Structural basis of novel TRP14, thioredoxin-related protein that regulates TNF-α signaling pathways

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Thioredoxin (Trx) is a small redox protein that is ubiquitously distributed from achaes to human. In diverse organisms, the protein is involved in various physiological roles by acting as electron donor and regulators of transcription and apoptosis as well as antioxidants. Sequences of Trx within various species are 27~69% identical to that of *E.coli* and all Trx proteins have the same overall fold, which consists of central five β strands surrounded by four α helices. The N-terminal cysteine in WCGPC motif of Trx is redox sensitive and the motif is highly conserved. Compared with general cysteine, the N-terminal cysteine has low pKa value. The result leads to increased reduction activity of protein. Recently, novel thioredoxin-related protein (TRP14) was found from rat brain. TRP14 acts as disulfide reductase like Trx1, and its redox potential and pKa are similar to those of Trx1. However, TRP14 takes up electrons from cytosolic thioredoxin reductase (TrxR1), not from the mitochondrial thioredoxin reductase (TrxR2). Biological roles of TRP14 were reported to be involved in regulating TNF-α induced signaling pathways in different manner with Trx1. In depletion experiments, depletion of TRP14 increased TNF-α induced phosphorylation and degradation of IκBα more than the depletion Trx1 did. It also facilitated activation of JNK and p38 MAP kinase induced by TNF-α. Unlike Trx1, TRP14 shows neither interaction nor interference with ASK1. Here, we determined three-dimensional crystal structure of TRP14 by MAD method at 1.8Å. The structure reveals that the conserved cis-Pro (Pro90) and active site –W-C-X-X-C motif, which may be involved in substrate recognition similar to Trx1, are located at the beginning position of strand β4 and helix α2, respectively. The TRP14 structure also shows that surface of TRP14 in the vicinity of the active site, which is surrounded by an extended flexible loop and an additional short α helix, is different from that of Trx1. In addition, the structure exhibits that TRP14 interact with a distinct target proteins compared with Trx1 and the binding may depend mainly on hydrophobic and charge interactions. Consequently, the structure supports biological data that the TRP14 is involved in regulating TNF-α induced signaling pathways in different manner with Trx1.