Characterization of poplar GrxS14 in different structural forms

Dear Editor,

Glutaredoxins (Grxs) are glutathione-dependent thiol disulfide oxidoreductases of the thioredoxin family present in all organisms from bacteria to human (Noguera et al., 2005). Depending on their active site sequence, Grxs are essentially classified into three families: the dithiol Grxs, the monothiol Grxs and the CC type restricted to plants (Rouhier et al., 2008). Grxs play important biological functions in plants, such as oxidative stress responses, iron-sulfur (FeS) cluster assembly, and cell signaling, etc. (Rouhier et al., 2008). There are totally 31 Grx isoforms in Arabidopsis thaliana, and 19 Grx isoforms in Populus trichocarpa (Rouhier et al., 2004). For Grxs of Populus trichocarpa, structures of GrxC1, GrxC4 and GrxS12 have been resolved, all belong to dithiol Grxs (Noguera et al., 2005; Feng et al., 2006; Rouhier et al., 2007; Couturier et al., 2009). The only structure available for monothiol Grxs in plants is that of Arabidopsis Grxcp, which is also called GrxS14 or CAXIP1 (Cheng and Hirschi, 2003; Li et al., 2010).

It was found that Arabidopsis GrxS14 is a new class of signaling molecules in plants that can regulate the Ca^{2+} transport activity of CAX1 (cation exchangers) by interacting with the N-terminal region of CAX1 (Cheng and Hirschi, 2003). It was suggested that Arabidopsis GrxS14 functions to protecting cells against protein oxidative damage (Cheng et al., 2006). Both Arabidopsis and poplar GrxS14 are monothiol Grxs located in the chloroplasts, which exist as an apo form and a holof form bridged by a [2Fe-2S] cluster with two external glutathione (GSH) ligands, and they can complement a yeast grx5 mutant defective in FeS cluster assembly in vivo (Bandyopadhyay et al., 2008). It was proposed that Arabidopsis and poplar GrxS14 may function as scaffold protein for the assembly of [2Fe-2S] cluster, as GrxS14 can transfer intact cluster to physiologically relevant acceptor proteins which is regulated by GSH (Bandyopadhyay et al., 2008; Wang et al., 2012; Liu et al., 2013).

Here we report the solution structure of reduced monomeric GrxS14 using NMR data collected on protein samples in the presence of 20 mmol/L GSH. A summary of structural restraints used in the structure calculation and statistics for the structure ensemble is listed in Table S1. Residues 5–109 of apo GrxS14 form a compact thioredoxin fold structure while the first four residues are flexible (Fig. 1A). It comprises five α-helices and four β-strands constitute a mixed β-sheet as the core of structure (Fig. 1B). Helices α1 and α3 are packed on one side of the β-sheet, while α2, α4 and α5 are on the other side.

The overall fold of poplar GrxS14 is similar to other Grxs. The RMSD of backbone heavy atoms in secondary structure regions is 1.7 Å between poplar and Arabidopsis GrxS14 which shares an 80% sequence identity (Figs. 1D and S3A). The relatively large RMSD between the two may be due to that the poplar apo GrxS14 was determined in the presence of GSH, while no GSH is in the crystal structure of Arabidopsis GrxS14. When comparing to dithiol Grxs, the major difference is at the loop region between β1 and α2: poplar GrxS14 contains ten amino acid residues, while only four residues in poplar GrxC1 and human Grx2 (Fig. 1C). Sequence alignment indicated that monothiol Grxs all possess a long loop in this region, whereas the dithiol Grxs usually have a short loop (Fig. S3). This long loop before the active site (CGFS) is a structural characteristic of monothiol Grxs.
analysis revealed that the amount of dimer fraction is significantly reduced for the two mutants without GSH (Figs. S2C and S2D), indicating that the aromatic stacking and the salt bridges are critical for the dimerization. Thus, the docking model of the GrxS14 dimer is valid.

While most Grxs are found to be monomeric protein (Lillig et al., 2008), it was reported that the reduced poplar GrxC4 can self-associate into dimers with $K_d$ in mmol/L range (Noguera et al., 2005). Comparing the dimer interface between GrxS14 and GrxC4, the residues involved aromatic stacking (F35 in GrxS14 and Y29 in GrxC4) and electrostatic interactions (D88, E92 in GrxS14 and D85, E89 in GrxC4) are conserved in sequences (Fig. S3B), and the dimer interface of GrxS14 should be similar to that of GrxC4 (Noguera et al., 2005). Although there is only one molecule in the crystallographic asymmetric unit for the crystal structure of Arabidopsis GrxS14 (Li et al., 2010), we found that a similar dimer interface exists between two molecules in two asymmetric units (Fig. S5B). The dimer interface of Arabidopsis GrxS14 is mainly involved in aromatic contact between two F99 residue and electrostatic interaction between K130 and E156, similar to those in poplar apo GrxS14 dimer (Fig. S5). However, it is expected that there are differences for the details of the two dimer interfaces, since our poplar apo GrxS14 dimer structure is a docking model and the dimer interface for Arabidopsis GrxS14 may be distorted due to crystal packing.

We have also performed NMR titration experiments to monitor the interaction between apo GrxS14 and GSH. The perturbation of GSH on NH signals of apo GrxS14 is rather dramatic, as residues with significant combined NH chemical shift changes (>0.05 ppm) between free protein and that with 230-fold of GSH are distributed on all secondary structure elements (Figs. 2C and S6). This is consistent with the above mentioned relatively large RMSD between Arabidopsis GrxS14 and poplar apo GrxS14, as our poplar apo GrxS14 structure is determined in the presence of GSH (Fig. 1D). This may suggest that GSH binding can trigger relatively global conformation adjustment for poplar apo GrxS14.

NH signals from residues at different areas were chosen to fit the dissociation constant and similar $K_d$ values (apparent $K_d$ ~5 mmol/L) were obtained, which suggests that only one GSH binds GrxS14 (Fig. S7). Based on the chemical shift perturbation of GSH, we calculated structure models of GSH bound GrxS14 using HADDOCK 2.0 (supplementary methods), which reveal that GSH does bind at the conserved GSH binding motif of Grxs (Lillig et al., 2008). In the models, GSH mainly contacts three regions of GrxS14 (Fig. S4). One is the S2D, indicating that the aromatic stacking and the salt bridges are critical for the dimerization. Thus, the docking model of the GrxS14 dimer is valid.

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the cis configuration of P75, should provide enhanced favorable contacts with GSH (Couturier et al., 2009). The structural model for GrxS14/GSH complex may provide a recognition mechanism for Grxs to target glutathionylated protein substrates in the reversible protein glutathionylation process. In addition, as the GSH binding site was included in the dimer interface of apo GrxS14 (Figs. 2B, 2D and S8C), the binding of GSH is incompatible with the dimerization of GrxS14, which explains why GSH can prevent the dimerization of GrxS14 (Fig. S2B).

Grx structures with GSH covalently linked to the active site cysteine by disulfide have been reported for E. coli Grx3 (PDB 3GRX), human Grx1 (PDB 1B4Q), yeast Grx1 (PDB 3C1R), Arabidopsis GrxC5 (PDB 3RHB) and poplar GrxS12 (PDB 3FZ9). Grx structures with non-covalently bound GSH are also available for human Grx2 (PDB 2FLS), poplar GrxC4 (Noguera et al., 2005) and yeast Grx6 (PDB 3L4N), all belong to the classical dithiol Grxs. It is found that the GSH binding modes are quite similar at the conserved GSH binding motif (three regions mentioned above) for both monothiol and dithiol Grxs.
whether GSH bound non-covalently or covalently with mixed disulfide (Figs. S8A and S8B). Meanwhile, ligand GSHs in holo Grxs also show very similar binding modes at the conserved GSH binding motif (Fig. S8B).

Furthermore, we have characterized the holo GrxS14 with NMR spectroscopy. The backbone resonance assignments of holo GrxS14 were determined for ~90% of the total residues (Wang et al., 2011). A comparison of the 2D 1H–15N HSQC spectra of apo and holo GrxS14 is shown in Fig. 2E. NH peaks of residues G26, C33, F35, Q37, T38, V39, V40, W71 (side chain NH), F74, G86 and D88 are missing (Fig. 2E). Most of 13Cα chemical shifts are obtained for holo GrxS14, except for residues P31, C33, G34, T73 and G86. On the other hand, only 5 residues have significant NH chemical shift changes (δ > 0.05 ppm) among the 86 assigned NH signals: S36, L56, K66, W71, G85 and V91 (Figs. 2F and S9A). NH signals of residues M24, T27, K28, Q41, I55, Q63, L77, V91 and K95 show significantly intensity reduction compared to the apo form (Figs. 2F and S9B). The 13Cα chemical shift changes are quite small for most residues except for G26, F35, S36, V39, E92 and K95 (δ13Cα < 0.3 ppm) (Figs. 2F and S9C).

Most of missing and weaker NH signals should be due to the paramagnetic effect of the [2Fe-2S] cluster, as they are from residues located around the CGFS active site and/or the GSH binding site, where the [2Fe-2S] cluster is presumably coordinated by the active site cysteines and the two GSH cysteines (Fig. 2F). The paramagnetism of the [2Fe-2S] cluster could cause NMR signals broadened and/or hyperfine-shifted for residues over 10 Å away from the cluster, dependent on the magnetic susceptibility tensor of the cluster (Feng et al., 2006). Thus, although some of the residues are not very close to the active site, their NH signals are also affected. Since most NH peaks in the 2D 1H–15N HSQC spectrum of holo GrxS14 can be superimposed on those of apo GrxS14, and the residues without significant 13Cα chemical shift changes are distributed in all secondary structure elements (Fig. 2E and 2F), the structure of each subunit in the holo GrxS14 should largely remain the same as the apo protein.

As a summary, we have determined the solution structure of apo GrxS14, and investigated the structures of the apo GrxS14 dimer, its complex with GSH, and holo GrxS14. The conserved GSH binding site in apo GrxS14 implies the recognition mechanism of monothiol Grxs to various glutathionylated proteins as substrates. While GSH serves as FeS cluster ligand in holo GrxS14, GSH also inhibits the non-covalent dimerization of apo GrxS14. As the Kd values of GSH binding is in mmol/L level and the concentration of GSH is fluctuating in the mmol/L range in plant chloroplast (Rouhier et al., 2008), GSH may have a regulation effect on the dimerization of GrxS14 in vivo. Therefore, it seems that GSH may play a more complicated role in the structure and function of GrxS14, as we have previously reported that GSH can regulate the FeS cluster transfer from holo GrxS14 to apo ferredoxin (Wang et al., 2012). Further studies are needed to uncover the hidden physiological roles of GSH on GrxS14.

FOOTNOTES

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Lei Wang, Yifei Li, Jean-Pierre Jacquot, Nicolas Rouhier, and Bin Xia declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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