Obligate chemolithotrophy

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SUMMARY

Bacteria play a dominant role in the sulfur cyle. Not only are they responsible for the production of hydrogen sulfide in a variety of processes but also for the oxidation of this compound. The latter reaction is catalyzed by the colourless sulfur bacteria which oxidize reduced inorganic sulfur compounds under aerobic conditions, whereas under anaerobic conditions in the light phototrophic bacteria are mainly responsible for the H_2S oxidation. The aim of this thesis was to characterize in detail a group of organisms belonging to the colourless sulfur bacteria, the obligately chemolithotrophic thiobacilli, in order to understand their competitiveness in the natural environment. Obligately chemolithotrophic thiobacilli are able to generate energy from the oxidation of reduced sulfur compounds only, and not from the oxidation of organic compounds. Organic carbon is synthesized by these bacteria from CO_2 via the reductive pentose phosphate- or Calvin cycle. Organic carbon sources can be assimilated by these bacteria only to a very limited extent. Considering the fact that this group of bacteria has to compete in nature for reduced sulfur compounds with facultatively chemolithotrophic thiobacilli and chemolithoheterotrophs it is hard to understand that it has survival value to leave organic substrates to the competitors. Facultative chemolithotrophs are not only able to grow autotrophically with reduced sulfur compounds as energy source but are also capable of heterotrophic growth on organic substrates. Simultaneous oxidation of reduced sulfur compounds and organic compounds during mixotrophic growth, can also be carried out by this type of organisms. Chemolithoheterotrophs are able to oxidize reduced sulfur compounds but are unable to grow autotrophically. The occurrence and coexistence of these metabolic types in the same habitat prompted us to compare the different physiological characteristics of these types of organisms. In this thesis attention is focussed particularly on the relevant properties of obligate chemolithotrophs which were compared to those of the facultative chemolithotrophs.

The obligate chemolithotrophs such as Thiobacillus neapolitanus do not respond to the appearance of organic substrates in the growth medium by an induction of relevant enzymes for the metabolism of these compounds. This suggested that the metabolic regulation in T. neapolitanus would be rigid. A generally rigid physiology in bacteria would seem to be a competitive drawback in a natural environment which is continuously changing. Therefore the question was raised whether this would apply to all organic carbon compounds and furthermore also to inorganic nutrients. Our investigations on the effects of fluctuating inorganic parameters such as CO_2 and nitrogen showed, however, that T. neapolitanus appeared to be very flexible with respect to the assimilation of these compounds.

The activity of the CO_2-fixing enzyme D-ribulose-1,5-bisphosphate carboxylase (RuBPCase) as measured in CO_2-limited cultures was about 5-fold higher than the activity of cells

grown under conditions with high concentrations of CO_2 (Chapter II). Quantification of RuBPCase protein by application of immunochemical methods showed that the increase in RuBPCase activity parallelled the increase in RuBPCase protein (Chapter III). RuBPCase protein amounted to maximally about 17% of total cell protein under CO_2-limiting conditions. Such an increase in enzyme concentration would result in a higher affinity of the organism for the growth limiting substrate CO_2. Different concentrations of CO_2 in the growth medium also were shown to affect the ultrastructure of T. neapolitanus cells. Low concentrations of CO_2 resulted in formation of many virus-like polyhedral bodies, the so-termed carboxysomes (Chapters II, III, IV, V). These organelles contain RuBPCase which is localized in the cytoplasm of these organisms as well. The carboxysomal and cytoplasmic enzyme appeared to be immunologically identical and to exhibit a comparable potential CO_2-fixing activity per mg RuBPCase protein (Chapter III). The possible funcxtion of carboxysomes which occur in almost all obligate chemolithotrophs and cyanobacteria has been extensively investigated in this dissertation. Besides RuBPCase all other Calvin cycle enzymes were detected in isolated carboxysomes. In addition significant activities of malate dehydrogenase, aspartate aminotransferase and adenylate kinase were shown to be present in these bodies. The presence in the carboxysomes of one of the Calvin cycle enzymes, fructose-1,6-bisphosphatase, was also demonstrated with cytochemical techniques (Chapter V). The functioning of the Calvin cycle requires reducing power (NADH) and energy (ATP). CO_2 fixation in isolated carboxysomes was stimulated by ATP but not by NADH whereas addition of malate to the organelles resulted in an increased CO_2 fixing activity. A hypothetical model for carboxysome functioning has been presented in which reducing power travels in the form of malate into the carboxysomes; subsequent oxidation catalyzed by malate dehydrogenase results in the formation of NADH which may be used for the Calvin cycle. The lack of stimulation of CO_2 fixation by NADH indicated that the carboxysomal shell is impermeable to NADH. This complicated compartmentation would prevent oxidation of NADH in the cytoplasm by NADH oxidase activity. Obligately chemolithotrophic bacteria have to synthesize NADH by an energy-consuming reversed electron transport since the oxidation-reduction potential of most inorganic energy sources is too high to allow for direct NAD^+ reduction. The reducing power formed by reversed electron transport is used to reduce oxaloacetate yielding malate. It is our hypothesis that in this way NADH oxidation through the electron transport chain would be minimized. Work from other authors has shown that carboxysomes are surrounded by a 3.5 nm thick monolayer consisting of glycoproteins devoid of any lipids. These results were substantiated by results of freeze-etching experiments showing the absence of hydrophobic (lipid) layers in the carboxysomal shell (Chapter V). At present it is not understood in which way the carboxysomal shell effectuates selective permeability. Apparently the carboxysome is the first clear example of an organelle in prokaryotes designed for CO_2 fixation. The carboxysome might thus be termed "Calvinosome". The presence of these organelles in

T. neapolitanus is another example of its extreme specialization to autotrophic growth.

The effects of varying concentrations and sources of nitrogen on the metabolism of T. neapolitanus were examined as second example of fluctuating inorganic parameters. The organism appeared to be able to use ammonia, nitrate and urea as nitrogen sources. Under N-limited growth conditions T. neapolitanus derepressed the synthesis of glutamine synthetase which exhibits a high affinity for the growth limiting ammonia. This enzyme lost its activity rapidly after addition of high concentrations of ammonia to N-limited organisms. Short-term loss of glutamine synthetase activity appeared to be due to inhibition by low molecular weight compounds; loss of activity during the first hour turned out to be due to a reversible modification of the enzyme into an inactive state. Long-term loss of enzyme activity apparently was due to repression of the synthesis of the enzyme. Under energy-limiting conditions with abundance of ammonia, alanine dehydrogenase was induced. This is an enzyme which according to the literature exhibits a lower affinity for ammonia as compared to glutamine synthetase but the action of which consumed less energy (Chapter VII).

N-limited cells of T. neapolitanus contained an intracellular polymer which after isolation and characterization appeared to consist of glucose units. Polyglucose formation by this obligately chemolithotrophic bacterium is of interest since the organism is not able to grow with exogenous glucose as energy source. T. neapolitanus cells were able to make use of polyglucose as a storage-energy- and storage-carbon source under aerobic conditions (Chapter VIII). Anaerobically, polyglucose was fermented by this bacterium to ethanol, lactate and CO_2 via the heterolactic fermentation pathway which hitherto was detected only in heterotrophic bacteria (Chapter IX).

From this short survey of results it can already be concluded that the obligately chemolithotrophic T. neapolitanus cannot be considered to be metabolically rigid with respect to the assimilation of inorganic compounds and even is able to accumulate and metabolize an organic storage compound. It seems as if during evolution only the flexibility with respect to the assimilation of exogenous supplied organic compounds has been given up by these bacteria. But further work showed that this also applied to glycollate, an organic compound which is formed intracellularly. Glycollate is formed in T. neapolitanus by oxygenase activity of RuBPCase, and is metabolized to malate. The key enzymes for the metabolism of glycollate showed a constant activity which was independent of the rate of glycollate formation. This resulted in excretion of this compound into the environment (Chapter VI). This rigid physiology with respect to the metabolism of organic compounds is accompanied by a very high oxidation capacity for reduced sulfur compounds. Compared to the mixotrophic Thiobacillus A2, T. neapolitanus is able to oxidize far less different substrates but the specialized obligately chemolithotrophic thiobacillus is able to oxidize reduced sulfur compounds e.g. under thiosulfate limitation in the chemostat at a much higher rate and consequently pos[illegible]sses a

higher maximal specific growth rate. T. A2 on the other hand is characterized by a very flexible physiology; the organism is able to grow continuously under alternate growth limitation of 4 hours sulfide and 4 hours acetate. This growth under alternate conditions is characterized by a rapid induction and repression of relevant enzymes; during growth on acetate for example the oxidation capacity for reduced sulfur compounds disappeared in part. This flexible strategy appears to be of disadvantage to the organism during competition in mixed cultures with T. neapolitanus during alternate supply of sulfide and acetate. When after a 4 hour period in the presence of acetate the addition of reduced sulfur compounds was resumed the specialistic organism appeared to be able to oxidize the sulfide faster than T. A2. In the presence of acetate the specialist retained its full capacity to oxidize reduced sulfur compounds and as a consequence it was able to grow at a higher rate as compared to the mixotroph which has to induce the required enzymes. This interpretation of the data could be substantiated by measurements of the concentration of growth limiting sulfide undr alternating growth conditions in pure cultures of both organisms. The specialist T. neapolitanus appeared to be able to maintain a far lower concentration of the growth limiting substrate under such conditions in the chemostat as compared to T. A2.

A comparison of many characteristics of both types of thiobacilli showed that the organisms are adapted to different ecological niches. The strategy of survival of T. A2 is aimed at growth under simultaneous limitation by reduced sulfur compounds and organic substrates (mixotrophic growth). T. neapolitanus on the other hand will thrive in environments where the turn-over rate of reduced sulfur compounds is high relative to that of organic substrates. The obligate chemolithotrophs are also very well suited for growth under alternate substrate supply since they easily survive starvation periods and retain a very high capacity to respire sulfur compounds (Chapter X).

Apparently thiobacilli were forced during the evolution to choose between the ability to oxidize many substrates simultaneously at a relatively low rate (T. A2) or to specialize their physiology to the metabolism of a few chemically related compounds (T. neapolitanus) which can be oxidized at a relatively high rate. For both types of strategy, which are incompatible, there exists an ecological niche.