Construction and structural characterization of a unique dimer of ferredoxin from *Thermotoga maritima*

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The addition of persulfide to a reactive cysteine in a protein is called persulfidation.[1] *Thermotoga maritima* (*T. maritima*) ferredoxin is consisted of a 60-amino acid polypeptide chain with a single [4Fe–4S] cluster and is expressed in *E. coli* in a monomeric form.[2] In this study, we have found that *T. maritima* ferredoxin forms a unique dimer with a polysulfide bond.

Ferredoxin oligomerized by incubation at pH 10.0 and 40 °C for 20 hrs. The dimer yield increased by incorporation of guanidine hydrochloride (GuHCl) during the incubation (Fig. 1). The ferredoxin dimer was stable up to 70 ℃, and the dimers dissociated to monomers by an addition of dithiothreitol. In the crystal structure of the dimer, the bond between Cys13 and the iron ion of the iron-sulfur cluster was cleaved and a new intermolecular bond was formed between two Cys13 sulfur atoms with a bridging sulfur atom (Fig. 2). In addition, the iron-sulfur cluster changed from [4Fe–4S] in the monomer to [3Fe–4S] in dimer. The ESI-QTOF mass spectrum supported the hypothesis of a bridging sulfur atom in the dimer.

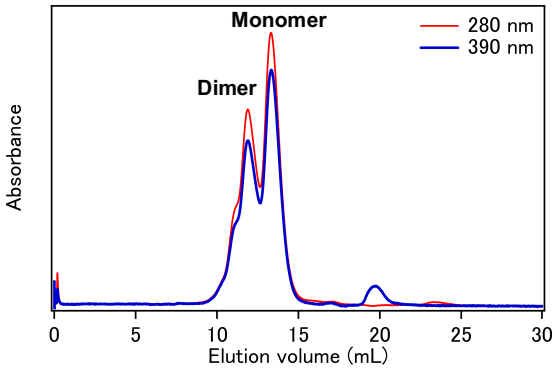

Fig. 1. Size exclusion chromatogram (Superdex 75 column) of oxidized ferredoxin after incubation in the presence of 50 mM GuHCl at pH 10.0 and 40 ℃ for 20 hrs. Absorbance was monitored at 280 and 390 nm.

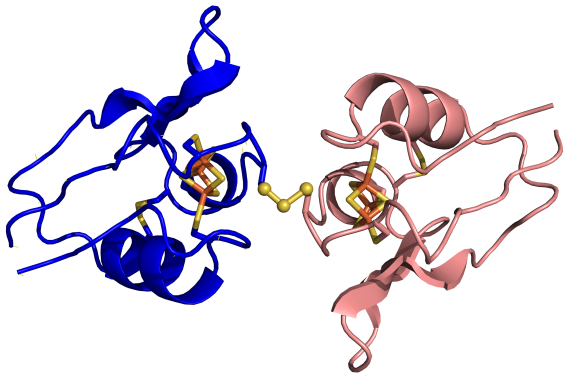

Fig. 2. Crystal structure of ferredoxin dimer. The iron-sufer clusters are shown in stick models. The polysulfide bond is shown in a ball-and-stick model.

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