Redox modulation on chloroplast ATP synthase

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In higher plants, chloroplast ATP synthase has a unique redox switch on its ϒ subunit, which modulates the enzyme activity to limit the ATP hydrolysis at night. To understand the mechanistic details of the redox modulation, we used single-particle cryo-EM to determine the structures of spinach chloroplast ATP synthase in both reduced and oxidized states. We modulated the redox state of the enzyme using external redox agents, dithiothreitol and iodosobenzoate, and characterized the activities of ATP synthesis. The oxidized structure is the same as the previous cryo-EM structure. The disulfide linkage of the oxidized ϒ subunit introduces a torsional constraint to stabilize the two β hairpin structures, interacting with adjacent subunits extensively. It results in a long pause while the central shaft rotates. Because the reduced form is active and flexible, we added an uncompetitive inhibitor, tentoxin, in the reduced sample to limit the flexibility of the enzyme and obtained high-resolution details. In our structures of the reduced state, we found one ADP molecule bound in the βO binding site, and it is likely that we captured the ADP entry state for the subsequent ATP synthesis. Once the ϒ subunit is reduced, free cysteines alleviate this constraint and exert a conformational change to destabilize the long β hairpin structure. The short β hairpin structure is disrupted by a newly formed one-turn helix, destabilizing the interactions of the EDE motifs with adjacent subunits. Our results also showed that the proportions between rotary states of the reduced enzyme are more even than those of the oxidized states. This implies that the reduced enzyme has a smooth transition between rotary states, allowing fast rotation. These result in a concerted movement between subunits within the enzyme complex to facilitate ATP synthesis. Our cryo-EM structures provide mechanistic insight into the redox modulation on the chloroplast ATP synthase for its energy regulation.