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Properties of Oxaloacetate Decarboxylase from *Veillonella parvula*

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Oxaloacetate decarboxylase was purified to 136-fold from the oral anaerobe *Veillonella parvula*. The purified enzyme was substantially free of contaminating enzymes or proteins. Maximum activity of the enzyme was exhibited at pH 7.0 for both carboxylation and decarboxylation. At this pH, the K_m values for oxaloacetate and Mg^{2+} were at 0.06 and 0.17 mM, respectively, whereas the K_m values for pyruvate, CO_2, and Mg^{2+} were at 3.3, 1.74, and 1.85 mM, respectively. Hyperbolic kinetics were observed with all of the aforementioned compounds. The Keq' was 2.13×10^{-3} mM^{-1} favoring the decarboxylation of oxaloacetate. In the carboxylation step, avidin, acetyl coenzyme A, biotin, and coenzyme A were not required. ADP and NADH had no effect on either the carboxylation or decarboxylation step, but ATP inhibited the carboxylation step competitively and the decarboxylation step noncompetitively. These types of inhibition fitted well with the overall lactate metabolism of the non-carbohydrate-fermenting anaerobe.

In 1951, Johns showed that propionate formation by *Veillonella gazogenes* (now *V. alcalescens*) was proportional to the amount of CO_2 available (12). From this he proposed a scheme for propionate synthesis in *Veillonella* species, which included oxaloacetate (OAA) formation from pyruvate via CO_2 fixation, similar to that outlined earlier by Kalnitsy and Werkman (13) and Delwiche (7) for propionic acid bacteria. We have previously shown that the human oral isolates of *Veillonella parvula* do not require exogenous carbon dioxide for lactate metabolism (19). Despite this apparent contradiction, it was observed that cell-free extracts of *V. parvula* could readily convert pyruvate to OAA via CO_2 fixation. Because CO_2 fixation could proceed in the presence or absence of ATP, it was proposed that *V. parvula* possessed two pyruvate carboxylases (21). The enzyme not requiring ATP for the fixation of CO_2 was called ATP-independent pyruvate carboxylase. de Vries et al., working with *V. alcalescens*, had also observed similar activity (8). The data obtained with *V. alcalescens* were comparable to those obtained with *V. parvula* because the neotype strain of *V. alcalescens* (ATCC 17745) has a high degree of DNA-DNA homology with the neotype strain of *V. parvula* (ATCC 10790), and thus *V. alcalescens* was considered to be a synonym of *V. parvula* (18). However, upon further investigation, it was determined that the ATP-independent enzyme in *V. parvula* was actually OAA decarboxylase (OAADC). In this paper, data will be presented to substantiate this fact.

OAADC (EC 4.1.1.3) was first reported in *Micrococcus lysodeikticus* by Krampitz and Werkman (14). Since then, similar enzymes have been reported in other bacterial species (3, 9, 10, 13, 22, 23, 25). In general, the enzyme specifically catalyzes the decarboxylation of OAA to pyruvate in the presence of Mn^{2+} (10) or Mg^{2+} (22) or both (22). The cellular regulation of the enzyme varied depending on the microorganism from which the enzyme was isolated. Reports indicated that compounds such as acetyl coenzyme A inhibited the OAADC isolated from *Pseudomonas ovalis* (10) and *P. citronellolis* (22), whereas malate inhibited enzymes from *Acetobacter xylinum* (4) and *Azotobacter vinelandii* (23). The enzyme isolated from *Acetobacter aceti* was not regulated by any of the above metabolites; however, its specific activity was affected by the type of substrate the bacteria were metabolizing (25).

The purpose of this paper is to report findings demonstrating the enzyme OAADC in *V. parvula*. Upon purification from the anaerobe, the enzyme was observed to be regulated by some metabolic intermediates.

MATERIALS AND METHODS

Bacteriological and cell extract preparation. Cells of *V. parvula* were grown in Rogosa's 1% lactate broth (24) and harvested by continuous centrifugation; crude extracts were prepared through sonication as previously described (19). After sonication the enzyme was

treated with 0.2% (wt/vol) protamine sulfate. At this stage the OAADC in the crude extracts was observed to be unstable even in the presence of 20 mM β-mercaptoethanol and under an N_2 atmosphere. However, upon treatment of the extracts with 0.2% (wt/vol) Norit A-activated charcoal for 30 min at 4°C and subsequently dialyzed overnight against 50 mM phosphate buffer (pH 7.0) containing 20 mM β-mercaptoethanol, the OAADC activity was stable for at least 2 months at 4°C under a nitrogen atmosphere. As 20 mM β-mercaptoethanol was required to stabilize the activity of OAADC, all buffers employed in subsequent purification procedures contained this reducing agent.

Purification of OAADC. (i) Ammonium sulfate precipitation. Preliminary data had established that 95% of the OAADC activity in the crude extracts could be found in the fraction precipitating between 30 and 50% saturation with ammonium sulfate. The pellet thus obtained was dissolved in a minimal volume of phosphate buffer (50 mM, pH 7.0) and was stably stored at 4°C in screw-capped tubes under nitrogen atmosphere until further use.

(ii) Column chromatograph. Further purification of the enzyme was achieved via Sephadex G-100 gel filtration and carboxymethyl (CM)-cellulose ion-exchange column chromatography. In the gel filtration procedures, the OAADC enzyme was eluted from a 2.5- by 90-cm Sephadex column with 50 mM phosphate buffer (pH 7.0). The fractions containing OAADC were pooled and concentrated by the Diaflo ultrafiltration cell (Amicon Ultrafiltration, Lexington, Ky.) with a UM-10 filter. Upon concentration, the partially purified enzyme solution was immediately applied to a 2.5- by 40-cm CM-cellulose column and initially eluted off the column with a 0 to 0.3 M NaCl gradient in 50 mM phosphate buffer (pH 7.0). The fractions containing the OAADC activity were concentrated with a Diaflo ultrafiltration cell and further purified by elution through a second CM-cellulose column. This column was eluted with a less steep gradient of 0 to 0.15 M NaCl in 50 mM phosphate buffer (pH 7.0). The fractions containing the enzyme were again pooled and concentrated and stored at 4°C under nitrogen atmosphere. The enzyme preparation was used for kinetic studies.

Enzyme assay. The CO_2-exchange method of Benziman and Heller (3) was not applicable to the *V. parvula* system because members of *Veillonella* spp. have been shown to possess an indigenous CO_2-pyruvate exchange reaction (20, 31) that was shown to be distinctly different from the OAADC-catalyzed reaction in that biotin was required as a cofactor (8). For this reason other methods had been employed to assay the activities of OAADC purified from *V. parvula*. In general, the OAADC activity in *V. parvula* was assayed by two methods, namely, the spectrophotometric and the radioactive methods.

In the spectrophotometric method (method A) the reaction mixture contained the following: 10 mM substrate, 0.1 mM NADH, 5 mM $MgSO_4$, 50 mM phosphate buffer (pH 7.0), 0.2 μg of substrate dehydrogenase, and 0.2 μg of extracts or enzyme in a volume of 1 ml. The substrate employed was dependent upon the direction in which the OAADC enzyme was assayed, that is, in the decarboxylation or carboxylation direction. In the decarboxylation assays, the substrate was OAA, and lactate dehydrogenase was used to convert the pyruvate formed to lactate. The resultant oxidation of NADH at 37°C was monitored continuously at 340 nm with a Pye-Unicam SP 800 recording spectrophotometer.

The decarboxylation assay was also employed in a noncontinuous fashion to monitor the OAADC activities in fractions collected via column chromatography. In this noncontinuous assay 200 μl of each collected fraction was employed. The substrate concentration in the noncontinuous assay was set at 3 mM. After 10 min of incubation at 37°C the reaction in each tube was terminated by the addition of 1 ml of 0.5 N NaOH in 50% ethanol. The content was mixed thoroughly and centrifuged at 7,000 rpm for 5 min in a model 59 Fisher centrifuge. The oxidation of NADH, monitored at 340 nm, was employed as an indication of the presence of OAADC enzyme. However, since extracts of *V. parvula* do contain substantial activity of malate dehydrogenase, control assay tubes that contained only OAA and NADH were incorporated to ensure that the NADH-oxidizing fractions were indeed OAADC.

In the carboxylation assay, the substrates were pyruvate and sodium bicarbonate at 10 mM each, and malate dehydrogenase was used to convert the OAA formed to malate. The activities of the OAADC and malate dehydrogenase enzymes were again monitored in the same manner as that of the decarboxylation assay. When crude extracts, protamine sulfate, or ammonium sulfate-treated preparations were used in the assay 10 mM sodium arsenite was added to inhibit the endogenous pyruvate dehydrogenase activity (19).

The radioactive method (method B) was similar to method A, except that either [3-^{14}C]pyruvate or [^{14}C]bicarbonate was employed as a tracer compound. The reaction was incubated at 37°C and was terminated by the addition of 0.2 ml of 2% 2,4-dinitrophenylhydrazine in 17.6% H_2SO_4 to each assay tube. The contents were mixed, covered with Parafilm "M" (American Can Co., Greenwich, Conn.) and allowed to stand at 4°C for 1 h to permit complete precipitation of the α-ketoacid hydrazones. After centrifugation of the tubes at 15,000 × *g* for 15 min at 4°C, the resultant supernatants were discarded, and the hydrazone precipitates were washed twice with 0.5 ml of 2% 2,4-dinitrophenylhydrazine. The washed precipitates were dissolved in 0.2 ml of ethyl acetate, and portions were analyzed by paper chromatography (20). The radioactive spots on the chromatogram were cut out, and radioactivity was determined by liquid scintillation counting by the method of Wang and Jones (30). Since radioactive OAA was not commercially available, this method was employed to measure the ability of OAADC to catalyze the carboxylation of radioactive pyruvate to OAA. In situations where crude extracts or protamine sulfate- or ammonium sulfate-treated preparations were used, 10 mM sodium malonate was added to the assay mixture to inhibit the endogenous malate and succinate dehydrogenase activities (19).

Kinetic studies. The pH optimum of OAADC was established by measuring the enzyme activity (2 μg of protein) in 0.05 M phosphate buffer ranging from pH 5.0 to 8.5. Depending on which direction the enzyme activity was measured, either method A or B was employed.

In the K_m studies for pyruvate, bicarbonate, magnesium, and OAA the enzyme activity was measured in

various concentrations of respective substrates while the other substrate parameters were kept at 15 mM or saturating concentration. To calculate the true K_m of each substrate, all data were replotted with the slope-intercept method (27).

Other enzymes. The presence of malate dehydrogenase activity in preparations of OAADC was determined by measuring NADH oxidation at 37°C in the presence of OAA. The reaction contained the following: 5 mM oxaloacetate, 0.5 mM NADH, 50 mM phosphate buffer (pH 7.0), and 2 μg of the OAADC enzyme preparation in a volume of 1 ml.

As malate-lactate transhydrogenase is unique to *Veillonella* spp. (1, 19), the presence of this enzyme was assayed in the system containing the following: 4 mM pyruvate, 4 mM malate, 50 mM Tris-hydrochloride (pH 7.5), and 2 μg of the OAADC preparation in a volume of 1 ml. The reaction was stopped by the addition of NaOH-glycine buffer (pH 10.5) containing NAD and 2 μg of commercial lactic dehydrogenase. At this pH, the lactate formed by the transhydrogenase was reconverted back to pyruvate, and the reduction of NAD was measured at 340 nm.

Phosphoenolpyruvate carboxykinase activity in various preparation was determined by the conversion of phosphoenolpyruvate to OAA in the presence of ADP as previously reported (21). Both the NAD- and the NADP-specific malic enzymes were assayed by measuring the oxidation of 0.5 mM NADH and NADPH, respectively, in the presence of pyruvate and sodium bicarbonate as previously reported (22).

Reaction equilibrium of OAADC. The equilibrium constant (Keq) of OAA decarboxylation was studied by incubating the purified enzyme at 25°C in a reaction mixture containing the following: 20 mM pyruvate, 10 mM HCO_3^-, 5 mM Mg^{2+}, 5.0 mM phosphate buffer (pH 7.0), and 2 μg of purified enzyme in 1 ml. After 60 min of incubation, 0.2 ml of 5 N NaOH was added to stop the reaction. The contents were then centrifuged at 35,000 × *g* at 4°C for 15 min after neutralization. The supernatant was divided into two portions; one portion was assayed for OAA by the malate dehydrogenase assay, whereas the second was assayed for the remaining pyruvate by the lactic dehydrogenase method. To ensure that the reaction had reached equilibrium, the assays were incubated for 90, 120, and 150 min. In all cases, equilibrium was reached by 120 min. The amount of HCO_3^- remaining at equilibrium was calculated from the amount of OAA formed. A boiled enzyme solution was incorporated as a control in all enzyme assays.

Other analyses. Protein was assayed by the method of Lowry et al. (17), but in the case where 20 mM β-mercaptoethanol was present in the solution, both the spectrophotometric procedure of Layne (16) and the dye-binding method of Bradford (5) were employed.

Materials. All radioactive materials were purchased either from New England Nuclear Ltd. (Montreal, Canada) or from the Radiochemical Centre (Amersham, Oakville, Canada). Commercial enzymes and metabolites were obtained from Boehringer-Mannheim Corp. (New York, N.Y.) or from Sigma Chemical Co. (St. Louis, Mo.). Column materials were purchased from Pharmacia (Montreal) or Mandel Scientific Co. (Montreal). The chemicals for gel electrophoresis were purchased from Bio-Rad Laboratories (Mississauga, Canada).

RESULTS

Ammonium sulfate fractionation. After protamine sulfate and activated charcoal treatment, the extract was subjected to ammonium sulfate fractionation in increments of 10% saturation. The majority of the OAADC activity was precipitated between 30 and 40% saturation with ammonium sulfate, whereas most of the pyruvate carboxylase and malate dehydrogenase activities were precipitated at the 60 and 20% concentrations, respectively. With these data in mind, further purification of the OAADC enzyme was undertaken with the protein precipitated between 30 and 50% ammonium sulfate.

Sephadex G-100 column chromatography. The ammonium sulfate-precipitated proteins were separated into four major peaks by Sephadex G-100 gel filtration. The OAADC assay was detected in the first peak, which was also the major protein peak of the separation. As shown in Table 1 this process was the most significant step in the purification of OAADC from *V. parvula*. The enzyme was purified 58-fold with only a slight loss of the original enzyme activity (approximately 12%). Care was taken not to overload the Sephadex column; in all cases, not more than 0.6 g of protein was loaded onto the column.

CM-cellulose ion-exchange chromatography. The concentrated protein peak off the G-100 column was separated into three peaks when eluted off CM-cellulose with 50 mM phosphate buffer and a gradient of 0 to 0.3 M NaCl. The major and first peak of the elution profile was eluted by the 0.075 to 0.090 M NaCl gradient and contained the OAADC activity. As shown in Table 1, there was a 48% reduction in protein

TABLE 1. Purification of OAADC from *V. parvula*[a]

Fraction	Protein (mg)	Sp act[b]	Purification (fold)	Total activity (× 10^3)	Other enzyme activities	
					Pc[c]	MDH[d]
Crude extract	4,800	72	1.0	346	90	80
Protamine sulfate	4,318	79	1.1	341	55	62
Ammonium sulfate (30 to 50%)	2,875	113	1.6	325	400	1,000
Sephadex G-100	46	6,590	92	303	22	77
CM-cellulose (first run)	24	9,700	135	233	0.5	3.8
CM-cellulose (second run)	18	9,760	136	176		0.8

[a] Assayed by the NADH-malate dehydrogenase method as described in the text.

[b] Micromoles of NADH oxidized per milligram of protein per 10 min.

[c] Pyruvate carboxylase.

[d] Malate dehydrogenase.

concentration after the CM-cellulose procedure. The first peak was reapplied to a second CM-cellulose column and further separated into two peaks with the OAADC activity appearing in the first peak eluted by the 0.03 to 0.05 M NaCl gradient. At this stage the OAADC from *V. parvula* was purified 136-fold.

Contamination by other enzyme activities. Substantial amounts of pyruvate carboxylase and malate dehydrogenase enzymes were coprecipitated at the ammonium sulfate step with the OAADC. For this reason, the activities of pyruvate carboxylase and malate dehydrogenase were also assayed along with that of OAADC in further purification steps. As shown in Table 1, after the second CM-cellulose step, there was no detectable activity of pyruvate carboxylase, and only a residual amount of malate dehydrogenase activity was present. However, this small amount of malate dehydrogenase activity was only 0.008% of the total OAADC activity and therefore was considered insignificant. Besides the malate dehydrogenase and pyruvate carboxylase activities, the 136-fold-purified OAADC preparation was also free of malate-lactate transhydrogenase and phosphoenolpyruvate carboxykinase activities.

Kinetic parameters of OAADC. In the course of the study, it was observed that the stability of the OAADC enzyme was dependent upon the buffer in which the enzyme was dissolved. In 50 mM of phosphate buffer or 0.5 mM arsenate buffer (both at pH 7.0 and containing 20 mM β-mercaptoethanol), the enzyme was stable at 4°C under a nitrogen atmosphere for more than 2 months. However, in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, morpholineethanesulfonic acid, or *N*-tris(hydroxymethyl)-methyl-2-aminoethane sulfonic acid buffers (pH 7.0), the enzyme was not stable and lost 23% of its activity within 24 h. Therefore, the enzyme obtained from second CM-cellulose purification step was always dialyzed against 0.5 mM arsenate buffer. The arsenate buffer was employed because the arsenate ions potentially inhibited microbial growth and stabilized the enzyme activity. Since only a small sample (usually less than 20 μl) of the concentrated enzyme was employed in kinetic studies, the amount of arsenate ion in the reaction mixture was negligible. Besides the effect of buffer solution, it was observed that either Mg^{2+} or Mn^{2+} was required for both the carboxylation and decarboxylation reactions.

In terms of pH effect, both the decarboxylation and the carboxylation reactions catalyzed by OAADC showed maximum activity at pH 7.0. At this pH, the decarboxylation reaction was sixfold higher than the carboxylation reaction.

It was observed that OAADC exhibited hyperbolic kinetics with a maximum velocity of 115 μmol/10 min per mg of protein. The double-reciprocal and slope-intercept plots showed that the K_m for OAA was 6.0×10^{-5} M. In the same decarboxylation direction, the enzyme also exhibited hyperbolic kinetics with various concentrations of Mg^{2+} producing a K_m of 1.7×10^{-4} M.

In the carboxylation direction, hyperbolic kinetics was also observed with various concentrations of pyruvate and HCO_3^-. The K_m obtained for pyruvate was 3.3×10^{-3} M, whereas K_m values obtained for HCO_3^- and Mg^{2+} were 1.7×10^{-3} and 1.8×10^{-3} M, respectively.

By comparing the K_m values obtained, it was concluded that OAA and Mg^{2+} ions bound more readily to the enzyme in the decarboxylation step than did pyruvate, bicarbonate, and Mg^{2+} in the carboxylation direction. These data also suggested that the enzyme purified from *V. parvula* functioned more readily as a decarboxylase than as a carboxylase enzyme. For this reason, the apparent equilibrium constant (Keq') for the OAADC was determined. From the respective equilibrium concentrations of 19.6 mM for pyruvate, 9.6 mM for HCO_3^-, and 0.4 mM for OAA, a Keq' of 2.1×10^{-3} was obtained, and from the Keq' data the standard free energy change (ΔG^0) for the carboxylation step was +4.5 kilocalories (ca. +18.84 kJ).

Effect of metabolites. The presence of avidin, acetyl coenzyme A, biotin, ADP, coenzyme A, and NADH produced little or no effect on the carboxylation activity of OAADC. However, ATP exerted an inhibitory effect on the carboxylation reaction of OAADC. In the presence of 5 mM ATP, only 64% of the carboxylation activity remained. Mindful of the fact that the purified enzyme functioned normally in the decarboxylation reaction, further studies were made to elucidate the regulatory role of ATP on the enzyme, both in its carboxylation and decarboxylation mode.

At various concentrations of ATP and OAA and in the presence of a saturating concentration of Mg^{2+}, ATP inhibited OAA decarboxylation noncompetitively (Fig. 1A). The K_i obtained from the plot was observed to be 2.2×10^{-3} M. The noncompetitive nature of the inhibition was confirmed by replotting the data by the method of Hunter and Downs (11; Fig. 1B). Since ATP also inhibited the carboxylation reaction, further study on the type of inhibition were made. As shown in Fig. 2A, with various concentrations of ATP and pyruvate, ATP inhibited the OAA formation competitively with a K_i 4.8×10^{-3} M. This value was again confirmed by replotting the data by the method of Hunter and Downs (11; Fig. 2B).

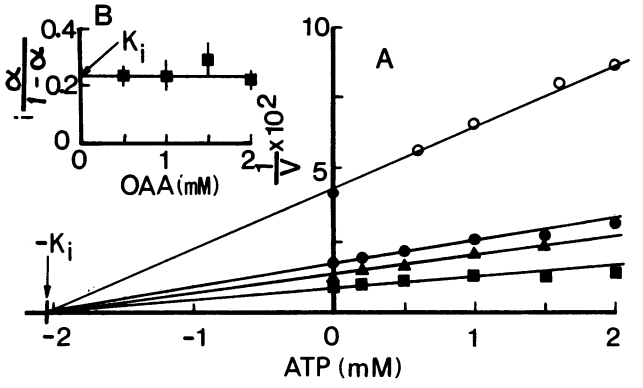

FIG. 1. Effect of ATP on the decarboxylation activity of OAADC at various concentrations of OAA. A, Dixon plot; B, Hunter and Downs plot. The spectrophotometric method employed was as described in the text, except that 10 mM Mg^{2+} was employed in each assay. Symbols: ■, 2.0 mM OAA; ▲, 1.5 mM OAA; ●, 1.0 mM OAA; ○, 0.5 mM OAA.

DISCUSSION

The results presented here indicate that *V. parvula* M_4, in fact, contains three enzymes capable of fixing CO_2 with pyruvate: one forming malate, and two forming OAA. The fact that the latter two enzymes, one requiring ATP (pyruvate carboxylase; 26) and the other active without ATP (OAADC), are separate proteins is evident from the purification data in Table 1. The absence of malic enzyme in the purified OAADC enzyme is apparent from the inability of that preparation to form substantial [^{14}C]malate from [3-^{14}C]pyruvate and bicarbonate in the presence of NADH.

Thus far, except for *M. lysodeikticus* (now *M. luteus* [9]), all of the OAADCs studied have been isolated from rod-shaped bacteria such as *Acetobacter aceti* (24), *Acetobacter xylinum* (4), *Azotobacter vinelandii* (23), *P. ovalis* (now *Pseudomonas putida* [10]), *P. citronellolis* (29) and *Escherichia coli* (13). All of these procaryotes are aerobic or facultatively anaerobic bacteria. Except for this report, nothing is known concerning OAADC from obligate anaerobes. However, except for enzyme from *A. aceti*, it seems that most of the OAADC would require only divalent ions such as Mg^{2+} or Mn^{2+} or both to decarboxylate the substrate.

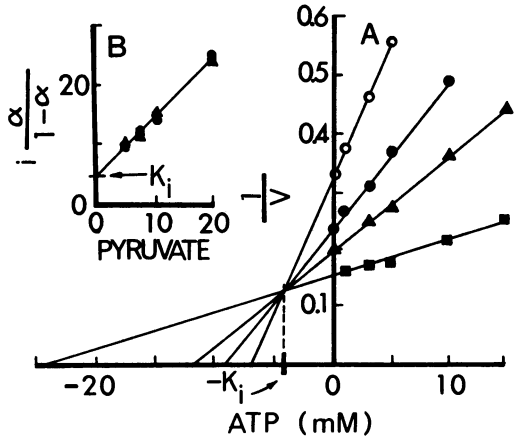

FIG. 2. Effect of ATP on the carboxylation activity of OAADC at various concentrations of pyruvate. A, Dixon plot; B, Hunter and Downs plot. The spectrophotometric method employed was as described in the text, except that 10 mM Mg^{2+} was employed in each assay. Symbols: ■, 20 mM pyruvate; ▲, 10 mM pyruvate; ●, 7.5 mM pyruvate; ○, 5 mM pyruvate.

In general the OAADC of bacteria can be divided into two optimal pH groups: the first is the neutral to basic group which includes *A. vinelandii* (pH 6.8), *P. citronellolis* (pH 7.0), *A. aceti* (pH 7.5), and *P. ovalis* (pH 8.0); and the second is the acidic group which includes *A. xylinum* (pH 5.6) and *M. lysodeikticus* (pH 5.4). The enzyme from *V. parvula* functions optimally at pH 7.0 for both its carboxylation and decarboxylation reaction; thus, it belongs to the first group. Methodologies employed in assaying for the OAADC vary between laboratories, so it is difficult to compare the kinetic parameters of this enzyme from various sources. But the K_m for OAA has been reported to range from 2.2×10^{-4} M (25) to 2.8×10^{-3} M (22). The K_m for the *V. parvula* enzyme is the lowest at 6.0×10^{-5} M. There are no data regarding the K_m for pyruvate from other bacteria; the K_m of *V. parvula* for pyruvate was 3.3×10^{-3} M. The K_m of the *V. parvula* enzyme for Mg^{2+} depends on whether the enzyme is in a carboxylation or decarboxylation mode. In the carboxylation mode, the K_m for Mg^{2+} is higher than that of the reverse reaction. Comparable data are not available for OAADC from other bacteria. The carboxylation activity has been tested with the *A.*

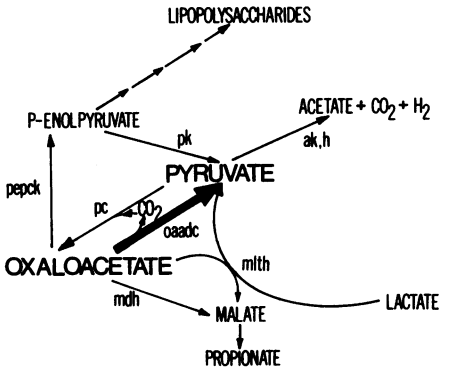

FIG. 3. Lactate metabolism by *V. parvula*. Abbreviations: ak, acetate kinase; h, hydrogenase; mdh, malate dehydrogenase; mlth, malate-lactate transhydrogenase; pc, pyruvate carboxylase; pepck, phosphoenolpyruvate carboxykinase. The thick arrow is for emphasis only.

xylinum enzyme, with data indicating that no net carboxylation of pyruvate occurs. The Keq' and ΔG^0 obtained for the *V. parvula* enzyme have clearly suggested that the carboxylation reaction is not a normal function of the enzyme. But bearing in mind that when the OAADC within the cell is coupled with the exergonic malate dehydrogenase enzyme, readily available high concentrations of pyruvate, and carbon dioxide, such a carboxylation reaction is not impossible. The data presented here clearly suggest that carboxylation of pyruvate can be catalyzed by the *V. parvula* enzyme.

The OAADC purified from *V. parvula* is similar to that of *A. vinelandii* (23) in that they are both inhibited by ATP in the decarboxylation step. According to Krebs (15) and Steinberger and Westheimer (28), metal ion is involved in the decarboxylation of OAA. In this context, it is possible that ATP inhibits via binding with Mg^{2+} ion in the reaction system. Since there is only a maximum OAA concentration 2 mM, and there is ample Mg^{2+} (10 mM) in the system to discount this possibility. At 10 mM Mg^{2+}, there is no inhibition in either the carboxylation or decarboxylation process.

The data presented in this paper have shown that two enzymes are involved in the amphibolic CO_2 fixation process in *V. parvula*. Since these enzyme activities have been detected in bacteria harvested in the midlogarithmic phase of growth, the data have also suggested that both enzymes are being synthesized during the active growth of the anaerobe. If indeed the OAADC of *V. parvula*, in conjunction with the pyruvate carboxylase enzyme, can readily function as a CO_2-fixing enzyme, then one of the two enzymes appears redundant. On the other hand, if OAADC can decarboxylate the OAA without any regulation, this would make the interconversion of OAA and pyruvate one of the most futile pathways in the anaerobe. Therefore, purely on telenomic grounds, the cellular regulation of OAADC is desirable (6). Experimental data have certainly supported such a notion because all of the OAADC reported thus far is being regulated either by metabolic intermediates (4, 10, 22, 23) or the substrate the bacteria are metabolizing (28). The data presented here have indicated that the *V. parvula* enzyme is being regulated by ATP and suggest that the regulation of OAADC follows the energy charge system proposed by Atkinson (2). Although the exact mechanism is not known, the inhibition by ATP fits well with the metabolism of the anaerobe. As shown in Fig. 3, the fixation of pyruvate to form OAA is crucial to the lactate metabolism of *V. parvula* because we have shown earlier that majority of the catabolic processes proceed via propionate formation (19). In the context of ATP regulation, we proposed that in situations where ATP content of the anaerobe is low, e.g., in the lag or initial phase of growth, CO_2 fixation via the classical pyruvate carboxylase enzyme may not be feasible. The CO_2 fixation process would then be catalyzed by the OAADC enzyme. Such a process can be futile since OAADC will also decarboxylate the OAA formed. To ensure net OAA formation, the cellular concentrations of pyruvate and CO_2 must be high, and this is possible only when the cellular lactate concentration is high, i.e., at the initial stage of growth.

In situations where ATP content is high, the CO_2 fixation process can be catalyzed by the pyruvate carboxylase enzyme, and the competitive nature of ATP inhibition on the OAADC would drastically decrease the carboxylation process via the latter enzyme. In other words, the redundancy of having two enzymes catalyze the same metabolic process is being reduced. The inhibition of OAADC by ATP would also further prevent the decarboxylation of OAA formed via the pyruvate carboxylase enzyme. Obviously, the ability of OAADC to decarboxylate OAA would depend upon the availability of OAA within the cell. The presence of active malate dehydrogenase and phosphoenolpyruvate carboxykinase (H. Chau and S. Ng, submitted for publication) enzymes in *V. parvula* will certainly result in a low cellular concentration of OAA.

To substantiate the above argument, data concerning the cellular concentration of ATP in various stages of *V. parvula's* growth are necessary. Investigation in this area is in progress.

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