Molecular Anatomy of Plant Photoprotective Switches: The Sensitivity of PsbS to the Environment, Residue by Residue

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Supporting Information

ABSTRACT: Under strong sunlight, plants avoid photooxidation by quenching the excess absorbed energy. Quenching is triggered by PsbS, a membrane protein that is activated and deactivated by the light-dependent pH changes in the thylakoid lumen. The mechanism of action of this protein is unknown, but it was suggested that several glutamates act as pH sensors. However, the pKₐ of glutamate is several pH units below the physiological values in the lumen. Thus, how can PsbS sense the pH of the lumen, and how does it respond to it? By applying a nonstandard molecular dynamics method that treats pH explicitly, we show that the lumen-exposed glutamates of PsbS have strongly shifted pKₐ values and that such shifts are crucial for the pH sensitivity in physiological conditions. We also demonstrate that protonation drives a systematic unfolding of a region key for protein–protein interactions, indicating that PsbS response to pH is a functional conformational switch.

Plants avoid photooxidation by dissipating as heat the energy absorbed in excess in what is called energy-dependent quenching or qE.1 The light-harvesting complexes (LHCs) are the site of qE.2 Under low or normal light conditions, the LHCs transfer excitation energy to the core of photosystem II (PSII). Here, photochemistry is initiated and protons are pumped into the thylakoid lumen, an aqueous compartment enclosed by the photosynthetic membrane. Under high light, because of the increased rate of electron transfer, a proton gradient is established between the stromal compartment enclosed by the photosynthetic membrane.

To understand the structural basis of the pH sensitivity and the effect of pH changes on PsbS, we applied a state-of-the-art method known as constant-pH molecular dynamics (CpHMD).11,12 CpHMD simulations are able to capture with atomistic resolution the time-dependent structural transitions of proteins at a given pH. In brief, during each CpHMD trajectory, protonation states for the titratable residues of the protein are reset iteratively at given time intervals, depending on the local environment to which each residue is exposed and on the set pH. To do so, CpHMD combines molecular dynamics (MD) simulations with continuum electrostatics (Poisson–Boltzmann or PB) and Monte Carlo (MC) methods. Via the MD simulations, the protein is allowed to rearrange its structure in function of the protonation pattern at a given pH. Via the PB/MC calculations, the protonation pattern is then regularly updated throughout the MD simulation, allowing for the equilibration of the protein structure at the investigated pH value and the collection of quantitative information about the pH sensitivity of every residue. Although the calculation of pKₐ values is a challenging task,13 the CpHMD method has proven to be able to capture (i) the atypical pKₐ values that some residues exhibit depending on the local environment,14 (ii) the coupling between selected protein conformations and protonation states,12,15 and (iii) the entropy contribution of every possible protonation state at each titratable site.16

In this work, the dynamics of a monomer of PsbS in a simulated native-mimicking membrane has been investigated at 6 different pH values ranging from 3 to 8. At each pH value investigated, 5 independent replicas were run for a total of 30 independent simulations and about 5 μs of total simulated time. This titration allowed us to determine the pKₐ values of each protonable residue of PsbS and to identify the pH-dependent conformational changes of the protein.
In order to determine the sensitivity of PsbS to pH, residue by residue, we calculated the frequency of protonation at a given pH for every titratable site over each CpHMD trajectory at equilibrium. By doing so, complete titration curves were reconstructed for the whole set of (protonable) residues. For comparison, the frequency of protonation at the different pH values was also computed on the crystal structure of PsbS embedded in the same equilibrated membrane−water system used for the CpHMD simulations (see Methods in the Supporting Information).

The titration curves for the whole set of titratable amino acids of PsbS are reported in Figure S1. Figures 1 and 2 focus on the glutamic acids (Glu) because several Glu residues have been proposed to be responsible for the pH sensitivity of the protein.17,18 We found that all of the pK_a values of the stroma-exposed Glu (Glu13, Glu20, Glu35, Glu37, Glu105, Glu111, and Glu141) have a small shift (within one pH unit, Figure 2) from the pK_a of Glu in water (4.3,19 green curve in Figures 1 and S1). This means that their pK_a values are not strongly influenced by the protein environment and/or the lipid membrane. At striking variance with the stromal side, most of the lumenal exposed Glu have a pK_a that is strongly shifted with respect to the value in water. These pK_a shifts, up to 3.4 pH units in the case of Glu182 (Figure 2), make the majority of the lumenal exposed Glu (Glu69, Glu76, Glu78, Glu173, Glu180, and Glu182) sensitive to the range of physiological pH values that are critical for the activation of qE. This means that when light saturates the photosynthetic machinery, the lumen-exposed Glu sense the associated change in pH allowing PsbS to respond to light fluctuations. The fact that multiple glutamates contribute to the sensitivity of PsbS is in agreement with experimental results17 showing that single or double mutations of Glu69, Glu78, Glu173, and Glu182 all resulted in plant lines with reduced qE levels. Our results show that all these residues have pK_a values in the range of the thylakoid lumen. This is not the case for the other two lumen-exposed residues Glu55 and Glu159, which have a very low probability to be protonated at pH > 5. Interestingly, these residues were found to be essential for the proper folding and stability of PsbS.17 We found that the unprotonated form of these residues, which is present at all physiological pH values, preserves their role in the anchoring of the central transmembrane helices TM1 and TM3 via hydrogen bonds to the...
Figure S3 shows that a reasonable relation is found between the pKα shifts of PsbS Glu residues and the pKα of Glu computed via the CpHMD method by fitting the titration curves of the simulated structures in the Hill approximation (see Methods in the Supporting Information). The different colors associated with the Glu residues represent the difference between the computed pKα and the standard pKα value of Glu in water (4.3). pKα shifts below 0 and above 3 are colored in white and dark red, respectively. Glu residues are shown as van der Waals spheres. Glu55 (shown in gray) does not have a well-defined single midpoint pKα value (indicated as ND in the figure), as seen in its titration curve (Figure 1).

The pKα shifts in proteins are well-studied, including in Glu residues,20,21 being a common benchmark for pKα prediction methods.15 They are caused by desolvation and/or electrostatics, both possibly modulated through pH-dependent structural changes. Desolvation effects occur when a protein ionizable residue is not as exposed to the solvent as it would be when alone in aqueous solution, meaning that its charged form would be less stabilized by surrounding water dipoles and, therefore, the neutral–charged equilibrium would be shifted toward the neutral form. In the case of acidic groups (Glu, Asp, etc.), this would correspond to a pKα higher than when alone in solution. Therefore, if a Glu residue has a high pKα due only to desolvation, we would expect its solvent-accessible surface area (SASA) to be unusually low when the residue is neutral (the residue will always tend to get exposed when charged, even though that may happen only at unusually high pH). Figure S3 shows that a reasonable relation is found between SASA and pKα, except for Glu-37, Glu-141, and Glu-159. Therefore, desolvation seems to explain a part of the observed high pKα of Glu residues, but electrostatics must also be playing a role.

Electrostatic effects occur when a group experiences substantial electrostatic interactions from other charged groups, either because they are close to it or, if more distant, they can strongly interact through a region of low dielectric constant (e.g., the hydrophobic core of a protein or membrane). In the case of two acidic groups, such an interaction induces a positive pKα shift and, if they happen to titrate around the same pH region, the shift can be reciprocal, i.e., both groups will increase its pKα. When these mutual shifts take place, the protonation of the two groups tends to be opposite, i.e., negatively correlated: their joint state is either charged/neutral or neutral/charged. Furthermore, when such strong interactions involve more than two residues, unexpected positive correlations may occur, with some pairs of groups exhibiting a charged/charged or neutral/neutral joint state, possibly associated with nonmonotonic “up-and-down” titration curves like those of Glu-55 and Glu-141, as experimentally observed in small compounds.22 The “outliers” in Figure S3, Glu-37, Glu-141, and Glu-159, are indeed found to engage in an extensive network of correlations with multiple residues, as illustrated in Figure S4. Therefore, the high pKα values of these Glu residues seem to be due to this network of electrostatic interactions.

It is important to note that, without the combination of continuum electrostatics and molecular dynamics (CpHMD), it would not have been possible to predict most of the pKα shifts, as it can be inferred from the titration curves computed on the crystal (Figures 1 and S1). This difference with the crystal structure strongly suggests that conformational dynamics are crucial in determining the pKα values of PsbS and, therefore, its functionality.

Similar to the stromal Glu, the aspartate residues, which are all present at the stromal side of PsbS, also showed pKα shifts of <1 unit with respect to the value in water (Figure S1), meaning that their pKα values remain outside of the physiologically relevant pH range. The pKα computed for the other titratable residues considered in this work (lysine and tyrosine) did not show deviation from the values estimated in water and were all above 8 (Figure S1).

We conclude that the ability of PsbS to sense the pH range relevant for the activation of qE, which is 1–3 pH units higher than the standard pKα of an acid residue, comes from the significant shift in pKα of its lumenal exposed Glu. This is particularly interesting considering that it has been proposed that the main role of PsbS is to raise the pH at which qE is triggered to a value which is physiologically attainable in the lumen.23

What is the response of PsbS to the different pH values? To answer this question, we characterized the conformational space of PsbS at the different pH values. In order to capture the pH-dependent flexibility of PsbS, we computed the pH-dependent root-mean-square fluctuation (RMSF) that is a measure of the conformational freedom of single residues at equilibrium. The sets of RMSFs per different pH values are shown in Figure S5. The RMSF analysis revealed that PsbS is characterized, at all pH values, by a rigid transmembrane region and by highly flexible solvent-exposed domains, namely, the termini and the first (I) and second (II) luminal loops (see Figure 3A for a structural reference). To better distinguish which regions respond to pH, we calculated the root-mean-square deviation (RMSD) per residue of the average protein structures at a given pH with respect to the average structure equilibrated at pH 5, corresponding to the pH value at which the protein was crystallized.3 The different RMSD profiles are reported in Figure S5. The most substantial deviations were found at the termini and at the I- and II-lumen loops, where the highest RMSF was also found. This means that the apparent deviations from the structure at pH 5 registered at these regions might originate from (i) an intrinsic disorder of these regions which results in high variance at all pH values or (ii) a real deviation from the structure at pH 5.

To discriminate between these possibilities, we determined the equilibrated secondary structure of PsbS at the different pH values. In Figure S6, the frequency of the occurrence of specific types of local structures is reported for each protein domain. The possible types of local structure follow the DSSP...
algorithm assignment (see Methods in the Supporting Information) and are 3_{10} helix, α-helix, π-helix, β-bridge, β-sheet, turn, or coil. As reported in Figure S6, we found that, except for the I-lumen loop which contains the short amphipathic helixes H1 and H3 (Figure 3A), all other protein domains largely conserved at every investigated pH value (3−8) the type of secondary structure that they have in the crystal.8 Among these well-conserved domains are the C- and N-terminus and the II-lumen loop containing helix H2, suggesting that their high RMSD (Figure S5) is due to an intrinsic disorder of these regions without a change of secondary structure. In the case of H1, instead, the simulations showed that the α-helix present in the crystal structure had the tendency to change to either a 3_{10} helix or a turn, but without a clear pH dependence (values of α-helix probability of 99%, 95%, 73%, 62%, 93%, and 84% in the pH range 3−8, respectively).

What then is the response of PsbS to pH? We systematically found that at high pH values (pH 7−8) the helical H3 domain switched to a lumen exposed loop (Figure 3C). In the crystal, H3 is hydrogen bonded via the backbones of Ile74 and Tyr75 to the short amphipathic helix H2 belonging to the other monomer in the PsbS crystal unit8 (Figure 3A). Fan et al. suggested that these hydrogen bonds are crucial in stabilizing the monomer–monomer interactions.9 The residue on H2 responsible for the hydrogen bond is Glu1738 (Figure 3A), one of the putative pH-sensitive sites of PsbS.17 Interestingly, as shown in Figure S6, protonation of Glu173 did not induce a conformational change in H2, and it conserved (together with the whole second (II) lumen loop) its structure at all pH values. On the other hand, high pH values (7 and 8) induced a complete unfolding of the amphipathic helix H3: this domain changed from a 3_{10} helix to a turn/coil (Figures 3B and S6). The representative conformations of PsbS at the different pH values are reported in Figure 3C.

What could be the functional effect on qE of the conformational switch of PsbS? The change in the structure of the H3 would result in the disruption of the ordered hydrogen bond network that anchors H2 to H3 in the dimer and weaken the interactions between monomers (Figure 3A, C and Supplementary Video 1). Our findings can explain the experimental results of Fan et al., who observed that the luminal loops of the two PsbS monomers in the dimer are located at a larger distance at high pH.8 Because at low pH PsbS is supposed to interact with other LHCs,6,9,24 we speculated that the creation of a pH-sensitive docking mechanism through a network of tunable electrostatic interactions could be the basis of the mechanism of action of PsbS. Indeed, the alignment of any high-resolution structure of the homologous LHCs25,26 onto the central helices (the most

Figure 3. pH-dependent conformational change of PsbS. (A) Crystal structure of PsbS monomeric subunit (chain A, upper) and of PsbS dimer (lower). The amphipathic helices are indicated as well as the network of interactions between H2 and H3 in the inset. In cyan, the sequence reconstructed via the sequence homology model (see Methods in the Supporting Information) is reported. Chain B has been also reconstructed in the figure for the parts not resolved in the original structure.7 (B) pH-dependent structure of H3. (C) Selected structures of PsbS monomer extracted from the CpHMD at different pH values as indicated in the legend and aligned on top of chain A to the crystal structure of PsbS dimer.
monomers in the dimeric crystal unit, results in dimers (Figure S7A–C) in which the PsbS H3 domain is facing the luminal amphipathic helix of the other subunit, i.e., helix 4 for Lhcb and H2 for PsbS in the crystal. Such an organization (amphipathic helix 4 facing the amphipathic helix between the first and second transmembrane helices) is typical of dimeric LHCs in plants (Figure S7D). This would suggest that PsbS can stably dock to LHCs only at low pH, when the H3 is structured (see Supplementary Video 2 for an example of the PsbS dimer at low pH). In this respect, the presence of PsbS dimers in the crystal structure (resolved at pH 5) can be the result of the high capacity of PsbS to interact with other proteins at low pH.

Overall, our work suggests that a conformational reorganization is the basis of the mechanism of action of PsbS in plants. This conformational reorganization is triggered by the protonation of multiple Glu residues exposed to the thylakoid lumen. We found that this is possible because the PsbS design adapted to match the range of physiological pH values of the thylakoid lumen by fine-tuning the pKₐ of its lumen-exposed residues.

**COMPUTATIONAL METHODS**

The methods are reported in the Supporting Information.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcllett.9b00437.

PsbS monomer aligned to the chain A in the PsbS crystallized dimer and simulated here via CpHMD at pH 8, showing the progressive loss of structure of its I-lumen loop at the interface between the two monomeric subunits, while the II-lumen loop maintains its structure (AVI)

PsbS monomer aligned to the chain A in the PsbS crystallized dimer and simulated here via CpHMD at pH 5, showing the conservation of structure of its I- and II-lumen loops at the interface between the two monomeric subunits (AVI)

Fully detailed methods and supplementary figures (PDF)

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Notes

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