University of Groningen

Metabolism of methylated amines in Hyphomicrobium spp.

Meiberg, Johannes Bernardus Maria

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

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

Publication date:
1979

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Meiberg, J. B. M. (1979). *Metabolism of methylated amines in Hyphomicrobium spp.* s.n.

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 03-06-2022

SUMMARY

At first sight hyphomicrobia are of only minor importance in the metabolism of C_1-compounds (organic carbon compounds which do not contain carbon-carbon bonds) in nature. Their growth yields and growth rates are relatively low. Consequently, in aerobic enrichments on C_1-compounds hyphomicrobia are rapidly overgrown by other microorganisms. However, as has been amplified by the present investigations, they are able to successfully compete with other organisms under specific environmental conditions. At the start of the investigations described in this thesis growth of hyphomicrobia had only been reported on methanol, methylamine and formate. In addition, some species were found to utilize the C_2-compounds ethanol and acetate. Growth on higher organic carbon compounds (containing two or more carbon-carbon bonds) was not possible. Despite their limited growth capacities hyphomicrobia are widely distributed in nature. They can be isolated from a variety of soil and water samples, mainly from rather nutrient-poor environments but from eutrophic environments as well. However, little is known about the quantitative distribution of hyphomicrobia in nature.

In chapter II a general introduction is given to the biology, physiology and biochemistry of hyphomicrobia. Chapter II also presents some of my own investigations which relate to factors possibly involved in the competition between hyphomicrobia and other aerobic C_1-utilizers: A high affinity for its growth substrates for example would give the organism a selective advantage over other microorganisms. Furthermore, the potential to utilize non-growth substrates as additional carbon sources would enable them to utilize their carbon- and energy source more economically. However, the results reported in this chapter do not sustain either of these hypotheses.

Hyphomicrobia are not only able to grow aerobically but they can also utilize nitrate instead of oxygen as a terminal electron acceptor. These organisms may find an appropriate ecological niche in environments where they can utilize their combined ability to grow on C_1-compounds and to denitrify. C_2-compounds are probably less important in this respect due to severe competition for these substrates in natural environments (for example by denitrifying *Pseudomonas* species).

In addition to methanol, methylamine and formate, which are suitable growth substrates for hyphomicrobia, many more typical C_1-compounds occur in nature. A variety of higher methylated organic compounds occur in living material. Degradation of these compounds will produce various C_1-compounds, which might be potential growth substrates for hyphomicrobia. One class of compounds, namely methylated amines, was chosen for further studies on the physiology and biochemistry of hyphomicrobia. The occurrence, formation and utilization of methylated amines as carbon-, nitrogen- and energy source and the oxidative pathways of these compounds in bacteria have been described in chapter III.

Hyphomicrobia could be selectively enriched on trimethylamine and dimethylamine under anaerobic conditions with nitrate as terminal electron acceptor (Chapter IV). Hyphomicrobia, which had been previously isolated (on methanol) by other investigators,

were also found to grow on trimethylamine and dimethylamine. From a wide range of substituted amines only some C_1- and C_2-compounds such as mono-, di- and trimethylamine, (di)ethylamine, methylethylamine and (methyl)ethanolamine, were shown to sustain growth of hyphomicrobia (Chapter IV).

The aerobic metabolism of methylated amines has been investigated in many microorganisms and as a consequence the degradative pathways and enzymes involved are fairly well known (Chapter III). In contrast, little attention had been paid to the anaerobic metabolism of these compounds. The presence of mono-oxygenases is a characteristic feature of the aerobic metabolic routes for methylated amines. Molecular oxygen is a substrate for these enzymes. It was therefore decided to study the anaerobic metabolism of methylated amines in hyphomicrobia, since this had to be different from the aerobic metabolism. *Hyphomicrobium* X was chosen for this study since the physiology and biochemistry of this organism was well documented (Chapter II).

On the basis of enzyme activities in cell-free extracts a metabolic pathway for the anaerobic degradation of trimethylamine, dimethylamine and methylamine in *Hyphomicrobium* X is presented (Chapter V). During anaerobic growth on trimethylamine and dimethylamine two enzymes are involved in the initial attack of these compounds. These enzymes (trimethylamine dehydrogenase and dimethylamine dehydrogenase) were partially purified and some properties have been determined (Chapter VI). Trimethylamine dehydrogenase is an enzyme which is identical to other trimethylamine dehydrogenases, described in the literature. The enzyme oxidizes both trimethylamine and dimethylamine with the production of formaldehyde and dimethylamine or methylamine, respectively. The comparatively slow rate of oxidation of dimethylamine by this enzyme is of little significance in vivo. Dimethylamine dehydrogenase is a new enzyme which had not been described before. The enzyme has a molecular weight of 176,000 and contains two, probably identical subunits. Dimethylamine is converted stoicheiometrically, into methylamine and formaldehyde. The affinity constant (apparent K_m) of the enzyme for dimethylamine was 15.6 μM. Trimethylamine was a potent competitive inhibitor of the enzyme (K_i 7.1 μM).

The kinetic properties of trimethylamine dehydrogenase and dimethylamine dehydrogenase allow an explanation of the peculiar substrate utilization pattern, observed during anaerobic growth of *Hyphomicrobium* X on trimethylamine in batch culture. In the initial growth phase trimethylamine was utilized as the substrate with concomitant accumulation of stoicheiometric amounts of dimethylamine in the medium. Obviously, this is due to inhibition of dimethylamine dehydrogenase by trimethylamine. Only when the trimethylamine concentration in the medium had become very low, dimethylamine oxidation was observed (Fig. 1, Chapter V). This explanation implies that dimethylamine dehydrogenase, at the site of its localization, is exposed to trimethylamine concentrations sufficiently high to cause inhibition. This means that dimethylamine dehydrogenase must be easily accessible to this substrate and therefore be located in the cytoplasmic membrane.

A study on the localization of dimethylamine dehydrogenase using a cytochemical staining technique revealed the membrane-bound nature of the enzyme (Chapter VII). Not only the cytoplasmic

membrane but also intracellular membrane structures appeared to stain positively for this enzyme. It was concluded from electron micrographs of freeze-etch replicas that these internal membranes are invaginations of the cytoplasmic membrane. The precise localization of dimethylamine dehydrogenase (i.e. in the inner- or the outer side of the membranes) could not be established.

In chapter VIII the aerobic metabolism of trimethylamine and dimethylamine is described. During aerobic growth dimethylamine mono-oxygenase appeared to be functional in conjunction with dimethylamine dehydrogenase. As compared to dimethylamine dehydrogenase (K_i 7.1 μM) dimethylamine mono-oxygenase was only scarcely inhibited in its activity by trimethylamine (K_i 4.2 mM); as a consequence, during aerobic growth on trimethylamine (when dimethylamine mono-oxygenase was present) dimethylamine did not accumulate in the medium. The affinity constant of dimethylamine mono-oxygenase for oxygen was relatively high (23.2 μM). This indicates that the *activity* of the enzyme is low at low dissolved oxygen tensions. When *Hyphomicrobium* X was grown in continuous culture on trimethylamine or dimethylamine at different dissolved oxygen tensions also the *synthesis* of dimethylamine mono-oxygenase was dependent on oxygen tension. At lower dissolved oxygen tensions the specific activity of the mono-oxygenase decreased, whereas the synthesis of dimethylamine dehydrogenase increased. Assuming that dimethylamine mono-oxygenase is the universal enzyme for dimethylamine oxidation in all aerobic microorganisms able to grow on trimethylamine or dimethylamine, the added property of having dimethylamine dehydrogenase will favour hyphomicrobia above other microorganisms, because this enzyme will enable them to grow on trimethylamine or dimethylamine at low oxygen tensions or under anaerobic conditions (with nitrate present).

It was demonstrated in continuous culture experiments that *Hyphomicrobium* X was able to carry out denitrification and aerobic respiration simultaneously. At low growth rates (low substrate concentrations) the synthesis of nitrate reductase was already significant at relatively high dissolved oxygen concentrations. In addition to the oligocarbophilic nature of hyphomicrobia this capacity for "aerobic" denitrification may be of ecological importance.