BACTERIOCHLOROPHYLL FORMATION BY FACULTATIVE METHYLOTROPHS, *PROTAMINOBACTER RUBER* AND *PSEUDOMONAS* AM 1

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Received 5 November 1977

1. Introduction

The unique physiological characteristic of the photosynthetic bacteria is their ability to grow anaerobically in the light, a property conferred upon them by their photosynthetic pigment systems [1]. Facultative methylotrophs, *Protaminobacter ruber* [2,3] and *Pseudomonas* AM 1 [4] are obviously different from such photosynthetic bacteria, since they grow aerobically and do not grow anaerobically in the light. Nevertheless, they produce bacteriochlorophyll under certain conditions. As far as the author is aware, this is the first report on the formation of bacteriochlorophyll by the microorganisms classified as non-photosynthetic bacteria.

Several microorganisms such as *Mycobacterium kansassi* and *Neurospora crassa* are known as photochromogens and can synthesize carotenoids photoinducibly [5,6]. However, there is no report on photoinduced bacteriochlorophyll formation by non-photosynthetic bacteria. This paper presents evidence for photoinduced, or photo-enhanced, bacteriochlorophyll formation by the methylotrophs, *Protaminobacter ruber* and *Pseudomonas* AM 1.

2. Materials and methods

Protaminobacter ruber was isolated by us [2,3] and *Pseudomonas* AM 1 was kindly supplied by Professor J. R. Quayly. *P. ruber* was grown in the medium described [2] and both bacteria were also grown in a similar medium in which DL-1,2-propanediol was used as sole carbon and energy source instead of methanol and the pH of the medium was adjusted to 7.5. Test tubes containing 10 ml medium were shaken at 28°C on the reciprocating shaker operating at 100 strokes/min. When illumination was supplied, 200 W tungsten lamp was used at 60 V at a distance of about 60 cm (900–1100 lux). The intervals of illumination are described in table 1.

The pigment was extracted from the cells with chloroform–methanol (1:1, v/v), 4 ml/tube and its A_{775} max was measured with a Hitachi 624 digital spectrophotometer. The content of bacteriochlorophyll was tentatively determined by assuming the molar extinction coefficient as 1×10^5 [7,8]. Growth was measured turbidimetrically at 660 nm with an Akiyama D.S. 374 Fuji spectrophotometer. Dry weight of the cells was calculated by using the relationships between ADS values and dry weight of the cells obtained in separate experiments.

In order to isolate the pigment *P. ruber* was grown for 4 days in 500 ml Sakaguchi flasks containing 200 ml medium by shaking on the reciprocating shaker. The cultures were illuminated for a period of 8–20 h under the above conditions, followed by a dark period of 16–20 h. This was repeated until the cells were collected. The cells thus obtained from 400 ml cultures were suspended in 150 ml chloroform–methanol (1:1, v/v) and the pigment was extracted for about 2 h in the dark at room temperature in an atmosphere of nitrogen by shaking the vessel occasionally. All subsequent operations were carried out in dim light and whenever possible, under nitrogen. After centrifugation, the pigment in the supernatant was transferred into diethylether by shaking the supernatant with 400 ml ether and 500 ml

Table 1
Growth and bacteriochlorophyll formation in *Protaminobacter ruber*

Conditions[a]	Growth substrate			
	Methanol		1,2-Propanediol	
	Cells, dry wt (mg/ml culture)	Bacteriochlorophyll (nmol/ml culture)	Cells, dry wt (mg/ml culture)	Bacteriochlorophyll (nmol/ml culture)
No light	2.8	ND	4.1	0.24
Intermittent light (light, 8 h/day)	3.1	ND*	4.3	0.70
Continuous light	3.0	ND	4.6	ND*

[a] The bacterium was cultivated for 3 days at 28°C aerobically

ND, not detected; ND*, negligibly small, if any

10% potassium chloride. The ether layer was washed with another 500 ml 10% potassium chloride and dehydrated with anhydrous sodium sulfate. After filtration and concentration to dryness of the extract, the pigment dissolved in chloroform was loaded on to a column (2.2 × 2.5 cm) of Yamani silica gel MH-250, which was the best material among several adsorbents tested, including sugars and other commercially available silica gel. First, an orangish pigment was eluted with 30 ml chloroform and then a bluish pigment (bluish color on the column and greenish color in the eluate), which was identified as bacteriochlorophyll as shown later, was collected in a 50 ml fraction. Further development with 1% methanol in chloroform eluted a reddish pigment in a 50 ml fraction and again a bluish pigment in a 60 ml fraction.

The bluish pigment eluted with chloroform was concentrated to dryness and dissolved in diethyl-ether. It was shaken with 1.0 N HCl according to the methods for the preparation of bacteriopheophytin (magnesium-free bacteriochlorophyll) [7]. The ether layer was allowed to stand at 4°C overnight and its ether layer was shaken with 1.0 N HCl and distilled water.

All the solvents were distilled before use and Yamani silica gel was kindly supplied from Yamani Pharmaceutical (Osaka, Japan).

3. Results

P. ruber produces the bluish pigment assumed as bacteriochlorophyll, which has approx. A_{775} max in

Table 2
Growth and bacteriochlorophyll formation in *Pseudomonas* AM 1

Conditions[a]	Cells, dry wt (mg/ml culture)	Bacteriochlorophyll (nmol/ml culture)
No light	2.5	ND*
Intermittent light (light, 8 h/day)	2.2	0.10
Continuous light	1.9	ND

[a] The bacterium was cultivated in the medium containing DL-1,2-propanediol as growth substrate for 6 days at 28°C aerobically

ND, not detected; ND*, negligibly small, if any

chloroform–methanol (1:1, v/v). When *P. ruber* was cultured in the medium containing methanol as the growth substrate, the formation of the bluish pigment was not detected clearly even under the condition of periodic light (table 1). However, when the bacterium was cultured in the medium containing DL-1,2-propanediol as sole carbon and energy source, a significant amount of pigment was formed. The intermittent illumination enhanced the formation of the pigment, but in continuous illumination no pigment was formed.

Table 2 shows that *Pseudomonas* AM 1 similarly produced the bluish pigment only under the condition of periodic light.

As shown in fig.1, the absorption spectrum of the pigment purified with silica gel is very much like that of the well-known bacteriochlorophyll [9,10]. The peak position of the pigment from *P. ruber* resembles that of bacteriochlorophyll from *Chromatium vinosum* [9]. Furthermore, the spectrum of the pigment treated with HCl is very similar to that of bacteriopheophytin, magnesium-free bacteriochlorophyll. It was thus confirmed that the bluish pigment was a bacteriochlorophyll, which is known to be produced in photosynthetic bacteria. The bluish pigment of the later fraction eluted with 1% methanol in chloroform also had a spectrum similar to fig.1 except that it had a small but clear peak at 677 nm and the peak in the ultraviolet region was higher. It is probable that this compound was formed by modification of the pigment of the faster fraction during the isolation.

4. Discussion

Methanol-utilization by photosynthetic bacterium, *Rhodopseudomonas acidophila* was reported [11] and the similarity of the membrane structure between the photosynthetic bacteria and methylotrophs was observed with the electron microscope [12]. This

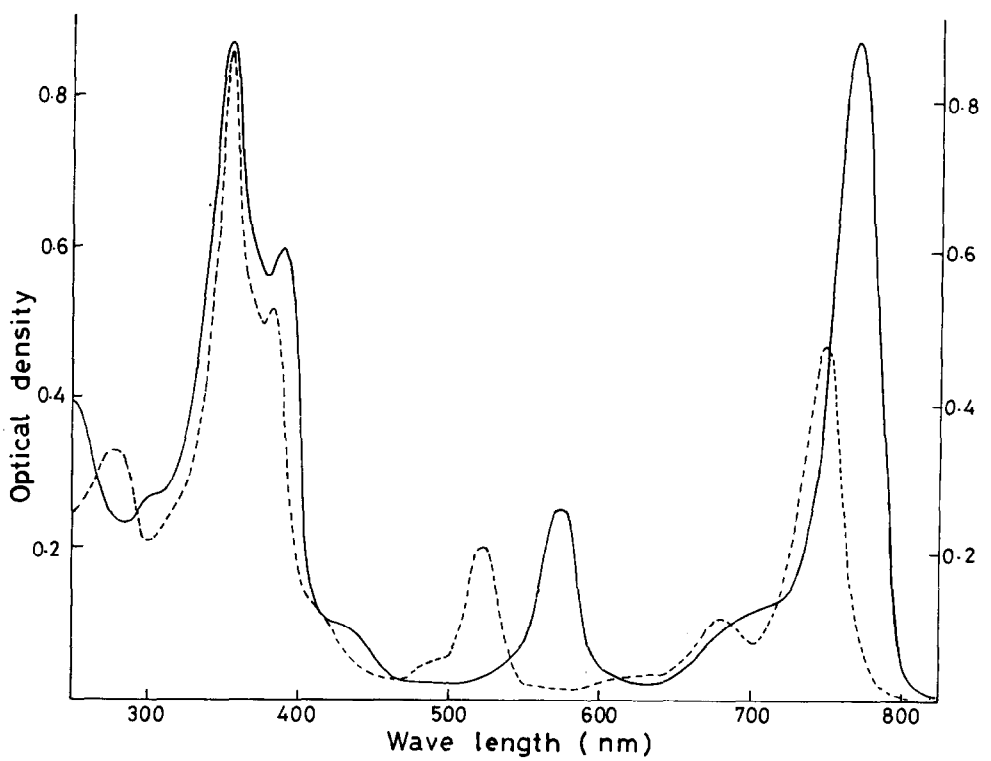

Fig.1. Absorption spectra of the bluish pigment (——) isolated from *P. ruber* and the pigment (– – – –) treated with HCl, both of which were dissolved in diethylether.

may reflect a close relationship between photosynthetic bacteria and non-photosynthetic methylotrophs. Such correlation seems to have become higher, since bacteriochlorophyll was formed by *P. ruber* and *Pseudomonas* AM 1 (fig.1, table 1,2). There is a possibility that at least some of the methylotrophs and related bacteria might be descended from photosynthetic bacteria that have adapted themselves to an oxygen-rich environment. The discovery of the mutant of a photosynthetic bacterium [13], which grows aerobically in the dark and produces bacteriochlorophyll but does not grow anaerobically in the light, also suggests the possibility that methylotrophs may have descended from photosynthetic bacteria.

The formation of bacteriochlorophyll in methylotrophs would be important as a clue in taxonomic studies. Furthermore, it would be informative to study the mechanism of photoinduced, or rather, photoenhanced formation of bacteriochlorophyll as well as photoinduced carotenoid synthesis in photochromogens [6]. The effect of light and culture conditions on bacteriochlorophyll formation will be described elsewhere.

Acknowledgements

I am grateful to Professor S. Shimizu for his interest and encouragement and indebted to Mr M. Hiraoka for his technical assistance in parts of this work. I also wish to thank Professor J. R. Quayle, Department of Microbiology, University of Sheffield, Sheffield, England, for supplying *Pseudomonas* AM 1 and Dr M. E. Coates, National Institute for Research for Dairying, England, for the critical reading of this manuscript.

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