PHYLOGENETIC ANALYSIS OF EXTRINSTIC PROTEINS OF PHOTOSYSTEM II

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Abstract
A phylogenetic analysis of extrinsic proteins associated with the oxygen evolving complex of Photosystem II was performed by generating trees for each extrinsic subchain of PSII. Subsequently these subtrees were written into one resultant tree. These investigations provide hints about the evolution of the oxygen evolving complex, helping in understanding functionally important and conserved parts of the protein structures. The detailed description of the evolution of oxygen evolving complex and the extrinsic proteins on basis of the presented data is a first step in a wider analysis to describe the interactions of the extrinsic proteins within the oxygen evolving complex and of the complex itself with PSII. In contrast with previous our study contains all extrinsic proteins including PsbP-like and PsbQ-like and we try to build resultant a tree containing all species, not just those having the same gene.

Introduction

Oxygenic photosynthesis is an energy-transducing process whereby light energy is trapped and converted into biochemical energy. This multi-step process encompasses a series of electron transfer reactions from water molecules to $NADP^+$, coupled to ATP synthesis [1]. Four types of protein complexes participate in this process going on in thylakoid membrane of cyanobacteria, red and green algae and in green plants. One of these complexes – photosystem II (PSII) – mediates electron transfer from water to plastoquinones, as a result of the photo induced charge separation between a primary chlorophyll donor P680 and a pheophytin acceptor molecule [1]. It forms a dimeric structure. Each of almost identical monomer units consist of more than 25 subunits.We focus on the extrinsic proteins of PSII that can be found in lumenal face of thylakoid membrane. PsbO protein – sometimes called 33 kDa protein according to its apparent molecular mass of spinach protein on SDS-gel – is common for all mentioned types of organisms. It is directly bound to intrinsic proteins of PSII, especially to CP47, but participation of other subunits can not be excluded. It is in charge of stabilization of manganese cluster and protecting it from attack by endogenous reductants. It also effects the conformation of the lumenal side of intrinsic PSII subunits which are responsible for binding PsbP and PsbQ [2]. Other extrinsic proteins differ among mentioned groups of organisms. Extrinsic subunits of green algae and green plants contains moreover PsbP (23 kDa protein) and PsbQ (16 kDa protein) subunits which are bound to PSII through PsbO protein, binding of PsbQ also requires presence of PsbP. These subunits create a high affinity Cl^- binding site, protect the manganese cluster from chemical attack and stabilize it. PsbP protein moreover prevents Ca^{2+} from being released from PSII during the S-state turnover.On the other hand extrinsic region of PSII of cyanobacteria contains proteins PsbO, PsbU (14 kDa protein) and PsbV (cytochrome c-550). PsbV has a function similar to that of O subunit in O_2 evolution. The function of PsbU remains unknown [1]. Recently it was shown [3] that cyanobacteria have also homologues of PsbP and PsbQ proteins of higher plants (homology of about 30 %). They take part in modifying the $CaCl_2$ requirements for PSII activity.Extrinsic proteins of PSII of red algae contain PsbO, PsbV, PsbU and protein which is called PsbQ like protein (20 kDa protein). The last protein does not function directly in oxygen evolution but is required for maximum binding of PsbV and PsbU protein which are required for optimum activity of oxygen evolution [4]. Our work was inspired by [5], some of their experiments were retaken with broader group organisms and we examined phylogeny of all extrinsic protein.

Methods

All available primary sequences of extrinsic proteins of PSII were extracted from databases using PubMed internet interface [6]. The abbreviation of used species can be seen in Table 1. If necessary, transit peptides were removed. Multiple alignment of sequences of each protein was done by ClustalX [7]. Later these alignments were used for computation of phylogenetic trees using PHYLIP software package [8] version 3.6. Two methods – Fitch-Margoliash with the assumption of molecular clock and protein parsimony – were used. To examine the stability of tree branches bootstrap for 1000 times was used; the order of protein sequences was jumbled for 50 times. Finally trees were rooted, the most ancient species was chosen as the root. For determining the relationship between different species trees calculated by Fitch-Margoliash with the assumption of molecular clock without branch length were used. Branches with bootstrap scores higher than 50 % were taken as conclusive. The resultant tree was drawn using CorelDraw software by adding branches to PsbO protein tree. If some species were presented at different places in different trees the one with the highest bootstrap value was used.To demonstrate the time distance of single species the same trees using the branch length were calculated. We used protein parsimony method to compare and confirm the results (especially „most important“ branches).

Results and Discussion

Our study is the first study where all available genes of extrinsic proteins of oxygen evolving complex were used. First we built phylogenetic tree for each gene separately and than we composed these trees into one containing all available genes.Our results clearly demonstrate that green plants (figures 1 to 6) form the closest group of species. This is in agreement with the fact that this group is evolutionary the youngest one. Also higher plants are multicellular organisms and thus less prone to mutation compared with unicellular organisms. On the other hand, the evolutionary oldest group of unicellular cyanobacteria is expected to be the most „diffuse" group of examined species. It can be divided into two branches from one of which the green plants had developed.Green algae form another evolutionary important group; they are relative close to green plants. These finding are supported by the composition of extrinsic proteins of PSII – both green plants and green algae have PsbO, PsbP and PsbQ. The group of green algae can be divided into two subgroups, a more consistent subgroup of multicellular green algae and more „diffuse" subgroup of unicellular green.Other unique group of genes are red algae that appear to be an interlink between the upper mentioned green algae and green plants and cyanobacteria. Extrinsic subpart of PSII contains chains PsbO, PsbU, PsbV and PsbQ homolog of green plants (homology of about 30 %). This means red algae is the only group of examined genomes (i.e. green plants and algae, red algae and cyanobacteria) lacking the gene encoding the PsbP chain. As can be seen in the phylogenetic trees of PsbO that red algae separated from the common ancestor at about the same time as green algae clearly demonstrating that red algae is not an ancestor of green algae. Also it seems that red algae did not develop from that time on. This supports the presumption that PsbP up rates the water oxidation and coupled reactions. Interestingly the subgroup of cyanobacteria adjacent to red algae lacks PsbQ proteins. So it seems that nature was trying many ways how to reduce protein chains necessary for water oxidation (the more protein the more energy that could be used more effectively is needed). Probably some when in the past there were some organisms having PsbO, PsbP, PsbQ

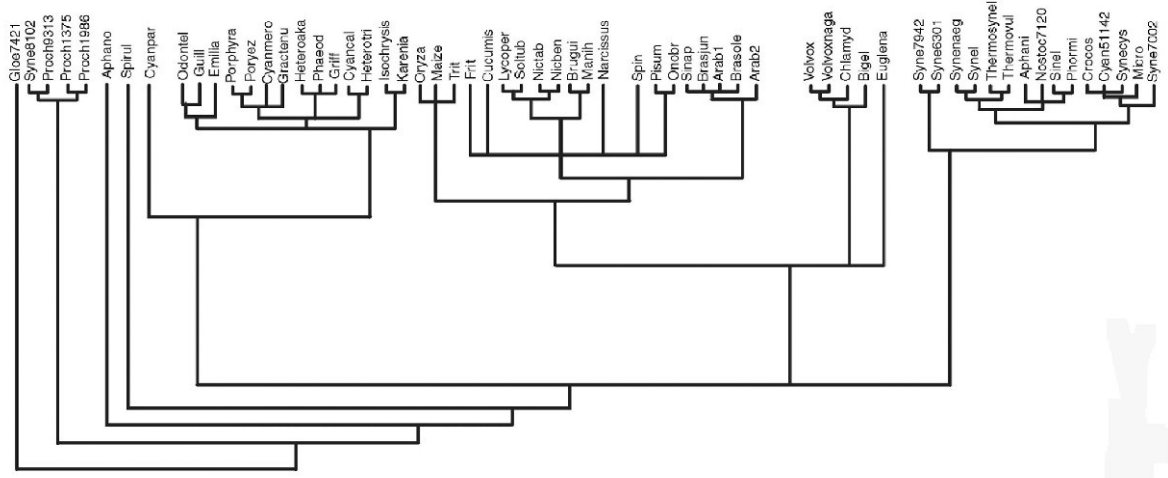

Figure 1. Resultant phylogenetic tree containing all 65 used species. As the root of this tree was used the most acient organism Gloeobacter violaceus PCC 7421.

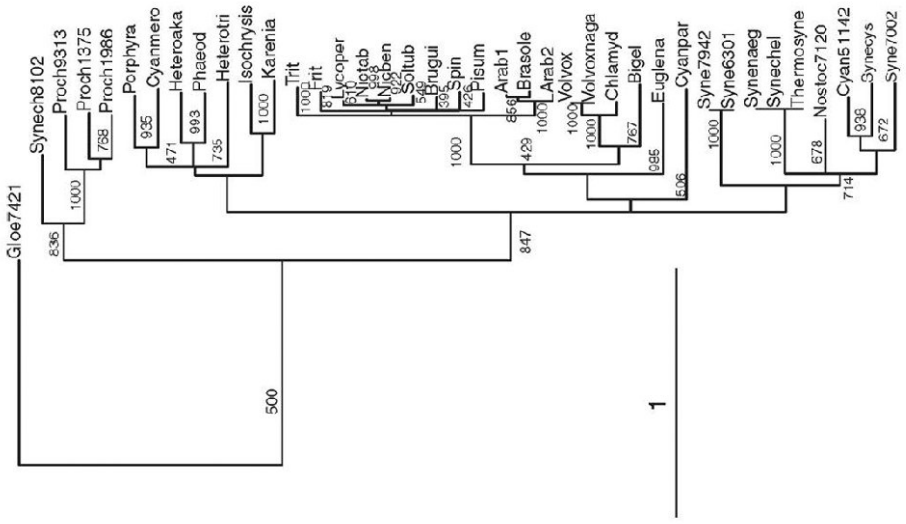

Figure 2. Phylogenetic analysis of 29 available PsbP sequences. Image shows the rooted tree using Gloeobacter as outgroup, giving the bootstrapping scores with scales.

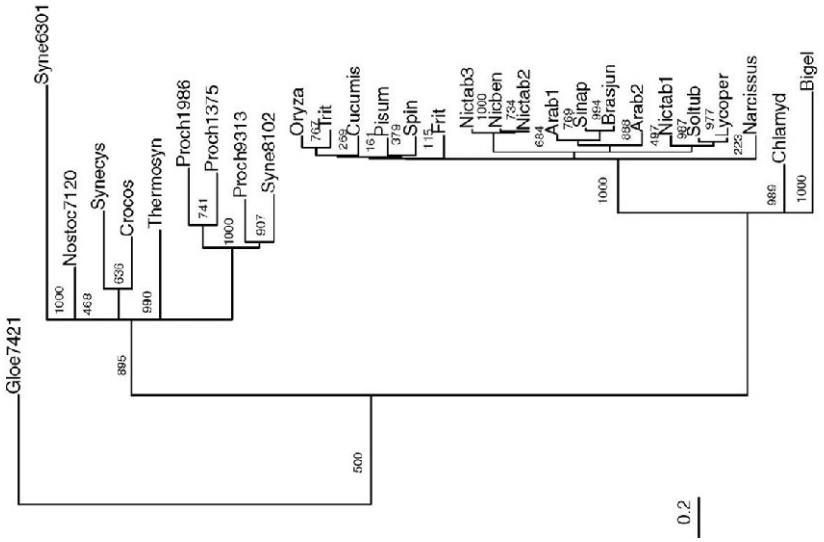

Figure 3. Phylogenetic analysis of 39 available PsbO sequences. Image shows the rooted tree using Gloeobacter as outgroup, giving the bootstrapping scores with scales.

Figure 4. Phylogenetic analysis of 15 available PsbU sequences. Image shows the rooted tree using Gloeobacter as out-group, giving the bootstrapping scores with scales.

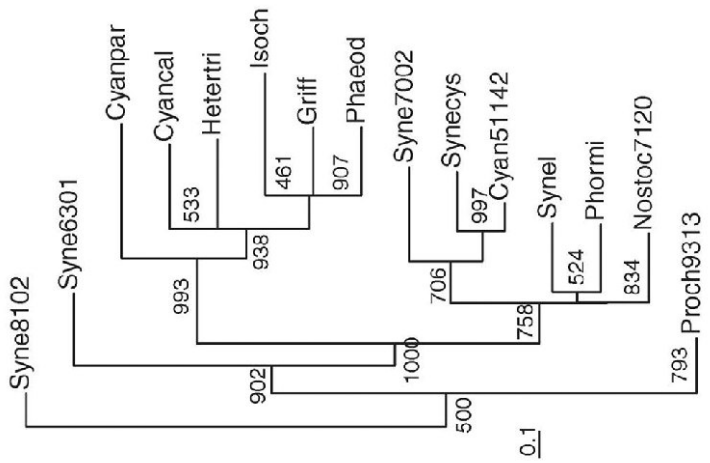

Figure 5. Phylogenetic analysis of 20 available PsbQ sequences. Image shows the rooted tree using Gloeobacter as outgroup, giving the bootstrapping scores with scales.

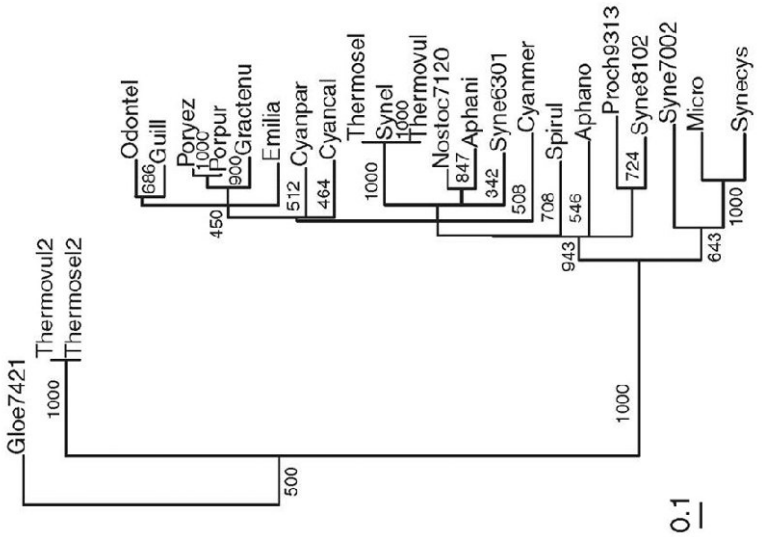

Figure 6. Phylogenetic analysis of 25 available PsbV sequences. Image shows the rooted tree using Gloeobacter as out-group, giving the bootstrapping scores with scales.

proteins and missing one of PsbU or PsbV proteins. There are known sequences of both PsbP and PsbQ proteins from the other subgroup of cyanobacteria; these cyanobacteria have also PsbU and PsbV proteins which play the crucial role in water oxidation. Later during the evolution (green algae and plants) the fundamental role of water oxidation was shifted to PsbP and PsbQ subunits and PsbV and PsbU subchains disappeared. In the case of PsbP it seems to be that first developed the PsbP-like protein and much later the PsbP protein in green algae and in higher plants. However, in case of PsbQ, the gene encoding the PsbQ chain in green algae is much closer to the PsbQ-like gene of cyanobacteria than to the higher plant one. Therefore it can be stated that PsbQ developed earlier, taking over the role of PsbU and PsbV, and then later PsbP developed to make oxygenic photosynthesis more efficient.

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