JOURNAL OF BACTERIOLOGY, Nov. 1976, p. 616-622

Vol. 128, No. 2
Printed in U.S.A.

Arginine Catabolism by *Treponema denticola*

R. P. BLAKEMORE AND E. CANALE-PAROLA*

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01002

Received for publication 2 August 1976

Treponema denticola, an anaerobe commonly present in the human mouth, ferments various amino acids and glucose. Amino acid analyses indicated that substrate amounts of arginine were utilized by *T. denticola* growing in a complex, serum-containing medium. Cell suspensions metabolized L-arginine to citrulline, NH_3, CO_2, proline, and small amounts of ornithine. CO_2, NH_3, ornithine, and proline were produced from L-citrulline by cell suspensions. Determinations of radioactivity in products formed from L-[U-^{14}C]ornithine indicated that cell suspensions converted this amino acid to proline. Furthermore, proline was excreted by cells growing in a complex, arginine-containing medium. Arginine iminohydrolase (deiminase) and ornithine carbamoyltransferase activities were detected in *T. denticola* cell extracts. Carbamoylphosphate dissimilation by extracts yielded adenosine triphosphate. The data indicate that *T. denticola* derives energy by dissimilating L-arginine via the arginine iminohydrolase pathway. However, unlike some of the other bacteria that utilize this pathway, *T. denticola* converts to proline much of the ornithine derived from L-arginine.

Treponema denticola is a common inhabitant of the human gingival crevice region. This region, which comprises the narrow fissure between the marginal gingiva and the tooth enamel (2), is populated by an enormous number and wide variety of microorganisms, many of which have not been cultivated. Microscopic counts of material from the gingival crevice have shown that an average of over 10^{11} microorganisms are present per g (wet weight) of gingival debris (21).

We are studying the energy-yielding pathways and other metabolic processes of *T. denticola*, with the intent of elucidating the factors that enable this obligately anaerobic spirochete to thrive and persist in the extremely competitive natural environment in which it occurs. *T. denticola* grows in complex, serum-containing media which support yields of approximately 5 $\times$ 10^8 cells/ml. Previous work (12) has shown that *T. denticola* cells growing in these media not only ferment amino acids such as L-cysteine, L-serine, L-alanine, and glycine, but also glucose. Glucose is degraded via the Embden-Meyerhof pathway coupled to a clostridial-type clastic system for pyruvate metabolism.

The work described in this paper was prompted by our finding that the level of arginine in culture fluid decreased markedly during growth of *T. denticola*. Thus, it seemed possible that this amino acid served as an energy source for the spirochete. The present report deals with our studies on the pathway of L-arginine catabolism in *T. denticola*.

(A preliminary report of this work was presented at the 75th Annual Meeting of the American Society for Microbiology in New York, 27 April-2 May 1975.)

MATERIALS AND METHODS

Organism and cultural conditions. Strain 10 of *T. denticola* (12) was used in this investigation. *T. denticola* was cultured at 36°C in medium GM-1, which had the following composition (g/100 ml of distilled water): Trypticase (Baltimore Biological Laboratories), 1.5; yeast extract (Difco), 0.5; NaCl, 0.25; sodium thioglycolate, 0.05; and L-cysteine hydrochloride, 0.1. The pH was adjusted to 7.4 with KOH, and the medium was autoclaved. The medium was rapidly cooled (to ca. 35°C) in a cold-water bath and was immediately inoculated after adding the following supplements (ml/100 ml): 0.2% (wt/vol) thiamine pyrophosphate, 0.3; volatile fatty acids solution, 0.5; 10% (wt/vol) sodium bicarbonate, 0.5; and heat-inactivated normal rabbit serum (Flow Laboratories), 0.3. These supplements were filter-sterilized, except for the bicarbonate solution, which was sterilized by autoclaving. The volatile fatty acids solution consisted of 0.5 ml each of isobutyric, DL-2-methylbutyric, isovaleric, and valeric acids dissolved in 100 ml of 0.1 N KOH. The final pH of medium GM-1 was 7.2 to 7.3. Amino acid analysis of a batch of medium GM-1 indicated that it contained (micromoles per milliliter, total concentration): arginine, 2.7; ornithine, 0.7; proline, 11.2.

Treponemes were mass cultured in cotton-stoppered Florence flasks (1 to 3 liters) filled to the neck

with medium. Inocula consisted of 5 to 10 ml (3×10^8 to 5×10^8 cells/ml) per liter of medium.

Cell suspensions. Cells were harvested from cultures (cell density, 2×10^8 cells/ml) by centrifugation at 5,000 × *g* for 20 min at 5°C. Cells (ca. 4.5 g, wet weight) harvested from 3 liters of culture were gently suspended in 100 ml of 0.06 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer solution (pH 7.0 or 7.4) or in potassium phosphate buffer solution (pH 7.0 or 7.4), each buffer solution containing 0.21% (wt/vol) NaCl and 4×10^{-3} M dithiothreitol (DTT). The suspension was centrifuged, and the cells were washed again by suspending them in 100 ml of fresh buffer and sedimenting them by centrifugation. Finally, the washed cells were suspended in 5 to 10 ml of fresh buffer, stored at 5°C under argon, and were used within 1 h. All buffers, immediately before use, were boiled and then cooled to approximately 5°C while being sparged with argon. Treponemes washed in this manner were motile and uniformly coiled. Cell densities were monitored using a Klett-Summerson photoelectric colorimeter. The protein content of cell suspensions was determined by the method of Lowry et al. (16).

Cell extracts. Treponemes were disrupted at 5°C by extruding washed cell suspensions through a French pressure cell at 10,000 lb/in² (7×10^6 kg/m²). Cellular debris was removed by centrifugation at 4°C for 15 min at 27,000 × *g*. The supernatant liquid was used in experiments. Protein content of the extracts was determined by the method of Warburg and Christian (25).

Amino acid analyses. Free amino acids as well as total (free and bound, e.g., in peptides) amino acids present in samples were determined chromatographically using automatic analyzers, according to conventional methods (22). Samples for automatic analyses or for analyses on cation-exchange columns (see below) were deproteinized prior to determination of free amino acids. Deproteinization of samples low in peptides and proteins (e.g., supernatant fluids from reaction mixtures in which washed cells were used) was accomplished by the method of Gerritsen et al. (7). Supernatant fluid from cultures was deproteinized by adding 0.05 ml of 70% perchloric acid to each 2-ml sample, allowing them to stand at room temperature for several minutes, and adjusting the pH to 7.6 with 70% (wt/vol) KOH. After centrifugation, samples deproteinized by either method were diluted at least 1:10 in 0.2 M lithium citrate buffer (pH 2.2) before application to columns. For determinations of total amino acids in samples containing protein and peptides, the samples were hydrolyzed prior to chromatographic analysis. Culture medium samples were hydrolyzed in 6 N HCl by refluxing in a constant stream of argon for 24 h. All other samples were hydrolyzed in 6 N HCl for 24 h or longer at 100°C in argon-filled, sealed glass ampoules. In determinations of the amino acid composition of *T. denticola* cells, corrections were made for hydrolytic destruction of amino acids, as well as for resistance to hydrolysis, by analyzing samples hydrolyzed for 24, 48, and 72 h. Free amino acids were determined using a Technicon autoanalyzer modified for discrete two-buffer methodology (22), employing spherical resins (Hamilton HP-AN). Acid-hydrolyzed samples were analyzed using a Beckman Spinco model 120C automatic amino acid analyzer, according to standard procedures for acid hydrolysates (22).

Amino acids in various samples were separated and identified by thin-layer chromatography. Silica Gel G chromatography plates (20 by 20 cm) were used, with a phenol:water (3:1, vol/vol) solvent system. After drying, the chromatograms were developed by spraying with ninhydrin reagent and heating (8).

In experiments with radiolabeled ornithine, amino acids were separated on columns (0.8 by 40 cm) of Dowex 50-X8, 100- to 200-mesh resin (Na^+ form). The amino acids were eluted from the columns at room temperature using a sodium citrate buffer gradient increasing in Na^+ concentration (0.2 to 0.8 N) and in pH (2.9 to 5). The gradient was established with a Technicon Autograd gradient maker. One-milliliter volumes of deproteinized sample were applied to the columns, and 5-ml fractions were collected. The flow rate through the columns was maintained at 36 ml/h using a peristaltic pump. This system effectively separated individual amino acids present in a solution containing 0.2 μmol each of L-proline, L-citrulline, L-ornithine, and L-arginine.

In addition to chromatographic methods, colorimetric assays were used to determine amino acids in reaction mixtures of experiments with cell suspensions and cell extracts. Arginine was assayed by means of van Pilsum's modification of the Sakaguchi reaction (24). Citrulline was determined by the method of Hunninghake and Grisolia (13). Ornithine and proline were separated either by thin-layer or column chromatography prior to colorimetric assay (3, 18).

Amino acid metabolism by cell suspensions. Reaction mixtures (total volume, 3.2 ml) were incubated in double-side-arm Warburg vessels for 3.5 h, at 36°C, in argon.

To determine products of L-arginine metabolism, the following reaction mixture was used (micromoles): potassium phosphate buffer (pH 7.5), 120; L-arginine, 40; DTT, 8; H_2SO_4 (side arm), 600; 30% (wt/vol) aqueous KOH (center well, where appropriate), 0.3 ml; and 6.5×10^{10} washed cells. An identical reaction mixture was used to study L-citrulline metabolism, except that L-citrulline (40 μmol) was used instead of L-arginine.

Products of L-ornithine metabolism were investigated by using the following reaction mixture (micromoles): L-[U-^{14}C]ornithine, 21.2 (specific activity, 26,000 cpm/μmol); potassium phosphate buffer (pH 7.4), 120; DTT, 8; H_2SO_4 (side arm, where appropriate), 600; 1 M hydroxide of Hyamine 10X (center well, where appropriate), 0.2 ml; and 1.4×10^{11} cells.

Enzymatic activities in cell extracts. Activity resulting in the formation of citrulline from L-arginine was assayed by a method similar to that used by Schimke et al. (19) to assay for arginine iminohydrolase (arginine deiminase, EC 3.5.3.6). The reaction mixture contained (micromoles): potassium phos-

phate buffer (pH 7.5), 300; DTT, 1.5; L-arginine, 40; cell extract (3.8 mg of protein); in a total volume of 2 ml. Incubation was for 80 min at 36°C in an air atmosphere.

Ornithine carbamoyltransferase (EC 2.1.3.3) activity was assayed in the direction of citrulline degradation by the technique of Slade et al. (20) in a reaction mixture consisting of (micromoles): HEPES buffer (pH 7.5), 130; DTT, 1.5; potassium phosphate buffer (pH 7.5), 20; adenosine diphosphate, 5; $MgCl_2$, 10; L-citrulline, 40; 30% (wt/vol) KOH (in center well, where appropriate), 0.3 ml; cell extract (7 mg of protein); and water to a final volume of 3.2 ml. The reaction mixture was incubated in Warburg vessels for 60 min at 36°C in an argon atmosphere.

We also assayed ornithine carbamoyltransferase activity in the direction of citrulline synthesis, using the method of Nakamura and Jones (17). Components of the reaction mixture were (micromoles): tris(hydroxymethyl)aminomethane - hydrochloride (Tris) buffer (pH 8.5), 500; DTT, 5; carbamoylphosphate (lithium salt), 50; L-ornithine, 50; and cell extract (3 mg of protein); in a total volume of 1.7 ml. Incubation was for 3 min at 36°C in an air atmosphere.

Activity resulting in adenosine triphosphate (ATP) generation in the presence of carbamoylphosphate was assayed as described by Schimke et al. (19), using a Gilford continuous recording spectrophotometer equipped with a temperature-controlled cell compartment. The reaction was coupled to the formation of glucose-6-PO_4 in the presence of glucose and hexokinase. Glucose-6-PO_4 was detected by assaying for pyridine nucleotide reduction, which occurred in the presence of added glucose-6-PO_4 dehydrogenase. The reaction mixture consisted of: hexokinase (Sigma), 40 U; glucose, 10 μmol; glucose-6-PO_4 dehydrogenase (Sigma, type V), 5 U; nicotinamide adenine dinucleotide phosphate, 1 μmol; carbamoylphosphate (lithium salt), 10 μmol; adenosine diphosphate, 5 μmol; Tris, 10 μmol; $MgCl_2$, 10 μmol; cell extract (0.03 mg of protein); in a final volume of 1 ml. The reaction was followed by recording change in optical density at 340 nm and 37°C.

The extracts were assayed for their ability to catalyze citrulline formation in the presence of NH_4^+, HCO_3^-, ATP, and L-ornithine (14). The reaction mixture consisted of (micromoles): Tris (pH 8.5), 50; NH_4Cl, 100; $NaHCO_3$, 100; $MgCl_2$, 5; L-ornithine, 20; DTT, 2; ATP, 10; cell extract (11 mg of protein); in a final volume of 1.5 ml. Incubation was for 5 min at 25°C in an air atmosphere. Citrulline formed was assayed colorimetrically (13).

Other experimental procedures. Ammonia was determined under conditions suitable for use in the presence of labile ammonia-forming material (1, 11).

Amines were extracted from samples in the manner of Goldschmidt et al. (8) and were co-chromatographed with amine standards on Silica Gel G thin-layer chromatographic plates using a phenol:acetic acid:water (6:1:6, vol/vol/vol) solvent system. Dowex 50-X8 resin columns (0.6 by 15 cm) were used to separate amino acids from amines. These columns (Na^+ form) were eluted batchwise with sodium citrate buffer (0.3 N Na^+, pH 5) to remove amino acids. Subsequently, amines such as putrescine were eluted with 0.1 N NaOH.

Urease activity was assayed by manometric measurements of gas evolved from urea (40 μmol) by whole cells suspended in a reaction mixture similar to that used to determine products formed by cell suspensions from L-arginine, except that the latter amino acid was omitted. Urease activity was also measured by determining pH changes in reaction mixtures including urea and whole cells.

Urea production by cell suspensions metabolizing arginine was determined using reaction mixtures incubated in Warburg vessels, as described above. The reaction mixtures were similar to those for the determination of products of arginine metabolism (see above). After incubation, samples of the supernatant fluid were applied to thin layers of cellulose MN 300 (Brinkmann Instruments, Inc., Westbury, N.Y.). Separation was achieved by electrophoresis (2 h, 19 V/cm) in glacial acetic acid:water (2:98, vol/vol) using a Desaga (Heidelberg) chamber, model 121210. Ninhydrin and Ehrlich reagents (10) were used for color development.

Radioactivity measurements were performed using a Packard Tri-Carb liquid scintillation spectrometer, according to conventional methodology (9).

Conventional manometric techniques were used (23).

All chemicals used were of the highest purity obtainable commercially.

RESULTS

Amino acid analyses showed that *T. denticola* cells growing in medium GM-1 dissimilated significant amounts of arginine (Table 1). The metabolic activities of the growing cells brought about a marked decrease in the levels of free arginine present in the medium and of arginine bound in medium components (e.g., peptides). Furthermore, substantial amounts of ornithine and proline accumulated in the culture's supernatant fluid (Table 1).

Washed cell suspensions incubated with L-arginine formed NH_3, CO_2, proline, citrulline, and ornithine (Table 2). The ratios of NH_3 and CO_2 produced to arginine utilized suggested that two deamination steps and one decarboxylation step occurred during arginine catabolism. Urea was not produced from arginine under the conditions used (Table 2).

Experiments were conducted to determine whether the three amino acids produced by cell suspensions from L-arginine were metabolized by *T. denticola*. Neither cell suspensions nor cell extracts dissimilated L-proline under a variety of conditions tested, such as in the presence of potential energy sources, electron donors, or acceptors, at different pH values, and using cells harvested at different stages of growth. The same amount of proline added to

TABLE 1. *Arginine utilization by growing cells of T. denticola*

Arginine	μmol/ml[a] after incubation for:			μmol/ml of culture[b] that were:		
	0 h	44 h	172 h	Utilized	In cell material[c]	Dissimilated
Free	1.99	1.62	0.55	–[d]	–	–
Bound	0.71	0.40	0.23	–	–	–
Total[e]	2.70	2.02	0.78	1.92	0.02	1.90

[a] Present in culture supernatant fluid. Cells growing in medium GM-1.

[b] After 172 h of incubation. No cell lysis detected during this period of incubation. Cell yield after 172 h: 4×10^8 cells/ml.

[c] Determined from the arginine content of cell hydrolysates.

[d] –, Not applicable.

[e] Includes bound (e.g., in peptides) and free amino acids. The following amino acids increased by the amounts indicated (micromoles per milliliter, total concentration; 172-h incubation) in the culture supernatant fluid: ornithine, 0.96; proline, 1.27.

cell suspensions was recovered at the end of incubation, and gaseous or nongaseous products (e.g., CO_2, NH_3, amino acids, delta-aminovalerate, keto acids, lactate) were not detected after incubation with cell extracts or whole cells.

L-Citrulline was utilized by *T. denticola* cell suspensions (Table 3) at a faster rate than L-arginine. Roughly 1 μmol each of CO_2 and NH_3 was produced per μmol of L-citrulline metabolized. Comparison of these CO_2 and NH_3 yields with those resulting from L-arginine (Table 2) indicated that a single deamination of arginine preceded citrulline formation. When cells were supplied with either arginine (Table 2) or citrulline (Table 3), C and N recoveries in products were incomplete. Apparently a product(s) had not been recovered. The analyses revealed that no amino acid other than those listed in Tables 2 and 3 was formed in significant amounts from arginine or citrulline. Thus, the unaccounted for product(s) was not an amino acid.

Incubation of *T. denticola* cell suspensions with L-ornithine yielded proline as the only amino acid produced in significant amounts. Furthermore, CO_2 was formed from L-ornithine. To determine whether proline was, in fact, derived from L-ornithine, washed cell suspensions of *T. denticola* were incubated with uniformly labeled L-[^{14}C]ornithine, and the amount of label in products formed and in residual L-ornithine was determined. The greatest portion of the label in amino acids recovered by cation-exchange column chromatography was in the unused L-ornithine and in the proline that was formed (Fig. 1, Table 4). The specific activity of proline recovered was approximately the same as that of L-ornithine (Table 4). The results showed that *T. denticola* cells utilized the carbon skeleton of L-ornithine to produce proline. Approximately 46% of the radioactive label from the ornithine metabolized by the cells remained associated with the column after elution of all amino acids (Table 4). This radioactive material was eluted at alkaline pH and high sodium ion concentration, using 0.1 N NaOH. The eluted material was ninhydrin positive and, because of its behavior when chromatographed on thin layers and on ion-exchange columns, was believed to be putrescine. A material with similar chromatographic behavior was produced by growing cells. Formation of CO_2 from L-ornithine (Table 4) is consistent with the observation that putrescine, the decarboxylation product of ornithine, was produced by the cells. The low C and N recoveries in analyses of products from L-arginine (Table 2) and L-citrulline (Table 3) may be explained by formation of putrescine from these amino acids.

TABLE 2. *Products of L-arginine metabolism by T. denticola cell suspensions*[a]

Compound	μmol recovered/100 μmol of L-arginine utilized
NH_3	177.5
CO_2	92.3
Proline	69.2
Citrulline	13.7
Ornithine	4.9

[a] All values corrected for endogenous activity. Under the experimental conditions used (see Materials and Methods), the cells utilized 18.2 μmol of L-arginine. C recovery, 90.8%; N recovery, 74.4%. The cells did not have urea-hydrolyzing ability.

TABLE 3. *Products of L-citrulline metabolism by T. denticola cell suspensions*

Compound	μmol recovered/100 μmol of L-citrulline utilized[a]
NH_3	105.7
CO_2	125.8
Proline	65.4
Ornithine	3.0

[a] All values corrected for endogenous activity. Under the experimental conditions used (see Materials and Methods), the cells utilized 29.8 μmol of L-citrulline. Arginine was not formed from citrulline in detectable amounts. C recovery, 78%; N recovery, 59%.

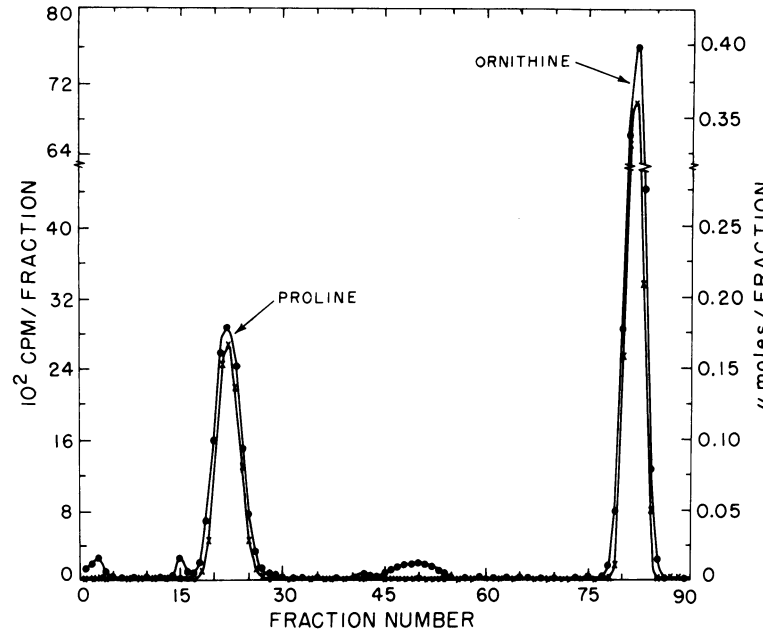

FIG. 1. *Column chromatography (Dowex 50-X8) elution profile of supernatant fluid from a reaction mixture in which T. denticola cell suspensions were incubated with* L-[U-^{14}C]*ornithine. Corrected for endogenous activity. Five-milliliter fractions were collected. See Materials and Methods. Symbols: counts per minute (cpm),* ●; *micromoles,* ×.

TABLE 4. *Metabolism of* L-[U-^{14}C]*ornithine by T. denticola cell suspensions*[a]

Compound	Total cpm	Total μmol	Sp act (cpm/μmol)[b]
Ornithine utilized	381,300	14.4	26,480
Proline formed	77,370	2.9	26,680
CO_2 formed	72,530	10.2	7,110
Minor products eluted	16,080		
Residue on column (presumably putrescine)	175,400		

[a] Values corrected for endogenous activity.

[b] Calculated using the values in the table. Specific activity of L-[U-^{14}C]ornithine added to the reaction mixture was determined to be 26,000 cpm/μmol.

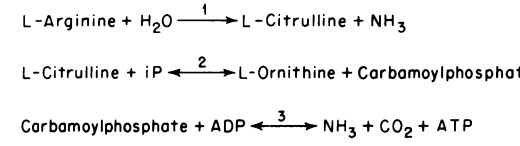

FIG. 2. *Arginine iminohydrolase pathway. Reaction 1 is catalyzed by arginine iminohydrolase (deiminase), reaction 2 by ornithine carbamoyltransferase, and reaction 3 by carbamate kinase.*

The results of experiments with cell suspensions, as well as the observation that arginine disappeared whereas ornithine accumulated in *T. denticola* culture fluids, were in agreement with the possibility that this spirochete dissimilated arginine via an arginine iminohydrolase pathway similar to that demonstrated in other bacteria (Fig. 2). To test this possibility, we assayed *T. denticola* cell extracts for enzymatic activities associated with this pathway.

Cell extracts converted L-arginine to citrulline (Table 5). The molar amounts of L-arginine utilized by the extracts were greater than the amounts of citrulline detected, suggesting that a portion of the citrulline produced from arginine was degraded to other products. These results, and those obtained with cell suspensions (Table 2), indicated that arginine iminohydrolase activity was present in *T. denticola* cells.

Ornithine carbamoyltransferase activity was detected in *T. denticola* cell extracts (Table 6). Using L-citrulline as the substrate (Table 6, reaction A), the activity was measured as CO_2 evolution resulting from the coupling of carbamoylphosphate-degrading activity in the extract to the carbamoyltransferase activity. CO_2 evolution was dependent upon adenosine diphosphate and inorganic phosphate. Addition of L-ornithine to the reaction mixture strikingly

TABLE 5. *Formation of citrulline from L-arginine by cell extracts*

Assay conditions[a]	Assay A (μmoles of L-arginine utilized)	Assay B (μmoles of citrulline detected)
Complete	23.4	7.1
Boiled cell extract	0.0	0.0
L-Arginine omitted		0.0
Added 1.9 mg of extract protein	10.2	4.6
Added 7.6 mg of extract protein		16.0

[a] See Materials and Methods.

TABLE 6. *Ornithine carbamoyltransferase activity in T. denticola cell extracts*

Assay conditions[a]	Reaction A (μmol of CO_2 formed)[b]	Reaction B (μmol of citrulline detected)
Complete	4.0	3.0
Ornithine omitted		0.2
Phosphate omitted	3.0	
ADP omitted	2.9	
Phosphate, ADP, $MgCl_2$ omitted	1.1	

[a] See Materials and Methods. ADP, Adenosine diphosphate.

[b] Values corrected for endogenous activity.

inhibited CO_2 evolution, whereas added proline had no effect. Furthermore, extracts formed citrulline from L-ornithine and carbamoylphosphate (Table 6, reaction B).

Activity resulting in ATP generation in the presence of carbamoylphosphate was detected in cell extracts of *T. denticola*. This activity was assayed using a glucose-hexokinase trapping system (see Materials and Methods). The specific activity of the carbamoylphosphate-degrading system corresponded to 343 nmol of pyridine nucleotide reduced per min/mg of extract protein. *T. denticola* cell extracts catalyzed synthesis of citrulline (37 nmol/min per mg of extract protein) in the presence of ammonium ions, bicarbonate, ATP, and L-ornithine, an indication of the reversibility of the enzymatic system.

DISCUSSION

The data indicate that *T. denticola* converts L-arginine to citrulline and ammonia through a reaction catalyzed by arginine iminohydrolase (deiminase). The citrulline formed is metabolized via ornithine carbamoyltransferase activity, a reaction that yields ornithine and carbamoylphosphate. We did not detect urea formation by cell suspensions metabolizing arginine to citrulline and to other products (Table 2). Furthermore, urease activity was not found in *T. denticola* cell suspensions. This indicates that, under the experimental conditions used, the cells lacked arginase (EC 3.5.3.1) activity.

T. denticola cell extracts incubated with carbamoylphosphate yielded ATP. It is likely that this reaction was catalyzed by carbamate kinase, rather than by carbamoyl-phosphate synthase (EC 2.7.2.5) activity. The latter enzyme is known to function in a biosynthetic direction toward arginine synthesis (4) and was reported to be subject to end product repression in *Escherichia coli* (15).

The evidence presented in this paper indicates that *T. denticola* derives carbamoylphosphate from L-arginine through the activity of arginine iminohydrolase and ornithine carbamoyltransferase. The carbamoylphosphate formed by these processes is degraded in an ATP-yielding reaction. Thus, *T. denticola* generates ATP by catabolizing L-arginine via an arginine iminohydrolase pathway similar to that demonstrated in other bacteria (Fig. 2; 6, and references therein). However, results of experiments with growing cells (Table 1) and with cell suspensions (Tables 2–4) indicate that *T. denticola*, unlike other bacteria utilizing the arginine iminohydrolase pathway, converts much of the ornithine formed from arginine to proline, CO_2, and to an amine presumed to be putrescine. Experiments in which [^{14}C]ornithine was converted to proline by cell suspensions showed that the specific activity of the proline recovered was essentially identical to that of the ornithine utilized. Thus, it appears that the ornithine carbon skeleton is used for the synthesis of proline, and that there is no condensation with carbon from other compounds. A pathway similar to that operating in certain clostridia for the conversion of ornithine to proline (5) may be present in *T. denticola*.

The extent to which L-arginine dissimilation contributes ATP for growth processes of *T. denticola* may be controlled by complex regulatory mechanisms, which are probably affected by the availability of other substrates and by other environmental conditions. Nevertheless, the ability of *T. denticola* to use arginine as an energy source undoubtedly adds to the metabolic versatility of this spirochete and is one of the factors that enable it to compete successfully with other microorganisms in its natural habitat.

ACKNOWLEDGMENTS

We thank Peter L. Pellett for invaluable advice on amino acid analyses and for the use of his equipment.

This research was supported by Public Health Service grants AI-12482 and AI-08248 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Archibald, R. M.** 1943. Quantitative microdetermination of ammonia in the presence of glutamine and other labile substances. J. Biol. Chem. 151:141-148.
2. **Burnett, G. W., and H. W. Scherp.** 1968. Oral microbiology and infectious disease, 3rd ed. The Williams & Wilkins Co., Baltimore.
3. **Chinard, F. P.** 1952. Photometric estimation of proline and ornithine. J. Biol. Chem. 199:91-95.
4. **Cohen, P. P.** 1962. Carbamyl group synthesis, p. 477-494. *In* P. D. Boyer, H. Lardy, and K. Myrbäck (ed.), The enzymes, 2nd ed., vol. 6. Academic Press Inc., New York.
5. **Costilow, R. N., and L. Laycock.** 1969. Reactions involved in the conversion of ornithine to proline in clostridia. J. Bacteriol. 100:662-667.
6. **Doelle, H. W.** 1975. Bacterial metabolism, 2nd ed., p. 465, 649. Academic Press Inc., New York.
7. **Gerritsen, T., M. L. Rehberg, and H. A. Waisman.** 1965. On the determination of free amino acids in serum. Anal. Biochem. 11:460-466.
8. **Goldschmidt, M. C., B. M. Lockhart, and K. Perry.** 1971. Rapid methods for determining decarboxylase activity: ornithine and lysine decarboxylases. Appl. Microbiol. 22:344-349.
9. **Hash, J. H.** 1972. Liquid scintillation counting in microbiology, p. 109-155. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 6B. Academic Press Inc., New York.
10. **Heathcote, J. G., D. M. Davies, and C. Haworth.** 1971. An improved technique for the analysis of amino acids and related compounds on thin layers of cellulose. V. The quantitative determination of urea in urine. J. Chromatogr. 60:103-109.
11. **Herbert, D., P. J. Phipps, and R. E. Strange.** 1971. Chemical analysis of microbial cells, p. 209-344. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 5B. Academic Press Inc., New York.
12. **Hespell, R. B., and E. Canale-Parola.** 1971. Amino acid and glucose fermentation by *Treponema denticola*. Arch. Mikrobiol. 78:234-251.
13. **Hunninghake, D., and S. Grisolia.** 1966. A sensitive and convenient micromethod for estimation of urea, citrulline, and carbamyl derivatives. Anal. Biochem. 16:200-205.
14. **Jones, M. E.** 1962. Carbamyl phosphate synthesis and utilization, p. 903-925. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 5. Academic Press Inc., New York.
15. **Kalman, S. M., P. H. Duffield, and T. Brzozowski.** 1966. Purification and properties of a bacterial carbamyl phosphate synthetase. J. Biol. Chem. 241:1871-1877.
16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
17. **Nakamura, M., and M. E. Jones.** 1970. Ornithine carbamyltransferase (*Streptococcus faecalis*), p. 286-294. *In* H. Tabor and C. W. Tabor (ed.), Methods in enzymology, vol. 17A. Academic Press Inc., New York.
18. **Ratner, S.** 1962. Transamidinase, p. 843-848. *In* S. P. Colowick and H. O. Kaplan (ed.), Methods in enzymology, vol. 5. Academic Press Inc., New York.
19. **Schimke, R. T., C. M. Berlin, E. W. Sweeney, and W. R. Carroll.** 1966. The generation of energy by the arginine dihydrolase pathway in *Mycoplasma hominis* 07. J. Biol. Chem. 241:2228-2236.
20. **Slade, H. D., C. C. Doughty, and W. C. Slamp.** 1954. The synthesis of high-energy phosphate in the citrulline ureidase reaction by soluble enzymes of *Pseudomonas*. Arch. Biochem. Biophys. 48:338-346.
21. **Socransky, S. S.** 1970. Relationship of bacteria to the etiology of periodontal disease. J. Dent. Res. 49:203-222.
22. **Spackman, D. H., W. H. Stein, and S. Moore.** 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30:1190-1206.
23. **Umbreit, W. W., R. H. Burris, and J. F. Stauffer.** 1964. Manometric techniques, 4th ed. Burgess Publishing Co., Minneapolis.
24. **Van Pilsum, J. F., R. P. Martin, E. Kito, and J. Hess.** 1956. Determination of creatine, creatinine, arginine, guanidinoacetic acid, guanidine, and methyl guanidine in biological fluids. J. Biol. Chem. 222:225-236.
25. **Warburg, O., and W. Christian.** 1941. Isolierung und Kristallisation des Gärungsferments Enolase. Biochem. Z. 310:384-421.