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Report:

Thioredoxin 2 (TXN-2) is a mitochondrial protein that contains the active-site proper to thioredoxins: W**C**GP**C**K.Both cysteines belonging to that site are involved in the biochemical mechanism of thioredoxins. They take part in the reduction of the disulfide bond of the target protein.The reduced form TXN-$(SH)_2$ can directly reduce the disulfide bond from its substrate and the thioredoxin takes its oxidized form, characterized by the formation of an intramolecular disulfide bond, TXN-S_2. The oxidized mitochondrial peroxiredoxin molecules are part of the possible substrates.In the TXN-1 molecules additional Cys residues are present, whereas they do not exist in the sequence of TXN-2. Those Cys residues induce, in TXN-1, the creation of homodimers by the formation of intermolecular disulfide bonds. Those homodimers could play the part of a regulator.

In a previously determined structure of TXN2, despite the crystallization in presence of DTT, we observed respectively the following distances between the S^{γ} atoms of Cys31 and Cys34, the two catalytic residues: 3.01, 2.92, 3.36, 3.38, 3.18, 3.13 Å. These values are to be compared to the typical distance observed in a disulfide bridge, 2.05 Å and to the sum of the Van der Waals radii of two sulfur atoms, 3.6 Å, which should correspond to the minimum distance when the disulfide bridge is not present. With the exception of molecules C and D which are on the whole reduced, all the other molecules show distances intermediate between the values expected in the reduced form and the oxidized form. It must be concluded that in the crystal, some molecules were reduced while the other ones were oxidised and that the sulfur atoms appear in some average electron density. Since all the attempts to grow crystals in absence of DTT were unsuccessful

and considering that the two sulfur atoms are not too far away from each other, it was supposed that oxidation could take place in already grown crystals.The TXN2 protein crystals were grown in 0.2M ammonium sulfate, 0.1M sodium acetate buffer (pH 4.6), 22% (w/v) PEG 3350, 1mM 1,4-dithio-dl-threitol (DTT) as antioxidant, and 0.02% (w/v) sodium azide by the hanging drop vapour diffusion method at 18°C, using a protein concentration of 6mg ml^{-1}. Crystals appear after 3 days. They look like tin plates and grow to a size of 0.4 x 0.4 x 0.05 mm^3. The oxidized form of TXN2 was obtained by soaking already grown crystals in 1mM H_2O_2 for 90 seconds. The oxidized form of TXN2 crystallizes in space group P1 with 6 molecules in the asymmetric unit. A high degree of completeness (99.3%) was obtained by collecting data of the triclinic crystal in two different orientations. A resolution of 1.8 Å was achieved. The structure was solved by molecular replacement and refined to R = 0.169 and Rfree = 0.218. The resulting S^{γ}-S^{γ} distances are: 2.07, 2.12, 2.08, 2.08, 2.08, 2.11 Å.

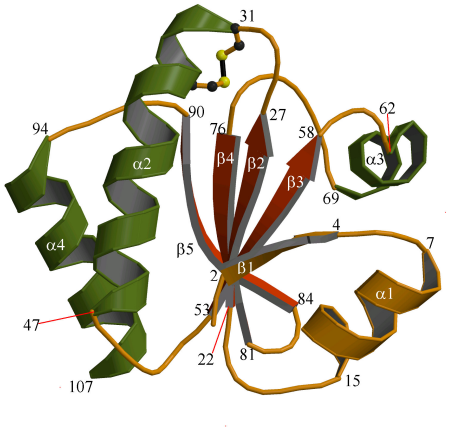

Fig. 1

Fig. 1 shows a ribbon diagram of one molecule in the oxidized state. The secondary structural elements are labelled as well as the beginning and the end of each of them. The disulfide bridge between Cys31 and Cys34 is represented. The three helices and the four β-strands belonging to the thioredoxin fold are coloured green and red, respectively, while the remaining elements are coloured yellow.

In the crystal structure of TXN1, the formation of regulatory homodimers is described, in which two monomers are related through a crystallographic twofold axis. These dimers exist in the oxidized and in the reduced states and are stabilized by a disulfide bond between residues Cys73 of two monomers. Similar dimers (A-B, C-D and E-F) are also present in TXN2 in spite of the impossibility to form the disulfide bond since Cys73 is not present in the sequence of TXN2 and the corresponding residue in the structural alignment is an alanine (Ala73). About 490 $Å^2$ per monomer are buried in this dimerization. The contacts are mainly hydrophobic, Trp30 is the most concerned residue and Ile59, Ala66, Ile67, Val74 are also involved.