Three-Dimensional Model of a Novel Photosynthetic Ferredoxin Protein from *Gardenia jasminoides*

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Abstract. Chloroplast ferredoxins (Fd) are small proteins containing an iron-sulfur cluster in photosynthetic organisms. Fd functions as transferring electrons from photosystem I (PSI) to $NADP^+$ via ferredoxin: $NADPH^+$ reductase (FNR). Fd is also the low-potential electron donor to many metabolic and regulatory pathways of the chloroplast. It plays a dual role in primary and secondary metabolism. In this work, we aimed to isolate a gene of Fd from *Gardenia jasminoides*. A *G. jasminoides* fruit cDNA library was constructed, and the GjFd cDNA was isolated from the cDNA library by sequencing method. The GjFd cDNA contains a predicted 444 bp open reading frame that encodes 147 amino acids. A bioinformatics analysis indicated that GjFd has conserved four iron-binding sites and FNR binding residues. A three-dimensional dimmer model of GjFd was built, the structure is similar to Fds from maize and spinach. The results suggest that GjFd is a typical photosynthetic ferredoxin protein mainly responsible for linear photosynthetic electron transort pathway.

1. Introduction

Photosynthesis is an important event for autotrophic plants, which synthesis carbohydrates and ATP for life activities. Oxygenic photosynthesis involves light-driven electron transfer reactions. In photosynthetic electron transport (PET), electrons are originally generated by the splitting of water at photo system II (PSII), transferred to the cytochrome b6f complex (Cytb6f) by plastoquinone (PQ), and from there through the thylakoid lumen to photosystem I (PSI), via plastocyanin (PC). The final events in PET involve the transfer of excited electrons from PSI to the small (11kD), soluble [2Fe-2S] protein ferredoxin (Fd) (Fig.1). The electrons may either follow a linear pathway (linear electron flow, LEF) from water to $NADP^+$ requiring PSI and PS Ⅱ in which Fd serves to reduce ferredoxin $NADP^+$ oxidoreductase (FNR) to produces NADPH and ATP needed for the Calvin cycle [1]; or by a cyclic electron flow (CEF) pathway around photosystem I in which ferredoxin serves to reduce the PQ pool without NADPH generation or water oxidation, its important for balancing the proportion of ATP:NADPH produced by PET [2].

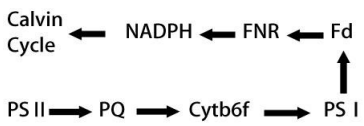

Fig 1.Fd function in electron transfer pathway. Linear electron transfer from water via PSII, plastoquinone (PQ) pool, Cytb6f complex, PSI, Fd and FNR to $NADP^+$, the NADPH may be used for carbon assimilation (Calvin cycle).

Plant-type ferredoxin is a soluble, acidic protein distributed in various plant plastids. Ferredoxin proteins have conserved [2Fe-2S] clusters and binding four Cys residues. Fds are composed of three to six β-strands and one to three α-helices [3]. Fd is encoded as multiple copies in the genomes of most photosynthetic organisms. All the higher plant Fds are categorized into leaf, root and extended C-terminus type, and each type may be involved in different function [4]. In leaves, at least two

isoforms conduct linear and cyclic photosynthetic electron transort around photosystem I [5]. Six Fds have been identified in Arabidopsis [6-7]. Fd1 (minor) and Fd2 (major) represent about 10% and 90% of the total photosynthetic Fd pool, respectively. Fd1makes a specifically higher contribution to CET, Fd2 is mainly responsible for LEF [8], Fd3 located in the roots, which may has function in nitrate and sulfate assimilation [9]; Fd4 present in the leaves, with unknown function; FdC1 and FdC2 are special ferredoxins which contain C-terminal extensions, which is critical for interaction with the C, D, and E subunits of PSI during photosynthetic electron transfer [10].

In addition to the primary function in photosynthesis, Ferredoxin plays a pivatol role in plant cell metabolism, such as biosynthesis of chlorophyll, phytochrome and fatty acids, assimilation of sulphur and nitrogen, and also in redox signaling [4,11]. Overexpression of Fd in plant was found to enhance tolerance to abiotic and biotic stress [12]. It was because that Fd could down-regulate reactive oxygen species (ROS) level produced under adverse environments through a ROS-scavenging pathway [13]. In hydrogen produced green algae *Chlamydomonas reinhardtii*, the relatively crucial enzyme is [FeFe]-hydrogenase (HydA), is considered a promising source of renewable clean energy. When the Fd-HydA fusion gene is expressed in micro-algal cells of *C. reinhardtii*, the fusion enzyme is able to intercept photosynthetic electrons and use them for eicient hydrogen production[14]. Cytochrome P450s (P450s) are key enzymes in the synthesis of bioactive natural products in plants. The fusion of Fd with P450CYP79A1 from *Sorghum bicolor*, which catalyzes the initial step in the pathway to cyanogenic glucoside dhurrin, the electron transfer from photosystem Ⅰto CYP79A1 has been enhanced. The fusion strategy therefore forms the basis for using photosynthetic reducing power toward P450-dependent biosynthesis of important natural products[15].

Gardenia jasminoides originates in Asia and has been in cultivation for at least a thousand years. The fruit of *G. jasminoides* is used in Asian countries as a natural colorant, and as a traditional herbal medicine. Crocin, crocetin and geniposide are the main secondary metabolites in the fruit, and they all exhibit a wide range of pharmacological activities [16]. In this paper, we identified and analyzed a photosynthetic ferredoxin protein in *G. jasminoides*.

2. Materials and Methods

Plant and Growth Conditions. *G.jasminoides* plants cultivated at Guangdong Pharmaceutical University were used as materials. Fruits were collected at development stageII, closed with yellowish green exocarp and orange mesocarp. The samples were stored at–80℃ until required.

CDNA Library Construction，ESTs Sequencing and Cloning of GjFd. Total RNA was extracted from Gardenia fruit (stageII), using a modified CTAB (hexadecyl trimethyl ammonium bromide) based extraction protocol [17]. From total RNA, the cDNA library construction and amplification were performed following the users manual of the CreatorTM SMARTTM cDNA Library construction Kit (Clontech, USA). The SMART cDNAs were ligated into SfiI-digested pDNR-LIB vector and transformed into Escherichia coli strain DH5α. Colonies were randomly picked, inoculate each colony to a separated PCR reaction solutions. The colony was lysised by heating the mixed solutions at 95℃ in a PTC-200 Thermocycler (MJ Research, USA) for 5 min. After then, went to PCR amplification procedure with M13 primers provided by the CreatorTM SMARTTM cDNA Library construction Kit. The amplified PCR products (ESTs, expressed sequence tags) were analyzed by 1.2% agarose gel electrophoresis. When the amplified PCR products were longer than 1000 bp, incubated the isolated colonies and sequenced the ESTs. There are 40 ESTs were sequenced. After sequencing and analysis, the colony containing the predicted pDNR-LIB-GjFd was isolated.

3. Results and Discussion

We identified novel Fd homologues in *Gardenia jasminoides* (named GjFd) by exploiting the fruit cDNA library of *G. jasminoides*. The The full-length GjFd cDNA (Genbank accession No.

KM371232) was obtained. The cDNA contains a predicted 444 bp ORF that encodes 147 amino acids. The predicted protein sequence of GjFd (Genbank accession No. AIX10940) was compared to Genbank database, multiple sequence alignment was performed using the program Clustal Omega, the best homology was found to Fd-like protein of *Coffea canephora*. The two proteins share 74.8% identical amino acids. And the homology to Fd proteins from other species are range from 70.9% to 35.1%. GjFd is more closed to major Fds from many species, which consists of barley HvFd1, rice OsFd1, maize ZmFd1. The potential chloroplast transit peptides (cTP) in GjFd were predicted using the ChloroP1.1 server (http://www.cbs.dtu.dk/services/ChloroP). The cTP was located between amino acid residues 1 and 50, a potential cTP cleavage site was predicted at the residue 51. GjFd have a common Fd domain, and four iron binding sites and several catalytic loops in this domain. GjFd is composed of five β-strands and four α-helices. The main differences are at the N-terminal. Phylogenetic analysis of GjFd (using MEGA4) with representative Fd proteins from database indicated that GjFd is clustered into the leaf type subgroup which consists of barley HvFd1, rice OsFd leaf type, maize ZmFd1 and 2, and Arabisopsis AtFd1 and 2 (not shown). In Arabidopsis, under conditions thought to stimulate CEF, such as drought or high light, transcripts of AtFd1 increase significantly [18]. Knockout mutation of the major isoform AtFd2 in Arabidopsis led to higher expression of nonphotosynthetic Fd (Fd3 and Fdc1), whereas AtFd1, the minor photosynthetic isoform preferentially engaged in CEF, was up-regulated only when plants were exposed to high light irradiation [13]. Studies in pea indicated that the minor pea Fd isoform (PsFd1) promotes enhanced cyclic flow around photosystem I [5].

The 3D (three dimension) model structure of GjFd was predicted using SWISS-PDB software, the X-Ray diffraction at resolutions down to 1.7 Å of Zea mays Fd1 (residues from 53-148) complexes with Fe_2S_2 (PBD code 3B2F) was used as template [19] (Fig. 2A), the GjFd includes residues 51-146 was predicated. The structure was successfully built as a monomer (Fig. 2D), the fold of GjFd consists of four α-helices flanked by five β-sheet. Indicating a high level of structural similarity with maize Fd1, both Fd have similar four iron-binding sites and four active loops [23].

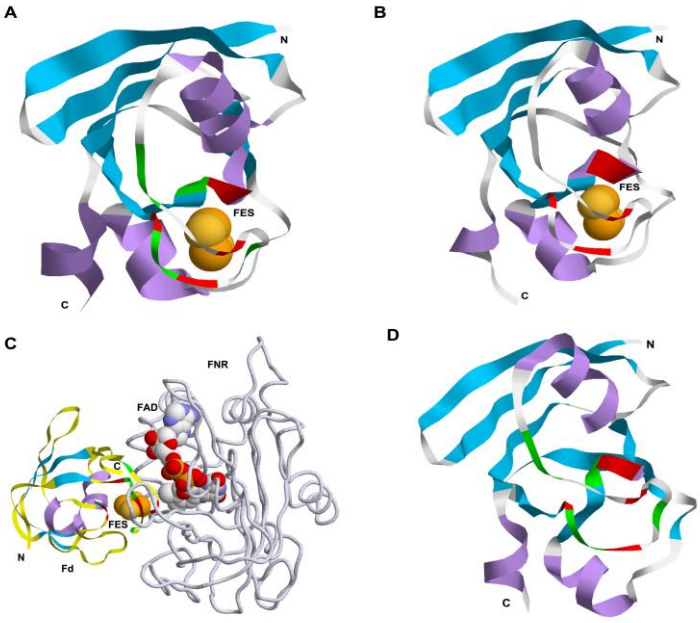

Figure 2. Three-dimensional structure of Fd. (A): Crystal structure of dimer Fd1 from *Zea mays* (53-148) complex with FES, PBD code: 3B2F. (B): Crystal structure of Fd1 from *Spinacia oleracea* (51-147) complex with FES, PBD code: 1A70. (C): Crystal structure of dimer Fd1 from *Zea mays* (53-148) complex with FNR, PBD code: 1GAQ. (D): Model structure of GjFd (51-146 residues; Genbank accession no. AIX10940), predited by Swiss-PDB software. In (A-C): FES in Fd are shown in sphere diagram, prosthetic group FAD in FNR is shown in sphere diagram, FNR is shown in trace diagram In (A, C and D): catalytic loops site shown in green. In all: Fd are shown in ribbon diagram, α-helix are shown in purple, β-sheet are shown in light blue, iron-binding site shown in red.

Crystal structure of Fd1 from Spinacia oleracea (51-147) complex with Fe_2S_2 (FES), PBD code: 1A70 (Fig. 2B), and the X-Ray diffraction at resolutions down to 2.59 Å of Zea mays dimmer Fd1(53-150) complexes with FNR (19-314, PBD code 1GAQ, Fig. 2C) , ware compared to GjFd. The fold of GjFd has structural similarity with 1A70 and Fd in 1GAQ. In 1GAQ, The redox centers of FNR and Fd are in close proximity, benifit for direct electron transfer through space between the two prosthetic groups. Five pairs of which (glu 29, Are 40, Asp 61, and Asp65 in Fd, shown in red in Fig 2, and Lys 304, Glu154, Lys 33, and Lys 91 in FNR, not shown) form intermolecular salt bridegs [20]. The corresponding four residues and their 3D position in GjFd is highly concerved.

4. Summary

Chloroplast Fds are small, two-iron two-sulfur proteins that serve as electron donors in various metabolic pathways other than photosynthesis, such as nitrogen and sulfur assimilation. Although the molecular features that determine Fd preference for LEF or CEF-PSI have yet to be determined, it is conceivable that evolution has refined the properties of these eclectron carrier proteins to optimize energy use efficiency and protection under changing environments, controlling their relatve abundance, affinities, and electron transfer rates.

This work suggests that, GjFd may belong to typical photosynthetic Fd proteins and it's a major Fd in photosynthetic mainly responsible for LEF.

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