Metabolic regulation in the facultative methylotroph arthrobacter P1

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Document Version
Publisher’s PDF, also known as Version of record

Publication date:
1985

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
SUMMARY

Many microorganisms are able to utilize C₁ compounds, i.e., compounds which do not contain carbon-carbon bonds, as carbon- and energy sources for growth. In order to synthesize cell constituents from these C₁ compounds, special metabolic pathways are employed by such organisms. Although a great deal of knowledge currently exists on the physiology and biochemistry of microorganisms able to grow on C₁ compounds (autotrophs and methylotrophs), relatively little is known about the regulation of the synthesis of enzymes specifically involved in the various C₁ assimilation pathways. About ten years ago such regulatory studies were initiated in our laboratory. As a first approach the regulation of the synthesis of Calvin (RuBP) cycle enzymes in autotrophic bacteria was investigated and a considerable body of information on this subject is currently available. In many bacteria able to grow aerobically on C₁ compounds, however, carbon assimilation is initiated by fixation of formaldehyde via the ribulose monophosphate (RuMP) cycle. The regulation of the synthesis of RuMP cycle enzymes constitutes the main topic of this thesis.

A survey of the literature available on methylotrophic microorganisms, which assimilate carbon in the RuMP cycle, revealed that no suitable model organisms for these studies had yet been described (chapter 1). Therefore, attempts were made to isolate such bacteria from enrichment cultures containing the C₁ compounds methylene or trimethylamine as sole source of carbon and energy. After screening several of the isolates obtained in this way for fast growth on C₁ compounds, their ability to grow on various substrates containing carbon-carbon bonds, such as sugars and organic acids, and for the presence of RuMP cycle enzymes, an Arthrobacter species was chosen for further physiological and biochemical studies (chapter 2). During growth of this bacterium (Arthrobacter P1) on methyamine it appeared that the enzyme amine oxidase performs a key role in the metabolism of this compound. This enzyme catalyzes the oxidation of methyamine with molecular oxygen to produce formaldehyde, hydrogen peroxide and ammonia. Cytochemical staining experiments showed that amine oxidase activity is mainly located on invaginations of the cytoplasmic membrane. Assimilation of the formaldehyde, produced from the oxidation of methyamine, is accomplished by the enzymes hexulose phosphate synthase (HFS) and hexulose phosphate isomerase (HPI). Since enzymes for the linear oxidation of formaldehyde (via formate to carbon dioxide) were undetectable in methyamine-grown cells of this organism, it was speculated that during growth of Arthrobacter P1 on C₁ compounds energy generation proceeds via the so-called dissimilatory RuMP cycle. The operation of this cycle, involving the RuMP cycle enzymes mentioned above and enzymes of the oxidative pentose phosphate pathway, is rather common among RuMP cycle methylotrophs.

In these studies it was also observed that the enzymes specifically...
involved in C₁ metabolism in *Arthrobacter* P1 are normally not found in cells grown on "heterotrophic" substrates, such as glucose or acetate, indicating that the synthesis of these enzymes in this facultative methylotroph is regulated in an efficient manner.

Further studies of methylamine metabolism in *Arthrobacter* P1 revealed that conversion of formaldehyde into C₃ compounds, the precursors for the biosynthesis of cell material, proceeds via the energetically most favourable variant of the RUMP cycle. The results (chapter 3) suggest the operation of the FBP aldolase (Embden-Meyerhof) route for sugar phosphate cleavage and the transketolase/transaldolase sequence of regeneration of the formaldehyde-acceptor molecule ribulose monophosphate.

Generally, in methylotrophic microorganisms only one particular C₁ assimilation pathway operates as the major route of C₁ carbon leading to the formation of cell constituents. In chapter 4 enzymatic evidence is presented which indicates that in *Arthrobacter* P1 the exceptional situation exists that both the RUMP cycle as well as (part of) the serine pathway are involved in the metabolism of choline. In this organism choline is broken down in a series of reactions yielding glycine and formaldehyde in a ratio of one to three. It is postulated that glycine and one of the formaldehyde molecules combine to yield serine, which is subsequently converted to phosphoglycerate through typical serine pathway enzymes, whereas the remaining formaldehyde molecules are fixed in the RUMP cycle.

In chapters 5 and 6 results of studies on the regulation of the synthesis of key enzymes in the specific methylotrophic and heterotrophic carbon assimilation pathways in *Arthrobacter* P1 are presented, obtained in substrate transition experiments, from methylamine to ethylamine, and vice versa, and during growth on mixtures of methylamine and acetate, in batch as well as continuous cultures. In this organism the C₂ compound ethylamine and the C₁ compound methylamine are metabolized initially in an identical fashion, via the respective aldehydes. Transfer of ethylamine-grown cells into a medium with methylamine resulted in immediate excretion of low levels of formaldehyde (max. 0.5 mM). In the reverse experiment excretion of much higher acetaldehyde levels were observed. In further experiments, some of which involved mutants of *Arthrobacter* P1 blocked in aldehyde utilization, it became clear that in transient state situations accumulation of toxic levels of formaldehyde from methylamine oxidation is prevented because of a rapid synthesis of HPS up to high levels and by feedback inhibition of formaldehyde on the activities of the methylamine uptake system and amine oxidase.

When *Arthrobacter* P1 was grown on mixtures of acetate and methylamine in batch culture in the presence of ammonia, acetate turned out to be the preferred growth substrate irrespective of the way cells were pregrown. Under these conditions acetate did not (fully) repress the synthesis of enzymes specific for methylamine metabolism. The observed sequential utilization of these two substrates was subsequently shown to be due to a strong (non-competitive) inhibition of methylamine. When ammonium plus methylamine the organism ammonium to fulfill its growth on ammonia to fulfill its growth cells in this medium. The very poor rate of mixtures of acetate and acetate plus continuous cultures was undetectably low. The two substrates at dilute mixtures. In these experiments dependent on the ratio of the metabolism of the C₁ substrates of the enzymes involved in acetate, indicating that acetate is the energy source. At D = 0 it only occurred when acetate was the feed of a methylamine medium.

Since formaldehyde and ethylamine and its subsequent fixation (chapters 5 and 6) it was controlling the synthesis of enzymes involved in experiments involving each other as growth substrate. In view of making it virtually impossible to determine in batch culture, *Arthrobacter* P1 cultures (chapter 7), it was clear that enzymes involved in converting formaldehyde into ethylamine transport system and membrane of methylamine, whereas by formaldehyde, present in the cell from the nitrogen of catabolite repression mechanism was subsequently repressed (glucose or acetate). The results in simultaneous utilization of acetate and a complete system of HPS. This indicated that the enzyme is indeed sensitive to the metabolism of these "heterotrophic" substrates. The enzyme HPS (chapter 8).
petitive) inhibition exerted by acetate on the uptake and oxidation of methyamine. When ammonia is omitted from the medium containing acetate plus methyamine the organism is dependent on the oxidation of methyamine to ammonia to fulfil its need for nitrogen. Incubation of methyamine-pre-grown cells in this medium did result in simultaneous utilization of both substrates, but the growth rate was very low and most likely determined by the very poor rate of ammonia generation. When Arthrobacter P1 was grown on mixtures of acetate plus methyamine in carbon- and energy source-limited continuous cultures the residual concentration of acetate in the culture was undetectably low. This allowed simultaneous and complete utilization of both substrates at dilution rates below the \( V_{\text{max}} \) on either of these substrates. In these experiments, however, the observation was made that, dependent on the ratio of acetate and methyamine in the medium supply, the metabolism of the C\(_1\) substrate methyamine was able to repress the synthesis of the enzymes involved in carbon assimilation from the C\(_2\) substrate acetate, indicating that under these conditions acetate was only used as an energy source. At \( D = 0.10 \, h^{-1} \) synthesis of the glyoxylate cycle enzymes only occurred when acetate was added at concentrations above 7.5 - 10 mM to the feed of a methyamine-limited (\( S_R = 25 \, \text{mM} \)) chemostat culture.

Since formaldehyde and acetate strongly influence the uptake of methyamine and its subsequent oxidation to formaldehyde in Arthrobacter P1 (chapters 5 and 6), it was realized that a clear picture of the mechanisms, controlling the synthesis of the RuMP cycle enzymes, might only be obtained in experiments involving formaldehyde itself rather than methyamine as the growth substrate. In view of the extremely toxic nature of formaldehyde, making it virtually impossible to grow microorganisms on this compound in batch culture, Arthrobacter P1 was grown in formaldehyde-limited continuous cultures (chapter 7). Enzyme analyses revealed that the synthesis of enzymes involved in conversion of methyamine into formaldehyde and in formaldehyde fixation is induced sequentially in this organism. The methyamine transport system and amine oxidase are only synthesized in the presence of methyamine, whereas the RuMP cycle enzymes are apparently induced by formaldehyde, present in the medium reservoir or following its intracellular production from methyamine. The possible involvement of a mechanism of catabolite repression in regulating the synthesis of the RuMP cycle enzymes was subsequently studied by adding excess of a "heterotrophic" substrate (glucose or acetate) directly to these continuous cultures. This resulted in simultaneous utilization of both formaldehyde and glucose or acetate and a complete switch-off of the synthesis of the RuMP cycle enzyme HPS. This indicated that, under these conditions, the synthesis of this enzyme is indeed sensitive to catabolite repression exerted by the metabolism of these "heterotrophic" substrates. In experiments with resting cell suspensions of Arthrobacter P1 clear evidence was obtained that formaldehyde is indeed the inducing signal for the synthesis of the RuMP cycle enzyme HPS (chapter 8). The relative importance of induction and repression
mechanisms in regulating the synthesis of HPS was subsequently investigated in experiments where formaldehyde was added at a low, constant rate to batch cultures of Arthrobacter P1 growing exponentially on glucose or acetate (chapter 8). In each case this resulted in the immediate and very rapid synthesis of HPS, while no significant accumulation of formaldehyde occurred. It is therefore concluded that induction by formaldehyde is the overriding control mechanism for the synthesis of HPS. Repression may act as an auxiliary mechanism and its main function may be found under conditions, characterized by high (excess) levels of HPS, requiring rapid adaptation of the cells to a changing environment.

The present study established the presence of transaldolase isoenzymes in Arthrobacter P1 (chapter 9). This enzyme is involved in the pentose phosphate pathway as well as in the RuMP cycle. One of these isoenzymes appeared to be synthesized constitutively, whereas the second is specifically induced during growth on C1 compounds. A mutant lacking the "constitutive" transaldolase was unable to grow on gluconate or xylose, since this enzyme is obligatory in the metabolism of these compounds (chapter 3). Studies with this mutant revealed that normal growth on gluconate was, however, possible provided methylamine was supplied as a nitrogen source. This resulted in the induction of the "methylotrophic" transaldolase to high levels. This indicates that, functionally, there are no differences between both isoenzymes. To study this in more detail, the transaldolases were partially purified and it was subsequently shown that they did not differ with respect to kinetic properties. However, the molecular weights appeared to be slightly different and, moreover, the "methylotrophic" transaldolase was very heat sensitive. It is postulated that during growth on C1 compounds the "methylotrophic" transaldolase serves to ensure a sufficiently high rate of regeneration of the formaldehyde-acceptor molecule ribulose monophosphate.

Vele microorganismen zijn in staat om koolstofbronnen te verwerken die geen koolstofbron van koolstofhoudende celbestanddelen te synthetiseren. Over de fysiologie en biochemie kunnen groepen (autotrofe bacteriën, over de regulering van de assimilatieroutes betrokken, laboratorium een begin geven). Een benadering werd de regulatie van de enzymen in autotrofe bacteriën in laboratorium een begin gegeven. Informatie over dit onderwerp is te vinden in het voornamste onderwerp.

Bestudering van de enzymen, die hun koolstofbron voor groei te voeren waren beschreven (Hoofdstuk 2). Het bleek dat bacteriën die koolstof tevoeren waren beschreven (Hoofdstuk 2). Het bleek dat...