Biosynthesis of Bacteriochlorophyll-c *via* the Glutamate C-5 Pathway in *Chloroflexus aurantiacus*

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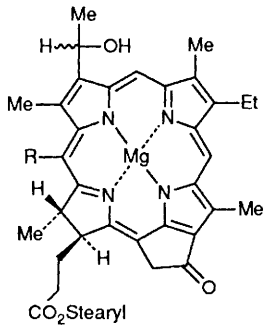
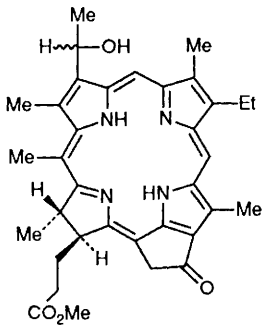
Administration of L-[1-^{13}C]glutamic acid to growing cultures of *Chloroflexus aurantiacus* (J-10-fl) causes enrichment of the corresponding carbons in the macrocycle of the resulting bacteriochlorophyll-c pigment, indicating that δ-aminolevulinic acid in this gliding filamentous bacterium is biosynthesized by way of the C-5 pathway; studies using carbon-13 enriched glycine and methionine are also compatible with this conclusion.

Tetrapyrrolic natural products are known to be biosynthesized from δ-aminolevulinic acid (ALA) in all organisms. For haem biosynthesis in animals, fungi, and some bacteria, ALA is synthesized *via* the 'Shemin pathway' which involves the condensation of succinyl CoA and glycine catalysed by ALA synthase.[1] For chlorophyll (Chl) biosynthesis in plants an alternative pathway for ALA formation has been demonstrated which involves the C-5 pathway.[2,3] In this process, glutamate is converted enzymatically into ALA, probably by way of glutamate semi-aldehyde. Research has shown that phototrophic prokaryotes use both types of pathway. Several species of the nonsulphur purple bacteria (*Rhodospirillaceae*) synthesize ALA from succinate and glycine.[4,5] Recently bacteriochlorophyll-a (BChl-a) formation in *Chromatium*[6] and bacteriochlorophyll-d (BChl-d) and BChl-c formation in *Chlorobium limicola* and *vibrioforme*[5,7] have been shown to proceed *via* the C-5 pathway. The thermotropic photosynthetic bacterium *Chloroflexus aurantiacus*, which has received great attention with regard to its photosynthetic apparatus,[8] features the BChl-c **1** as its major pigment,[9] though minor amounts of the de-methyl compound **2** have also recently been identified in the cultures.[10,11] Evidence regarding the pathway for formation of ALA and the BChl-c **1** in *Chloroflexus aurantiacus* is much more ambiguous; whereas neither glycine nor glutamate was shown to be effective biosynthetic precursors of ALA in *Chloroflexus*,[5] the biosynthesis of ALA in a cell-free preparation from this organism was inhibited by RNAse,[12] and BChl-c **1** synthesis was also inhibited by gabaculine (3-amino-2,3-dihydrobenzoic acid),[13] a known suppressor of the C-5 pathway.[14,15] In this paper we provide evidence that BChl-c formation in *Chloroflexus aurantiacus* (J-10-fl) occurs through the C-5 glutamate pathway.



1 R = Me
2 R = H



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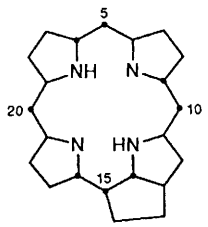

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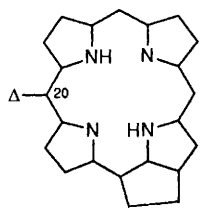

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Incorporation of C-1 labelled glutamic acid, through ALA into the BChl-c should give labelling of the carbon atoms circled in structure **3**. Fig. 1*a* shows the natural abundance carbon-13 NMR spectrum† of methyl bacteriopheophorbide-c (Bmph-c) **4** isolated from *Chloroflexus*.‡ Incubation of the bacteria in the presence of L-[1-^{13}C]glutamic acid provided BChl-c which was enriched in ^{13}C. Fig. 1*b* shows the spectrum of the Bmph-c from the carbon-13 enriched glutamic acid feeding experiment; on the basis of our previously published carbon-13 assignments[17,18] it is clear that the 1-carbon of glutamate is incorporated into the macrocyclic carbon skeleton **1** of the BChl-c, according to the pattern shown in **3**.§

Incubation of *Chloroflexus* with [2-^{13}C]glycine showed incorporation only at the 20-methyl carbon (structure **5**) as determined from the carbon-13 NMR spectrum (Fig. 1*c*). Biosynthesis of BChl-c *via* the 'Shemin pathway,' (*i.e.*, condensation of glycine and succinyl CoA eventually to form ALA), would give the same incorporation pattern as presented in structure **3**. Since there was no evidence of incorporation of the C-2 of glycine into the skeleton (*meso* or quaternary carbons, as in structure **3**, any possibility that ALA formation might result from a dual pathway is ruled out and the 'Shemin pathway' is inoperative for BChl-c (and by inference, ALA) biosynthesis in *Chloroflexus aurantiacus*. Feeding studies using L-[^{13}CH$_3$-S-]methionine gave carbon-13 NMR results (Fig. 1*d*) identical with those of the glycine feeding experiment. Interestingly, incorporation of glycine-derived methionine into the δ-methyl of BChl-c has also been demonstrated in the *Chlorobium* bacteria.[7]

Our studies have demonstrated unequivocally that ALA biosynthesis in *Chloroflexus aurantiacus*, strain J-10-fl, proceeds by way of the C-5 pathway, and that this organism can be added to the growing list of those using glutamate for

† Carbon-13 NMR spectra were measured on a General Electric QE300 spectrometer at 75.47 MHz in $CDCl_3$ with the $CDCl_3$ as internal reference at 77.00 p.p.m. Typically, spectra were run overnight with approx. 20000 acquisitions and a sweep width of 20 kHz (32k data points). Pulse width was 3.5 μs (90°) with acquisition time 0.8 s and delay of 1.0 s.

‡ The *Chloroflexus aurantiacus*, strain J-10-fl, was a generous gift from Professor R. E. Blankenship (Arizona State University), and was grown photosynthetically at 55 °C in the medium described by Pierson and Castenholtz.[16]

§ Enriched peaks are observed (Fig. 1*b*) at δ 97.409 (C-5), 102.448 (C-10), 105.573 (C-15), 105.879 (C-20), 132.697 (C-4), 147.500 (C-9), 151.415 (C-14) and 159.186 (C-16). In Fig. 1*c* the enriched peak appears at δ 20.394 (C-20-CH_3), and in Fig. 1*d*, at δ 20.405 (C-20-CH_3).

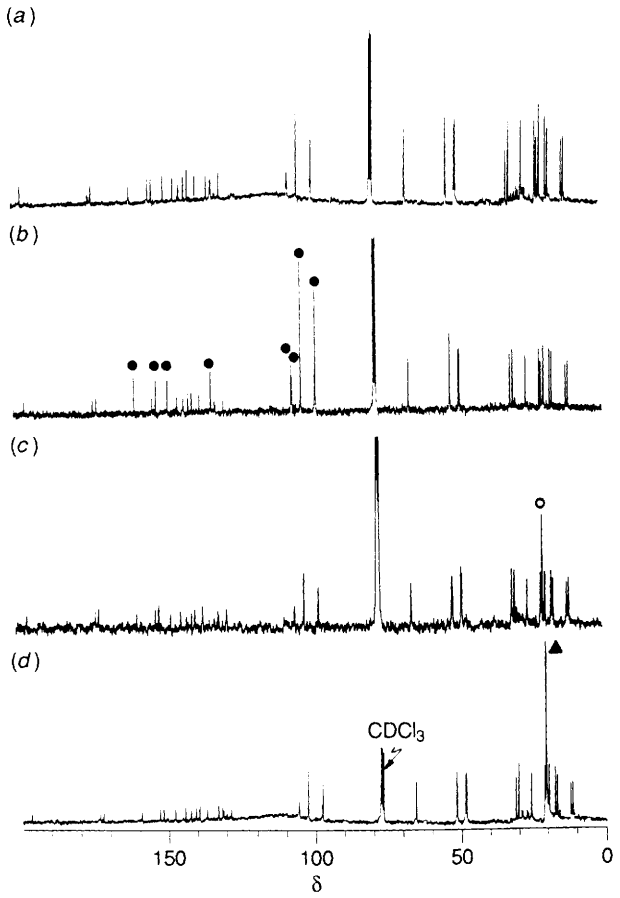

Fig. 1 Carbon-13 NMR spectra of Bmph-c from *Chloroflexus aurantiacus* (J-10-fl) grown (*a*) without any carbon enriched source; (*b*) with 99% L-[1-^{13}C]glutamic acid; (*c*) with 99% [2-^{13}C]glycine and (*d*) with L-[$^{13}CH_3$-S-]methionine. Incorporations of carbon-13 in the appropriate spectra are indicated with eight black dots for C-1 of glutamate, with an open circle for incorporation of glycine, and with a black triangle for incorporation of methionine. Assignments§ are based on previous literature work.[17,18] Owing to dilution of the administered labelled glutamate with endogenous precursors, no ^{13}C–^{13}C couplings of adjacent labelled carbons are observed.

synthesis of their Chl or BChl macrocycles. The results definitively establish a further link between *Chloroflexus* and the *Chlorobium* bacteria. Earlier work suggested the possibility of a novel mechanism for ALA formation in the organism because neither ^{14}C-glycine nor ^{14}C-glutamate were apparently incorporated into ALA isolated from it. The reasons for these conflicting observations are not entirely clear, but doubt was already cast upon them by recent inhibition experiments where the initial t-RNA step[12] and the final transamination step[13] in the C-5 pathway to ALA were inhibited in *Chloroflexus*.

This work was supported by a grant from the National Science Foundation (CHE-90-01381).

Received, 14th August 1990; Com. 0/03740K

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