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Regulation of Autotrophic and Heterotrophic Metabolism in *Pseudomonas oxalaticus* OX1: Growth on Mixtures of Acetate and Formate in Continuous Culture

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Abstract. Growth of *Pseudomonas oxalaticus* in carbon- and energy-limited continuous cultures with mixtures of acetate and formate resulted in the simultaneous utilization of both substrates at all dilution rates tested. During growth on these mixtures, acetate repressed the synthesis of ribulosebisphosphate carboxylase. The degree of this repression was dependent on the dilution rate and on the ratio of acetate and formate in the medium reservoir. At fixed acetate and formate concentrations in the inflowing medium of 30 and 100 mM, respectively, and dilution rates above 0.10 h^{-1}, the severe repression of autotrophic enzymes resulted in a marked increase in bacterial dry weight compared to the growth yield of the organisms on the two substrates separately. Also, at these dilution rates a significant increase in isocitrate lyase activity was observed in the cells as compared to growth on acetate alone. This indicated that under these conditions more acetate was assimilated and less dissimilated since acetate was partly replaced by formate as the energy source. When formate was added to the reservoir of an acetate-limited culture (S_R = 30 mM), derepression of RuBPCase synthesis was observed at formate concentrations of 50 mM and above. Below this concentration formate only served as an energy source for acetate assimilation; when its concentration was increased above 50 mM a progressively increasing contribution of carbon dioxide fixation to the total carbon assimilation was observed as the activity of RuBPCase in the cells increased. It is concluded that in *Pseudomonas oxalaticus* the synthesis of enzymes involved in autotrophic carbon dioxide fixation via the Calvin cycle is regulated by a repression/derepression mechanism.

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Abbreviations: RuBPCase = ribulosebisphosphate carboxylase; PMS = phenazine methosulphate; DCPIP = 2,6-dichlorophenol-indophenol; FDH = formate dehydrogenase; S_R = concentration of growth-limiting substrate in reservoir

Key words: *Pseudomonas oxalaticus* OX1 – Formate and acetate – Mixed substrates – Continuous culture – Calvin cycle – Derepression – Glyoxylate cycle.

When the bacterium *Pseudomonas oxalaticus* grows on acetate, energy is generated during its oxidation in the tricarboxylic acid cycle, while carbon is assimilated via the glyoxylate cycle (Dijkhuizen et al., 1978). It is not known in which way the synthesis of the two key enzymes of the glyoxylate cycle is controlled in this organism. In *Escherichia coli* the synthesis of one of these enzymes, namely isocitrate lyase, is presumably not induced by acetate itself (Kornberg, 1966). Instead, acetate seems to play a role in lowering the concentration of the repressor molecule involved and the available evidence indicates that in this organism phosphoenolpyruvate, or a closely related metabolite, controls both the activity and the synthesis of isocitrate lyase (Kornberg, 1966).

When *P. oxalaticus* is grown on formate, carbon is assimilated autotrophically via the Calvin cycle while energy and reducing power is generated by the oxidation of formate to carbon dioxide (Quayle, 1961). In batch culture studies on the regulation of the synthesis of Calvin cycle enzymes in *P. oxalaticus*, acetate, when present in mixtures with the "autotrophic" substrate formate, showed to be an excellent source of catabolite repression (Dijkhuizen et al., 1978). Although in these mixtures simultaneous utilization of the two substrates occurred, formate only served as an ancillary energy source in the metabolism of acetate and RuBPCase synthesis was totally repressed.

In the preceeding paper (Dijkhuizen and Harder, 1979) it was shown that during growth of *P. oxalaticus* on mixtures of oxalate and formate in continuous culture, the repression of RuBPCase synthesis was

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dependent on the dilution rate and on the ratio of oxalate and formate in the medium reservoir. The results suggested that in this organism the synthesis of enzymes involved in autotrophic CO_2 fixation via the Calvin cycle is regulated by a repression/derepression mechanism. If this suggestion is correct then it is to be expected that derepression of RuBPCase may also occur during growth of *P. oxalaticus* on mixtures of acetate and formate in continuous culture. In this paper we report an analysis of the growth of *P. oxalaticus* on mixtures of these two substrates in carbon- and energy-limited continuous cultures. The results further strengthen the above suggestion and also clarify the relationship between growth rate and degree of repression of Calvin cycle enzymes in this organism during growth on a mixture of formate and a substrate which is a potent source of catabolite repressor(s) in batch culture. Preliminary reports of this work have been presented elsewhere (Dijkhuizen and Harder, 1978a, b).

Materials and Methods

Organism

The organism used, *Pseudomonas oxalaticus* OX1, and its maintenance has been described previously (Dijkhuizen and Harder, 1975).

Continuous Culture Experiments

The organism was grown in carbon- and energy-limited continuous cultures, in a fermenter (working volume 1,0 l), as described previously (Dijkhuizen et al., 1977b). Dry weight was determined with a carbon analyzer (Beckman, model 915A), connected to an infrared analyzer (Beckman, model 865). Q_{O_2} values, rate of $^{14}CO_2$ fixation by whole cells, enzyme activities, residual substrate concentrations, and protein were measured in culture samples using the methods described by Dijkhuizen et al., 1978).

Calculations

The rate of CO_2 fixation necessary to explain the growth rate of *P. oxalaticus* on formate (R_r) and the capacity or RuBPCase to fix CO_2 at various dilution rates in a formate-limited continuous culture (R_p) were calculated as described previously (Dijkhuizen and Harder, 1979).

Results

Growth on Single Substrates

The relationship between dilution rate and the specific activities of key enzymes of the dissimilatory and assimilatory pathways during growth of *Pseudomonas oxalaticus* in formate-limited continuous cultures have been presented in the preceeding paper (Dijkhuizen and Harder, 1979). When *P. oxalaticus* was grown in acetate-limited continuous cultures, the specific activity of isocitrate lyase, a key enzyme of the glyoxylate cycle, increased with dilution rate, reached a maximum at a dilution rate of $0.20\,h^{-1}$ and decreased slightly as the dilution rate was further increased to $0.30\,h^{-1}$ (Fig. 2). The molar growth yield of the organism on acetate is much higher than that on formate (21.4 and 3.64 g/mol, respectively, at $D = 0.10\,h^{-1}$). The bacterial dry weight values, uncorrected for ash content, during growth on either 100 mM (S_R) formate or 30 mM (S_R) acetate at the various dilution rates are shown in Fig. 3 (see below).

Growth on Mixtures of Acetate and Formate

The effect of increasing concentrations of acetate in the medium reservoir of a formate-limited continuous culture of *P. oxalaticus*, growing at a constant dilution rate of $0.10\,h^{-1}$, is shown in Fig. 1A. The various measurements were made after the culture had reached a steady state. The results obtained were similar to those of experiments in which oxalate was added to the reservoir of a formate-limited culture of the organism (Dijkhuizen and Harder, 1979). With all the mixtures of acetate and formate tested, both substrates were completely utilized at steady state. An increase in the concentration of acetate in the medium resulted in a progressive increase in the specific activity of isocitrate lyase, the key enzyme in the assimilation of acetate-carbon. When the acetate concentration had reached a value of 27.5 mM, the specific activity of this enzyme was about 27% higher than that observed during growth on 30 mM acetate alone (see Fig. 2). As the acetate concentration was increased, the synthesis of RuBPCase and the CO_2 fixation rate were progressively repressed. Complete repression was not observed under these conditions. This is contrary to the situation in batch culture where during growth on a mixture of acetate and formate RuBPCase synthesis was fully repressed and formate served only as an ancillary energy source. A calculation of the specific activity of RuBPCase, necessary to synthesize the same amount of cell material from CO_2 during growth on the mixture as during growth on formate alone, shows that the remaining activity of RuBPCase is still sufficiently high to fulfill this task (Table 1). Under these experimental conditions repression is exerted to such an extent that at all acetate concentrations in the feed the RuBPCase still has an activity slightly in excess of that required. This indicates that at all ratios of acetate and formate in the feed both heterotrophic and autotrophic carbon assimilation pathways may function simultaneously. The data (Fig. 1A) suggest that this potential of the cells to fix CO_2 is in fact used during growth on the mixture, because the amount of bacterial dry weight produced on the mixture almost equals the sum of the dry weights obtained during growth on acetate and

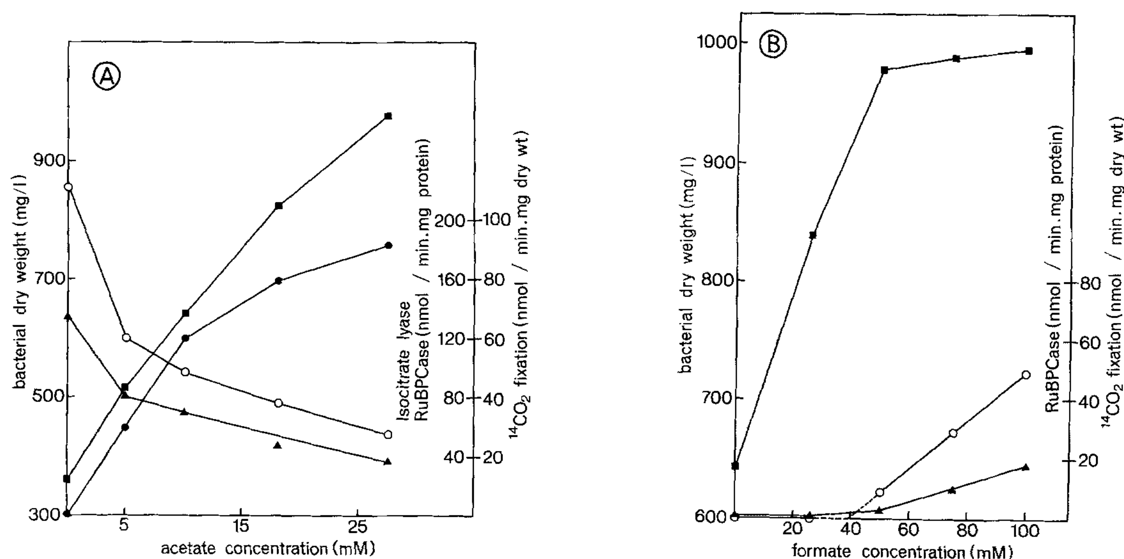

Fig. 1. A Effect of increasing concentrations of acetate (0–30 mM) in the reservoir of a formate-limited (S_R = 100 mM) continuous culture of *Pseudomonas oxalaticus* OX1 at $D = 0.10\,h^{-1}$ on a number of steady state culture parameters. ■ bacterial dry weight; ● isocitrate lyase; ○ RuBPCase; ▲ $^{14}CO_2$ fixation. **B** Effect of increasing concentrations of formate (0–100 mM) in the reservoir of an acetate-limited (S_R = 30 mM) continuous culture of *Pseudomonas oxalaticus* OX1 at $D = 0.10\,h^{-1}$ on a number of steady state culture parameters. Symbols as in Fig. 1A

Table 1. The capacity of RuBPCase to fix CO_2 at various concentrations of acetate in the reservoir of a formate-limited culture of *Pseudomonas oxalaticus* at a dilution rate of $0.10\,h^{-1}$

Acetate concentration (mM)	0	5	10	18.4	27.5
Rp: potential rate of CO_2 fixation	2.52	1.93	1.93	1.99	1.70
R_r: required rate of CO_2 fixation[a]	1.43	1.43	1.43	1.43	1.43
R_p/R_r: capacity of RuBPCase	1.76	1.35	1.35	1.39	1.19

[a] The required rate of CO_2 fixation was calculated on the assumption that the same amount of cell material was synthesized from CO_2 during growth on the mixture as during growth of the organism on formate alone

formate separately. At all acetate concentrations tested, the dry weight on the mixture is approximately 5% higher than the sum of the dry weights produced on the single substrates, indicating that the amount of acetate-carbon assimilated was only slightly increased during growth on the mixture, compared to the growth on acetate alone. This was also reflected by the slight increase in isocitrate lyase activity already mentioned above. The energy required for this small redistribution of acetate-carbon must have been obtained from formate. It is therefore concluded that only a small fraction of the formate present in the mixture was used as an ancillary energy source for acetate assimilation under these conditions.

In these experiments the specific activities of the NAD-FDH and the PMS/DCPIP linked FDH decreased with increasing acetate concentrations in a parallel fashion. However, per liter of culture the activities of the two enzymes to oxidize formate were fairly constant. Recently it has been reported that the soluble NAD-FDH of *P. oxalaticus* also reacts to some extent with the redox-dyes PMS and DCPIP (Müller et al., 1978). No correction for this effect was made in the present work, so that an unknown part of the activity of the PMS/DCPIP-linked FDH reported here may be due to NAD-FDH.

The results obtained in the reverse experiment in which the effect of increasing concentrations of formate in the reservoir of an acetate-limited culture of *P. oxalaticus* was studied at a dilution rate of $0.10\,h^{-1}$ are shown in Fig. 1B. At all ratios of formate and acetate in the feed tested, both substrates were utilized completely. Activity of RuBPCase and the capacity to fix CO_2 were detected at formate concentrations in the medium of 50 mM and higher. Once these activities appeared in the culture, they increased linearly and in a similar fashion with increasing formate concentrations. When 50 mM formate was present in the medium reservoir a 50% increase in the dry weight of the culture was observed (Fig. 1B). Since acetate was the only available carbon source, acetate-carbon must have been redistributed over the dissimilatory and assimi-

Table 2. The capacity of RuBPCase to fix CO_2 at various concentrations of formate in the reservoir of an acetate-limited culture of *Pseudomonas oxalaticus* at a dilution rate of $0.10\,h^{-1}$

Formate concentration (mM)	25	50	75	100
R_p: potential rate of CO_2 fixation	0	0.31	0.90	1.53
R_r: required rate of CO_2 fixation[a]	0.36	0.71	1.07	1.43
R_p/R_r: capacity of RuBPCase	0	0.44	0.84	1.07

[a] The required rate of CO_2 fixation was calculated on the assumption that the same amount of cell material was synthesized from CO_2 during growth on the mixture as during growth of the organism on formate alone

latory pathways, i.e. more acetate was assimilated and less was dissimilated under these conditions. This progressive redistribution of acetate-carbon as the formate concentration was increased is reflected in a change in the specific activity of isocitrate lyase; it increased gradually from 0.143 units/mg protein (at 0 mM formate) to 0.165 units/mg protein (at 50 mM formate). Moreover, within the same range of formate concentrations, the maximum capacity of the cells to oxidize acetate decreased from 186 to 149 µl O_2/mg dry weight · h. These results indicate that at formate concentrations in the mixture below 50 mM, it only served as an ancillary energy source for the assimilation of acetate-carbon. When the formate concentration was increased above 50 mM, the increase in dry weight per mmol of added formate dropped remarkably. This reflected the increasing contribution of the energetically expensive autotrophic CO_2 fixation to the biosynthesis of cell material (Table 2; see also DeCicco and Stukus, 1968; Rittenberg and Goodman, 1969). It can be calculated that, when the formate concentration in the mixture was 25 mM, the bacterial dry weight produced is 14% higher than expected when both acetate and formate were metabolized as if the other substrate were not present; at 50 mM it was 19% higher and at 75 and 100 mM 8 and 0% higher, respectively. These calculations indicate that once RuBPCase activity is present in the culture, the contribution of autotrophic CO_2 fixation to the total carbon assimilation progressively increased as the formate concentration in the medium is increased, until at a formate concentration of 100 mM the rate of CO_2 fixation by the cells is independent of the presence of acetate. This is further supported by the increasing ratio of the potential rate of CO_2 fixation (R_p) and the required rate of CO_2 fixation (R_r) of the culture (Table 2). This ratio reached the value of 1 at a formate concentration in the reservoir of 100 mM. As was found during growth of the organism on mixtures of oxalate and formate (Dijkhuizen and Harder, 1979), these results indicate that if RuBPCase is present in the cells during growth on mixtures of acetate and formate, the Calvin cycle does contribute to the in vivo carbon assimilation to an extent which is mainly governed by the activity of this enzyme. In the experiment of Fig. 1B the NAD-FDH and the PMS/DCPIP-FDH were synthesized immediately in response to the presence of formate in the medium reservoir. At a formate concentration of 100 mM in the mixture, the specific activities of these enzymes were about 40% of the values observed during growth with formate alone. However, per liter of culture the capacity to oxidize formate during growth on the mixture was comparable to that during growth on formate alone.

Figures 2 and 3 show the results obtained during growth of *P. oxalaticus* in a carbon- and energy-limited continuous culture with 30 mM acetate, 100 mM formate and with a constant mixture of acetate and formate (30 and 100 mM, respectively) in the reservoir, at various dilution rates. The specific activity of RuBPCase and the rate of CO_2 fixation reached a maximum at a dilution rate of $0.05\,h^{-1}$ (Fig. 2). At dilution rates below $0.10\,h^{-1}$ the specific activities of RuBPCase required to synthesize the same amount of cell material from CO_2 as occurs during growth with formate alone is sufficient to allow the cells to assimilate CO_2 via the Calvin cycle as if acetate were not present in the mixture (Table 3). The growth yield data (Fig. 3) indicate that this is actually the case, since the dry weight of organisms produced on the mixture of acetate and formate is almost exactly the same as the sum of the dry weights found during growth on the single substrates. At dilution rates above $0.10\,h^{-1}$ the RuBPCase activity is too low to accomplish this (Table 3). This results in the preferential utilization of formate as an ancillary energy source in the assimilation of acetate. In this respect this dilution rate of $0.10\,h^{-1}$ seems to represent a border-line where a limited redistribution of acetate-carbon, as mentioned earlier in relation to Fig. 1, had already occurred. This conclusion is supported by two facts. Firstly, at dilution rates above $0.05\,h^{-1}$ the specific activities of isocitrate lyase were higher during growth on the mixture as compared to growth with acetate alone (Fig. 2). Also the maximum capacity of the cells to oxidize acetate was lower during growth on the mixture. This indicates that more acetate is assimilated and less is dissimilated and that it is partly replaced by formate as an energy

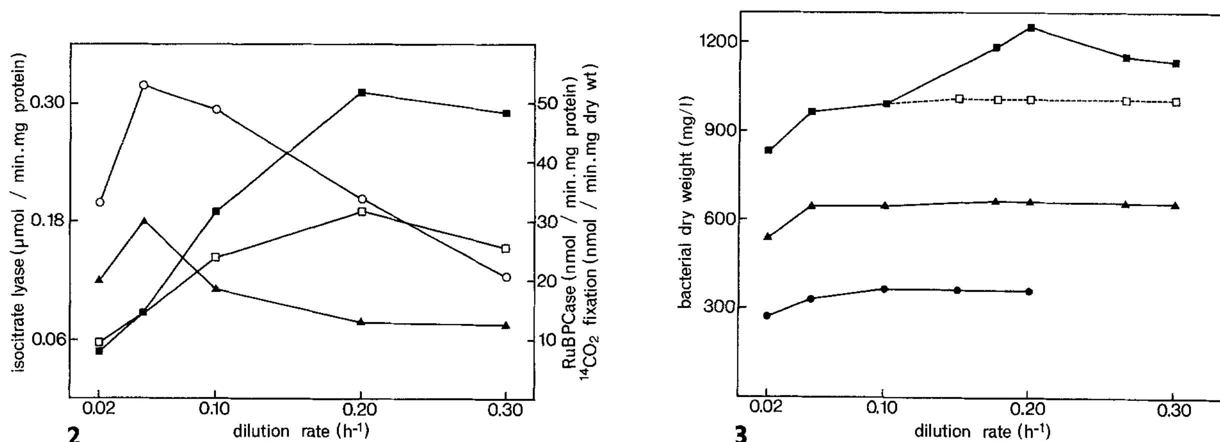

Fig. 2. Relationship between enzyme activity and dilution rate of *Pseudomonas oxalaticus* OX1 during growth on acetate (S_R = 30 mM) alone and on a mixture of acetate (S_R = 30 mM) and formate (S_R = 100 mM). Acetate alone: □ isocitrate lyase. Mixture: ○ RuBPCase; ▲ $^{14}CO_2$ fixation; ■ isocitrate lyase

Fig. 3. Relationship between bacterial dry weight and dilution rate of *Pseudomonas oxalaticus* OX1 during growth on: ● formate (S_R = 100 mM); ▲ acetate (S_R = 30 mM); ■ mixture of formate (S_R = 100 mM) and acetate (S_R = 30 mM); □ sum of the bacterial dry weight obtained during growth on formate and acetate separately

Table 3. The capacity of RuBPCase to fix CO_2 during growth of *Pseudomonas oxalaticus* on a mixture of acetate (30 mM) and formate (100 mM) in the reservoir of a carbon source-limited continuous culture at different dilution rates

Dilution rate (h^{-1})	0.02	0.05	0.10	0.20	0.30
R_p: potential rate of CO_2 fixation	0.86	1.62	1.53	1.34	0.75
R_r: required rate of CO_2 fixation[a]	0.21	0.66	1.43	2.86	4.28
R_p/R_r: capacity of RuBPCase	4.10	2.46	1.07	0.47	0.18

[a] The required rate of CO_2 fixation was calculated on the assumption that the same amount of cell material was synthesized from CO_2 during growth on the mixture as during growth of the organism on formate alone

source. Secondly, at dilution rates above $0.10\,h^{-1}$, the bacterial dry weights produced are significantly higher than expected when the metabolism of acetate and formate were unimpaired (Fig. 3). At a dilution rate of $0.20\,h^{-1}$ the increase is about 23%. Once more this reflected the energy saving effect of fixing less CO_2 via the Calvin cycle. However, even at the highest dilution rate tested, there was still a significant activity of RuBPCase present in the cells. The available data indicate that at dilution rates above $0.10\,h^{-1}$ there was a small contribution of the autotrophic CO_2 fixation to the total carbon assimilation by the cells. This contribution gradually decreased as the dilution rate is increased above $0.10\,h^{-1}$ and its relative contribution to the total carbon assimilation is probably mainly governed by the capacity of RuBPCase (Table 3).

The maximum growth rate of *P. oxalaticus* on formate is close to $0.20\,h^{-1}$. During growth of the organism on mixtures of formate and acetate, formate was also utilized at dilution rates above $0.20\,h^{-1}$, albeit not completely. The residual formate concentration in the culture increased from 0 mM (at $D = 0.20\,h^{-1}$) up to 14.1 mM (at $D = 0.27\,h^{-1}$) and 15.9 mM (at $D = 0.30\,h^{-1}$). In this experiment the specific activities of the two formate-oxidizing enzymes increased with increasing dilution rates to approximately the same extent and reached a maximum at a dilution rate of $0.20\,h^{-1}$. At higher dilution rates both enzymes were strongly repressed; at $D = 0.30\,h^{-1}$ only 25–30% of the maximum activity observed at $D = 0.20\,h^{-1}$ remained. This may explain the presence of residual formate in the culture at high dilution rates. Low activities of the formate-oxidizing enzymes were also observed in batch cultures on mixtures of acetate and formate (Dijkhuizen et al., 1978).

Discussion

The regulation of the utilization of mixtures of acetate and formate by *Pseudomonas oxalaticus* in carbon- and energy-limited continuous cultures shared many features in common with that observed during growth on mixtures of oxalate and formate reported before (Dijkhuizen and Harder, 1979). In both cases, the

presence of the "heterotrophic" substrate, acetate or oxalate, in the reservoir of a formate-limited culture strongly repressed the synthesis of RuBPCase and the rate of autotrophic CO_2 fixation. However, per millimol of substrate added, the repressive effect of acetate was much stronger than that of oxalate. Whereas in batch cultures during growth on a mixture of acetate and formate the synthesis of RuBPCase was totally repressed (Dijkhuizen et al., 1978), in continuous culture repression was dependent on the acetate concentration in the reservoir (Fig. 1A) and on the dilution rate (Fig. 2). When acetate was added to the reservoir of a formate-limited culture of *P. oxalaticus*, growing at a dilution rate of $0.10\,h^{-1}$, a progressive repression of RuBPCase synthesis and of the rate of CO_2 fixation was observed as the acetate concentration was increased. However, even at the highest acetate concentration used in the mixture (27.5 mM) the RuBPCase activity remaining was sufficient for the cells to be able to synthesize the same amount of cell material from CO_2 during growth on the mixture as during growth on formate alone (Table 1). The dry weight of organisms recorded in these experiments indicated that in vivo in fact both the autotrophic and heterotrophic carbon assimilation pathways functioned almost independently, i.e. as if the other substrate were not present. A similar conclusion was reached in experiments in which oxalate was added to the reservoir of a formate-limited culture of the organism growing at a dilution rate of $0.10\,h^{-1}$ (Dijkhuizen and Harder, 1979). The effect of the dilution rate on the regulation of autotrophic CO_2 fixation during growth on the mixture was studied at a fixed ratio of acetate ($S_R = 30$ mM) and formate ($S_R = 100$ mM) in the medium reservoir. The activity of RuBPCase and the rate of CO_2 fixation reached a maximum at a dilution rate of $0.05\,h^{-1}$ and decreased at both lower and higher dilution rates (Fig. 2). Progressive repression of RuBPCase synthesis at dilution rates above $0.05\,h^{-1}$ is probably due to an increase of the intracellular repressor concentration along with an increasing concentration of the growth-limiting carbon source(s) in the culture (Herbert et al., 1956) as the dilution rate is increased. The repression of RuBPCase is strongest at the highest dilution rate tested. In this range of dilution rates the growth rate approaches that observed in batch culture, where the synthesis of the enzyme is fully repressed. The extent of repression of the formate-oxidizing enzymes was also characteristic of that observed in batch culture (Dijkhuizen et al., 1978). In all the experiments presented above the activities of the two formate-oxidizing enzymes varied in a parallel manner, indicating that both enzymes are regulated in a similar fashion. The decrease of RuBPCase activity at dilution rates below $0.05\,h^{-1}$ was also observed during growth of the organism on formate alone (Dijkhuizen and Harder, 1979). It is important to point out that, despite the decrease in its specific activity in the culture (Fig. 2), the capacity of the enzyme to fix CO_2 increased from 2.46 to 4.10 as the dilution rate was decreased from $0.05\,h^{-1}$ to $0.02\,h^{-1}$ (Table 3).

From the results obtained during growth of *P. oxalaticus* in continuous cultures on mixtures of oxalate and formate (Dijkhuizen and Harder, 1979) it was concluded that synthesis of Calvin cycle enzymes is regulated by a repression/derepression mechanism. The inducer-like effect exerted by formate on the synthesis of RuBPCase during growth on formate alone and in mixtures with "heterotrophic" substrates (e.g. the higher the ratio formate/oxalate or formate/acetate in the medium reservoir the higher the level of RuBPCase) is probably caused by the fact that its only metabolic function is that of an energy source (compare the function of hydrogen in the hydrogen bacteria). This is most clearly seen in the experiments in which the effect of the addition of formate to the reservoir of an acetate-limited culture of *P. oxalaticus* was investigated (Fig. 1B). At the lower concentrations of formate, the NADH and ATP produced from its oxidation is used to increase the flow of carbon that is assimilated from the "heterotrophic" substrate acetate. This is indicated by the increase in dry weight of cells produced and may have been made possible by virtue of a higher specific activity of isocitrate lyase, the key enzyme in the carbon assimilation pathway from acetate, along with a decrease in the capacity to oxidize acetate. As the concentration of formate in the medium reservoir is increased, the increased rate of NADH (and ATP) production from formate (functioning as an ancillary energy source) enhances the flow of acetate carbon towards the biosynthesis of cell material (Fig. 1B), thereby possibly also gradually lowering the intracellular concentration of metabolic intermediates. This will also cause the concentration of the repressor(s) of RuBPCase to decrease, until at a formate concentration of about 50 mM full repression of RuBPCase synthesis can no longer be maintained. As was the case during growth of the organism on mixtures of oxalate and formate (Dijkhuizen and Harder, 1979), once derepression of RuBPCase occurred on the mixture of acetate and formate, both autotrophic and heterotrophic carbon assimilation pathways functioned simultaneously. This was indicated by the amount of bacterial dry weight produced and is clearly visible in Fig. 1B. Above a formate concentration of 50 mM in the reservoir, the increase in bacterial dry weight produced per mmol of formate added dropped remarkably because of the high energy demand of autotrophic CO_2 fixation. The extent at which repres-

sion was exerted during growth of *P. oxalaticus* on the mixture of acetate plus formate steadily increased with dilution rate (Fig. 2), contrary to the results obtained with the mixture of oxalate and formate (Dijkhuizen and Harder, 1979). This indicates that in the metabolism of acetate the growth-limiting reaction is probably not in the conversion of the substrate to isocitrate which is situated at the branch point of dissimilation and assimilation of this compound. Consequently, with increasing dilution rates, the potential of the metabolism of acetate to produce intermediates for biosynthesis is probably increased, which is reflected by a stronger repression of the synthesis of RuBPCase. These results may also explain the absence of any RuBPCase in batch cultures during growth of *P. oxalaticus* on mixtures of formate with one of the so-called "fast-growth" substrates: acetate, lactate, and glycollate (Dijkhuizen et al., 1978).

From the available evidence it is not possible to conclude whether the synthesis of isocitrate lyase in *P. oxalaticus* is regulated by induction/repression or by a repression/derepression mechanism. The addition of acetate to the reservoir of a formate-limited culture resulted in the rapid synthesis of this enzyme (Fig. 1A). A similar rapid response has been reported when acetate was added directly to a glucose-limited culture of *Nocardia salmonicolor* (Sariaslani et al., 1975). However, similar to the postulated role of formate in the synthesis of Calvin cycle enzymes, acetate could play a role in lowering the concentration of the repressor molecule involved in the regulation of the synthesis of isocitrate lyase. The possible involvement of a repression/derepression mechanism in the regulation of the synthesis of this enzyme is strongly supported by the observation that it is present in cells of *Pseudomonas ovalis* grown in a succinate-limited culture (Tempest, 1970). In the present study an increase in the specific activity of isocitrate lyase was observed when formate was added to the reservoir of an acetate-limited continuous culture (Fig. 2). In this case the synthesis of isocitrate lyase may be explained in a similar way as the synthesis of RuBPCase discussed above, i.e. the increased activity of isocitrate lyase may have occurred because the internal concentration of the repressor molecule for the synthesis of this enzyme was reduced by an extra pull towards biosynthesis of the cell material exerted by the energy and reducing power generated from formate. Data have been reported which indicated that phosphoenolpyruvate, the major end-product of the glyoxylate cycle, or a closely related metabolite, controls both the activity and the synthesis of isocitrate lyase in *Escherichia coli* (Kornberg, 1966). One might speculate that in *P. oxalaticus* phosphoenolpyruvate also represses the synthesis of isocitrate lyase. Similarly, the major end-product of the Calvin-cycle, 3-phosphoglycerate, might repress the synthesis of RuBPCase. However, if that is the case there appears to be a difference in sensitivity of these two important enzymes to repression by the closely related end-products of the metabolic route in which they play a role (Fig. 2). From a bioenergetic point of view this could be of great importance to the organism, since a large difference exists in the energy and reducing power requirement of the glyoxylate cycle and the Calvin cycle.

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