CYTOCHROMES OF TWO METHANE-UTILIZING BACTERIA

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1. Introduction

Methane-utilizing bacteria (methylotrophs) are obligately dependent on C-1 substrates for growth. The two organisms selected for this study, *Methylomonas albus* and *Methylosinus trichosporium*, represent two groups of methylotrophs which have previously been shown to differ with respect to the structural organisation of their cytoplasmic membranes [1] and pathways of methane assimilation [2]. This report describes the spectrophotometric examination of the cytochromes present in these two bacteria and discusses the possible involvement of a carbon monoxide-sensitive cytochrome in the early stage(s) of methane oxidation.

2. Methods

The organisms were grown in 400 ml amounts in 2 litre shake flasks, using the nitrate mineral salts described by Whittenburry et al. [3] with methane as sole source of carbon and energy. After harvesting, the cells were suspended in 30 mM phosphate buffer (pH 6.9) to the desired concentration (about 6 mg dry weight/ml). Spectra were recorded using an Aminco-Chance Dual Wavelength/Split Beam Recording Spectrophotometer. The procedure of Rieske [4] as modified by Peterson [5] was used to assay pyridine haemochromogens, although by this method we did not detect the pyridine haemochromogen of haeme *a* (587 nm approximately).

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3. Results and discussion

Table 1 shows the cytochrome content of the two organisms, no pyridine haemochromogen was detected at 587 nm which could be attributed to a cytochrome *a*. From the spectra discussed below, it is evident that some haeme *a* should be detectable, at least in the case of *M. trichosporium*. This organism possessed slightly less cytochrome *c* than did *M. albus*, but appeared to contain fractionally more protohaeme. In *M. albus*, the amount of cytochrome *c* greatly exceeded the amount of protohaeme, but this difference was not nearly so marked in *M. trichosporium*.

Fig. 1 shows the reduced minus oxidised difference spectrum of *M. albus*. As suggested by the results in table 1, a cytochrome(s) of the *c*-type is the predominant species, having a Soret band at 423 nm and α-band at 553 nm. The symmetry of the α-band suggests that there is little or no contribution from any *b*-type cytochrome. However, the small peaks at 443 nm and in the region of 608 nm suggest the presence of a relatively low concentration of an *a*-type cytochrome.

Using similar cell concentrations, *M. trichosporium* gave the reduced minus oxidised difference spectrum shown in fig. 2 (dashed line). The same *c*-type Soret band was observed at 423 nm as with *M. albus*. but in this case the Soret region of the spectrum was complicated by the presence of a strong chromophore at 443 nm. This again can be attributed to an *a*-type cytochrome, in this case present in much higher concentration. There is again no evidence to suggest the presence of any appreciable amount of a *b*-type cytochrome apart from a slight shoulder on the longer wavelength side of the 553 nm α-band of the *c*-type. Any Soret band of a *b*-type cytochrome would un-

Table 1
Cytochrome content of *M. albus* and *M. trichosporium**.

Cytochrome	*M. albus*	*M. trichosporium*
Cytochrome *c*	0.13	0.09
Protohaeme	0.02	0.03

* The content of cytochrome *c* and protohaeme was determined according to Peterson [5] using E_m values of 21.0×10^3 (550 nm/540 nm) and 34.1×10^3 (557 nm/575 nm) respectively. Values are expressed as nmoles of cytochrome/mg dry weight of cells.

doubtedly be obscured by those of the *c*- and *a*-types. Fig. 2 also shows the reduced plus carbon monoxide (CO) minus reduced difference spectrum of *M. trichosporium* (solid line). It appears that the *a*-type cytochrome, or one of the *a*-types, is of the oxidase type (see ref. [6]), giving a new band (appearing in this case as a shoulder) around 425 nm, with a corresponding trough at 445 nm. The strongest band of the spectrum appeared at about 416 nm, and corresponds to that observed for the complex of CO with a cytochrome *o*. A similar study with *M. albus* proved to be erratic, and, while the cytochrome *o*–CO band was evident in some preparations, in others it was not detectable. The band corresponding to the cytochrome *a*–CO complex was, however, invariably present.

These results suggest that the two organisms studied possess qualitatively similar cytochromes, although quantitatively they appear to be rather different. It is possible that these differences further justify the grouping of methylotropic organisms on the basis of their cytoplasmic membrane structure and pathway of methane assimilation. It is moreover, interesting to speculate on the function of the cytochromes found in these organisms. Cytochrome *o* has been implicated as a terminal oxidase in a number of bacteria [7] and it has recently been suggested that it may function as an 'oxygen carrier' in a manner similar to another CO-sensitive pigment, cytochrome *P*-450 [5]. Methane-oxidizing bacteria have been shown to utilise molecular oxygen in the oxidative step(s) from methane to methanol; it is currently believed that this reaction may be energetically expensive, most likely involving a mixed function oxidase [8]. Current studies (unpublished work) suggest that the respiration of methane by whole cells is preferably inhibited in the presence of CO whereas the respiration of methanol

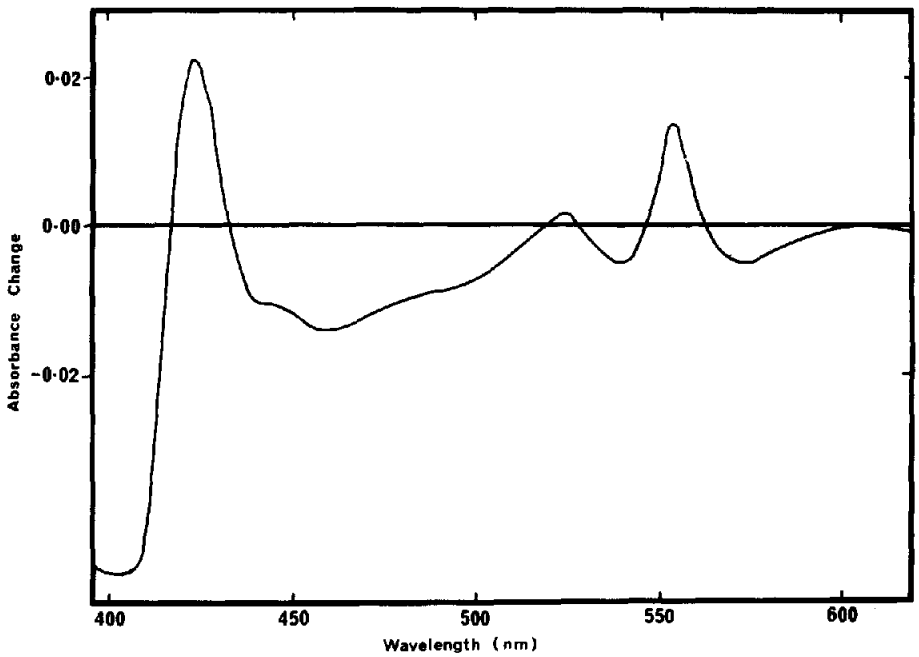

Fig. 1. Difference spectrum of *M. albus*. The cells were diluted to a concentration of 6 mg dry weight/ml with phosphate buffer. A few milligrams of sodium dithionite were added to the sample cuvette and the spectrum recorded.

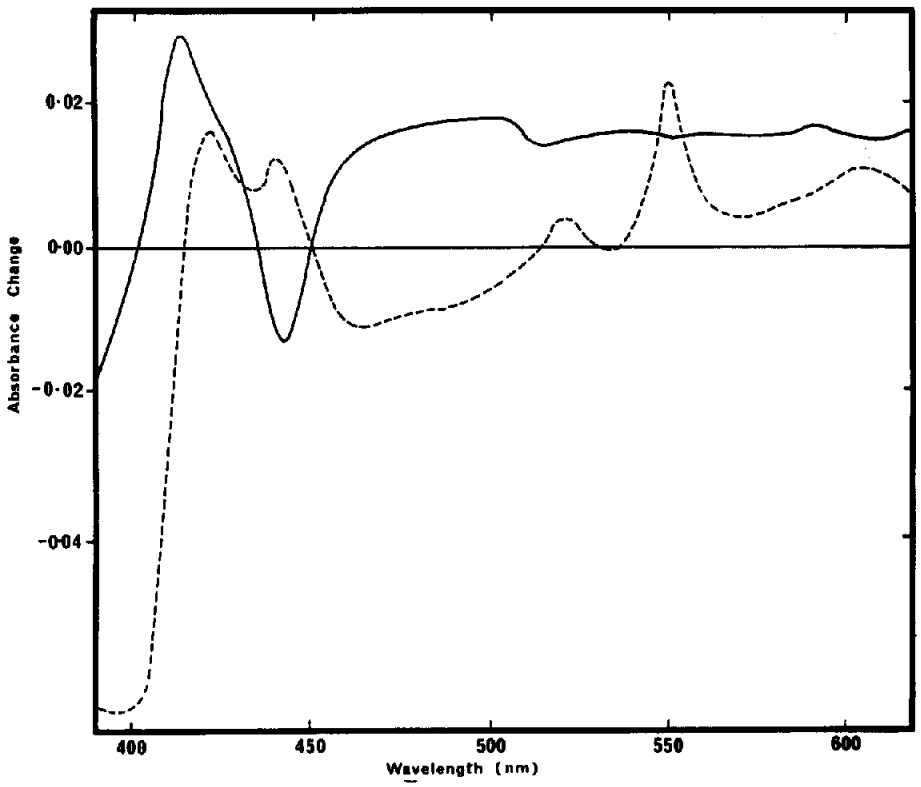

Fig. 2. Difference spectra of *M. trichosporium*. The reduced minus oxidized difference spectrum was recorded as described for fig. 1 (– – – –). After recording the latter spectrum, the cuvettes were mixed and the baseline recorded. The sample was then bubbled with carbon monoxide for 20 sec and the reduced plus CO minus reduced difference spectrum recorded (———).

or formaldehyde (proposed intermediates), does not appear to be affected. This strongly indicates the involvement of a CO-sensitive pigment early in the oxidation of methane. It is possible however to suggest that the CO-sensitive *a*-type cytochrome might perform this function, in which case the cytochrome *o* may serve as terminal electron acceptor. The problem is further complicated by the apparent lack of inhibition by CO of respiration on methanol and other substrates, although this may be explained as being indicative of a terminal electron acceptor which combines very slowly or loosely with CO. Further work is in progress to determine the functionality of these pigments in the bacterial oxidation of methane.

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