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Structural Basis for Delivery of the Intact [Fe2S2] Cluster by Monothiol Glutaredoxin†

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ABSTRACT: Glutaredoxins (GRX) are redox proteins which use glutathione as a cofactor and are divided into two classes, monothiol and dithiol. In each class, several GRX have been shown to form [Fe2S2] cluster coordinating homodimers. The dithiol GRX homodimer is proposed to serve as a sequestration form and its iron−sulfur cluster as an oxidative stress sensor. In contrast, the monothiol GRX homodimer has been suggested to act as a scaffold for [Fe2S2] cluster delivery. We present here the structure of a monothiol GRX homodimer (*Escherichia coli* GRX4) coordinating a [Fe2S2] cluster that reveals the structural basis of intact iron−sulfur cluster delivery.

Glutaredoxins (GRX) are redox proteins widely present in both prokaryotes and eukaryotes which share the thioredoxin fold and use glutathione as a cofactor. These proteins are divided into two classes, monothiol and dithiol, on the basis of the presence of one and two cysteines in the active site (C-G-F-S and C-P-Y-C, respectively). Whereas dithiol GRX have been characterized as reducers of disulfides (protein−S−S) or glutathione mixed disulfides (protein−S−SG) thereby serving important roles in redox homeostasis and signal transduction, the mechanism and function of monothiol GRX remain puzzling (for a review, see ref (*1*)). It is noteworthy that some dithiol glutaredoxins that contain a serine or a glycine in their active site (C-S/G-Y-C) instead of the canonical proline can form homodimers which coordinate a [Fe2S2] cluster (*2*−*4*). Each iron atom of the cluster is bound to the glutaredoxin N-terminal cysteine and the cysteine moiety of the noncovalently bound glutathione. This [Fe2S2] cluster thus plays the part of a lock at the dimer interface (*4*, *5*). It has been proposed that this homodimer centered on the [Fe2S2] cluster constitutes a sequestration form of the active monomeric GRX which would be liberated upon degradation of the cluster under oxidative conditions (*2*). Monothiol GRX are inactive in dithiol specific activity assays but have been demonstrated to be crucial to iron−sulfur cluster biosynthesis (for a review, see ref (*6*)). Furthermore, monothiol GRX of bacteria, cyanobacteria, and eukaryotes have recently been shown to coordinate an iron−sulfur cluster (*7*, *8*) and proposed to act as scaffold for the delivery of the [Fe2S2] cluster to acceptor proteins, although in vivo data are still missing (*9*). However, it is still unclear how the differences between [Fe2S2] cluster binding dithiol and monothiol GRX biochemical properties can be explained, all the more since both classes bind the cluster the same way using the N-terminal active site cysteine and the cysteine moiety of the noncovalently bound glutathione. We here report the first dimeric structure of a monothiol GRX (*Escherichia coli* GRX4) bound to two glutathione molecules and coordinating a [Fe2S2] cluster. The comparison of this structure with those of monomeric GRX4 and both monomeric and dimeric dithiol human GRX2 provides a conformational-change-requiring mechanism for possible monothiol GRX-dependent transfer of the intact [Fe2S2] cluster to other proteins.

E. coli GRX4 was overexpressed as a His-tagged recombinant protein and first purified on a nickel affinity resin under aerobic conditions. It is noteworthy that the His tag has been shown to impair neither the function of yeast GRX5 in the biogenesis of Fe/S clusters nor the rescue of the GRX5-less yeast mutant by *E. coli* GRX4 (*10*). The protein was then transferred to anaerobic conditions in a glovebox for gel filtration purification and subsequent crystallization trials. The protein was brownish and crystallized into dark red crystals which were flash-frozen under anaerobic conditions. They diffracted up to 1.9 Å in the $P4_32_12$ space group. After anomalous phasing at the iron edge and refinement, the structure exhibited an R_{free} of 23.4% (see the Material and Methods in the Supporting Information)

The asymmetric unit contains one GRX4 homodimer bound to two molecules of glutathione and a [Fe2S2] cluster. The two protomers follow the canonical thioredoxin fold constituted by a central sheet made of four β-strands surrounded by five α-helices (Figure 1). They are very similar as illustrated by their low root-mean-square deviation (0.22 Å^2 for the 104 Cα atoms over 108). The homodimer is centered on a [Fe2S2] cluster, and each iron atom is tetracoordinated by the two inorganic sulfurs, the N-terminal cysteine of the protein (Cys30), and the cysteine moiety of the glutathione in a manner similar to that of the human dithiol HsGRX2 dimer (*5*) (Figures 1 and 2). The glutathione molecule

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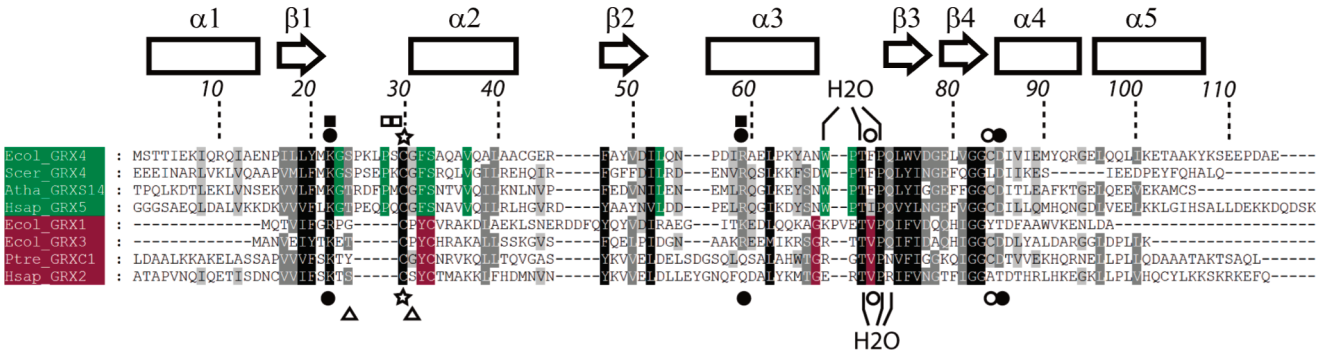

FIGURE 1: Sequence alignment of monothiol (green) and dithiol (purple) glutaredoxins. Sequences are from *E. coli* (Ecol), *Saccharomyces cerevisiae* (Scer), *Arabidopsis thaliana* (Atha), *Populus tremuloides* (Ptre), and human (Hsap). Conserved residues over the two classes are highlighted in black (high degree of conservation) or gray (low degree of conservation). Residues specifically conserved in monothiol or dithiol GRX are colored green or purple, respectively. Structural elements are depicted in boxes (α-helices) and arrows (*β*-sheets). The N-terminal cysteine that contacts the [Fe2S2] cluster iron is denoted with a star. Squares indicate *E. coli* GRX4 residues that participate in the contacts between the two protomers [(■) one protomer and (□) the other]. Circles indicate *E. coli* GRX4 and human GRX2 residues that contact the glutathione molecule [(●) side chain involved and (○) main chain involved]. The triangles indicate human GRX2 residues that interact with the glutathione molecule nested in the partner protomer groove.

nests in the same groove as and in a position similar to that of HsGRX2 and forms hydrogen bonds with the same residues, namely, Lys22, Arg59, Phe70, Cys84, and Asp85. However, whereas the orientation of a protomer toward the other is very similar in both dithiol GRX dimer structures [HsGRX2 and poplar GRX C2 (*4*)], the *E. coli* GRX4 structure presents a contrasted orientation consisting of a 90° rotation of a protomer relative to the other as depicted in Figure 1 of the Supporting Information. As a consequence, GRX4 protomers establish original direct interactions with each other, implying residues localized in the loop connecting *β*-sheet 1 to α-helix 2 and in α-helix 3 (Lys22, Arg59, Pro28, and Ser29), whereas the interface of HsGRX2 is composed of the cluster and the two glutathione molecules to the exclusion of any direct contact between the two protomers. This structural difference has important consequences for the electronic environment of the cluster which would explain the differences observed by Bandyopadhyay and co-workers (*9*) between monothiol and dithiol GRX in UV−visible absorption as well as circular dichroism and resonance Raman spectra.

Although the overall structures of the monomeric [Protein Data Bank entry 1YKA (*11*)] and dimeric forms of GRX4 look similar, a closer comparison of GRX4 dimer and monomer structures reveals important differences (Figure 2A). Briefly, whereas all helix and sheet positions are conserved between these structures, the *β*1−α2, *β*2−α3, and α3−*β*3 loops present noticeable changes. The position of the α3−*β*3 loop in the dimeric conformation allows the adequate positioning of three residues (Trp68, Thr70, and Pro72) that contact an ordered water molecule interacting with the carboxylate of the glutathione glutamate, whereas the Phe71 aromatic part can pack against the aliphatic portion of this glutamate. The *β*1−α2 loop, which corresponds to a specific insertion of monothiol glutaredoxins, presents even more contrasted positions, as depicted in Figure 2A. This leads to important differences in the positions of Lys22 and Cys30 which are both crucial for iron−sulfur cluster binding as we showed previously (*7*). Indeed, Cys30 coordinates an iron atom, while Lys22 directly contacts both the carboxylate function of the glutathione glycine residue and the adjacent protein. By contrast, in the monomer structure, Cys30 thiolate and Lys22 amine are 7 and 6.4 Å, respectively, from their positions in the homodimer and thereby cannot perform the interactions described above. It is noteworthy that Ser33 which is specific to the monothiol active site and conserved in the whole class seems to play a pivotal role by establishing two different contacts in monomeric and dimeric conformations. This residue performs an interaction with Lys22 backbone amine function in the homodimer structure, most probably stabilizing the peculiar position of *β*1−α2 loop (Figure 2 of the Supporting Information). This contact no longer exists in the monomer structure where Ser33 interacts with Ser29. Interestingly, replacement of Ser33 with an alanine renders GRX4 insoluble (*7*). Finally, the *β*2−α3 loop adapts to *β*1−α2 positions in either the monomeric or dimeric conformation to avoid steric clashes.

The GRX4 monomer conformation thus prevents the binding of the glutathione molecule and the [Fe2S2] cluster and thus precludes the formation of a homodimer. This suggests that the formation of the GRX4 dimer harboring a glutathione-liganded iron−sulfur cluster requires important conformational adaptations of the three loops listed. In other words, this means that GRX molecules switching from dimeric to monomeric conformation should release their [Fe2S2] cluster. We thus propose that the mechanism that might allow GRX4 to deliver an intact iron−sulfur cluster to acceptor proteins could rely on a switch between these two conformations which might be triggered by the cluster acceptor proteins and/or chaperones implicated in cluster transfer. The conservation of both length and composition of the monothiol specific α2−*β*2 loop in the other monothiol glutaredoxins strongly suggests that this mechanism would be conserved in this whole class.

Interestingly, comparison of the structures of monomeric GRX2 (NMR structure, 2CQ9, RIKEN Structural Genomics/Proteomics Initiative, no publication available), monomeric GRX2 bound to glutathione (crystal structure, 2FLS), and dimeric GRX2 (crystal structure, 2HT9) reveals that all three conformations superimpose almost perfectly with subtle accommodations of few side chains in contact with the cluster or the glutathione molecule (Figure 2B). The only noticeable difference lies in the *β*1−α2 loop which moves ~4 Å to accommodate the glutathione.

Taken together, these data suggest a structural basis for the two diametrically opposite roles of monothiol and dithiol

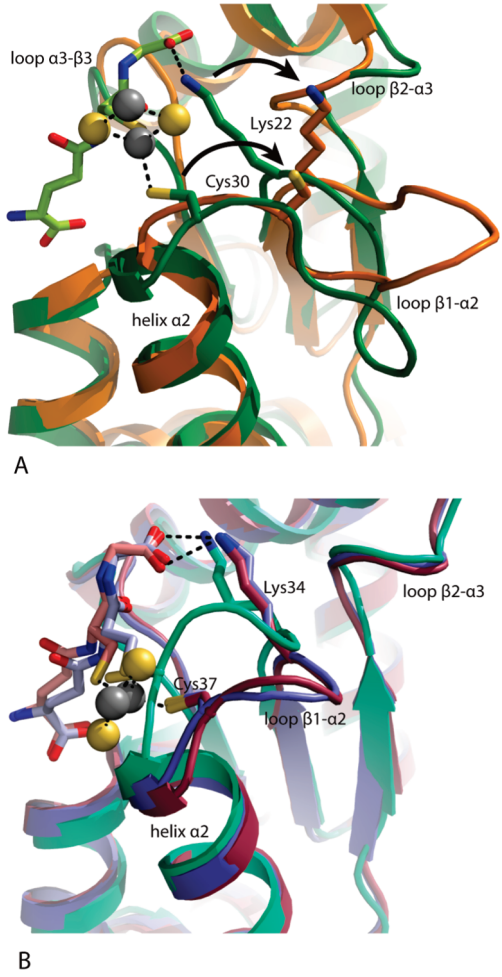

FIGURE 2: Structure of monothiol *E. coli* GRX4 and dithiol human GRX2 active sites. (A) Superimposition of monomer (orange) vs homodimer (green) structures of *E. coli* GRX4 (Protein Data Bank entries 1YKA and 2WCI, respectively). (B) Superimposition of monomer (cyan), monomer bound to glutathione (blue), and homodimer (purple) structures of human GRX2 (Protein Data Bank entries 2CQ9, 2FLS, and 2HT9, respectively). The glutathione molecule present in the homodimer structure is shown as sticks (pale green for GRX4 and pale blue or pink for GRX2), and the cluster atoms are represented by gray (iron) and yellow (sulfur) spheres. The second protomer of the homodimer structures and its related glutathione have been omitted for the sake of clarity. Side chains of selected residues are depicted as sticks. The black arrows highlight the major conformational change occurring between the dimer and monomer conformation.

glutaredoxin homodimers. The monothiol GRX dimer would serve as an iron−sulfur cluster sequestration form, whereas the dithiol one acts as a sequestration form for active GRX−glutathione complexes. We propose that in monothiol dimers the protein undergoes major conformational changes to release the intact [Fe2S2] cluster, thus serving roles of storage, transport, and delivery to acceptor proteins, whereas in dithiol GRX, this cluster in contrast serves as a sensor and is destroyed under oxidizing conditions to liberate dithiol GRX, of which the structure remains almost unchanged (*2*, *12*, *13*).

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SUPPORTING INFORMATION AVAILABLE

Supplementary figures, detailed experimental procedures, and crystallographic statistics. This material is available free of charge via the Internet at http://pubs.acs.org.

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