Review of Studies on the Last Enzymes in Bacteriochlorophyll (Bchl) and Chlorophyll (Chl) Biosynthesis

Silvio M. D. Nascimento, Yuanyuan Zou, Qi Cheng*

Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Email: chengqi@vip.126.com

Received 22 June 2016; accepted 21 August 2016; published 24 August 2016

Copyright © 2016 by authors and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY).

http://creativecommons.org/licenses/by/4.0/

Abstract

This paper summarizes the information available online related to mechanisms of Chlorophyll and Bacteriochlorophyll biosynthesis, emphasizing four enzymes in its last steps. The biosynthesis of Chlorophyll (Chl)/Bacteriochlorophyll (Bchl) is essential for the occurrence of photosynthesis. Four enzymes catalyze parts of the last chemical reactions steps to biosynthesize Bchl/Chl; they are: The Light-Dependent Protochlorophyllide (Pchlide) Oxidoreductase (LPOR: EC 1.3.1.33), the Light-Independent Pchlide oxidoreductase (DPOR: EC 1.3.7.7), Chlide Reductase (COR: EC 1.3.99.35) and the Divinyl Reductase (DVR: EC 1.3.1.75). These enzymes catalyze the reductions reactions of tetrapyrrrole’s rings in the Chlorophyll and Bacteriochlorophyll biosynthesis process. This review has the aim to organize, analyze and compare the most important discoveries related to these four enzymes discovered so far. The comparisons are made with the information from the bibliography used, and the sequence of these enzymes got from online database. Sequence alignment, phylogenetic and molecular evolutionary analysis of all the four enzymes was conducted to find their levels of similarities.

Keywords

Photosynthesis Chlorophyll, Bacteriochlorophyll Biosynthesis, Enzymes DVR, DPOR, LPOR and COR

1. Introduction

There is no consensus about when the process of Photosynthesis started. There are a number of different lines of
evidence which point to an origin: Carbon isotope data, other chemical evidence and the recent fossil record [1] [2]. They all indicate that the process of photosynthesis originated early in Earth’s history, more than 3 billion years ago. They each suggest that photosynthesis evolved to its current mechanistic diversity and phylogenetic distribution by a complex, nonlinear process. Photosynthesis is the processes responsible for the evolution of life, by converting photons of light, the basic source of energy that “feed” the process of photosynthesis, into chemical potential energy. It is dependent on a conserved NADPH₂, ATP and light-capturing made by the pigments Bacteriochlorophyll (Bchl)/Chlorophyll (Chl), which are the primary electron donors that drive the conversion of light into chemical energy to be conserved in NADPH₂ and ATP [3]. These pigments are incorporated within antenna complexes of plants, algae and phototrophic bacteria, including also other apparatus as electron transfer complexes protein-pigment complexes known as reaction centers and carbon fixation machinery, allowing the harvest of the light energy, and perform photochemical reactions that lead to stable charge separation [1] [4]-[6]. Structural modifications to the tetrapyrrole macrocycle, that has four pyrrole rings (designated A to E) which are ligated into a tetrapyrrole ring with a magnesium atom in the center having the ring D esterified with phytol, are responsible for the specific absorption and energy-transfer features of the light-harvesting apparatus, influencing both pigment-pigment and pigment-protein interactions within the antenna complexes [7] [8]. The Bchl biosynthetic pathway is multibranchled and represents the template of a Chl-protein biosynthesis center where photosystem (PS) I, PSII, and light-harvesting Chl-protein complexes are assembled into functional photosynthetic units [9]. The Chl and Bchl biosynthetic pathway from protoporphyrin IX to Chl begins with the insertion of the Mg²⁺ ion [10]-[12]. After insertion of the Mg²⁺ ion in the tetrapyrrole structure, the enzyme “magnesium-protoporphyrin methyltransferase” esterifies the propionic side chain of ring C in preparation for the cyclization reaction that produces ring E [12]. These rings are synthesized in the Chloroplast from eight molecules of 5-aminolevulinic acid. The porphyrin ring with its conjugated double bonds is assembled in the Chloroplast from eight molecules of 5-aminolevulinic acid, a highly reactive nonprotein amino acid (5-amino, 4-keto pentanoic acid) [3]. The spectral range of these complexes is extended by modifications, Bchls absorb at longer wavelengths than Chls [13]. Modifications as presence of ethyl and vinyl groups, can extend or confine the delocalized π-electron system of the Bchl macrocycle [14] to the Bchl macrocycle, which influence pigment-pigment and pigment-protein interactions within the antenna complexes [15]-[17]. Data shows that these parts have not had the same evolutionary history in all organisms. Therefore, the photosynthetic apparatus is best viewed as a mosaic made up of a number of substructures each with its own unique evolutionary history where the evolutionary histories of the various classes of antenna/light-harvesting complexes appear to be completely independent [2]. The transition from anoxygenic to oxygenic photosynthesis took place when the cyanobacteria started to use water as an electron donor for carbon dioxide reduction [2], marking a key point, and perhaps the point of the origin of the Photosynthesis process. Only later, when a predecessor to modern cyanobacteria acquired the ability to synthesize singly reduced pigments did Chl appear first serving as an antennae pigment (Chlorins in the antennaes of modern Chlorobium) and later as a component of the reaction center [13]. Protochlorophyllide (Pchlide) is known as the main metabolite for the biosynthesis of Chl and Bchls [6]. Traveling through the evolution process of the photosynthetic organisms, analyzing the Chl and the Bchl biosynthesis last steps, starting from Protoporphyrin IX, which is marked by the introduction of Mg metalloclouter, the first enzyme (of those studied in this work) to find is the: Divinyl Reductase (DVR), that can be find in the enzyme databases with the Enzyme entry number: “EC 1.3.1.75”; then Light-Dependent Protochlorophyllide oxidoreductase (LPOR: EC 1.3.1.33); Light-Independent (dark-operative) Protochlorophyllide oxidoreductase (DPOR: EC 1.3.7.7) and the last one is the Chlorophyllide a Reductase (COR: EC 1.3.99.35). This group of enzymes participate in these reducing steps reactions in different rings. The DVR converts the divinyl Chlorophyllide a (Chlide a) to Monovinyl Chlide a [9] [18]. After the action of DVR, the next step is the production of Chlide from Pchlide, which involves the reduction C₁₇ = C₁₈ in the D-ring (Figure 1) of the Mg-tetrapyrrrole intermediate, Pchlide [19] [20]. There are two homologous enzymes that do the same work, but in different light conditions, one of these homologous is Light-Dependent “LPOR” [21], which requires light for catalysis, and the other one is the Light-Independent “DPOR” [22], which operates in absence of light. These steps are shared with Bchl, having all phototrophs (except angiosperms that have only LPOR, and anoxygenic bacteria, Photosynthetic bacteria having only DPOR), to form respectively Chl, and Bchl. Bchl, that differs from Chl in the substituent rings A and B. The enzyme COR, is the last of these four enzymes of this study enter in action, which is specific of bacterio phototrophics (purple and green eubacteria). COR (called also CAO), perform the additional step that differentiate Bchl from Chl, the stereo-specific reduction of C₇ = C₈ in the B ring (Figure 1), this is chemically similar to the D ring reduction of...
Pchlide to form Chlide [23]. All this information about these enzymes has been put together in this paper. We have compared them with each other, summarized the most important discoveries to date, sequenced alignments and conducted phylogenetic and molecular evolutionary analysis of all the four enzymes.

1.1. Di-Vinyl Reductase (DVR)

This enzyme can be found with different denomination: Di-Vinyl Reductase; Divinyl Chlorophyllide a 8-Vinyl Reductase or 4Vinyl Chlorophyllide a Reductase, but here in this work it will be just “DVR”. DVR converts divinyl chlide a to monovinyl chlide a in the Chl biosynthetic route [9] [18]. The ethyl group at the C8 position (8E) of the macrocycle is produced by the reduction of a vinyl group (8V), catalyzed by an 8VR (8Vinyl reductase; EC 1.3.1.75), resulting in the production of an 8E pigment [17]. The DVR is divided into two types of unrelated enzymes (Figure 1), one in plant reductases, BciA belonging to the SDR family [24], and also having a ligand-binding site using NADPH as electron donors. And the other enzyme, which is in cyanobacterial reductases: BciB which uses ferredoxin as an electron donors and is present in some organisms belonging to Chlorobia, cyanobacteria, eukaryotes and Proteobacteria. BciB has high homology with coenzyme F420-reducing hydrogenase only found in methanogenic archaea [25]-[27]. The BciB tree is one of the few cases where Chloroflexi and Chlorobia are not sister groups, with Chloroflexi, closer to Allochromatium vinosum and Chloracidobacterium thermophilum sequences. Also, BciB shows separation between the major proteobacterial clade and the other photosynthetic taxa [28]. The orthologous (BciA and BciB) genes form a well-supported clade that can be subdivided into two groups, one comprising GSB and Proteobacteria sequences and another with cyanobacteria and eukaryotic sequences. The only exception is the Acaryochloris marina sequence that clusters within the proteobacterial clade. Because of the broad distribution of NAD(P)H-binding sites (Rossmann folds “GxGxxG”) in proteins, database searching retrieved many additional hits, namely at the lower part of the tree [28], but in the protein alignment here for the phylogenetic trees, the DVR, and the other nitrogenase-like enzymes, showed no significant presence of Rossmann folds, except in the case of the L subunit (DPOR).

1.2. Light-Dependent Protochlorophyllide Oxidoreductase (LPOR)

In the Chl biosynthesis, the final reaction that differentiates the angiosperms from the rest of the photosynthetic organisms [3] forms the chlorine structure of Chla at the Pchlide. This step is a key regulatory step in the Chl
1.3. Light-Independent Protochlorophyllide Oxidoreductase (DPOR)

Dark-operative (or Light-Independent) Protochlorophyllide Oxidoreductase (DPOR: EC 1.3.7.7) is the other enzyme that is involved in the reduction of the penultimate step of Bchl/Chl biosynthesis. [39], DPOR catalyzes the same stereo-specific reaction of $C_{17} = C_{18}$ double bond of Pchlide ring D (Figure 1) to form Chlde to produce Chlide [21] [29] [33]. This reaction, requires both light (absorption maximum 638 - 650 nm) and NADPH for activity [3] [21] [34]. The DPOR polypeptide binds both NADPH and Pchlide, but the reduction does not occur until the protochlorophyllide molecule absorbs light at 628 - 630 nm [35]. LPOR is composed of a single polypeptide that belongs to Short-chain Dehydrogenase/Reductase (SDR) family [30] [33]. LPOR is the enzyme responsible for the greening of angiosperms and is the only Pchlide reductase to operate in angiosperms [21] [31] [32] [36]. This starts to act when triggered by light, and plays an important regulatory role in angiosperm development, since it functionally acts as a gate in the biosynthetic pathway allowing Chl synthesis only when the plant is illuminated [37]. According to Yang & Cheng [38] the LPOR gene originated in the cyanobacterial genome before the divergence of eukaryotic photosynthetic organisms, and the photosynthetic eukaryotes obtained their LPOR homologues through endosymbiotic gene transfer proving the finding of Suzuki & Bauer [37], that which defended that LPOR evolved before the advent of eukaryotic photosynthesis and that LPOR did not arise to fulfill a function necessitated either by the endosymbiotic evolution of the chloroplast or by multicellularity; rather, it evolved to fulfill a fundamentally cell-autonomous role. Based on the protein sequence alignments, Wilks and Timko [30] identified two conserved residues (Tyr-275 and Lys-279) within the active site of the enzyme and also showed that they are critical for activity of LPOR.

Figure 1
angiosperms. Some authors [43] [57] [58] suggest that the ChIL protein is associated with membranes and that it may function as an adenosine triphosphate-dependent electron donor, and Reinbothe et al. [22] suggests the L-protein of DPOR might function to sense the partial oxygen pressure in response to the light environment, thus providing a molecular tool to switch from oxygen-sensitive to oxygen-insensitive Pchlide reduction, confirming again this oxygen sensitivity of the DPOR, specifically the L-protein subunit. NB-protein is stable in an aerobic condition [47]. Crystal structures of the DPOR components show that the shortest distance between an NB-cluster and Pchlide is 10.0 Å, which is close enough for permitting the through-space electron transfer reaction [59]. A primary, single electron transfer from the 4Fe-4S cluster of L-protein to NB-cluster of NB-protein occurs and it is similar to that from the 4Fe-4S cluster of Fe-protein to the P-cluster of MoFe-protein in the nitrogenase complex [43] [60]. The activity of DPOR is dependent upon both ATP hydrolysis and a reductant, which is most likely ferredoxin in vivo, and also dependent on the reductant dithionite [43] [44]. Crystallographic analysis of L-protein and NB-protein complex from Prochlorococcus marinus suggested that a water molecule just above C18 is the direct proton donor for C18 rather than the C17-propionate in the Prochlorococcus NB-protein [61], however contribution of the water molecule has not yet been experimentally proven to be critical for Pchlide reduction in the Prochlorococcus DPOR. Results obtained so far in Rodobacter capsulatus DPOR support the reaction mechanism of the C17-propionate as the proton donor to C18 [33].

1.4. Chlorophyllide α Reductase (COR)

In most photosynthetic organisms, the chlorin ring structure of Chla is formed by the reduction of the porphyrin D-ring by the DPOR. Subsequently, the chlorine ring B (Figure 1) is reduced in Bchl biosynthesis to form 3-vinyl bacteriochlorophyllide which has a bacteriochlorin ring structure [23]. This reduction is made by the enzyme: Chloride Reductase (COR: EC 1.3.99.35) which has three subunits: bchX, bchY and bchZ [53], and reduces the C7 = C8 double bond of Chlide [62]. According to Nomata et al. [23] structural changes in COR have special effects on the spectral properties of these compounds enabling them to absorb infrared light to perform anoxenic photosynthesis. COR from the bacterium Rhodobacter capsulatus is described to be able to also reduce the 8V group of Chlidelithus considered the third class of 8VR (DVR), referred as a COR-type reductase [62]. R. capsulatus also contains an orthologue of BciA (the translated sequence of which is 61% identical and 72% similar to BciA from R. sphaeroides). It is likely that organisms with 8E-BChls use COR to reduce the 8V group of any Chlide molecule that has bypassed the conventional 8VR. This mechanism may also account for the lack of any BciA or BciB orthologues in the genomes of 8E BChla-producing Roseiflexus species [63] of green non-sulfur bacteria.

1.5. Similarities between These Enzymes

The DPOR and the COR enzymes show the most similarities with each other (Table 1), both convert porphyrin (Pchlide) to bacteriochlorin (3-vinyl Bchlide a) in Bchl a biosynthesis [47] and both are composed of three subunits [40]. In the amino acid sequence alignments of BchX proteins and the closely related Bchlor ChIL subunits of DPOR, both cysteiny1 ligands responsible for 4Fe-4S cluster formation and residues for ATP binding are conserved [65]. Wätzlich et al. [49] presents, that COR subunit BchX forms a redox-active inter subunit cluster analogous to that described for DPOR subunits of Bchlor ChIL. In addition, the Tyrosine (Tyr127) is proved to be essential for DPOR catalysis, where this surface-exposed residue is directly involved in protein-protein interaction and is responsible for the inter subunit electron transfer [49]. Gene duplication of an ancient reductase gave rise to a nitrogenase (NifH) and a Bchl/Chl branch. This ancient reductase (Chl/Bchl) of the Bchlm/Chl path has thus evolved into the current BchlX and BchlX/Chl proteins. This evolutionary process came along with the appearance of subunits BchNB/Chlb and BchYZ, responsible for the specific reduction of rings B and D, respectively [49]. BchlX and Bchl share 34% amino acid sequence identity [53], also proved by the phylogenetic tree (Figure 2) where the two subunits are close to each other. Relatively to COR, which is also very similar related to DVR (Table 1), is also what was expected after know that it is able to reduce the 8 V group of Chlide, because of that is being considered the third class of DVR [62]. Anaerobic conditions are required for the maximum activity of DPOR to complement the loss of LPOR [66]. Analysis of DPOR and LPOR enzymatic activity, in a variety of species, has demonstrated several functional differences in these enzymes, showing that coordination and regulation of DPOR and LPOR activity are responsive to environmental conditions, which allow the
Figure 2. This phylogenetic tree has 68 sequences divided in all 4 enzymes together DPOR subunits (B, L and N), COR (X, Y and Z), DVR and LPOR (porA, porB and porC). This tree was generated using the neighbor-joining method after the alignment was made using MUSCLE also from the software package of MEGA6 [78].
amino acid sequence homology to the corresponding subunits of nitrogenase. Whereas subunits BchB, BchX, and BchZ enzymes, confirming some mentioned above and new ones. For example the situation of BchB protein shows more similarity with the nitrogenase homologous subunits: BchlA and BchlB (subunits): BchlB/ChlB, BchlC/ChlC and BchlN/ChlN. The reductions made by ferredoxin or flavodoxin and then the electrons are transferred between these two complexes. The other complex is the MoFe-protein, which provides the catalytic centers. It has two types of metalloclusters: the P-cluster (an [8Fe-7S] cluster) and the FeMo-cofactor comprising 1Mo-7Fe-9S-X homocitrate. The Fe-protein is reduced by ferredoxin or flavodoxin and then the electrons are transferred from the [4Fe-4S] cluster of the Fe-protein to the FeMo-cofactor of the MoFe-protein via the P-cluster. The enzymes DPOR and COR (Table 1) are considered twonitrogenase-like enzymes because they share significant amino acid sequence homology to the corresponding subunits of nitrogenase. Whereas subunits Bchl/Chll, BchlX and NifH exhibit a sequence identity at the amino acid level of 33%, subunits Bchl/Chll, BchlX and NifH, BchlY and BchlZ show more similar nitrogenase homologous MoFe protein (a NiFe-NifK heterotetramer) of nitrogenase [23] [31] [40] [43] [44] [46] [47] [53] [74], contrary, the L-protein (DPOR) is most structurally related to Fe-protein (a NifH homodimer) (33% identity and 50% similarity) [44]. Other authors [23] [33] [40] [43] [44] [60] discovered more cases of significant similarities between them such as all of them are dependent on the presence of ATP and reductant dithionite. Nomata et al. [23] showed that the in vitro assay catalytic mechanism of COR strongly resembles DPOR catalysis. X-ray crystallographic, electron paramagnetic resonance, mutagenesis studies and sequencing analysis showed more indepth information about the structure of some of these enzymes, confirming some mentioned above and new ones. For example the N and B proteins (DPOR) have shown that they do not have the requisite number of conserved Cys to assemble as a P-cluster or the FeMo cofactor of the nitrogenase NifH and NifD heterotetramer. Instead, the conserved Cys arrangement in Chll/NifH and Chll/BchlB seems to be more consistent with a cluster similar to the one found in the nitrogenase accessory proteins NifE and NifN [44]. These Cys residuesligate the intra subunit 4Fe-4S cluster, that is chelated by the two proto-mers of L-protein [40]. These Cysresidues are also conserved in Chll/BchlL what also happen to the nitrogenase NifH dimer [44] [45]. In all the nitrogenase iron protein’s sequences and in bchX, the position 100 is occupied by an Arginine (R) residue and in both Bchl and Chll it is Tyrosine (Y) [13]. The Tyr127 is found conserved in all BchI and Chl proteins, whereas the nitrogenase system, as well as the COR system makes use of an Arginine

### Table 1. Resume of some information of these four enzymes.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Oxygen</th>
<th>Light</th>
<th>Types</th>
<th>Encoded</th>
<th>Cofactor</th>
<th>Present in</th>
</tr>
</thead>
<tbody>
<tr>
<td>COR (EC 3.99.35)</td>
<td>Sensitive</td>
<td>Independent</td>
<td>BchX, BchY and BchZ</td>
<td>Chloroplast</td>
<td>ATP; NADPH; [Fe-S]</td>
<td>NS bacterium</td>
</tr>
<tr>
<td>DVR (EC 1.3.1.75)</td>
<td>Sensitive</td>
<td>Independent</td>
<td>BciA and BciB</td>
<td>Chloroplast (etio)plast</td>
<td>[4Fe-S]; FAD; NADPH</td>
<td>All Phototrophs</td>
</tr>
<tr>
<td>DPOR (EC 1.3.7.7)</td>
<td>Sensitive</td>
<td>Independent</td>
<td>(subunits): BchB/ChlB, BchlC/ChlC and BchlN/ChlN</td>
<td>Chloroplast</td>
<td>ATP; NADPH; [Fe-S]</td>
<td>Anoxigenic bacteria, Photos. bacteria, cyanobacteria, plants (except angiosperms)</td>
</tr>
<tr>
<td>LPOR (EC 1.3.1.33)</td>
<td>Insensitive</td>
<td>Dependent</td>
<td>(isoforms):porA, porB and porC</td>
<td>Nucleus</td>
<td>NADPH</td>
<td>cyanobacteria and plants</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Types</th>
<th>Encoded</th>
<th>Cofactor</th>
<th>Present in</th>
</tr>
</thead>
</table>
| ChlB                   |           |               | photosynthetic organisms to maintain a high level of Chl biosynthesis under distinct and changing conditions in their environment [67]. The reductions made by LPOR, and BciA (DVR) enzymes occur in the former process with NADPH as a hydride-donating cofactor (Table 1), while DPOR, BciB (DVR) and COR enzymes catalyze the reduction through the latter process with ferredoxin enzymatically reduced by NADPH as an electron donor [68]. After analyze at all the characteristics mentioned before, the comparison between these four enzymes shows that the LPOR is the one that has the less similarities related to the rest of this group (Table 1). LPOR is the most distinct according to the multiple sequence alignment with the other enzymes (DPOR, COR and DVR) which are closely related when comparing with each other. They have several characteristics in common (Table 1).  

### 1.6. Comparisons between These Enzymes and Nitrogenase

Nitrogenase (dinitrogenase) is a complex enzyme responsible for biological nitrogen fixation which converts the atmospheric dinitrogen (N2) into ammonia (NH3) [69]-[71], in N2-fixing organisms (diazotrophs). A typical nitrogenase is an εβε tetramer encoded by NifH, NifD and NifK and containing an iron-molybdenum cofactor, FeMoCo [72] [73]. Nitrogenase is an enzyme that is divided into two protein complexes, one is Fe-protein, which is an ATP-dependent reductase specific for the MoFe-protein and also carries one 4Fe-4S cluster bridged between these two complexes. The other complex is the MoFe-protein, which provides the catalytic centers. It has two types of metalloclusters: the P-cluster (an [8Fe-7S] cluster) and the FeMo-cofactor comprising 1Mo-7Fe-9S-X homocitrate. The Fe-protein is reduced by ferredoxin or flavodoxin and then the electrons are transferred from the [4Fe-4S] cluster of the Fe-protein to the FeMo-cofactor of the MoFe-protein via the P-cluster. The enzymes DPOR and COR (Table 1) are considered twonitrogenase-like enzymes because they share significant amino acid sequence homology to the corresponding subunits of nitrogenase. Whereas subunits Bchl/Chll, BchlX and NifH exhibit a sequence identity at the amino acid level of 33%, subunits Bchl/Chll, BchlX, NifD, and BchlB/ChlB, BchlZ, and NifK, respectively, show lower sequence identities of 15%. The DPOR’s NB-protein shows more similarity with the nitrogenase homologous MoFe protein (a NiFe-NifK heterotetramer) of nitrogenase [23] [31] [40] [43] [44] [46] [47] [53] [74], contrarily, the L-protein (DPOR) is most structurally related to Fe-protein (a NifH homodimer) (33% identity and 50% similarity) [44]. Other authors [23] [33] [40] [43] [44] [60] discovered more cases of significant similarities between them such as all of them are dependent on the presence of ATP and reductant dithionite. Nomata et al. [23] showed that the in vitro assay catalytic mechanism of COR strongly resembles DPOR catalysis. X-ray crystallographic, electron paramagnetic resonance, mutagenesis studies and sequencing analysis showed more indepth information about the structure of some of these enzymes, confirming some mentioned above and new ones. For example the N and B proteins (DPOR) have shown that they do not have the requisite number of conserved Cys to assemble a P-cluster or the FeMo cofactor of the nitrogenase NifH and NifD heterotetramer. Instead, the conserved Cys arrangement in Chll/NifH and Chll/BchlB seems to be more consistent with a cluster similar to the one found in the nitrogenase accessory proteins NifE and NifN [44]. These Cys residuesligate the intra subunit 4Fe-4S cluster, that is chelated by the two proto-mers of L-protein [40]. These Cysresidues are also conserved in Chll/BchlL what also happen to the nitrogenase NifH dimer [44] [45]. In all the nitrogenase iron protein’s sequences and in bchX, the position 100 is occupied by an Arginine (R) residue and in both Bchl and Chll it is Tyrosine (Y) [13]. The Tyr127 is found conserved in all Bchl and Chl proteins, whereas the nitrogenase system, as well as the COR system makes use of an Arginine
at the identical position [49]. These similarities happen because DPOR has evolved from ancestral genes common to nitrogenase and is distributed among anoxygenic photosynthetic bacteria, cyanobacteria, Chlorophytes, Pteridophytes, Bryophytes, and Gymnosperms, which until now has made them carry a lot of similarities [13] [75]. Another feature that Nitrogenase, COR and DPOR have is the oxygen sensitivity (Table 1), which can be irreversibly destructed by oxygen [40]. Through the evolutionary tree of the organisms, besides the fact of some domains being kept highly conserved [44] [45] [49], the DPOR have got smooth changes with the time, which in future can decrease significantly its oxygen sensibility, but still, the electrons from the [4Fe-4S] cluster of BchNB/ChlNB, that are transferred directly onto the Pchlide substrate at the active site of DPOR [49], a reaction that is potentially dangerous under oxygen-rich conditions because of the substrate radical that would be a source of reactive oxygen species that would cause severe damage to cells [40]. The rapid rise in global oxygen level may have also driven the evolution of heterocysts to protect nitrogenase from oxygen, that when reaches levels over 0.3% (v/v), the “Pasteur point”, considered the level above which the activity of DPOR is functionally insufficient, and the LPOR becomes essential to survive, in the organisms that have both DPOR and LPOR [66] [76] [77]. Some species, as cyanobacteria, to cope with environmental generated reactive oxygen species have developed effective protection mechanisms such as catalases, peroxidases, superoxide dismutases, A-type flavor proteins (Flv1 and Flv3) [66] An example, in Cyanobacterium sp. PCC6803 which are essential for photoreduction of oxygen to water and are probable candidates for the DPOR protection machinery and these mechanisms are believed to protect not only L-protein but also NB-protein from oxygen, upon exposure to air, where the gradual decrease in activity of purified NB-protein is much slower than that of L-protein [77]. DPOR seems to have become dependent on the protection mechanism rather than evolved to acquire oxygen tolerance, so, the presence of such mechanisms to protect DPOR could contribute to the evolutionary persistence of DPOR in oxygenic photosynthetic organisms. DPOR no longer operates in conditions where oxygenic photosynthesis is very active and cellular oxygen levels are very high [66].

2. Methods

The amino acid sequences were obtained from the NCBI’s online Protein database using the respective name of each subunit or each enzyme. All the names that are mentioned for each enzyme were used, to get the maximum number of sequences, and then the Protein-Protein Basic Local Alignment Sequence Tool (BLASTP) analysis was used to get even more similar sequences, where only the entries with more that 60% similarity were taken. Then all sequences from each enzyme were compared and the repeated ones were excluded, having only one exemplar of each subunit and stored in their respective group. Then the sequences were compared within subunit, within enzyme and also different enzymes were then compared with each other to get and confirm motifs that were already referenced in the literature. The sequence alignments were made with ClustleW, and then were made into trees using the Maximum Likelihood tool. Phylogenetic and molecular evolutionary analyses were conducted using the software: Molecular Evolutionary Genetics Analysis (MEGA) version 6 [78]. The tree tools were configurated to consider significantly better at 95% confidence (P < 0.05) [13]. Then the tree were analysed, and compared.

3. Conclusion

In this paper were confirmed the similarities between DPOR, DVR and COR are higher than LPOR, and specifically related to the subunits, the sub unit B of DPOR is highly similar with the sub unit Z (COR), also L (DPOR) is highly similar with X (COR) and N (DPOR) and Y (COR) also has high similarity, showing that COR and DPOR are the enzymes with higher similarities between these enzymes studied in this paper.

References

http://dx.doi.org/10.1007/BF00039173
http://dx.doi.org/10.1023/B:PRES.0000030457.06495.83
http://dx.doi.org/10.1105/tpc.7.7.1039

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC45935/
http://dx.doi.org/10.1073/pnas.90.5.1642

http://dx.doi.org/10.1016/S0014-5793(98)01245-9


http://rd.springer.com/article/10.1023%2FA%3A1007027005903


http://dx.doi.org/10.1073/pnas.90.15.7134

http://www.biochemj.org/content/243/1/23
http://dx.doi.org/10.1042/bj2430023

http://www.nature.com/nbt/journal/v1/n9/full/nbt1183-784.html
http://dx.doi.org/10.1038/361784a0

http://dx.doi.org/10.1042/BJ20121723

http://dx.doi.org/10.1042/BJ20140163


PCC6803 Is Essential for Conversion of 3,8-Divinyl(Proto)Chlorophyll(Ide) to 3-Monovinyl(Proto)Chlorophyll(Ide).

Conformational Changes in an Ultrafast Light-Driven Enzyme Determine Catalytic Activity.


and

http://dx.doi.org/10.1074/jbc.M601750200

S. M. D. Nascimento et al.


http://dx.doi.org/10.1105/tpc.4.8.929


Submit or recommend next manuscript to SCIRP and we will provide best service for you:

Accepting pre-submission inquiries through Email, Facebook, LinkedIn, Twitter, etc.
A wide selection of journals (inclusive of 9 subjects, more than 200 journals)
Providing 24-hour high-quality service
User-friendly online submission system
Fair and swift peer-review system
Efficient typesetting and proofreading procedure
Display of the result of downloads and visits, as well as the number of cited articles
Maximum dissemination of your research work
Submit your manuscript at: http://papersubmission.scirp.org/