Short communication

Site-directed mutagenesis of the CP 47 protein of photosystem II: alteration of conserved charged residues which lie within lethal deletions of the large extrinsic loop E

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Abstract

The intrinsic chlorophyll-protein CP 47 is a component of photosystem II which functions in both light-harvesting and oxygen evolution. The large extrinsic loop E of this protein has been shown to interact with the oxygen-evolving site. Previously, Vermaas and coworkers have produced a number of deletions within loop E which yielded mutants which were unable to grow photoautotrophically and which could not evolve oxygen at normal rates. During the course of our site-directed mutagenesis program in Synechocystis 6803, we have altered all of the conserved charged residues which were present within six of these deletions. All ten of these mutants were photoautotrophic and evolved oxygen at normal rates. We speculate that the severe phenotypes of the deletion mutants observed by Vermaas and coworkers is due to large structural perturbations in the extrinsic loop E of CP 47.

Photosystem II (PS II) is a multisubunit thylakoid membrane protein complex which catalyzes the light-driven oxidation of water to molecular oxygen and the reduction of plastoquinone to plastoquinol. Seven intrinsic PS II polypeptides appear to form the minimum complex capable of water oxidation [3, 4]: CP 47, CP 43, D1, D2, the α and β subunits of cytochrome b559, and the psbI gene product [3, 4, 5]. In the cyanobacterium Synechocystis 6803, an extrinsic 33 kDa protein is required for optimal rates of oxygen evolution at physiological salt concentrations [4] (in higher plants and algae, proteins of 33, 24 and 17 kDa are required). The inorganic cofactors manganese, calcium and chloride are also required for oxygen evolution activity. The binding sites for these cofactors within PS II are unknown [5].

The chlorophyll protein CP 47, a component of the interior antenna for PS II, is an integral membrane protein which is predicted to contain six membrane-spanning α-helical domains and three lumenally exposed loops, A, C, and E [2, 18], and is encoded by the psbB-gene [14]. Insertional [18] and deletion [6] mutagenesis of the psbB gene in the cyanobacterium Synechocystis 6803 leads to a PS II-minus phenotype and it has thus been hypothesized that CP 47 is required for PS II assembly [19]. In addition to its role in light-harvesting and PS II assembly, a variety of lines of evidence suggests that the large extrinsic loop E of this protein, 257W-450W [2], structurally interacts with the oxygen-evolving complex of PS II [1, 3, 7, 8, 9, 11, 15]. The functions of loops A and C have not been closely examined. In our laboratory, we are systematically modifying all of the conserved charged residues located in the lumenally exposed domains of CP 47. This effort was initiated in an attempt to identify residues which are important in the function
of CP 47 within the photosystem. These efforts have been quite successful. Five residues have been identified which affect PS II. 384R and 385R appear to form part of the binding domain for the extrinsic 33 kDa protein. Alteration of these residues leads to a marked increase in stability of the $S_2-[S_1]-S_1$ transition, and a decrease in the stability of binding of the 33 kDa protein [21]. 488R appears to be involved in the chloride requirement for PS II activity [17]. Mutant cyanobacterial strain with alterations at this residue (R448G and R448S; see Table 1 for mutant designations), normally exhibit moderate rates of photoautotrophic growth and oxygen evolution activity. They are, however, unable to grow at low chloride concentrations and assemble only small amounts of functional PS II reaction centers under these conditions. The residue 321K may also be involved in this chloride requirement. The mutant K321G exhibits a similar, though less extreme, phenotype to R448G (C. Putnam Evans and T.M. Bricker, in preparation). The residue 167W (which lies in loop C) appears to be required for the assembly and stability of the photosystem (J. Wu, C. Putnam-Evans, and T.M. Bricker, T. Assen). In addition to these studies, Vermaas and coworkers have produced a number of deletion mutations within the large extrinsic loop of CP 47 [6, 10]. Some of these have yielded mutants which cannot grow photoautotrophically and which evolve oxygen at severely depressed rates. During the course of our investigations we have altered all of the conserved charged residues, including those which lie within six of the severely affected deletions produced by Vermaas and coworkers [6, 10]. These residues are identical in all of the thirteen species for which sequence data is available [10].

Control and mutant strains of Synecochystis sp. PCC 6803 were grown in liquid BG-11 media as previously described. Restriction digests, cloning, growth transformation of bacterial strains, and isolation of DNA were performed according to standard procedures [13, 20]. Synecochystis transformation procedures and the characteristics of control strain K3 have been described elsewhere [16, 17]. The desired mutation was introduced into pTZ18K3 by oligonucleotide-directed mutagenesis using the procedure of Kunkel et al. [12]. The mutagenic primers shown in Table 1 were used to construct these ten mutations. These alterations were then confirmed by sequencing prior to transformation of the recipient strain DEL-1 [16]. Cell growth was assessed by measuring the optical density (A$_{730}$) of the cultures on nine consecutive days. PS II activity was measured by oxygen polarography using a Hansatech oxygen electrode. Cells were assayed in BG-11 media with 1 mM DCBQ added as an electron acceptor. Oxygen evolution was measured at a light intensity of 3000 mmol photons m$^{-2}$ s$^{-1}$ of copper sulfate-filtered white light at 25 °C. The chlorophyll concentration in all oxygen evolution assays was 10 mg/ml.

Figure 1 illustrates partial restriction maps of the bacterial strains and plasmids which we have used in this study. The deletion strain DEL-1, which possesses a truncated psbB gene, was an obligate phototrophic and exhibited no capacity for oxygen evolution (see Table 2). Table 1 summarizes the mutagenic oligonucleotides which were employed to introduce site-specific changes in the extrinsic loop of CP 47. After introduction of these mutations which were closely linked to a kanamycin resistance gene in the plasmid pTZ18K3 and sequencing to verify each mutation, the resulting plasmids were used to transform the partial deletion strain DEL-1. Transformants were screened for the loss of spectinomycin resistance and the acquisition of kanamycin resistance. After several rounds of streaking, mutant colonies were isolated, their genomic DNA extracted, and the 1400 bp KpnI/KpnI fragment containing the introduced mutation was amplified using the polymerase chain reaction. This fragment was cloned into the pGEM-T vector, and the resulting transformants were pooled and the entire 1400 bp fragment sequenced. The DNA sequence for each mutant demonstrated that they contained only the desired mutations.

Table 2 shows the results obtained examining both growth evolution activity for the ten mutants considered in this study. For comparison the growth and oxygen evolution rates reported for the deletion strains [6, 10] are also shown. Several hypotheses could explain the severe phenotype of the deletion strains. First, conserved charged residues within the deletions could be required for oxygen evolution, PS II assembly or PS II stability. Second, conserved charged residues could be required for these PS II functions. Finally, structural perturbations introduced by these deletions could affect the global structural and functional characteristics of CP 47 leading to a severe phenotype. In this communication, we have tested the first hypothesis. None of the site-directed alterations which we introduced at conserved acidic or basic residues resulted in any change of either the growth or oxygen evolution rates, as noted previously, other conserved charged residues within loop E have been identified which influence PS II activity [16, 17,