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Redox Modulation on Chloroplast ATP Synthase

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In higher plants, chloroplast ATP synthase has a unique redox switch on its □ subunit, which modulates the enzyme activity to limit the ATP hydrolysis at night (1, 2, 3). 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 (4). The disulfide linkage of the oxidized □ subunit introduces a torsional constraint to stabilize the two β hairpin structures, interacting 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 β_O binding site, and it is likely that we captured the ADP entry state for the subsequent ATP synthesis. Once the γ 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 the ATP synthesis. Our cryo-EM structures provide mechanistic insight into the redox modulation on the chloroplast ATP synthase for its energy regulation.

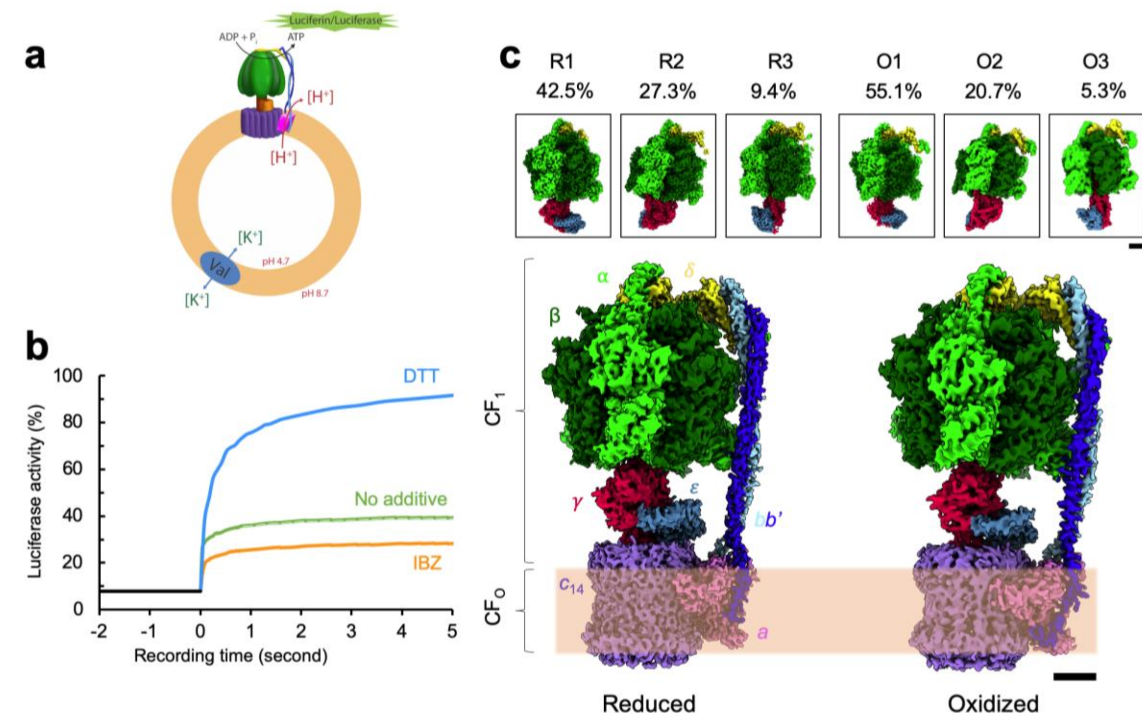

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Figure 1. Fig. 1 | Chloroplast ATP synthase (CF1FO) of Spinacia oleracea in different redox states. a, Schematics of the experimental design for measuring CF1FO function. Purified CF1FO was reconstituted into a liposome (orange) mixed with lipids of phosphatidylcholine and phosphatidic acid. The generated pH gradient across the membrane drove the reconstituted CF1FO to synthesize ATP molecules, which were detected using a luciferin/luciferase assay (green). Val indicates valinomycin. b, Profile of the CF1FO activity measurements of the CF1FO in different redox states. Blue curve represents the sample with dithiothreitol (DTT) (reduced), orange the sample with iodosobenzoate (IBZ) (oxidized), and green the sample with no redox agent added (control). c, Cryo-EM density maps of the oxidized and reduced forms of the CF1FO. Percentages of the particle images used are listed for individual rotary states. Scale bars indicate 25 nm. Color codes: α (light green), β (dark green), (yellow), bb' (blue and light blue), (crimson), (indigo), a (light pink), and c ring (purple). R indicates a reduced state, and O indicates an oxidized state. Membrane bilayer is indicated with the light orange band. The three-dimensional (3D) reconstructions are categorized into three different rotary states (states 1, 2, and 3). Upper insets are the density maps of the F1 domains.

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