Journal of Photochemistry and Photobiology A: Chemistry xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

Rapid C8-vinyl reduction of divinyl-chlorophyllide *a* by BciA from *Rhodobacter capsulatus*

Chihiro Azai[a], Manami Kobayashi[b], Tadashi Mizoguchi[b], Hitoshi Tamiaki[b], Kazuki Terauchi[a,b], Yusuke Tsukatani[c,*]

[a] College of Life Sciences, Ritsumeikan University, Shiga 525-8577, Japan
[b] Graduate School of Life Sciences, Ritsumeikan University, Shiga 525-8577, Japan
[c] Research and Development Center for Marine Biosciences, Japan Agency for Marine-Earth Science and Technology, Kanagawa 237-0061, Japan

ARTICLE INFO

Article history:
Received 11 July 2017
Received in revised form 28 August 2017
Accepted 3 September 2017
Available online xxx

Keywords:
Bacteriochlorophyll
BciA
Chlorophyllide oxidoreductase
Pigment biosynthesis
Photosynthesis

ABSTRACT

Divinyl-chlorophyllide *a* (DV-Chlide *a*) is a universal precursor for chlorophyll or bacteriochlorophyll biosynthesis in all photosynthetic organisms. Previous mutational analyses revealed that BciA works for reduction of the C8-vinyl group of DV-Chlide *a*. Chlorophyllide oxidoreductase (COR) reduces the C7=C8 double bond of Chlide *a* bearing the C8-ethyl group, but also potentially catalyzes the reduction of the C8-vinyl group of DV-Chlide *a*. In this study, we prepared a recombinant BciA protein from the purple photosynthetic bacterium *Rhodobacter capsulatus* and analyzed its C8-vinyl reduction activity towards DV-Chlide *a*. BciA formed a functional oligomeric complex consisting of at least three rigid dimers. BciA required NADPH as an electron donor for its C8-vinyl reduction activity. The enzymatic activity of BciA towards the substrate DV-Chlide *a* was much higher than that of COR towards Chlide *a*. Phylogenetic distribution and the enzymatic parameters of BciA and COR suggest that BciA is a more recently acquired auxiliary C8-vinyl reductase, attained to meet the high demand for bacteriochlorophylls used to produce large amounts of light-harvesting complexes.

1. Introduction

Chlorophyll (Chl) and bacteriochlorophyll (BChl) pigments are derivatives of a cyclic tetrapyrrole compound, divinyl-protochlorophyllide *a* (DV-PChlide *a*), and are essential for the primary photochemical reaction in natural photosynthesis (Fig. 1). Chl pigment species have a chlorin ring as a backbone structure and this ring contains a C17–C18 single bond. In contrast, BChl pigment species have a bacteriochlorin ring-based backbone structure that contains a C7–C8 as well as a C17–C18 single bond. Most Chl and BChl species, *e.g.*, Chl *a* and BChl *a*, have an ethyl group at the C8 position [1,2]. The C8-ethyl group is formed from the C8-vinyl group through catalysis by divinyl reductase.

BciA and BciB are the two major divinyl reductases and catalyze C8-vinyl reduction of the pigment intermediates DV-PChlide *a* or divinyl-chlorophyllide *a* (DV-Chlide *a*) [1–10] (Fig. 1). BciA is a plant-type NADPH-dependent divinyl reductase (EC 1.3.1.75) [3–5], whereas BciB is a cyanobacteria-type divinyl reductase (EC 1.3.7.13) which utilizes ferredoxin as an electron donor [6–9,11]. Most BChl *a*-producing anoxygenic photosynthetic bacteria have either BciA or BciB, although some strains have both. One exception to the distribution of divinyl reductase in BChl *a*-producing bacteria is *Roseiflexus (Rof.) castenholzii*, which lacks both BciA and BciB [6,12].

Chlorophyllide (Chlide) oxidoreductase, abbreviated COR, is a unique BChl biosynthesis enzyme which shows distinct substrate recognition and hydrogen addition activities, depending on the host strain. COR in BChl *a*-producing phototrophic bacteria (*a*-COR) catalyzes the C7=C8 double bond reduction of Chlide *a*, whereas COR in BChl *b*- and BChl *g*-producing bacteria (*b*/*g*-COR) forms the C8-ethylidene group using DV-Chlide *a* as the substrate [12–16]. In addition, it was recently reported that *a*-COR potentially has catalytic activity for C8-vinyl group reduction, and is therefore recognized as a third divinyl reductase [12,14]. Indeed, a recent genetic engineering study revealed that the ability of *a*-COR is the reason why *Rof. castenholzii* can synthesize BChl *a* without BciA or BciB [12].

COR participates in formation of the bacteriochlorin ring and is probably present in most of anoxygenic phototrophic bacteria. Especially, all the BChl *a*-producing anoxygenic phototrophic bacteria are considered to have BciA/B and also *a*-COR as a potential divinyl reductase, with the exception of *Rof. castenholzii*. Comparative analysis of the enzymatic parameters of these two,

* Corresponding author.
E-mail address: tsukatani@jamstec.go.jp (Y. Tsukatani).

http://dx.doi.org/10.1016/j.jphotochem.2017.09.010
1010-6030/

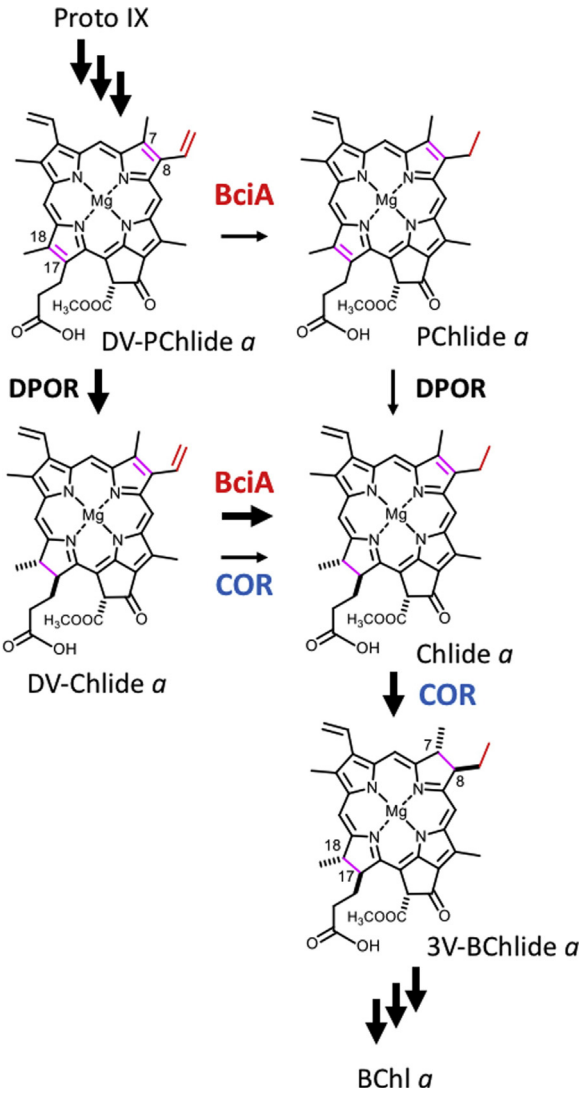

Fig. 1. A schematic diagram of the late steps of chlorophyllous pigment biosynthesis in anoxygenic phototrophs. Arrows between the compounds represent directions of biosynthetic reactions. The bold arrows indicate preferred routes *in vivo*. Abbreviations: Proto IX, protoporphyrin IX; DV-PChlide *a*, divinyl-protochlorophyllide *a*; PChlide *a*; protochlorophyllide *a*; DV-Chlide *a*; divinyl-chlorophyllide *a*; Chlide *a*; chlorophyllide *a*; 3V-BChlide *a*, 3-vinyl-bacteriochlorophyllide *a*.

very different divinyl reductases will provide new insights into pigment biosynthetic pathways. In this study, we prepared a recombinant BciA of *Rhodobacter (Rba.) capsulatus,* a BChl *a*-producing purple photosynthetic bacterium which has BciA and *a*-COR for the C8-vinyl reduction [12], and determined its kinetic parameters for the C8-vinyl reduction of DV-Chlide *a*. Compared to the enzymatic activity of *a*-COR from *Rba. capsulatus*, BciA has a lower affinity for DV-Chlide *a* but a much higher specific activity. The physiological significance of BciA for C8-vinyl reduction and evolutionary aspects of this enzyme are discussed.

2. Materials and methods

2.1. Bacterial strains and cultures

Rba. capsulatus was cultured at 30 °C in PYS medium [17]. The *E. coli* strains DH5α and Rosetta2 were used for DNA cloning and recombinant protein expression, respectively. The *E. coli* cells were routinely cultivated at 37 °C in LB medium supplemented with 100 μg/mL of ampicillin for selection and maintenance of the expression vector described below.

2.2. Construction of the E. coli expression vector for BciA

We cloned the *bciA* gene from *Rba. capsulatus* SB1003 (RCAP_rcc03260) into the plasmid vector pASK-IBA5plus (IBA, Göttingen, Germany) to express recombinant BciA protein with an N-terminal Strep-tag II in *E. coli*. The DNA fragment containing the *bciA* gene was amplified from the genomic DNA of *Rba. capsulatus* using the oligonucleotide primers Rcap-bciA-5F (ATGGTAGGTCTCAGCGCCAAAGATCTGAAATTCCGTGTGCTTT) and Rcap-bciA-5R (ATGGTAGGTCTCATATCAAAAGATCGCGTGATCGCCGCG). The amplified DNA fragment was digested with *Bsa*I and ligated with *Bsa*I-digested pASK-IBA5plus, resulting in the plasmid pASK-RcbciA. The nucleotide sequence of the cloned *bciA* gene was confirmed by Sanger DNA sequencing.

2.3. Expression and purification of the recombinant BciA protein

The expression vector pASK-RcbciA was transformed into *E. coli* strain Rosetta2 and pre-cultured at 37 °C for 16 h. The pre-culture was inoculated into 1 L of fresh LB medium to a turbidity of 0.2 at 600 nm. When the culture attained a turbidity of between 0.4 and 0.5 at 600 nm, 200 μg/L anhydrotetracycline was added to induce BciA protein expression. After 5-h cultivation of the induced culture at 37 °C, the cells were harvested by centrifugation at 10,000 × *g* for 15 min at 4 °C and frozen at −80 °C until use.

The cell pellet was thawed on ice, suspended in 50 mL Buffer A (100 mM Tris-HCl (pH 8.0), 1 mM EDTA, 150 mM NaCl), and disrupted by sonication. The following procedures were performed at 4 °C. After removing the cell debris by centrifugation at 17,900 × *g* for 10 min, the supernatant was loaded onto a 2-mL column of Strep-Tactin Sepharose (IBA, Göttingen, Germany). The column was washed with 100 mL Buffer A, and then the remaining proteins (mainly BciA) were eluted with 10 mL Buffer A supplemented with 2.5 mM desthiobiotin. For further purification, the BciA-enriched elution fraction was directly subjected to gel filtration chromatography using a HiPrep 16/60 Sephacryl S-200 HR column (120 mL column volume, GE Healthcare, Amersham, UK). The running buffer was Buffer A, and the flow rate was controlled at 0.4 mL per minute using an ÄKTA prime system (GE Healthcare). The column was calibrated with the following molecular mass standards (Sigma-Aldrich, St. Louis, MO, USA): blue dextran (to determine the void volume), horse spleen apoferritin (443 kDa), sweet potato β-amylase (200 kDa), yeast alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), bovine erythrocytes carbonic anhydrase (29 kDa) and horse heart cytochrome *c* (12 kDa). The fractions containing BciA were checked by SDS-PAGE and stored at −80 °C until use. For preparation under high-salt conditions, the NaCl concentration of Buffer A was 1.0 M throughout the entire procedure. SDS-PAGE was performed according to Laemmli's method [18].

2.4. Enzymatic assay for C8-vinyl reduction of DV-Chlide a

BciA activity was first assayed by HPLC analysis. Assay mixture (200 μL) containing 100 mM Tris-HCl (pH 8.0), 5.0 μM DV-Chlide *a*, 250 μg/mL BciA and 500 μM NADPH was incubated for 10 min at room temperature, then 800 μL acetone was added to stop the reaction. Equal volume of diethyl ether and then water were added to the solution, and the upper ether phase containing pigments was collected and dried under a stream of nitrogen gas. After dissolving in methanol, the pigments were separated on an HPLC system equipped with a photodiode-array detector (SPD-M20A, Shimadzu, Kyoto, Japan) using a C18 reverse-phase column

Please cite this article in press as: C. Azai, et al., Rapid C8-vinyl reduction of divinyl-chlorophyllide *a* by BciA from *Rhodobacter capsulatus*, J. Photochem. Photobiol. A: Chem. (2017), http://dx.doi.org/10.1016/j.jphotochem.2017.09.010