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Review

The importance of flavodoxin for environmental stress tolerance in photosynthetic microorganisms and transgenic plants. Mechanism, evolution and biotechnological potential

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ABSTRACT

Ferredoxins are electron shuttles harboring iron–sulfur clusters which participate in oxido-reductive pathways in organisms displaying very different lifestyles. Ferredoxin levels decline in plants and cyanobacteria exposed to environmental stress and iron starvation. Flavodoxin is an isofunctional flavoprotein present in cyanobacteria and algae (not plants) which is induced and replaces ferredoxin under stress. Expression of a chloroplast-targeted flavodoxin in plants confers tolerance to multiple stresses and iron deficit. We discuss herein the bases for functional equivalence between the two proteins, the reasons for ferredoxin conservation despite its susceptibility to aerobic stress and for the loss of flavodoxin as an adaptive trait in higher eukaryotes. We also propose a mechanism to explain the tolerance conferred by flavodoxin when expressed in plants.

1. Ferredoxins and flavodoxins

1.1. Electron shuttling: a common theme in oxido-reductive processes

Electron shuttling is a key feature of many redox pathways in all living organisms. This function is usually performed by diffusible electron carrier proteins which act as electronic switches between cellular sources of reducing power (i.e., light-driven reactions, pyridine nucleotides, sugars) and electron-consuming routes and processes. In organisms displaying oxygenic photosynthesis (plants, algae and cyanobacteria), ferredoxin (Fd) is a key player of electron shuttling [1]. There, Fds collect reducing equivalents generated in the photochemical reactions of the photosynthetic electron transport chain (PETC), and deliver them to a plethora of metabolic, regulatory, dissipative and developmental processes. A substantial fraction of photoreduced Fd is employed for the reduction of $NADP^+$ in an electron-hydride exchange reaction catalyzed by the flavoenzyme ferredoxin-$NADP^+$ reductase (FNR) [2,3]. The NADPH thus formed is subsequently employed for CO_2 fixation in the regenerative steps of the Calvin cycle and for other biosynthetic, regulatory and protective reactions. Reduced Fd molecules also act as electron donors for N and S assimilation, amino acid, fatty acid and secondary metabolism, reductive activation of enzymes, antioxidant regeneration, etc. (reviewed in [4]). A comprehensive list of known Fd partners is provided in Table 1.

Ferredoxins employ iron–sulfur clusters of different stoichiometry as prosthetic groups, with the photosynthetic Fd harboring a [2Fe–2S] center [1]. Canonical Fds have M_w of ~12 kDa and a midpoint redox potential of about −410 mV [5], which allows them to behave as low potential electron shuttles. Fd is found in a wide range of organisms pervading all kingdoms, aerobic and anaerobic, with plastid and mitochondrial variants in higher eukaryotes. Several isoforms are usually present in plants and cyanobacteria [6–9]. Expression of photosynthetic Fd is induced by light and declines under iron starvation [10–14]. Noteworthy, oxidative stress and adverse environmental situations (salt, extreme temperatures, water deficit) lead to down-regulation of Fd levels in both plants and cyanobacteria [14–18].

Many prokaryotes and some oceanic algae contain an isofunctional electron shuttle, flavodoxin (Fld), a small soluble protein (M_w = 15–22 kDa) which has a non-covalently bound FMN

Abbreviations: Fd, ferredoxin; Fld, flavodoxin; FNR, ferredoxin-$NADP^+$ reductase; Ga, billion years ago; GOGAT, glutamate–oxoglutarate amino transferase; PETC, photosynthetic electron transport chain; PSI, photosystem I; PSII, photosystem II; ROS, reactive oxygen species; sFNR, soluble FNR; WT, wild-type

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Table 1
Identified partners of Fd and Fld in chloroplasts and cyanobacteria.

Protein partners	Function	Metabolic pathway	Organisms	References
Ferredoxin				
Photosystem I (PSI)	Photosynthetic electron transport	Photosynthesis	Cyanobacteria, algae, plants	[82]
FNR	$NADP^+$ reduction	Photosynthesis	Cyanobacteria, plants	[1,83]
Nitrite reductase	Reduction of NO_2^- to $NH4^+$	Nitrogen assimilation	Cyanobacteria, algae, plants	[1,83]
Nitrate reductase	Reduction of NO_3^- to NO_2^-	Nitrogen assimilation	Cyanobacteria	[1,83]
Nitrogenase and pyruvate:Fd oxidoreductase or FNR	N_2 fixation	Nitrogen assimilation	Cyanobacteria	[84]
Hydrogenase	H_2 formation	Hydrogen metabolism	Cyanobacteria	[84]
Glutamate–oxoglutarate amino transferase (GOGAT)	Glutamate synthesis	Amino acid synthesis	Cyanobacteria, algae, plants	[1,83]
Sulfite reductase	Reduction of SO_3^{2-} to H_2S	Sulfur assimilation	Plants	[1]
Ferredoxin-thioredoxin reductase	Thioredoxin reduction	Redox regulation[a]	Cyanobacteria, algae, plants	[1,83]
Fatty acid desaturase	Double bond formation in fatty acids	Lipid metabolism	Cyanobacteria, plants	[83,85]
Monodehydroascorbate reductase	Ascorbate regeneration	Antioxidant defense	Plants	[86]
Heme oxigenase and phytochromobilin synthase	Phytochromobilin[b] synthesis	Development	Plants	[87,88]
Heme oxigenase and phycocyanobilin:Fd oxidoreductase	Phycocyanobilin[c] synthesis	Development	Cyanobacteria	[83,89,90]
PGRL1, PGR5, FNR and PSI	Cyclic electron flow	Photosynthesis	Algae, plants	[91,92]
Flavodoxin				
PSI	Photosynthetic electron transport	Photosynthesis	Cyanobacteria, algae	[82]
FNR	$NADP^+$ reduction	Photosynthesis	Cyanobacteria, algae	[22,93]
FNR and PSI	Cyclic electron flow	Photosynthesis	Cyanobacteria	[94]
Nitrogenase	N_2 fixation	Nitrogen assimilation	Cyanobacteria	[95]
Hydrogenase	H_2 formation	Hydrogen metabolism	Cyanobacteria	[84]

[a] Reduced thioredoxin activates key chloroplast enzymes of the Calvin cycle, the malate valve, etc.
[b] Plant chromophore of the light sensor phytochrome and intermediate in the synthesis of chlorophyll.
[c] Chromophore of the light sensor phytochrome in cyanobacteria and green algae, and precursor of the chromophores of the light-harvesting phycobiliproteins.

molecule as prosthetic group instead of an iron–sulfur cluster [19]. Unlike Fd, which is an obligatory one-electron carrier, the flavin group of Fld can in principle exchange one or two electrons, oscillating between the oxidized, the semiquinone and the hydroquinone states [20]. However, empirical evidence indicates that Fld behaves as a one-electron carrier under all circumstances, switching between the semiquinone/hydroquinone states [21]. This transition has a redox potential close to that of the Fe^{+3}/Fe^{+2} reaction of Fd, whereas the conversion of the oxidized form into the semiquinone is usually 200 mV less negative.

Fld properties as redox shuttle largely match those of Fd (Table 1), and the flavoprotein can replace the metalloprotein in most reactions [22]. In organisms in which both electron carriers are present, Fld is typically induced as an adaptive resource under environmental or nutritional hardships that compromise Fd expression or activity (i.e., iron limitation), allowing survival and reproduction under conditions that would be otherwise deleterious. However, a few Fld-specific metabolic routes have been described. Indeed, Fld is an essential gene in *Escherichia coli* and *Helicobacter pylori*, whereas Fd is not [23–25]. Both Fd and Fld are able to mediate $NADP^+$ photoreduction via FNR [26]. This reaction can proceed backwards, from NADPH to oxidized Fd/Fld, for instance in non-photosynthetic plant tissues (i.e., roots) and cyanobacterial heterocysts [3]. NADPH is the normal reductant in heterotrophic microorganisms and mitochondria, although carbohydrates can also be used as electron source to reduce Fd/Fld by committed enzymes such as the pyruvate-Fd reductase of *E. coli* [27].

Fld is present in prokaryotes (including cyanobacteria) and some algae, but has not been found in the genomes of plants (or animals), indicating that this adaptive resource was irreversibly lost in the long evolutionary history that led to current day streptophytes and metazoans [28].

1.2. The basis for functional equivalence: promiscuity as a virtue

Fld and Fd do not share any significant similarity in primary, secondary or tertiary structures, and yet they can interact productively with the same redox partners with comparable efficiency. The key to this apparent paradox resides in the very function of these proteins. The most desirable property of an electron shuttle is the ability to exchange reducing equivalents with the highest possible number of different redox partners. Accordingly, Fd and Fld have been tailored by evolution to be promiscuous in their interactions. Analysis of plant Fd binding sites in various Fd-dependent enzymes revealed no obvious homology [1]. Then, docking of Fd (and Fld) must be determined by general features of the proteins rather than contacts with specific conserved amino acids. The prosthetic groups of both proteins (flavin and [2Fe–2S]) are eccentric and surrounded by patches of negatively charged residues, while their enzyme partners harbor a crown of positively charged amino acids around their exposed cofactors [1]. Initial interactions are therefore steered by electrostatic attractions that help to stabilize the binary complexes, and serve to position the corresponding prosthetic groups at the proper distance to allow direct outer-sphere electron transfer between them. These charged regions are remarkably insensitive to mutations, and different accommodations of the two proteins (i.e., rotations) are allowed without losing the ability for efficient electron transfer [29–31].

Although Fd and Fld differ in nearly all structural features, they could be aligned on the basis of their Coulomb electrostatic potentials. Applying the Hodgkin index to evaluate their similarity in this sense, Ullmann et al. [32] obtained a significant overlapping. The cofactors, rather than their centers of mass coincided in the alignments. Both proteins have strong dipole moments (380–700 Debyes), with the vectors of the negative dipole pointing toward the flavin ring in Fld and the iron–sulfur cluster in Fd [21,33]. These considerations provided a conceptual framework to understand why these electron carriers are able to interact with so many different enzymes, and why they can be swapped without major loss in efficiency.

1.3. Ferredoxin: the Achilles' heel of aerobic life?

Iron–sulfur clusters are sensitive to oxidation and low iron availability, which are the hallmarks of aerobic environments. And yet they are probably the cofactors more diversely employed by contemporary organisms. Why aerobes rely so heavily on chemical groups which appear ill-suited for an oxygen-rich habitat? Iron–sulfur centers in general, and Fds in particular, are very ancient biocatalysts that were already present in early organisms. Life in Earth originated about 3.5 billion years ago (Ga) in an anaerobic environment where oxygen was largely absent and Fe^{+2} and sulfide, plentiful (Fig. 1A).