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Current views on the regulation of autotrophic carbon dioxide fixation via the Calvin cycle in bacteria

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The Calvin cycle of carbon dioxide fixation constitutes a biosynthetic pathway for the generation of (multi-carbon) intermediates of central metabolism from the one-carbon compound carbon dioxide. The product of this cycle can be used as a precursor for the synthesis of all components of cell material. Autotrophic carbon dioxide fixation is energetically expensive and it is therefore not surprising that in the various groups of autotrophic bacteria the operation of the cycle is under strict metabolic control. Synthesis of phosphoribulokinase and ribulose-1,5-bisphosphate carboxylase, the two enzymes specifically involved in the Calvin cycle, is regulated via end-product repression. In this control phosphoenolpyruvate most likely has an alarmone function. Studies of the enzymes isolated from various sources have indicated that phosphoribulokinase is the target enzyme for the control of the rate of carbon dioxide fixation via the Calvin cycle through modulation of existing enzyme activity. In general, this enzyme is strongly activated by NADH, whereas AMP and phosphoenolpyruvate are effective inhibitors. Recent studies of phosphoribulokinase in *Alcaligenes eutrophus* suggest that this enzyme may also be regulated via covalent modification.

INTRODUCTION

The ability of autotrophic growth, i.e. the use of CO_2 as the major carbon source for growth (Rittenberg, 1969), is widespread among bacteria. Over the years three different mechanisms of autotrophic CO_2 fixation have been elucidated in these organisms (for a review see Dijkhuizen and Harder, 1985). These

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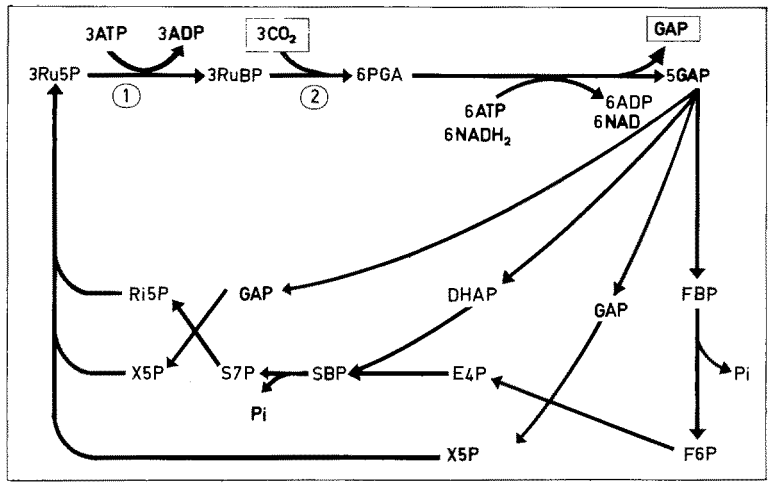

Fig. 1. The Calvin cycle. The enzymes specifically involved in its operation are (1) phosphoribulokinase and (2) ribulose-1,5-bisphosphate carboxylase. Abbreviations: Ru5P, ribulose-5-phosphate; RuBP, ribulose-1,5-bisphosphate; PGA, 3-phosphoglycerate; GAP, glyceraldehyde-3-phosphate; FBP, fructose-1,6-bisphosphate; F6P, fructose-6-phosphate; DHAP, dihydroxyacetonephosphate; E4P, erythrose-4-phosphate; X5P, xylulose-5-phosphate; SBP, sedoheptulose-1,7-bisphosphate; S7P, sedoheptulose-7-phosphate; Ri5P, ribose-5-phosphate. Modified after Calvin (1962).

are the Calvin cycle, found in the majority of autotrophic bacteria (Ohmann, 1979), the Reductive Carboxylic Acid cycle, in phototrophic bacteria belonging to the genus *Chlorobium* (Evans et al., 1966) and a linear pathway, encountered in non-phototrophic anaerobic bacteria (methanogens, acetogens and sulphate-reducing bacteria). Via this latter pathway the formation of acetyl-CoA from two molecules of CO_2 is accomplished (Zeikus, 1983; Fuchs and Stupperich, 1984). In this paper the current state of knowledge of the regulation of CO_2 fixation via the Calvin cycle is reviewed.

Autotrophic growth involving the Calvin cycle

The Calvin cycle in bacteria is similar in design to the cycle present in plants and algae (Fig. 1). Two enzymes are specifically involved in its operation, namely phosphoribulokinase (PRK) and ribulose-1,5-bisphosphate carboxylase (RuBPCase). Their combined activities result in the formation of two molecules of 3-phosphoglycerate from ribulose-5-phosphate and CO_2. Regeneration of ribulose-5-phosphate is accomplished via enzymes of the glycolytic and oxidative pentose phosphate pathways. The net effect of the Calvin cycle is the production of a C_3 compound from CO_2 according to the following stoichiometry:

$$3\ CO_2 + 9\ ATP + 6\ NADH_2 \rightarrow \text{glyceraldehyde-3-phosphate} + 9\ ADP + 6\ NAD + 8\ P_i$$

Table 1. Classification of bacteria on the basis of the energy sources used for the assimilation of carbon dioxide via the Calvin cycle

	Energy source	Representatives
Phototrophs	light	purple (non)sulphur bacteria cyanobacteria
Chemolithotrophs	inorganic compounds	hydrogen bacteria thiobacilli nitrifying bacteria CO-oxidizing bacteria
Methylotrophs	organic C_1 compounds	*Paracoccus denitrificans* *Pseudomonas oxalaticus* *Achromobacter* 1L *Pseudomonas* 8 *Mycobacterium* 50 *Xanthobacter autotrophicus* *Microcyclus aquaticus*

Carbon dioxide fixation via the Calvin cycle thus results in the production of intermediates of central metabolism, i.e. glyceraldehyde-3-phosphate or 3-phosphoglycerate, which can be used in all further biosynthetic pathways involved in cell growth and multiplication.

Although autotrophic bacteria have a common pathway for CO_2 fixation, they are diverse taxonomically and with respect to the energy sources used for growth. In this respect three main groups can be distinguished (Table 1), namely phototrophs (light energy), chemolithotrophs (oxidation of inorganic compounds) and methylotrophs (oxidation of organic C_1-compounds). The above equation clearly shows that fixation of CO_2 via the Calvin cycle is expensive in terms of energy expenditure. It is therefore not surprising that this process is under strict metabolic control. This control primarily affects the synthesis and activities of the two enzymes specific for the operation of the Calvin cycle, namely RuBPCase and PRK. From a comparison of the data now available in the literature it can be concluded that in the various groups of Calvin cycle organisms these regulatory mechanisms have many features in common and indeed may be based on identical molecular principles. This is discussed in more detail in the following sections.

RuBPCase: properties and regulation of activity

Studies on the mechanism and control of CO_2 fixation via the Calvin cycle have focused mainly on the enzyme involved in the actual CO_2-fixing step,

RuBPCase. This enzyme constitutes a major fraction of the total protein in extracts of both plant leaves and autotrophic bacteria and probably is the most abundant protein in nature (Ellis, 1979). A detailed study of the enzymes isolated from various sources has shown that, in most cases, it has a mol wt of around 550000 and consists of eight large (55000 dal) and eight small (15000 dal) subunits (L8S8) (Miziorko and Lorimer, 1983). An exceptional situation exists in the phototrophic bacterium *Rhodospirillum rubrum* in which RuBPCase occurs as a dimer of large subunits (L2) only and a mol wt of 114000 (Tabita and McFadden, 1974). Interestingly, in various members of the Rhodospirillaceae the presence of two RuBPCase proteins of different mol wt has been reported in one and the same organism (Gibson and Tabita, 1977; Sani et al., 1983). In *Rhodopseudomonas sphaeroides* for instance these enzymes have an L8S8- (556000 dal) and L6- (290000 dal) structure, respectively (Tabita, 1981; Tabita et al., 1983).

RuBPCase is a bifunctional enzyme and catalyses both the carboxylation and oxygenolysis of ribulose-bisphosphate (Miziorko and Lorimer, 1983; Tabita et al., 1984). This property has been observed with each and every RuBPCase studied so far, including those from anaerobically grown phototrophic bacteria (McFadden and Tabita, 1974) and the L2 enzyme of *Rsp. rubrum* (Storrø and McFadden, 1981). Since oxygenolysis of RuBP results in the production of one molecule of 3-phosphoglycerate and 2-phosphoglycolate each, and therefore in a decreased net rate of photosynthesis, the relationship between carboxylase and oxygenase activities has drawn considerable attention, especially in the field of plant engineering (Wildner, 1981). In these reactions CO_2 and O_2 compete in a linearly competitive manner and the amount of phosphoglycolate produced is thus determined by their relative concentrations in the gas atmosphere. This has for instance been demonstrated in continuous culture experiments with the chemolithotrophic bacterium *Thiobacillus neapolitanus* (Cohen et al., 1979). Numerous attempts to reduce the oxygenase activity specifically by the use of inhibitors (Ogren, 1984) or the isolation of mutationally altered species of the enzyme (Andersen, 1979) invariably also resulted in a comparable loss in carboxylase activity. This has led to the (still controversial) conclusion that the same active site is involved in both activities.

Kinetic studies of RuBPCase have been complicated by the failure to recognize early on that CO_2 is not only a substrate in the carboxylase reaction, and a competitive inhibitor of the oxygenase reaction, but also an activator for both these enzyme reactions. Maximal activities are only obtained after preincubation of the enzyme in the presence of CO_2 and Mg^{2+} ions. Interestingly, both activation and the catalytic process, in case of the carboxylase reaction, require CO_2 itself as the active species but involve different sites located on the large subunit. In recent years considerable progress has been made in the elucidation of the molecular mechanisms of these processes and a number of amino acids involved in the activation and catalytic sites have been identified (Miziorko and Lorimer, 1983). Activation by CO_2 is not restricted to enzymes with an L8S8-structure,

e.g. spinach chloroplast RuBPCase (Lorimer et al., 1976), but also affects the L2-enzyme in *Rsp. rubrum* (Whitman et al., 1979) and the L6-enzyme in *Rps. sphaeroides* (Gibson and Tabita, 1979). The precise function of the small subunits still remains to be established.

PRK: PROPERTIES AND REGULATION OF ACTIVITY

Compared to RuBPCase relatively little information is available on the structure and catalytic properties of PRK. The bacterial enzymes purified to homogeneity so far, from *Rps. capsulata* (Tabita, 1980), *Alcaligenes eutrophus* (Siebert et al., 1981) and the cyanobacterium *Chlorogloeopsis fritschii* (Marsden and Codd, 1984) were shown to be of comparable mol wt (220000–256000). The molecular structure of PRK in these organisms is relatively simple compared with that of RuBPCase. In each case a single size subunit (33000-40000 dal) has been reported, so that these enzymes presumably have a hexameric or octameric quaternary structure.

Investigations of the regulatory properties of the enzymes described above, and studies mainly performed with partially purified enzyme preparations from various sources (MacElroy et al., 1969; Kiesow et al., 1977; Tabita, 1980) have shown that in general PRK is strongly activated by NADH whereas AMP and phosphoenolpyruvate are effective inhibitors. Since the Calvin cycle requires considerable input of metabolic energy and reducing power, these effects indicate that PRK is the target enzyme for the in vivo control of the rate of CO_2 fixation. PRK from oxygen-evolving photosynthetic organisms, both prokaryotic, i.e. *C. fritschii* (Marsden and Codd, 1984) and *Anabaena* sp. (Tabita, 1980), and eukaryotic, i.e. *Chlorella sorokineana* (Tabita, 1980) and spinach chloroplast PRK (Kiesow et al., 1977) is not affected by NADH. This also applies to the enzymes from *Chromatium vinosum* (Hart and Gibson, 1971) and *T. neapolitanus* (MacElroy et al., 1972).

Evidence has been presented that in chloroplasts phosphoribulokinase, amongst other enzymes, is reductively activated in the light (Flügge et al., 1982). Recently, Leadbeater and Bowien (1984) observed that in *A. eutrophus* PRK is also regulated via an inactivation-reactivation mechanism. Addition of pyruvate to a culture of this organism growing autotrophically under a $H_2/CO_2/O_2$ atmosphere caused a rapid inactivation of PRK. Further experiments showed that not pyruvate itself but metabolites produced from it, phosphoenolpyruvate and ATP, most likely caused a chemical modification of the enzyme via (a) hitherto unknown reaction(s) (Bowien and Leadbeater, 1984). This mechanism is of obvious importance physiologically because PRK inactivation was paralleled by a decrease in the rate of CO_2 fixation by whole cells. The involvement of phosphoenolpyruvate in the control of PRK, both by inhibition and by inactivation, can most easily be understood on the basis of a feed-back type of mecha-

nism because this compound is metabolically closely related to 3-phosphoglycerate, the first product of CO_2 fixation via the Calvin cycle. How widespread the inactivation mechanism described above is among autotrophic bacteria, and whether it indeed provides an alternative to the light-dependent regulation of the chloroplast enzyme, remains to be investigated.

Regulation of enzyme synthesis

The extent to which autotrophic bacteria control the synthesis of Calvin cycle enzymes varies widely. There are species only able to grow under "autotrophic" conditions and at least some of these specialists have been shown to clearly regulate enzyme synthesis but to a limited extent (Smith and Hoare, 1977; Beudeker, 1981). Because of their rigid metabolism only few manipulations of the growth conditions of these organisms are appropriate for the study of regulatory phenomena. The presence of "heterotrophic" compounds in the medium, for instance, does not affect RuBPCase synthesis in *T. neapolitanus* growing on thiosulphate (Kuenen and Veldkamp, 1973). The most pronounced changes in the levels of this enzyme have been observed following changes in the autotrophic growth conditions themselves. Thus, growth of *T. neapolitanus* on thiosulphate in CO_2-limited continuous cultures resulted in a 3- to 5-fold increase in RuBPCase activity compared with thiosulphate-limited growth (Beudeker et al., 1980). Similar observations have been made with the cyanobacterium *Anacystis nidulans* (Karagouni and Slater, 1979), where again, CO_2 limitation resulted in a 15-fold increase in the specific activity of RuBPCase as compared with light-limited growth.

Many autotrophic bacteria, however, are metabolically versatile organisms and in response to changes in their environment large variations in the levels of the autotrophic enzymes may be observed. Their synthesis may even be completely switched off. In these experiments the specific activities of RuBPCase and PRK in nearly all cases vary in a comparable manner and this indicates that their synthesis is under coordinate control. During growth under autotrophic conditions in batch cultures the specific activities of RuBPCase and PRK generally are highest. In *Rsp. rubrum* this is for instance the case during photolithotrophic growth with H_2/CO_2 (Slater and Morris, 1973). After prolonged adaptation of this organism to these growth conditions approximately 50% of the total soluble protein was shown to consist of RuBPCase (Sarles and Tabita, 1983). During photoheterotrophic growth on malate however the level of this enzyme decreased to 0.3%, but growth under the same conditions on butyrate, a reduced substrate, plus CO_2 resulted in intermediate levels (14%; Tabita et al., 1983). Strong repression of the synthesis of the Calvin cycle enzymes was also observed during photoheterotrophic growth of *Rps. sphaeroides* (Tabita, 1981) and *Rps. blastica* (A. Sani, L. Dijkhuizen, and C. S. Dow, unpubl. results)

on various substrates. It is of interest that Tabita (1981), in a number of Rhodospirillaceae (*Rsp. rubrum, Rps. sphaeroides, Rps. capsulata*), only observed derepression of RuBPCase and PRK to high levels after these organisms reached the stationary growth phases in the butyrate/CO_2 media, which appeared to be due to the cells becoming limited for CO_2 (Tabita et al., 1983). This was subsequently investigated in more detail in *Rps. blastica* (Sani et al., unpubl. results). In the latter organism RuBPCase activity was very low during photoheterotrophic growth under malate-limiting conditions in continuous culture (2.4 nmoles·min^{-1}mg^{-1} protein) but indeed increased to 176 nmoles·min^{-1} mg^{-1} protein during CO_2-limited growth with butyrate under otherwise identical growth conditions. Friedrich (1982) showed that RuBPCase levels in cells of *A. eutrophus*, grown under autotrophic conditions ($H_2/CO_2/O_2$ atmosphere) in continuous culture, also strongly depended on the growth-limiting factor. In this organism again the highest specific activity of RuBPCase by far was observed under CO_2 limitation. Thus, both specialist (see above) and versatile phototrophs and chemolithotrophs respond to the state of CO_2 limitation by synthesizing increased levels of RuBPCase and PRK. The function of this control mechanism can most clearly be observed following incubation of *Rsp. rubrum* (Slater and Morris, 1973) and *Rps. sphaeroides* (Weaver and Tabita, 1983) in the light in the absence of an exogenous carbon source. In both organisms this resulted in a several-fold increase in CO_2-fixing activities.

Since in methylotrophs the oxidation of organic C_1 compounds such as methanol not only results in the production of metabolic energy and reducing power, but also in excess amounts of CO_2, comparable manipulations of autotrophic growth conditions in these organisms are not possible. Nevertheless, the available evidence indicates that in the three groups of autotrophic bacteria synthesis of the Calvin cycle enzymes is regulated in a similar fashion, namely via end-product repression in response to the intracellular concentration of 3-phosphoglycerate or a closely related compound in central metabolism (Dijkhuizen, 1979). Based on such a mechanism it is understandable that maximal derepression of CO_2-fixing enzymes must occur when CO_2 is the growth-limiting nutrient under autotrophic conditions (see above). Maximal repression on the other hand will occur during growth of these organisms with organic compounds which are metabolized heterotrophically and therefore already ensure a generous supply of intermediates of central metabolism. This repression phenomenon exerted by the metabolism of "heterotrophic" substrates has been observed not only in members of the Rhodospirillaceae during photoheterotrophic growth (see above), or aerobically in the dark, but also in hydrogen bacteria, i.e. *A. eutrophus* (Friedrich et al., 1981; Leadbeater et al., 1982), thiobacilli, i.e. *Thiobacillus* A2 (Gottschal and Kuenen, 1980), and methylotrophs, i.e. *Pseudomonas oxalaticus* (Dijkhuizen et al., 1978) and *Paracoccus denitrificans* (Van Verseveld et al., 1979).

In a number of these studies it also became clear that during growth on mix-

Table 2. Mixed substrate utilization by *Pseudomonas oxalaticus* in batch cultures

Mixture of formate and:	Doubling time on second substrate	Energy source	Carbon assimilation
succinate	2.0	both	heterotrophic
acetate	2.0	both	heterotrophic
glycolate	2.2	both	heterotrophic
glyoxylate	3.3	both	heterotrophic + autotrophic
fructose	2.3	both	heterotrophic + autotrophic
—	3.6	formate	autotrophic
oxalate	4.5	formate	autotrophic

tures of "heterotrophic" and "autotrophic" substrates in batch cultures the degree of repression varied with the organism under study and the organic compounds supplied. Thus, the presence of organic compounds has no effect on synthesis of Calvin cycle enzymes in specialist autotrophs, i.e. *T. neapolitanus* (Kuenen and Veldkamp, 1973), which may be due to the limited ability of the organisms to metabolize these compounds. In *P. oxalaticus* on the other hand, grown in batch cultures on mixtures of formate and a "heterotrophic" substrate, the general response was that of complete repression of the synthesis of the autotrophic CO_2-fixing enzymes (Dijkhuizen et al., 1978). As shown in Table 2, in case of acetate, glycolate and succinate this resulted in heterotrophic carbon assimilation only and utilization of both the "heterotrophic" substrates and formate (via formate dehydrogenases) as energy sources for growth. The other substrate mixtures are included in Table 2 because they represent exceptions to the general rule. Growth on mixtures of glyoxylate, or fructose (Dijkhuizen and Harder, 1984), with formate namely not only allowed simultaneous utilization of both substrates but also expression of both heterotrophic and autotrophic carbon assimilation pathways. Growth on formate plus oxalate, however, results in diauxie with formate being used first and complete suppression of oxalate metabolism. Thus, "heterotrophic" substrates not always cause strong repression of autotrophic CO_2 fixation. Even growth on single "heterotrophic" substrates, for instance *A. eutrophus* with fructose, gluconate or glycerol (Friedrich et al., 1981), may result in derepression of RuBPCase synthesis. Conceivably, in some organisms the maintenance of a sufficient flow of intermediates of central metabolism may be more of a problem than in others and may also be related to the individual "heterotrophic" substrate supplied. This in turn may be a reflection of differences in the properties of enzyme systems involved in the metabolism of these "heterotrophic" substrates, for instance the nature of the rate-limiting step. In some cases this may even result in switching on the autotrophic

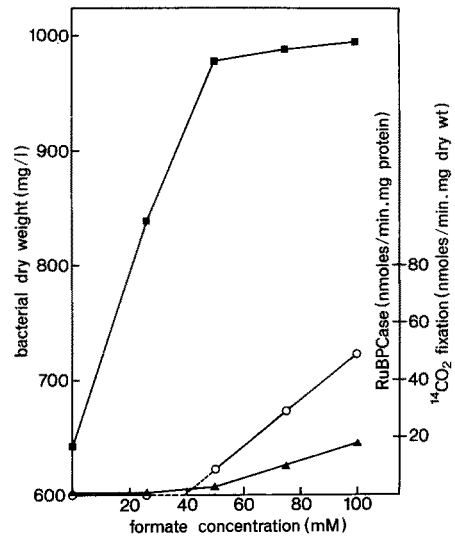

Fig. 2. Effect of increasing concentrations of formate (0–100 mM) in the reservoir of an acetate-limited (S_R = 30 mM) continuous culture of *Pseudomonas oxalaticus* OX1 at D = 0.10 h^{-1} on a number of steady-state culture parameters. ■ bacterial dry mass; ○ RuBPCase; ▲ $^{14}CO_2$ fixation rate by whole cells. Reproduced from Dijkhuizen and Harder (1979*a*) with permission.

CO_2-fixing system as an additional means of synthesizing compounds such as 3-phosphoglycerate and phosphoenolpyruvate. Alternatively, the utilization of autotrophic energy sources may completely suppress the metabolism of the "heterotrophic" substrate (i.e. oxalate) provided and thus in fact result in autotrophic growth conditions. Examples of this will be discussed below.

In view of the different effects of "heterotrophic" substrates on formate metabolism (or vice versa) in *P. oxalaticus* we made a comparative study of mixed substrate utilization in continuous cultures limited by the supply of these carbon and/or energy sources. Addition of formate (0–100 mM) to the medium reservoir of an acetate-limited continuous culture (D = 0.10 h^{-1}) resulted in simultaneous and complete utilization of the two substrates at steady state. Below 40 mM of formate its utilization, via formate dehydrogenases, did not result in synthesis of RuBPCase but nevertheless increased the bacterial dry mass in the culture by about 50% (Fig. 2). This was achieved by increased assimilation of acetate carbon by virtue of increased levels of isocitrate lyase. Above 40 mM of formate RuBPCase synthesis became derepressed and concomitantly the increase in the amount of dry mass synthesized per millimole of formate added dropped because of the energetically inefficient fixation of CO_2 via the Calvin cycle (Dijkhuizen and Harder, 1979*a*). These results show first of all that the metabolism of a "heterotrophic" substrate such as acetate is not rigid: the addition, and utilization, of increasing amounts of an additional energy source, i.e. formate, results in an increased flow of acetate carbon towards the biosynthesis of cell

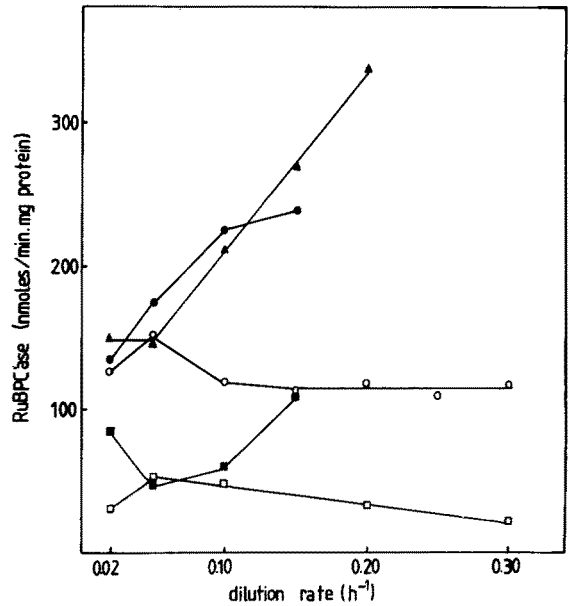

Fig. 3. Relationship between RuBPCase activity and dilution rate during growth of *Pseudomonas oxalaticus* OX1 in continuous culture on formate (S_R = 100 mM; ●) and on mixtures of formate (S_R = 100 mM) with acetate (S_R = 30 mM; □), fructose (S_R = 5 mM; ○), glyoxylate (S_R = 20 mM; ▲) or oxalate (S_R = 100 mM; ■). Modified after Dijkhuizen and Harder (1979*a*, *b*, 1984), unpubl. data of L. Dijkhuizen.

material. Thus, dependent on the amount of energy available, i.e. from formate oxidation, the flow over assimilatory (glyoxylate cycle) and dissimilatory (tricarboxylic acid cycle) pathways is adjusted accordingly, most likely to ensure sufficient intracellular pool sizes of for instance phosphoenolpyruvate, the end-product of the glyoxylate cycle. Above 40 mM of formate in the feed this redistribution mechanism is apparently not sufficient anymore; full repression of RuBPCase synthesis can no longer be maintained, and from this stage on both autotrophic and heterotrophic carbon assimilation pathways function simultaneously. The results also indicate that in carbon and energy source-limited continuous cultures at D = 0.10 h^{-1} repression of the synthesis of Calvin cycle enzymes is less severe than in batch culture and depends on the ratio of formate and acetate in the feed. In a further series of experiments, in which the organism was grown at different dilution rates in continuous cultures supplied with a constant mixture of acetate (30 mM) and formate (100 mM), repression of RuBPCase synthesis was strongest at the highest dilution rate tested (Fig. 3). This progressive repression of RuBPCase synthesis with increasing dilution rates is probably due to an increase in the intracellular concentration of intermediates of central metabolism, i.e. compounds such as 3-phosphoglycerate, along with an increasing concentration of the growth-limiting carbon source

in the culture as the dilution rate is increased (Herbert et al., 1956). These observations are in agreement with those made in batch culture during growth on this mixture, where RuBPCase synthesis is fully repressed, since at the higher dilution rates in continuous culture the growth rate approaches that in batch culture.

Fig. 3 also shows the results obtained during growth of *P. oxalaticus* in continuous culture on formate alone and on those mixtures which represented exceptional situations in batch culture. During growth on the latter mixtures in continuous culture the levels of RuBPCase did not decrease with increasing dilution rates. This is in agreement with the batch culture situation where the autotrophic carbon assimilation pathway was found to be functioning during growth on these mixtures. With the glyoxylate/formate mixture the activity of RuBPCase increased with dilution rates parallel to the levels of the enzyme observed during growth on formate alone. Although no RuBPCase synthesis occurred during growth on glyoxylate alone, either in batch or in continuous cultures, the data indicate that during growth on the mixture in continuous culture glyoxylate utilization does not cause repression of the synthesis of this enzyme. The explanation for this appears to be that the rate-limiting step in the metabolism of glyoxylate is in fact its uptake from the medium into the cell (Dijkhuizen, to be published). Thus, during growth on glyoxylate alone the intracellular levels of intermediates of central metabolism can be maintained sufficiently high to repress RuBPCase synthesis. Addition of formate will result in an increased rate of energy generation and an additional pull on these metabolites towards biosynthesis. This cannot be counteracted sufficiently by increased assimilation of glyoxylate carbon and therefore results in derepression of the enzymes of the Calvin cycle. With the fructose/formate mixture the level of RuBPCase remained nearly constant with dilution rate (Fig. 3). The most likely explanation for this response is that a balance exists between the activities and levels of glucose-6-phosphate dehydrogenase, key enzyme in fructose metabolism, and NADH and ATP generated in formate oxidation (Dijkhuizen and Harder, 1984). The latter compounds are effective inhibitors of glucose-6-phosphate dehydrogenase in *P. oxalaticus* and, in addition, the levels of this enzyme were found to decrease with increasing dilution rate during growth in the mixture but not with fructose alone (data not shown). With the formate/oxalate mixture repression of RuBPCase synthesis initially increased with increasing dilution rates. Above $D = 0.05$ h^{-1}, however, RuBPCase levels were found to increase again (Dijkhuizen and Harder, 1979*b*). Since in batch cultures (at μ_{max}) growth on this mixture for unknown reasons resulted in diauxie, it is conceivable that with increasing dilution rates oxalate metabolism is affected by formate itself, or indirectly by products of formate metabolism, in such a way that the capacity of oxalate to repress RuBPCase synthesis diminishes.

The studies described above on mixed substrate utilization offer intriguing examples of the complexity of the regulation of microbial metabolism. The data

show that dependent on the nature of the "heterotrophic" substrate under study great variations in the levels of autotrophic CO_2-fixing enzymes may occur. This variation most of all appears to be due to a diversity of regulatory mechanisms involved in the utilization of the "heterotrophic" substrates themselves. Care should therefore be taken in the interpretation of data obtained in one single mixture with respect to the regulation of the synthesis of Calvin cycle enzymes.

We have attempted to point out that in *P. oxalaticus*, and in other autotrophic bacteria, the extent of CO_2 fixation via the Calvin cycle is regulated depending on the availability of intermediates of central metabolism, closely related to compounds which can be designated as end-products of the Calvin cycle. Recent studies with *A. eutrophus* (Reutz et al., 1982; Im and Friedrich, 1983) suggest that phosphoenolpyruvate is the most prominent candidate for fulfilling an alarmone function in this mechanism of end-product repression. Via hitherto unknown reactions, increasing intracellular concentrations of this compound may cause activation of a repressor protein molecule effective in blocking transcription of the structural genes involved.

Concluding remarks

Autotrophic bacteria generally possess effective mechanisms for energy generation, employing either light, inorganic or one-carbon compounds. Provided that there is ample availability of these energy sources, carbon assimilation may proceed via CO_2 fixation in the Calvin cycle even in the presence of "heterotrophic" substrates. Both the rate of carbon dioxide fixation and synthesis of the specific enzymes involved however are carefully regulated via feed-back inhibition/repression mechanisms. Clearly, a complete elucidation of the regulatory mechanisms involved at the molecular level in controlling the rate of enzyme synthesis can only be obtained by a genetic analysis of this autotrophic growth system. Such studies have now been initiated in various research groups and are aimed at the isolation of a set of mutants blocked in autotrophic CO_2 fixation (Srivastava et al., 1982), the study of regulatory mutants (Weaver and Tabita, 1983) and cloning of the chromosomal genes involved (Curtis and Haselkorn, 1983; Shinozaki et al., 1983; Somerville and Somerville, 1984).

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