THE FORTUITOUS OXIDATION AND COMETABOLISM OF VARIOUS CARBON COMPOUNDS BY WHOLE-CELL SUSPENSIONS OF *METHYLOCOCCUS CAPSULATUS* (BATH)

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1. Introduction

Cell-free methane mono-oxygenase preparations from *Methylococcus capsulatus* (Bath) have been shown to catalyse the oxidation of a wide range of carbon compounds including, *n*-alkanes, *n*-alkenes, ethers, alicyclic and aromatic compounds [1]. This paper reports the restricted in vivo substrate specificity of the methane mono-oxygenase of *M. capsulatus* (Bath) and discusses reasons why so few compounds were oxidized when compared with the results obtained with cell-free preparations of the same organism. The term cometabolism is redefined in view of the results obtained.

2. Materials and Methods

2.1. Growth of bacteria and preparation of cell suspensions

Methylococcus capsulatus (Bath) [2] was grown at 45°C in O_2-limited continuous culture at a dilution rate of 0.05 h^{-1} on a basic mineral salts medium (MS), described previously [3], which was supplemented with 1 g $litre^{-1}$ ammonium chloride (AMS). Methane (20% v/v, in air) was the carbon source. Volumes of culture were centrifuged at 5000 × *g* for 10 min, washed once with ice-cold, 20 mM potassium phosphate buffer, pH 7.0 and resuspended in a small volume of the same buffer.

To ascertain whether *M. capsulatus* (Bath) could grow and replicate on any of the compounds which were oxidized during the oxidation studies, several 250 ml conical flasks were set up containing 25 ml of sterile AMS to which various amounts (25 μmol, 125 μmol, 250 μmol, 1.25 mmol) of test substrate were added. Appropriate controls either with methane as sole source of carbon or with no carbon source were prepared, then all the flasks were inoculated with 0.5 ml of a chemostat culture of *M. capsulatus* (Bath) as described above, giving an initial absorbance (A_{540}) of 0.24 in the flasks.

2.2. Oxidation assays and identification end estimation of products

The assays were done in 7 ml conical flasks essentially as described previously [1] except that cell-free extract was replaced with 0.53 mg dry weight cell suspension. Where appropriate, 4 μmol of formaldehyde was added to the reaction mixtures. The identification and estimation of products was as described previously for the cell-free extract studies [1].

3. Results and Discussion

All the 31 compounds which were oxidized by the cell-free methane monooxygenase preparations of *Methylococcus capsulatus* (Bath) [1] were tested for oxidation by whole-cell suspensions of the same organism. 19 of these compounds were not oxidized in the presence or absence of formaldehyde: iodomethane, dichloromethane, trichloromethane, tetrachloromethane, cyanomethane, nitromethane, methanethiol, trimethylamine, *n*-butane, *n*-pentane, *n*-hexane, *n*-heptane, *n*-octane, cyclohexane, benzene, tol-

uene, styrene, pyridine and L-phenylalanine. The compounds which were oxidized by whole cells are shown in Table 1, with the oxidation rates calculated from the amount of product formed or where the products were undetectable, from the amount of substrate consumed. In the absence of formaldehyde only 5 of the 31 potential substrates (chloromethane, bromomethane, dimethylether, ethene and propane) appeared to be oxidized by the methane mono-oxygenase in vivo. This may not be as surprising as first appears when one considers that the initial oxidation by the methane mono-oxygenase requires one molecule of NADH or reducing equivalent per molecule of substrate oxidized. Since many compounds are probably not further oxidized, thereby preventing the regeneration of reducing power, no oxidation will occur. Other possible reasons for negative results could have been the formation of oxidation products toxic to the organism, or the substrate itself may have been toxic, also some compounds may not have been able to enter the cell.

The same range of compounds were tested for oxidation by whole-cell suspensions of *M. capsulatus* (Bath) in the presence of 4 mM formaldehyde. As NAD^+-linked formaldehyde and formate dehydrogenases are present in *M. capsulatus* (Bath) [4], formaldehyde was included to generate reducing power which could be used for methane mono-oxygenase activity. Therefore any potential substrate which, due to its inability to regenerate reducing power, was not oxidized in the previous assays containing no formaldehyde, would now be oxidized. The results in column two of Table 1 show that 7 previously unoxidized compounds (carbon monoxide, diethylether, ethane, propane, 1-butene, *cis*-2-butene and *trans*-2-butene) were oxidized in the presence of exogenous formaldehyde.

None of the 12 compounds listed in Table 1 tested at various concentrations (1 mM, 5 mM, 10 mM and 50 mM), supported growth and replication of *M. capsulatus* (Bath) when incubated at 45°C for 10 days. Of the 5 compounds which were oxidized in the absence of formaldehyde, chloromethane and bromomethane gave identical oxidation rates in the presence of formaldehyde. Dimethyl ether, ethene and propene were oxidized three, five and four times more rapidly, respectively, in the presence of formaldehyde. This suggested that in the absence of an exogenous supply of reducing power, the oxidation rates of dimethyl ether, ethene and propene were limited by the poor regeneration of reducing power from the further oxidation of these compounds. The inability

TABLE 1

Oxidation of various carbon compounds by whole-cell suspensions of *Methylococcus capsulatus* (Bath)

Compound	Oxidation rate [μmol product formed min^{-1} mg dry weight cells]	
	no formaldehyde	+4mM formaldehyde
Chloromethane [a]	0.170	0.170
Bromomethane [a]	0.088	0.088
Dimethyl ether [a]	0.007	0.125
Diethyl ether [a]	0	0.016
Carbon monoxide	0	0.520 (carbon dioxides)
Ethane	0	0.025 (ethanal)
Propane	0	0.004 (1-propanol)
Ethene	0.004 (epoxyethane)	0.022 (epoxyethane)
Propene	0.006 (1,2-epoxypropane)	0.022 (1,2-epoxypropane)
1-Butene	0	0.023 (1,2-epoxybutane)
cis-2-Butene	0	0.014 (*cis*-2,3-epoxybutane)
	0	0.013 (*cis*-2-buten-1-ol)
trans-2-Butene	0	0.017 (*trans*-2,3-epoxybutane)
	0	0.035 (*trans*-2-buten-1-ol)

[a] Oxidation rates of these compounds are expressed as μmol substrate disappeared min^{-1} · (mg dry weight cells)$^{-1}$. Where product formation was measured the names of these products are given in parentheses.

of formaldehyde to stimulate the oxidation rates of either chloromethane or bromomethane suggested that the supply of reducing power was not the rate-determining factor in these cases.

The phenomenon whereby an actively growing micro-organism oxidizes a compound but cannot then utilize any carbon or energy derived from the oxidation was termed "co-oxidation" by Foster [5]. This definition was expanded by Jensen [6] to include reactions other than oxidations e.g. dehalogenations, and the obligate requirement for the presence of a growth substrate was dropped. He coined the term "cometabolism" to describe this amended definition. Both terms have been used ambiguously in the literature over the past few years and have become virtually synonymous. Furthermore the whole concept of co-oxidation/cometabolism has recently received strong criticism [7] on the basis that the above terms describe metabolic phenomena which are easily encompassed by the existing terms for metabolism, anabolism and catabolism.

The oxidations reported in Table 1 all fall under the heading of cometabolism as defined by Jensen [6], however, not all could be described as novel metabolic events (cf. anabolism and catabolism). The oxidation of the 5 non-growth substrates which were oxidized in the absence of formaldehyde did not constitute a new metabolic event but was merely a reflection of the non-specific nature of the methane monooxygenase which initiated the fortuitous metabolism of these compounds. This type of incomplete metabolism easily conforms with the original definition of catabolism by Foster in 1888 (see ref. 7) and essentially constitutes the partial fulfilment of a normal catabolic pathway. Therefore, it is suggested that the transformation of non-growth substrates in the absence of a co-substrate should be simply referred to as fortuitous oxidations, dehalogenations etc. and not be classed as novel metabolic events. It is also suggested that the term non-growth substrate be used to describe compounds which do not support cellular division (as opposed to an increase in cell mass) as it is possible that such compounds could be assimilated into cellular biosynthetic pathways but remain unable to support cellular division.

Co-oxidation as originally defined by Foster [5] describes an unusual metabolic phenomenon, although the actual enzymic route of any particular co-oxidative event still operates within the confines of the normal anabolic and catabolic pathways of the organism involved. However, it is felt that such phenomena are sufficiently interesting and unusual metabolic events to merit a term to describe them. It is proposed that the term cometabolism be redefined as: *the transformation of a compound, which is unable to support cell replication, in the requisite presence of another transformable compound (co-substrate).*

This definition remains true to the original definition of co-oxidation by Foster [5], but encompasses other reactions as well as oxidations and extends the range of co-substrates to include compounds which cannot support cell replication as well as growth substrates.

The 7 compounds in Table 1 which were only oxidized in the presence of formaldehyde can be regarded as cometabolic substrates as redefined above, in this case the co-substrate was the non-growth substrate formaldehyde.

An interesting aspect of these cometabolic and fortuitous oxidative events is the benefit, if any, which an organism receives from such metabolic activities. It is possible that although a substrate is unable to support cell replication it may contribute to the economy of the cell by producing energy and/or reducing power in some form. The production of energy from these substrates could be determined by techniques used to assay for nitrogenase activity in *M. capsulatus* (Bath) as previously shown for compounds such as ethanol, hydrogen, formaldehyde and formate [3]. In some cases the substrate could possibly contribute assimilable carbon but still be unable to sustain cell replication, e.g. ethanol.

There are a few examples of cometabolism, as redefined above, by methaneoxidizing bacteria in the literature, e.g. ethane, propane, *n*-butane [8] and carbon monoxide [9] by *Methylomonas methanica,* and ethane [2] and carbon monoxide [10] by *Methylomonas agile.* In comparison many more examples of the fortuitous oxidation of non-growth substrates by methane-oxidizing bacteria have been reported e.g. bromoethane by *M. methanica* [11] and *M. capsulatus* (Bath) [12], ethane [13] and carbon monoxide [9,10] by *Methylosinus trichosporium* OB3B, ethanol and formate by *M. capsulatus* (Texas) [14] and dimethyl ether by various unidentified methane-oxidizing bacteria [15].

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