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Ferredoxin 2 of *Chlamydomonas reinhardtii*: the crystal structure at 1.2 Å resolution and probing its interaction network

Ferredoxins are typically small, iron-sulfur cluster-containing proteins that act as electron shuttles in a multitude of metabolic redox reactions. The green alga *Chlamydomonas reinhardtii* contains at least six [2Fe2S] cluster ferredoxins, with FDX1 (or PetF) as the predominant isoform. FDX2 is highly homologous to FDX1 and has recently been shown to interact with known FDX1 interaction partners, i.e. FNR1, HYDA1 and PFOR, albeit promoting lower catalytic rates.
In order to determine and compare the characteristics of the two proteins and to be able to study their interaction with other proteins, we over-expressed both FDX1 and FDX2 in *E. coli* and purified the mature versions of the respective proteins by sequentially performing GST-affinity purification, TEV cleavage, Cobalt affinity purification and size exclusion chromatography. The purified proteins were used for UV/Vis, EPR and CD spectroscopy as well as for crystallization studies. Both UV/Vis and EPR spectroscopy revealed typical ferredoxin characteristics for both proteins, which displayed near identical spectra. CD spectroscopy confirmed that the purified proteins were in their folded state containing both α-helices and β-sheets, while recording a CD spectroscopy melting curve determined the T_m of both proteins to be around 60°C. Crystallization screens for different affinity-tagged versions of both proteins were setup, but only the cleaved form of FDX2-TEVcs-GST-His, which has 11 additional amino acid residues at its C-terminus, formed protein crystals. These protein crystals enabled us to solve the FDX2 structure at a resolution of 1.18 Å. This structure was then used to refine a FDX1 structure model and to compare the interaction surfaces on both proteins that are shared with known interactors, specifically the HydA1 hydrogenase. Pull-down experiments using FDX2 and FDX1 as bait and *Chlamydomonas reinhardtii* cell extracts grown under different conditions (i.e. aerobic, anaerobic, sulfur deprivation and KNO_3 instead of ammonium) as prey were performed to probe for known as well as potentially novel interaction partners. Furthermore, using a Clark-type electrode, we determined the hydrogen production rate for a FDX2 *Chlamydomonas* knock-out mutant strain and assessed FDX protein levels by immunoblotting in a variety of mutant strains (ΔFDX2, ΔHydA1 and ΔHydA2).

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