Bacteriochlorophyll-c Formation *via* the Glutamate C-5 Pathway in *Chlorobium* Bacteria

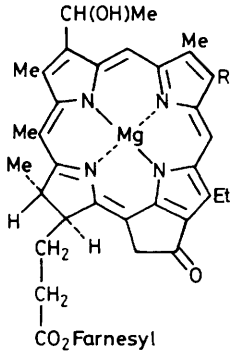
Kevin M. Smith* and Michael S. Huster

Department of Chemistry, University of California, Davis, California 95616, U.S.A.

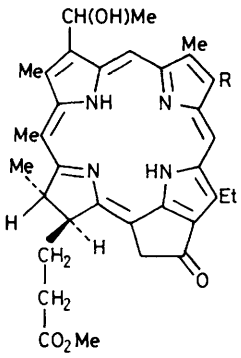
Using carbon-14 and carbon-13 labelled glycine and glutamate, biosynthesis of δ-aminolevulinic acid in *Chlorobium vibrioforme* (strain D), a bacteriochlorophyll-c producing bacterium, is shown to proceed *via* the so-called C-5 pathway; glycine is incorporated into the bacteriochlorophylls-c, but only into the *meso*-methyl and 4- and 5-side-chains by way of methionine.

There now exists abundant evidence to confirm that δ-aminolevulinic acid (ALA) is biosynthesized *via* the C-5 glutamate pathway for incorporation into plant chlorophylls, rather than by the ALA synthase (EC 2.3.1.27) 'Shemin' pathway *via* glycine and succinate precursors.[1] Indeed, exclusive operation of the C-5 pathway in tetrapyrrole biosynthesis in plants has recently been demonstrated,[2] and ALA has been shown to be derived from glutamate in certain algae.[3] ALA synthase activity has been demonstrated in a variety of photosynthetic and non-photosynthetic bacteria,[4] but recently bacteriochlorophyll-a (BChl-a) formation in *Chromatium* has been shown to proceed *via* the C-5 pathway.[5] In this paper we further demonstrate the biosynthetic similarity between plants and bacteria, and show that biosynthesis of BChl-c (**1**) in *Chlorobium vibrioforme* (strain D)†[6] occurs also *via* the C-5 glutamate pathway.

The *Chlorobium* bacteria were cultured in the normal way[7] in the presence of either L-[1-^{14}C]glutamate or [2-^{14}C]glycine to give the BChl-c. Incorporation of C-1-labelled glutamate or C-2-labelled glycine, through ALA, into the BChl-c should give labelling of the carbon atoms circled in (**2**). Both glutamate (1.6%) and glycine (0.6%) were incorporated‡ into

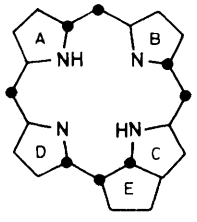


(**1**)

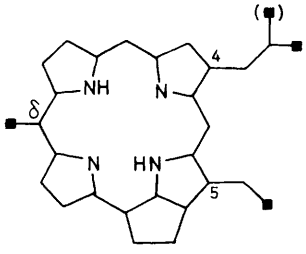


(**3**)

R = Et, Pr^n, or Bu^i



(**2**)



(**4**)

† *Chlorobium vibrioforme* normally produces BChl-d, but the U. C. Davis strain has undergone[6] a light-dependent adaptation/selection such that it now produces mostly (>85%) BChl-c (**1**). Approximate h.p.l.c. analysis shows that 14% of the BChl-c pigments in the Davis strain have R = Et, 64% R = Pr^n, and 22% R = Bu^i.

‡ Incorporation = 100 × (counts isolated/counts fed)%. These carbon-14 feedings were not further optimized.

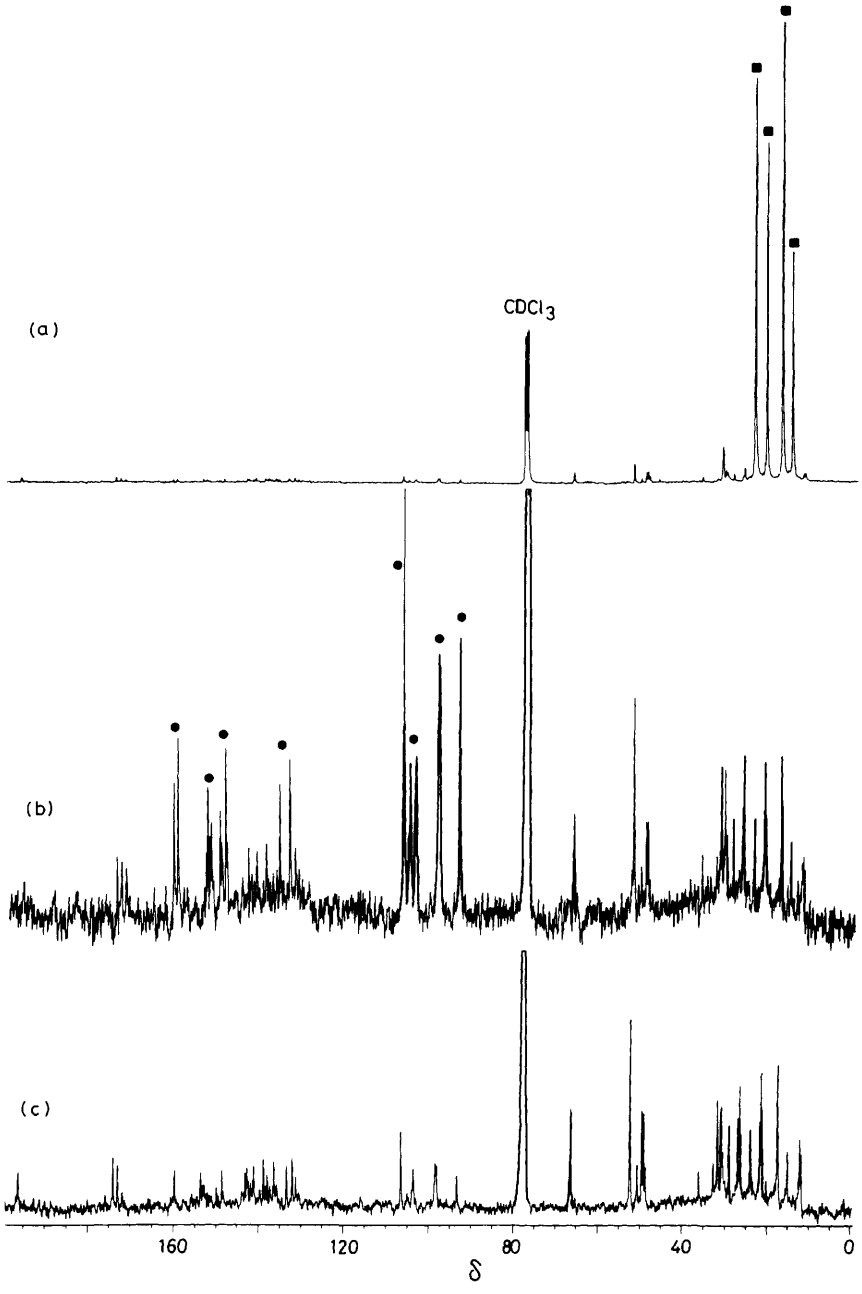


Figure 1. Carbon-13 n.m.r. spectra (90 MHz; Nicolet NT-360), in $CDCl_3$, of the Bmph-c§ from *Chlorobium vibrioforme* (Strain D) grown (a) without any carbon-enriched source; (b) with 99% enriched D,L-[1-^{13}C]glutamate; (c) with 99% enriched [2-^{13}C]glycine. Incorporations of carbon-13 are indicated with black circles for C-1 of glutamate and black squares for C-2 of glycine on the appropriate spectra. Multiple enriched peaks in the glutamate spectra are due to the presence of three pigments varying in the 4-substituent (R = Et, Pr^n, or Bu^i), and by minor amounts (<15%) of the Bmph-d.† Assignments are based on previous literature work[8,9] and on proton coupled spectra. Assignments of enriched peaks in Figure 1(c) are 4-$CH_2CH(CH_3)_2$, 23.21; δ-*meso*-CH_3, 20.47; 5-CH_2CH_3, 16.82; 4-$CH_2CH_2CH_3$, 14.46. The enhanced peak in Figure 1(c) at 30.76 is assigned to the terminal methyl groups of a 5-neopentyl substituent,[7] but final confirmation of this assignment must await h.p.l.c. separations.

the methyl bacteriopheophorbides-c [Bmph-c, (**3**)] isolated§ from the feeding experiments.

These apparent incorporations indicated the possible operation of a dual pathway. Since incorporations were significant, the two experiments were repeated with 99% D,L-[1-^{13}C]-glutamate and 99% [2-^{13}C]glycine (ICN Biomedical). Figure 1a shows the natural abundance carbon-13 n.m.r. spectrum of the mixture of Bmph-c produced by this strain of bacteria. Figure 1b shows the spectrum from the carbon-13 enriched glutamate feeding; on the basis of our previously published carbon-13 assignments[8,9] it is clear that the 1-carbon of glutamate is incorporated into the macrocyclic carbon skeleton (**2**) of the BChl-c, and therefore, *via* the C-5 pathway, into ALA. In contrast, the glycine feeding resulted (Figure 1c) in

§ The cells were filtered off on Celite and treated with acetone to release the pigments which, after evaporation, were treated with 5% sulphuric acid in methanol to give (**3**) after chromatography.

enrichment[8,9] of *only* the *meso*-methyl, the terminal carbons of the 4-propyl and 4-isobutyl, and the terminal carbon of the 5-ethyl group [structure (**4**)]. In expanded spectra, no evidence for incorporation of the C-2 of glycine into the skeleton [*meso* or quaternary carbons, as in structure (**2**)] was apparent.

Incorporation of glycine into the (methionine derived) 10-methoxycarbonyl methyl ester of BChl-a in *Chromatium* has previously been demonstrated.[5] Since we have already shown[8] by carbon-13 labelling that the extra methyl groups in the BChl-c are derived from methionine, our present work represents yet another example of incorporation of the 2-carbon in glycine *via* methionine, and an extremely efficient (Figure 1c) cross-over between these two one-carbon metabolic pathways in the *Chlorobiaceae*.

Added in proof: Oh-hama and coworkers (*Eur. J. Biochem.*, 1986, **159**, 189) have very recently demonstrated that the BChl-c from *Prosthecochloris aestuarii* are derived by the C-5 pathway, in accord with our findings for *Chlorobium vibrioforme*. However, they state, contrary to our findings (Figure 1c), that methionine biosynthesis from the C-2 of glycine is not operating in *Prosthecochloris*. Work in hand will reveal whether the difference in results is due to experimental conditions or due to differences in the strains of bacteria used in the two separate studies.

This research was supported by a grant from the National Science Foundation.

Received, 2nd September 1986; Com. 1267

References

1 P. A. Castelfranco and S. I. Beale, *Ann. Rev. Plant Physiol.*, 1983, **34**, 241.
2 M. A. Schneegurt and S. I. Beale, *Plant Physiol.*, in the press.
3 J. D. Weinstein and S. I. Beale, *Arch. Biochem. Biophys.*, 1985, **237**, 454; B. Gomez-Silva, M. P. Timko, and J. A. Schiff, *Planta*, 1985, **165**, 12.
4 G. D. Clark-Walker, B. Rittenberg, and J. Lascelles, *J. Bacteriol.*, 1967, **94**, 1648; I. A. Menon and D. Shemin, *Arch. Biochem. Biophys.*, 1967, **121**, 304; P. M. Jordan and D. Shemin, 'The Enzymes,' ed. P. D. Boyer, 3rd. edn., Vol. 7, Academic Press, New York, p. 339; G. H. Tait, *Biochem. J.*, 1973, **131**, 389.
5 T. Oh-Hama, H. Seto, and S. Miyachi, *Arch. Biochem. Biophys.*, 1986, **246**, 192.
6 K. M. Smith and F. W. Bobe, *J. Chem. Soc., Chem. Commun.*, in the press.
7 K. M. Smith and D. A. Goff, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1099.
8 G. W. Kenner, J. Rimmer, K. M. Smith, and J. F. Unsworth, *J. Chem. Soc., Perkin Trans. 1*, 1978, 845.
9 K. M. Smith, M. J. Bushell, J. Rimmer, and J. F. Unsworth, *J. Am. Chem. Soc.*, 1980, **102**, 2437.