dithiothreitol. Compared with *C. cylindrosporum*, experiments with *C. acidiurici* revealed a lower $^{14}C$ recovery in the fermentation products and an accompanying higher extent of glycine incorporation into cell material, which decreased the specific radioactivity in the fermentation products due to the unlabeled acetate and $CO_2$ derived from uric acid. However, the labeling pattern from [2-$^{14}C$]glycine indicated that both the methyl group of acetate and the $CO_2$ exhibited the highest specific labeling. This fact can be explained by a direct reduction of 0.75 mol of glycine to acetate concomitant with the oxidation of 0.25 mol of glycine to $CO_2$. This fermentation pattern yields, theoretically, three-fourths of the labeling in the methyl group of acetate and one-fourth in $CO_2$. The glycine-serine-pyruvate interconversion gives rise to unlabeled $CO_2$ and an acetate labeled highest in the carboxyl group. However, *C. acidiurici* carries out an efficient exchange reaction between $CO_2$ and the carboxyl group of glycine (44) that changes this pattern. In our experiments, the specific radioactivity of $CO_2$ was twice that of the carboxyl group of acetate and about half of that found in the methyl group. However, the reverse can be predicted for the glycine-serine-pyruvate pathway of acetate formation. Therefore, both *C. acidiurici* and *C. cylindrosporum* shift their main fermentation pathway toward a direct reduction of acetate by glycine reductase if selenium is available in sufficient amounts for these cells. Selenium is essential for growth of all known strains of these species (9, 16) on different purines or glycine in synthetic media (H. Schiefer-Ullrich, H. Lebertz, P. Dürre, R. Wagner, J. R. Andreesen, unpublished data). Our studies strengthened further the importance of glycine decarboxylase (glycine synthase) for these bacteria as found by Waber and Wood (44). However, the function of the active serine hydroxymethyltransferase and L-serine dehydratase as detected in both glycine- (16) and adenine-grown cells of *C. purinolyticum* can be regarded to be principally important for biosynthetic reactions.

The ratio of glycine to uric acid utilized by resting cells was six times higher in this study than that reported before for *C. acidiurici* and *C. cylindrosporum* (2). This correlates with the increasing amount of selenium present and the involvement of glycine reductase as the key enzyme of glycine metabolism. Since the latter system is more efficient in terms of energy (16), the higher ATP yield might enable the organisms to transport more glycine per mole of uric acid into the cells. According to previous labeling

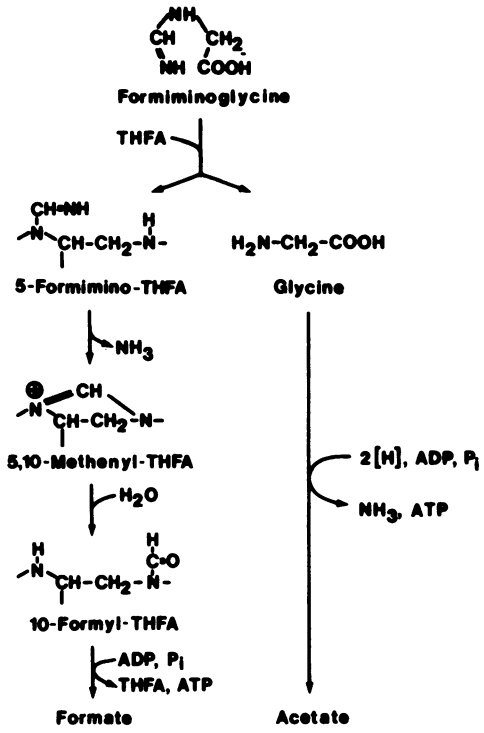


FIG. 3. Degradation of formiminoglycine as the last step of purine fermentation by *C. purinolyticum*. Abbreviations: THFA, tetrahydrofolic acid; 2[H], reducing equivalents.

data (21, 25), glycine reductase was only partly expressed in the cells. Since selenium is a component of all glycine reductases studied (16, 36, 39), its presence as contaminant in growth media can be presumed.

The requirement for selenium is more rigid for growth on glycine than on uric acid, as demonstrated for *C. purinolyticum* (14, 16). Therefore, the changes observed now in glycine metabolism can be explained by the different availability of selenium for the cells, which in turn affects the formation of an active glycine reductase.

According to our studies, the fermentation of purines is connected with two sites of energy conservation at the stage of formyltetrahydrofolate synthetase (27) and glycine reductase (Fig. 3). Thermodynamic data for hypoxanthine and uric acid fermentation indicate a free energy change of −166.5 kJ/mol (−39.8 kcal/mol) and −166.9 kJ/mol (−39.9 kcal/mol), respectively, according to the following equations (all data taken from reference 38).

$$1 \text{ hypoxanthine} + 9H_2O \rightarrow \text{acetate}^- + \text{formate}^- + 4NH_4^+ + 2HCO_3^- \quad (1)$$

$$1 \text{ uric acid} + 9H_2O \rightarrow 0.75 \text{ acetate}^- + 4NH_4^+ + 3.5HCO_3^- + 0.25H^+ \quad (2)$$

Since the formation of 1 mol of ATP from ADP and $P_i$ requires a free energy change of −41.8 to −50.2 kJ (−10 to −12 kcal) (38) these fermentations allow a net production of 2 mol of ATP per mol of purine, with a thermodynamic efficiency of 50 to 60%. Such values are characteristic for biological systems (38). Whether the three organisms gain additional ATP during xanthine hydrolysis to formiminoglycine at the level of 4-ureido-5-imidazolecarboxylate is not quite clear. This has been proposed as an energy conservation step (1). A carbamoyl phosphate-transferring enzyme, carbamate kinase, is present in *C. purinolyticum*. In addition, the formyltetrahydrofolate synthetase of *C. cylindrosporum* can catalyze a phosphoryl transfer from carbamoyl phosphate to ADP, yielding ATP (7). However, the formation of 3 mol of ATP per mol of purine would raise the thermodynamic efficiency to a level considered to be too high for a biological reaction.

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## ADDENDUM IN PROOF

*C. acidiurici* exhibited glycine reductase activity of 0.015 U/mg of protein by the standard test (36). However, it was quite essential to optimize further the glycine fermentation by whole cells and to incubate a dense cell population with glycine only before cell breakage (H. Lebertz and J. R. Andreesen, unpublished data).

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