Multiple Types of 8-Vinyl Reductases for (Bacterio)Chlorophyll Biosynthesis Occur in Many Green Sulfur Bacteria[▽]

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Two 8-vinyl reductases, BciA and BciB, have been identified in chlorophototrophs. The *bciA* gene of *Chlorobaculum tepidum* was replaced with genes similar to *bciB* from other green sulfur bacteria. Pigment analyses of the complemented strains showed that the *bciB* homologs encode 8-vinyl reductases similar to those of cyanobacteria.

Chlorophototrophic bacteria, algae, and plants synthesize chlorophylls (Chls) and/or bacteriochlorophylls (BChls) from the common substrate protoporphyrin IX (1, 4, 7, 12, 20). During the biosynthesis of most (B)Chls, the C-8 vinyl side chain on the tetrapyrrole B ring is reduced to an ethyl group, which may be further modified (7, 12). Exceptions include the 3,8-divinyl (DV) Chl *a* and *b* molecules that are synthesized by some *Cyanobacteria* (18).

Until AT5G18660 of *Arabidopsis thaliana* was shown to encode 8-vinyl reductase (14, 15), BchJ was mistakenly believed to be the enzyme responsible for the C-8 vinyl reduction in bacteria (6, 17). A homolog of the *A. thaliana* 8-vinyl reductase, designated *bciA*, is encoded by open reading frame (ORF) CT1063 in the green sulfur bacterium *Chlorobaculum tepidum*, and its product was also shown to have 8-vinyl reductase activity (6). However, many organisms which are known to synthesize monovinyl (MV) (B)Chls do not have BciA homologs (6); this means that another type of 8-vinyl reductase must occur in these organisms. In the cyanobacterium *Synechococystis* sp. strain PCC 6803, ORF slr1923 encodes a product essential for C-8 vinyl reduction (9, 10). Homologs of slr1923 are found in most but not all chlorophototrophic organisms without *bciA* homologs (10). However, heterologous expression of slr1923 in *Escherichia coli* was unsuccessful (10). Therefore, it was unclear whether the product of slr1923 was sufficient to catalyze the 8-vinyl reductase activity, required another protein(s) to form a multisubunit enzyme complex, or played some regulatory role. To acknowledge its role in (B)Chl biosynthesis, Bryant et al. designated slr1923 and its homologs as *bciB* (2).

Heterologous expression of slr1923 in *E. coli* could have failed to reveal the expected 8-vinyl reductase activity for many reasons, including improper folding or conformation, inability to introduce a cofactor, or absence of required electron donors. These factors might be corrected by expressing appropriate genes from a different expression platform. This idea was tested by constructing a

C. tepidum strain ($\Delta bciA::bciB^{Cher}-aadA$) in which the native *bciA* gene (CT1063) was replaced by *bciB* (*Ctha_1208*) from another green sulfur bacterium (GSB), *Chloroherpeton thalassium*, together with a selectable *aadA* antibiotic resistance cartridge (5) (Fig. 1A). A negative-control strain ($\Delta bciA::aadA$), in which the *bciA* gene was simply replaced with *aadA*, was also constructed (Fig. 1B). These constructs were made in plasmid pUC19 as described previously (11) and were designed so that *bciA* was completely replaced from start to stop codons. The resulting plasmids were linearized with AhdI and used to transform *C. tepidum* to spectinomycin/streptomycin resistance (3). Complete segregation of alleles was verified by PCR (Fig. 1C) and DNA sequencing of PCR products. Pigments were extracted from cells using acetone-methanol (7:2, vol/vol) and analyzed by reversed-phase high-pressure liquid chromatography (HPLC) as described previously (6).

Consistent with results obtained previously when *bciA* was insertionally inactivated (6), the $\Delta bciA::aadA$ mutant synthesized only two homologs of BChl *c_F* (BChl *c* esterified with farnesol), because the vinyl group at the C-8² position could no longer be methylated by BchQ (8). The Soret absorption peak of BChl *c_F* of the $\Delta bciA::aadA$ mutant was redshifted 10 nm to 445 nm compared to that of BChl *c_F* of wild-type cells (Fig. 2A, B, D, and E). Thus, replacement of *bciA* by *aadA* resulted in the loss of 8-vinyl reductase activity and resulted in the synthesis of [8-vinyl]-BChl *c_F*. However, the $\Delta bciA::bciB^{Cher}-aadA$ strain synthesized the same distribution of BChl *c_F* homologs as did the wild-type cells. The number of homologs, their abundance, and their absorption spectra were identical to those of wild-type cells (Fig. 2A, C, D, and F). These results demonstrated that BciB functionally complemented the loss of 8-vinyl reductase activity caused by deletion of the *bciA* gene. If BciB were a subunit of multisubunit enzyme complex, it would be extremely unlikely that *C. tepidum* would possess genes encoding (all) the other subunit(s) of that complex except BciB. Therefore, it is likely that BciB is sufficient to produce 8-vinyl reductase activity (in the metabolic context of a GSB cell). These experiments do not address the identity of the electron donor to BciB, but they suggest that a donor, perhaps the abundant [8Fe-8S] ferredoxins that are present in these cells (21), is available and efficiently donates electrons to BciB.

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environmental conditions (13, 16). It is possible that the BciA and BciB homologs have a similar complementary relationship, such that each is optimally active under different conditions. However, it is not yet clear what these conditions might be. BciA has no oxygen-sensitive redox cofactors and could be expressed in an active form in *E. coli* (6), but BciB is predicted to contain one or more Fe/S clusters, which might be oxygen sensitive. It is possible that Fe could be limiting in some environments, and the ability to switch to a protein lacking metal cofactors might be advantageous.

The occurrence of multiple divinyl reductases in some GSBs may also underscore the importance of reduced C-8 side chains for BChls in these organisms. In *Cyanobacteria*, mutant strains with DV-Chl *a* grew much slower than wild-type cells under high light (9, 10), because of rapid bleaching of DV-Chl *a* and dissociation of reaction center complexes (19). Disruption of *bciA* in *C. tepidum* caused significant differences in the supra-structure formed by BChl *c* in chlorosomes, which resulted in a 31-nm blueshift of the absorption maximum of the BChl *c* in chlorosomes (6). These changes resulted in less absorption per BChl *c* molecule (see reference 6), less-efficient energy transfer from BChl *c* to the BChl *a* associated with CsmA in the base plates because of the energy gap, and much greater spectral overlap with Chl *a*-containing organisms. All of these changes would lead to lower light-harvesting efficiency and lower growth rates. Although the $\Delta bciA::aadA$ mutant grew only slightly slower (13%) than the wild type under high-light conditions ($\sim 100 \mu\text{mol photons s}^{-1} \text{ m}^{-2}$), it grew at only 49% of the rate of the wild type under low-light conditions ($\sim 20 \mu\text{mol photons s}^{-1} \text{ m}^{-2}$; data not shown). Therefore, the ability to reduce the C-8 vinyl group represents a significant advantage for GSBs, which often live under low- and growth-limiting light conditions.

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