Docking analysis of models for 4-hydroxy-3-methylbut-2-enyl diphosphate reductase and a ferredoxin from Botryococcus braunii, race B

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Abstract The green microalga Botryococcus braunii Showa, which produces large amounts of triterpene hydrocarbons, exclusively uses the 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway for isoprenoid biosyntheses, and the terminal enzyme in this pathway, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR), is regarded as a light-dependent key regulatory enzyme. In order to investigate the possible association of HDR and ferredoxin in this organism, we constructed tertiary structure models of B. braunii HDR (BbHDR) and one of ferredoxin families in the alga, a photosynthetic electron transport F (BbPETF)-like protein, by using counterparts from E. coli and Chlamydomonas reinhardtii as templates, respectively, and performed docking analysis of these two proteins. After docked models are superimposed onto their counterpart proteins in a non-photosynthetic organism, Plasmodium falciparum, the BbPETF-like protein comes in contact with the backside of BbHDR, which was defined in a previous report (Rekittke et al. 2013), and the distance of the two Fe–S centers is 14.7 Å. This distance is in almost the same level as that for P. falciparum, 12.6 Å. To our knowledge, this is the first model suggesting the possible association of HDR with a ferredoxin in O2-evolving photosynthetic organisms.

Key words: 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, Botryococcus braunii, docking model, ferredoxin, photosynthetic electron transport F-like protein.

The colonial green microalga Botryococcus braunii B race can produce large amounts of triterpene hydrocarbons, namely botryococenes and methylsqualenes. However, key regulatory systems that produce precursors for these compounds are yet to be elucidated. B. braunii uses the 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway to provide universal precursors of isoprenoids to biosynthesize triterpenes (Sato et al. 2003). Though three isogenes of 1-deoxy-D-xylulose 5-phosphate synthase (DXS), the enzyme catalyzing the initial step in the MEP pathway, have been cloned and enzymatic properties of corresponding recombinant proteins were studied, catalytic efficiencies of these recombinant proteins were not particularly superior to those of DXSs in other organisms (Matsushima et al. 2012). Therefore, further studies on enzymes catalyzing other steps in the MEP pathway are required to better understand the mechanism that supplies isoprenoid precursors to achieve efficient hydrocarbon production by this alga. Figure 1 shows the outline of metabolic pathway of isoprenoids in green algae. Using pyruvate and glyceraldehyde 3-phosphate (GAP), which are provided from Calvin cycle, the universal precursors of all isoprenoids, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are produced in the MEP pathway. 4-Hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR) catalyzes the terminal reaction of the pathway. In higher plants, upregulation of gene expression for HDR resulted in a higher accumulation of carotenoids (Botella-Pavía et al. 2004). In the green alga Dunaliella salina, HDR is known to be a key enzyme for large accumulations of β-carotene in response to environmental cues (Ramos et al. 2009). As such reports, it can be estimated that B. braunii HDR (BbHDR) might be involved in the regulation of isoprenoid production, resulting in hydrocarbon yield.

Note

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Physiological analyses of MEP pathway enzymes have been well performed in a non-photosynthetic unicellular organism, *Plasmodium falciparum*. This organism is a protozoan parasite to cause malaria in humans. Inactivation of HDR in *P. falciparum* (*PfHDR*) is expected to alleviate membrane sterol production, which would inhibit cell division. A previous report showed that *PfHDR* was associated with an electron transport protein, ferredoxin (Röhrich et al. 2005).

Some recent studies have indicated that HDR is activated by light (Ershov 2007) and ferredoxin is transiently bound with photosystem I subunits in algae (Cashman et al. 2014). However, the concrete mechanisms of light regulation of this enzyme in photosynthetic organisms are yet to be revealed. In order to check the possible association of HDR with ferredoxin in *B. braunii*, we made models of *BbHDR* and one of ferredoxins, photosynthetic electron transport F (PETF)-like protein and performed docking analyses for them. The present study might provide insight to reveal regulation of the biosynthesis of precursors for triterpenes in *B. braunii*.

The *BbHDR* cDNA sequence information was retrieved from the DDBJ database with an accession number of LC090196. Entire region of *BbHDR* amino acid sequence possessed similarity and identity to *Plasmodium falciparum* 3D7 sequence (CAD49005.1, 69% and 28%, respectively) and to *Escherichia coli* K12 sequence (P62623.1, 69% and 24%, respectively) (Uchida et al. 2018a). In correspondence to the conserved cysteines in *E. coli* HDR sequence, *BbHDR* included three cysteines at the 140th, 232th and 363th residues (Uchida et al. 2018a). *BbHDR* sequence was uploaded into the SWISS-MODEL server (https://swissmodel.expasy.org/) (Biasini et al. 2014), which was accessed on July 27, 2016, and the three-dimensional structure model was built using a crystal structure of HDR from *E. coli* (PDB ID: 3F7T) as a template. Configurations of above-mentioned cysteine residues in *BbHDR* model and *E. coli* structure were well conserved (Supplemental data).

In order to find a ferredoxin homolog in *Botryococcus braunii* for our docking analysis, a transcriptome database constructed from RNAseq data (Uchida et al. 2015) was mined with TBLASTN using the amino acid sequence of *Chlamydomonas reinhardtii* photosynthetic electron transport F protein (*CrPETF*, protein ID 147787 in the *Chlamydomonas* genome v4.0) as a query. The reason why the PETF was selected as the representative from various members of ferredoxin is because it is coupled with the photosynthetic system I complex (Knaff and Hirasawa 1991). The highest score hit of a *B. braunii* contig, which encoded a putative protein with 81 amino acid residues, was obtained with an *E*-value of 1e-033. The cDNA nucleotide sequence for this contig was registered in DDBJ with the accession number of LC202849. The encoded protein was referred to as *B. braunii* PETF-like (*BbPETF-like*) protein.

The *BbPETF*-like protein was uploaded to SWISS-MODEL and a three-dimensional model was built using
CrPETF as a template. The models for BbPETF-like protein and BbHDR were then uploaded to ZDOCK 3.0.2 (http://zdock.umassmed.edu/, which was accessed on December 8, 2016) (Pierce et al. 2011), from which we obtained 10 predicted protein complexes. In these complexes, the root-mean-square deviations (RMSDs) between a complex and BbHDR were all the same. The same result was also obtained in RMSDs between complexes and the BbPETF-like protein. Using the UCSF chimera software (https://www.cgl.ucsf.edu/chimera/, which was accessed on December 6, 2016) (Pettersen et al. 2004), models for BbHDR or BbPETF-like were matched over complexes. Plasmodium falciparum-derived HDR (PfHDR) and ferredoxin including their Fe–S centers were further aligned onto each B. braunii model and the distances between these Fe–S centers were calculated using Pymol.

In six model complexes among the ten that were analyzed in ZDOCK, BbPETF came in contact with the front-side (Rekittke et al. 2013) of BbHDR, where helix 2 in domain 2 was observed (Figure 2A). In these complexes, the distances between the Fe atoms of HDR and ferredoxin ranged from 21.9 to 32.0 Å. In the other four models among the ten complexes, backside of BbHDR was docked with BbPETF (Figure 2C) and the distances between the Fe atoms of HDR and ferredoxin were 14.7 Å, 14.8 Å, 16.6 Å, and 18.0 Å, respectively. Using the model complex exhibiting the shortest distance between intra-Fe–S centers with a distance of 14.7 Å (Figure 2D), docking planes in reference to the Fe–S centers were observed in the trimmed models (Figure 2E–H). Two Fe–S centers
Docking models of HDR and ferredoxin in *Botryococcus braunii*

for the *Bb*HDR and *Bb*PETF-like protein models that were superimposed with *P. falciparum* structures were observed in the vicinity of the docking points, where marginal projections of the *Bb*PETF-like protein were inserted into *Bb*HDR cavities around the S atom of its Fe–S center (Figure 2G). Interestingly, two Fe atoms (Fe#2 and Fe#3 in Figure 2H, I) of the above-mentioned HDR were situated by an Fe atom in the docked PETF-like protein (Fe#1 in Figure 2F, I) at almost equal distances, at 14.7 Å and 14.8 Å, respectively.

In the apicomplexan *Plasmodium falciparum*, which does not possess a photosynthetic ability, docking analysis of HDR and ferredoxin has previously been reported (Rekittke et al. 2013), and the redox recycling system of HDR using ferredoxin has also been confirmed (Röhrich et al. 2005). Our docking model of *Bb*HDR and the *Bb*PETF-like protein, which were superimposed with corresponding protein structures of *P. falciparum*, revealed that the distance between two Fe–S centers of *Bb*HDR and *Bb*PETF-like protein as a functional ferredoxin was estimated to be 14.7 Å (Figure 2I). To our knowledge, this is the first report of the docking analysis of HDR and PETF-like protein from an O₂-evolving photosynthetic alga, and perhaps even among all O₂-evolving photosynthetic organisms. Our initial docking analysis of *Bb*HDR and *Bb*PETF-like protein could thus serve as key models for designing structure–activity analysis of key regulatory enzymes for terpenoid biosynthesis, and possibly light regulated activity analysis of key regulatory enzymes for terpenoid biosynthesis of various terpenoids. However, expression precursor biosynthesis, and possibly light regulated activity analysis of key regulatory enzymes for terpenoid biosynthesis as a target in identifying of new antibiotics, herbicides, and immunomodulators. *Appl Biochem Biotechnol* 1056: 93–125

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Knaff DB, Hirasawa M (1991), this role can be taken by flavodoxin in most bacterial reactions (Rogers 1987). This fact might suggest that HDR can also interact with flavodoxin, though flavodoxin and ferredoxin do not share common tertiary folds (Ullmann et al. 2000). This possibility should be investigated in the future studies.

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