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Short Review

PsbO, the manganese-stabilizing protein: Analysis of the structure–function relations that provide insights into its role in photosystem II

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ABSTRACT

The minireview presented here summarizes current information on the structure and function of PsbO, the photosystem II (PSII) manganese-stabilizing protein, with an emphasis on the protein's assembly into PSII, and its function in facilitating rapid turnovers of the oxygen evolving reaction. Two putative mechanisms for functional assembly of PsbO, which behaves as an intrinsically disordered polypeptide in solution, into PSII are proposed. Finally, a model is presented for the role of PsbO in relation to the function of the Mn, Ca^{2+}, and Cl^{-} cofactors that are required for water oxidation, as well as for the action of hydroxide and small Mn reductants that inhibit the function of the active site of the oxygen-evolving complex.

Contents

1. Introduction

The largest extrinsic subunit of the membrane-associated photosynthetic redox enzyme called photosystem II (PSII) is PsbO, a 26.5 kDa polypeptide also called the manganese-stabilizing protein, which is located on the lumen side of thylakoid membranes. The PSII reaction center consists of a core of intrinsic proteins:

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D1 and D2 bind the Chl a, Pheo a, and plastoquinone cofactors that participate in light-catalyzed charge separation; the larger chlorophyll-binding polypeptides CP47 and CP43 and cytochrome b559, along with a number of small protein subunits are also associated with the core structure of the photosystem [1,2]. Three inorganic cofactors (4 Mn, 1 Ca^{2+}, 1 Cl^{-}) and at least three extrinsic proteins (one of them PsbO) are bound to the PSII core proteins, and together they form a module called the oxygen-evolving complex (OEC), which is the catalytic center for water oxidation [3–6]. The OEC is assembled in a stepwise process termed "photoactivation", in which Mn^{2+} atoms are incorporated (photoligated) into the preassembled inactive apo-S-state complex that is activated by assembly of the Mn cluster [7,8]. Photoligation involves oxidation by light of Mn to states higher than +2 and binding of the metals to sites within the OEC. Calcium is not directly involved in the photoligation reaction, but it does function in Mn photoactivation [9] by preventing inhibitory ligation of the Mn ions in higher redox states ($Mn^{\geqslant 3+}$) [10]. On the other hand, PsbO is not essential for photoligation of Mn, but is absolutely required for maximal efficiency of the water-oxidation reaction [11]. The dispensability of PsbO in Mn photoligation experiments provides evidence to suggest that PsbO is not required for binding of Mn to PSII and that as such it has no amino acid residues that function as direct ligands to the Mn cluster.

While PsbO is unnecessary for binding of a substrate water molecule to the Mn cluster, at least in the S_1 state [12], its indispensable role in the oxygen evolution reaction has been demonstrated by the consequences of its extraction from PSII, which can be implemented by washing intact samples with alkaline-Tris [13], 1 M $CaCl_2$ [14] or $MgCl_2$ [15], or 0.2 M NaCl–2.6 M urea [16]. In the latter case, the combination of ionic strength and a chaotropic agent dissociates the protein under fairly mild conditions. In contrast, $CaCl_2$ and $MgCl_2$ at high concentrations disrupt electrostatic interactions between PsbO and PSII. A common feature of these manipulations is that in all cases, except for alkaline-Tris, the Mn cluster is retained in intact form. Tris at high pH exerts multiple effects on Mn and polypeptide binding, since both PsbO and the Mn cluster are released from PSII [17]. Caution is required in the use of $CaCl_2$-treated PSII where the role of Ca^{2+} is being characterized; residual concentrations of this cofactor can remain in a sample after the treatment [18]. Recently, Yu et al. [19] reported that PsbO can also be removed from spinach PSII by $HgCl_2$ at micromolar and higher concentrations without removing the PsbP and PsbQ extrinsic proteins. The authors hypothesized that Hg could react with the lone disulfide bridge of PsbO and replace it with the –S–Hg–S– motif, which would eventually result in a conformational change in the PsbO structure, and its release from PSII. Additional experiments are needed to confirm this interesting hypothesis. Since isolation of PSII usually employs spinach leaves [20], *in vitro* depletion of PsbO from such preparations reveals defects that are created in the activity of eukaryotic PSII. Typically, PsbO removal causes a decrease in the rate of O_2 evolution to ~20% of that observed with intact PSII, and the sample requires high concentrations of Ca^{2+} and especially Cl^{-} that prevents loss of two of four Mn atoms from the OEC [14,16,18].

In eukaryotic organisms, PsbO is encoded by nuclear DNA, and is imported into chloroplasts as a precursor [21]. Mutations that deleted PsbO from PSII were found to have different effects in prokaryotes and eukaryotes. The ΔPsbO mutant from *Synechocystis* sp. PCC 6803 assembles PSII, evolves oxygen at low rates, and grows slowly under photoautotrophic conditions, although it is light sensitive [22]. On the other hand, cells *Chlamydomonas reinhardtii* carrying the ΔpsbO mutation could not grow photoautotrophically, and did not accumulate PSII [23]. A similar phenotype was observed in *Arabidopsis thaliana*, where both genes for the PsbO isoforms PsbO-1 and PsbO-2 were suppressed by RNAi [24]. Expression of only the PsbO-2 protein in the *psbo1 A. thaliana* mutant caused retarded photoautotrophic growth [25], longer lifetimes of the S_2 and S_3 states, higher accumulation of $PSII_\beta$ reaction centers, and retardation of $Q_A \rightarrow Q_B$ electron transfer [26]. More details on the consequences of *in vitro* or *in vivo* removal of PsbO from PSII are presented in Section 3 (function of PsbO in PSII).

A prior PsbO minireview focused on its solution structure [27]. Since then, several reviews on PsbO that included analyses of the PsbO structure in solution or in the PSII-associated form were published [28–31]; many of them were based on the crystallographic models of PsbO from thermophilic cyanobacteria [2,32,33]. Here, information on PsbO is reviewed in the context of its behavior as a natively unfolded protein, and aspects of PsbO structure are examined that are most relevant to the protein's proper assembly and function in PSII. Two scenarios for a mechanism of functional association of PsbO's flexible loops into PSII are also proposed. With an emphasis on PsbO function in PSII, results of biochemical, spectroscopic, and mutagenesis experiments that provide some insights into the effect of PsbO on the inorganic cofactors, the S-states, and reduction of Y_Z in the OEC are reviewed. A schematic model summarizing the role of PsbO in relation to the function of the Mn, Ca^{2+}, and Cl^{-} cofactors and to the action of hydroxide and small Mn reductants, such as dimethylhydroxylamine, in the OEC active site is presented.

2. PsbO structure in solution and in the PSII-associated form

2.1. PsbO is a thermostable natively unfolded protein in solution

In solution, PsbO behaves as a natively unfolded or intrinsically disordered polypeptide [27,34,35]. Proteins that belong to this family (see the Disprot database (http://www.disprot.org)) possess several characteristics in common that cannot be found in normally-folded globular proteins. These properties include:

(1) Secondary structure that contains predominantly β-sheet and large amounts of turns and random coils (see [27,30,36,37]).
(2) Folding that is not assisted by chaperones, but rather by binding to a supermolecular complex of which the intrinsically disordered protein is a subunit [38,39].
(3) Overestimated molecular size based on SDS–PAGE or on gel filtration [40–43].
(4) Unusually large Stokes (hydrodynamic) radius [40,44].
(5) Extremely acidic or basic pI values (PsbO has pI of 5.2) [40,45].
(6) A high ratio of disorder promoting residues (A, R, G, Q, S, P, D, E, and K) to hydrophobic (W, C, F, I, Y, V, L, and N) residues [39,40,45].
(7) A resistance to heat denaturation that is unusual for mesophilic proteins [40,46–48].

Thermostability of PsbO and other natively unfolded mesophilic proteins could be a leftover from the era when the earth's atmosphere had a higher temperature compared to the present day, or it could be a consequence of an embedded physical/chemical property of amino acid sequences or overall protein charge. In the case of PsbO, there appears to be a subtle equilibrium between protein's thermostability and function. It has been shown recently that highly thermostable mutants, where Phe replaces W241 or Y242, exhibit low activity [49] because they cannot undergo overall folding that is important for their functional assembly into PSII [50,51]. However, removal of six N-terminal residues from W241F PsbO can significantly restore function to a W → F mutant, because it reduces its thermostability to a near-wild-type level [49,52]. Shutova