Journal of Photochemistry and Photobiology A: Chemistry 313 (2015) 52–59

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Inactivation of *bciD* and *bchU* genes in the green sulfur bacterium *Chlorobaculum limnaeum* and alteration of photosynthetic pigments in the resultant mutants

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ARTICLE INFO

Article history:
Received 17 March 2015
Received in revised form 2 June 2015
Accepted 9 June 2015
Available online 25 June 2015

Keywords:
Chlorosomes
Bacteriochlorophyll
Carotenoid
HPLC
Isorenieratene biosynthetic pathway
Photo-adaptation

ABSTRACT

The *bciD* and *bchU* genes were earlier identified as relating to the C7-formylation and to catalyzing the C20-methylation, respectively, of bacteriochlorophyll(BChl)-*e* biosynthesis in the green sulfur bacterium *Chlorobaculum* (*Cba.*) *limnaeum*. The resultant Δ*bciD* and Δ*bchU* mutants produced BChl-*c* and BChl-*f*, respectively, as the BChl pigments in the cultured cells. In this study, the mutant deleting both the genes was constructed and successfully accumulated BChl-*d* in the same species. The series of the mutants using the *Cba. limnaeum* RK-j-1 strain enabled us to synthesize the four different BChl pigments, BChls-*c*, *d*, *e*, and *f*, in the identical species. In order to evaluate photo-adaptation mechanisms of green sulfur bacteria, alteration of photosynthetic pigments including carotenoids (Cars) by the mutation as well as illuminated light intensity was investigated using HPLC. For the BChl pigments, inactivation of the *bciD* gene induced drastic changes in the composition of BChl homologs (further methylation at the 8^2-position from the 8-ethyl to isobutyl group) and epimers (inversion of the 3^1-stereochemistry from (3^1R)- to (3^1S)-configuration) compared to inactivation of the *bchU* gene; the total contents of the (3^1S)-epimers accounted for 23–36% in the wild type strain and for 87–90% in the Δ*bciD*/*bchU* double mutant, depending on illuminated light intensity. Such mutation did not affect Car biosynthesis, but the composition was strongly light-dependent. With an increase of illuminated light intensity, Cars possessing aromatic ϕ-end group(s), isorenieratene and β-isorenieratene that were characteristic of the *Cba. limnaeum* species, decreased for all three mutants as well as the wild type strain. Concomitantly, the contents of Cars possessing β-end groups, β-carotene and 7,8-dihydro-β-carotene, increased and were dominant under high-light illumination.

1. Introduction

Green sulfur bacteria are characterized by their light-harvesting systems called “chlorosomes”, which allow their growth under low-light conditions [1,2]. The core part of chlorosomes is composed of the self-aggregates of bacteriochlorophyll(BChl)-*c*, *d*, *e* or *f* molecules, depending on the bacterial strains; the BChl-*f* has been observed only in the genetically constructed mutant of the green sulfur bacterium *Chlorobaculum* (*Cba.*) *limnaeum* originally producing BChl-*e*, but not in nature [3,4]. These BChls are biosynthesized as a mixture of homologs bearing different degrees of methylation at the 8^2- and 12^1-positions as well as epimers at the chiral 3^1-position as shown in Scheme 1 [5–10]; notably, progression of the 8^2-methylation shifts the preferred 3^1-stereochemistry from *R*- to *S*-configuration [11]. The electronic absorption properties of chlorosomes, especially the most red-shifted Q_y absorption band, were regulated by alteration of the composition of the homologs and epimers. The chlorosome containing further methylated homologs with the (3^1S)-configuration exhibited a more red-shift of the Q_y maximum than that containing less methylated homologs with the (3^1R)-configuration [12–14]. This was confirmed by construction of the mutant lacking enzymes BchQ and BchR (methyltransferases) that were involved in the 8^2- and 12^1-methylation, respectively, of BChl-*c* biosynthesis in *Cba. tepidum*; the mutant gave a ∼15 nm blue-shifted Q_y

Abbreviations: APCI, atmospheric pressure chemical ionization; BChl, bacteriochlorophyll; Car, carotenoid; *Cba*, *Chlorobaculum*; LCMS, liquid chromatography mass spectrometry; PDA, photodiode array; R[E,M], (3^1R)-8-ethyl-12-methyl; R[E,E], (3^1R)-8,12-diethyl; R[I,E], (3^1R)-8-isobutyl-12-ethyl; R[P,E], (3^1R)-8-propyl-12-ethyl; S[E,E], (3^1S)-8,12-diethyl; S[I,E], (3^1S)-8-isobutyl-12-ethyl; S[N,E], (3^1S)-8-neopentyl-12-ethyl; S[P,E], (3^1S)-8-propyl-12-ethyl.

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http://dx.doi.org/10.1016/j.jphotochem.2015.06.012
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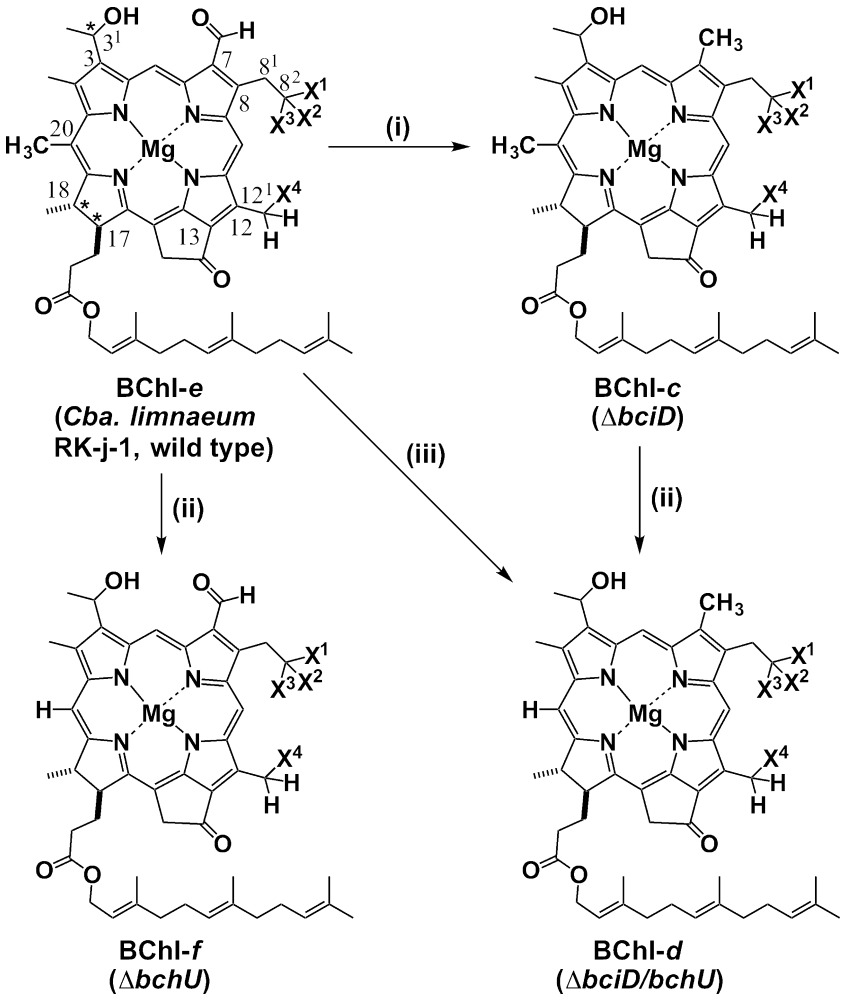

Scheme 1. Inactivation of the *bciD* and *bchU* genes of the BChl-*e* biosynthesis in *Cba. limnaeum* RK-j-1 strain and molecular structures of BChls in the resultant mutants: mutation of the *bciD* (i), *bchU* (ii) and *bciD*/*bchU* (iii) genes. Optically active carbons are indicated by asterisks in BChl-*e*. X^1, X^2, X^3 and X^4 = H or CH_3.

absorption compared to the corresponding wild type strain [14]. It was also demonstrated that the methylation proceeded with low-light illumination on bacterial cultivation [12,15,16], and thus the alteration of the composition of BChl homologs and epimers has been regarded as one of photo-adaptation of green sulfur bacteria.

In addition to the BChl pigments mentioned above, green sulfur bacteria are unique in their biosynthesis of carotenoid (Car) pigments characterized by an aromatic ϕ-end group. The bacteria producing BChl-*c* or BChl-*d* accumulate mono-cyclic chlorobactene, while those producing BChl-*e* biosynthesize di-cyclic isorenieratene and β-isorenieratene (see the structures in Scheme 2). Formation of the characteristic ϕ-end group was investigated using *Cba. limnaeum* species and the gene encoding its formation has already been identified [17,18]. However, Cars in green sulfur bacteria have been less studied compared to BChls, especially for the details of the composition as well as distribution in the photosynthetic apparatuses, since the contents of Cars in the organisms are much lower than those of BChls [19–21].

Some strains of *Cba. limnaeum* were isolated from the Black Sea at the depth of 80–140 m [1,22]. These strains revealed extreme low-light adaptation of growth compared to the other green sulfur bacteria containing BChl-*c* or BChl-*d*. Hirabayashi et al. reported that the composition of Cars in *Cba. limnaeum* was strongly light-dependent [19]. With an increase of illuminated light intensity on the species, its characteristic Cars with ϕ-end group(s), isorenieratene and β-isorenieratene, drastically decreased with the concomitant increase of Cars with cyclic β-end groups, β-carotene and 7,8-dihydro-β-carotene, whereas the composition of BChl-*e* homologs and epimers did not change. The compositional changes showed that *Cba. limnaeum* was photo-adapted by the alteration of the composition of Car pigments, but not BChls. Based on these observations, we have spotlighted *Cba. limnaeum* species and constructed its mutant deleting genes involved in BChl-*e* biosynthesis to produce the other BChls-*c*, *d* and *f* in the species. For example, inactivation of the *bciD* and *bchU* gene involved in the C7-formylation and the C20-methylation, respectively, of BChl-*e* biosynthesis, afforded BChl-*c* and BChl-*f* in *Cba. limnaeum* as shown in Scheme 1 [3,23]. In this study, we further constructed the mutant deleting both genes in order to produce BChl-*d* in the same strain. The series of the mutants of *Cba. limnaeum* as well as its wild type strain could produce four different BChls, BChl-*c*, *d*, *e* and *f*, in the identical species. To clarify photo-adaptation of green sulfur bacteria using chlorosomes for their efficient photosynthesis, alteration of the photosynthetic pigments including Cars induced by the mutation as well as by illuminated light intensity was investigated by HPLC.