Metabolic regulation in the facultative methylotroph arthrobacter P1

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SUMMARY

Many microorganisms are able to utilize C_1 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 C_1 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 C_1 compounds (autotrophs and methylotrophs), relatively little is known about the regulation of the synthesis of enzymes specifically involved in the various C_1 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 C_1 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 C_1 compounds methylamine or trimethylamine as sole source of carbon and energy. After screening several of the isolates obtained in this way for fast growth on C_1 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 methylamine it appeared that the enzyme amine oxidase performs a key role in the metabolism of this compound. This enzyme catalyzes the oxidation of methylamine 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 methylamine, is accomplished by the RuMP cycle via the enzymes hexulose phosphate synthase (HPS) and hexulose phosphate isomerase (HPI). Since enzymes for the linear oxidation of formaldehyde (via formate to carbon dioxide) were undetectable in methylamine-grown cells of this organism, it was speculated that during growth of Arthrobacter P1 on C_1 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 C_1 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 C_3 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 C_1 assimilation pathway operates as the major route of C_1 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 C_2 compound ethylamine and the C_1 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-com-

petitive) inhibition exerted by acetate on the uptake and oxidation of methylamine. When ammonia is omitted from the medium containing acetate plus methylamine the organism is dependent on the oxidation of methylamine to ammonia to fulfil its need for nitrogen. Incubation of methylamine-pregrown 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 methylamine 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 μ_{max} on either of these substrates. In these experiments, however, the observation was made that, dependent on the ratio of acetate and methylamine in the medium supply, the metabolism of the C_1 substrate methylamine 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 methylamine-limited (S_R = 25 mM) chemostat culture.

Since formaldehyde and acetate strongly influence the uptake of methylamine 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 methylamine 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 methylamine into formaldehyde and in formaldehyde fixation is induced sequentially in this organism. The methylamine transport system and amine oxidase are only synthesized in the presence of methylamine, whereas the RuMP cycle enzymes are apparently induced by formaldehyde, present in the medium reservoir or following its intracellular production from methylamine. 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 C_1 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 C_1 compounds the "methylotrophic" transaldolase serves to ensure a sufficiently high rate of regeneration of the formaldehyde-acceptor molecule ribulose monophosphate.