Poster Presentation

MS081.P04

Conversion of molecular assembly of peroxiredoxin

Tsutomu Nakamura[1], Maki Oshima[1], Koichi Uegaki[1]
[1]*National Institute of Advanced Industrial Science and Technology, Ikeda, Japan*
E-mail: nakamura-t@aist.go.jp

Peroxiredoxins (Prxs) are thiol peroxidases that reduce hydrogen peroxide and alkyl peroxides to water and to the corresponding alcohols, respectively. Prxs have a variety of quaternary structures involving monomer, dimer, decamer, and dodecamer. Decameric and dodecameric Prxs consist of ring-type arrangement of five and six dimers, respectively. In this study, we present conversion of molecular assembly of Prx from Pyrococcus horikoshii (PhPrx), which forms a decameric ring structure in the reduced state [1]. We designed dimeric PhPrx mutant by six-amino-acid substitutions. The resulting mutant (PhPrx6m) was confirmed to form a dimer in solution by gel-filtration chromatography. However, the six dimers assembled to form a dodecameric ring in the crystal structure. Although the hexagonal assembly of dimers is a crystallization artefact, the fact that six but not five PhPrx6m dimers formed a ring indicates that PhPrx dimer can potentially undergo both pentagonal and hexagonal assembly. In the crystal structure of PhPrx6m, the active site peroxidatic Cys was in sulfonic acid form and two Cys residues in the C-terminal region were linked with an intramolecular disulfide bond. Thus, we characterized wild type PhPrx overoxidized by hydrogen peroxide (PhPrxPer). PhPrxPer exhibited increased molecular mass in solution as revealed by analytical ultracentrifugation. This was confirmed by crystal structure of PhPrxPer which was a ring-type dodecamer of six dimers. Monomer structure of PhPrx is divided into a main domain and an arm domain [1, 2]. PhPrx and PhPrxPer were slightly different in the relative orientation of two the domains. The monomer structure of PhPrx6m was similar to that of PhPrxPer rather than to PhPrx. This difference was related to the number of dimers comprising the ring structure.
On the other hand, homologous Prx from Aeropyrum pernix (ApPrx) did not show the characteristics like PhPrx. ApPrx overoxidized by hydrogen peroxide (ApPrxPer) did not change its quaternary structure upon overoxidation to sulfonic acid form [3]. The mutant of ApPrx (ApPrx6m), which had been designed in the same way with PhPrx6m, was dimer both in solution and crystal. The monomer structures of ApPrx, ApPrx6m, and ApPrxPer were almost identical. These can be explained from the connection of the two domains in the ApPrx monomer structure. In PhPrx polypeptide, the N-terminal 162 residues (1-162) belong to the main domain and the C-terminal 64 residues (163-216) to the arm domain. ApPrx has a C-terminal extension which belongs to the main domain. Therefore, the main domain of ApPrx involves two separate polypeptide regions (1-167 and 220-250) and the arm domain (168-219) has two connections with the main domain. It is plausible that this strengthened connection between the two domains makes ApPrx insusceptible to conformational change in the monomer structure, which is related to molecular assembly of ring-type Prxs.
[1] Nakamura, T. et al., Acta Crystallogr. F 69, 719-722 (2013)
[2] Nakamura, T. et al., Proteins 62, 822-826 (2006)
[3] Nakamura, T. et al., Proc. Natl. Acad. Sci. USA 105, 6238-6242 (2008)
Keywords: peroxiredoxin, archaea, quaternary structure