FDX2 of *Chlamydomonas reinhardtii*
The crystal structure & probing its interaction with the hydrogenase

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Background

Ferredoxins (FDXs) are iron-sulfur proteins that are found in all living organisms. In photosynthetic organisms, FDXs act as "electron shuttles" that accept an electron from photosystem I (PSI) and under photoautotrophic growth conditions the ferredoxin-NADP$^+$ reductase (FNR) is the main electron sink. However, in *C. reinhardtii* cells grown under e.g. anaerobiosis or sulfur deprivation, hydrogenases (HYDA) can also accept electrons from a FDX (Figure 1).

C. *reinhardtii* contains six chloroplast FDXs and FDX1 (PetF) is the most important one. However, FDX2 is very homologous to FDX1 and has been shown to be involved in nitrogen assimilation[1]. Here we provide evidence that both FDX1 and FDX2 interact with the hydrogenases. We also present the FDX2 crystal structure at 1.2Å resolution and a HYDA1 interaction model for FDX1 and FDX2.

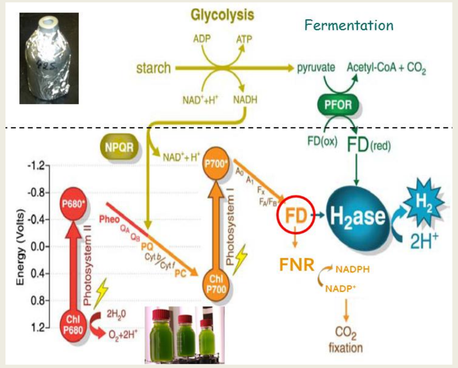
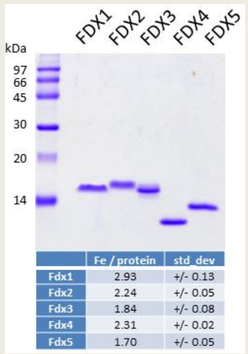


Figure 1: Electron pathways in *C. reinhardtii* (modified from Posewitz et al. *Chlamydomonas Source Book* 2007).

Research Aims

- **Over-expression and purification of *C. reinhardtii* FDXs.**
- **Probing the interaction of FDXs with the hydrogenase(s).**
- **Characterization of ferredoxins (spectroscopic, structural).**

FDX protein purification



	Fe / protein	std. dev
Fdx1	2.93	+/- 0.13
Fdx2	2.24	+/- 0.05
Fdx3	1.84	+/- 0.08
Fdx4	2.31	+/- 0.02
Fdx5	1.70	+/- 0.05

A variety of over-expression constructs for the mature version of each of the FDXs were engineered and used for purification. These included: N-terminal His-tag (with thrombin cleavage site), no-tag, N- or C-terminal GST-His-tag (with TEV cleavage site). Figure 2 shows cleaved FDXs that were obtained from C-terminal GST-His tagged fusion proteins. The iron content was determined and was typically around 2.

Figure 2: FDX purification and iron content determination.

FDX2 interacts with the hydrogenases

To probe for an interaction between FDX2 with the hydrogenases, we performed pull-down experiments (Table 1) and *in vitro* activity assays (Figure 3). For the pull-down experiments FDX2 and FDX1 were covalently coupled to CnBr activated sepharose beads and incubated with a variety of cell extracts. From sulfur deprived cell extracts, we were able to identify the hydrogenases (HYDA1 and HYDA2), the maturation factors (HYDG and HYDEF) as well as FDX5 as potential FDX2/FDX1 interactors (Table 1). In an *in vitro* photohydrogen production assay, FDX1 sustained a three fold higher rate than FDX2 (Figure 3).

Table 1: Selected potential interactors for FDX2 and FDX1. Given are peptide counts and calculated p-values (a measure for the hit to be wrong). Red numbers indicate hits with p-values > 0.2.

		FDX2			FDX1		
GI #	**Protein name**	**Sample**	**-Ctrl**	**p-value**	**Sample**	**-Ctrl**	**p-value**
159470457 159472741	HYDA1/HYDA2 - Iron hydrogenases	2	0	0.13	2	0	0.12
159466536	HYDEF - Iron hydrogenase assembly factor	4	1	0.17	1	1	0.93
159472741	HYDA2 - Iron hydrogenase	5	2	0.25	13	2	0.002
159466244	HYDG - Hydrogenase assembly factor	15	10	0.28	18	10	0.06
159470457	HYDA1 - Iron hydrogenase	5	4	0.71	8	4	0.17
159466834	FDX5 - Apoferredoxin	8	1	0.01	7	1	0.02

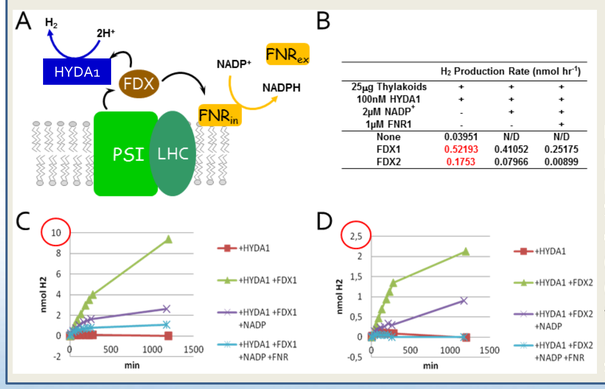


Figure 3: Competitive photohydrogen production assay. (A) Schematic model of the components involved. (B) Summary table and (C &D) rates observed for FDX1 and FDX2. Note the different scales of the Y-axis.

Conclusions

- **FDX1 to FDX5 were purified.**
- **Pull-down and *in vitro* assays provide evidence that FDX2 and FDX1 interact with the hydrogenase(s).**
- **The FDX2 crystal structure was solved at 1.2Å.**
- **FDX2 and FDX1 are very similar spectroscopically and structurally.**
- **FDX2 and FDX1 HYDA1 interaction model were generated and differences on the binding surface are observed.**

FDX2 structure and HydA1 interaction model

FDX2 and FDX1 were characterized by UV/Vis, EPR and CD spectroscopy. The UV spectra display typical ferredoxin peaks at around 330, 420 and 460 nm and the EPR spectroscopy yielded a strong rhombic signal characteristic for [2Fe2S] cluster proteins. CD spectroscopy confirmed that the proteins were folded (a mixture of α-helices and β-sheets) and contained the [2Fe2S] cluster. Both proteins are fairly thermostable with a T_m of around 55°C.

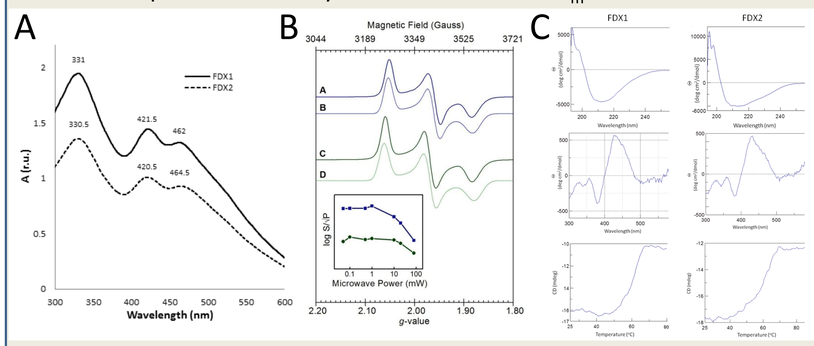


Figure 4: (A) UV/Vis, (B) EPR and (C) CD spectra for FDX1 and FDX2.

FDX2 protein crystals were obtained for the cleaved form of FDX2-TEV-GST-HT in 0.1 M HEPES pH = 7.0, 3.2 M Ammonium sulfate. The structure was solved at 1.2Å resolution and showed a typical ferredoxin fold (a β-sheet formed by five β-strands covered by a single α-helix). The surface charge is mostly negative (Figure 5B). The FDX1 structure could be modeled from homology and both structures were used to create HYDA1 binding models[2] (Figure 5C). There are several amino acid differences on the HYDA1 binding surface (Figure 5D), that might contribute to the different catalytic rates during photohydrogen production (Figure 3B).

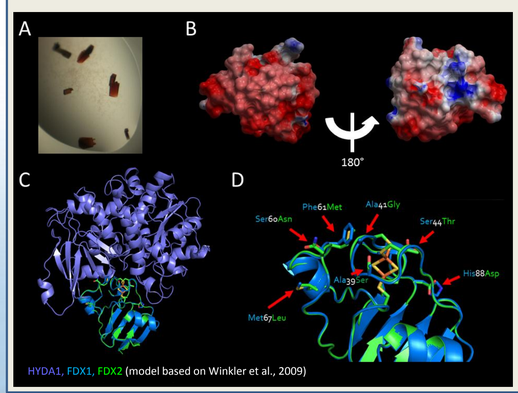


Figure 5: (A) FDX2 protein crystals, (B) FDX2 electrostatic surface charge model, (C) FDX2 and FDX1 HydA1 interaction models and (D) differences between FDX2 and FDX1 on the HYDA1 binding surface.

Acknowledgements

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References

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