Construction of Oligomers of *Thermotoga maritima* Ferredoxin

([1]*Division of Materials Science, Nara Institute of Science and Technology (NAIST)*)
○Nur Afiqah Azmi,[1] Masaru Yamanaka,[1] Shun Hirota[1]
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3D domain swapping (3D-DS) is a structural exchange process between proteins.[1] The structural element can be any part of the protein, such as an α-helix, a β-strand, or as large as an entire globular domain. Our group has reported that many metalloproteins can 3D-DS.[2] For example, cytochrome *c* and azurin that are electron transfer proteins can 3D-DS. However, the 3D-DS of ferredoxins, a major group of electron transfer proteins, has never been reported. *Thermotoga maritima* (*T. maritima*) ferredoxin is consisted of 60 amino acids in which a single 4Fe-4S cluster is incorporated. *T. maritima* ferredoxin can be expressed in *E.coli* in a monomeric form with the 4Fe-4S cluster assembled correctly. *T. maritima* ferredoxin forms a disulfide bond between Cys20 and Cys43, which holds the protein conformation strongly and makes it difficult to unfold when placed in an extreme environment. In this study, we searched for the ideal conditions for the oligomerization of *T. maritima* ferredoxin by modifying the reported 3D-DS procedure.[2]

It was necessary to reduce *T. maritima* ferredoxin at pH 10.0 with dithiothreitol (DTT) before the 3D-DS procedure to obtain oligomers, indicating that disulfide bond cleavage is obligatory for the oligomer formation of ferredoxin by the 3D-DS process. Next, we freeze-dried reduced ferredoxin at pH 10.0. The freeze-dried ferredoxin was re-dissolved with 50 mM borate buffer, pH 10.0, and incubated at 4 °C for 48 hours under air followed by incubation at 50 °C for 4 hours under air. The obtained solution of *T. maritima* ferredoxin was analyzed by size exclusion chromatography (SEC), showing formation of dimers (Fig. 1). The high pH during the 3D-DS procedure may be necessary to induce the unfolding of the protein and increase the intermolecular interactions.

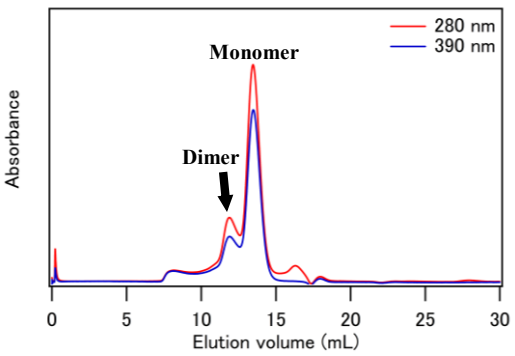

Fig. 1. SEC chromatogram of *T. maritima* ferredoxin after the 3D-DS procedure of DTT-reduced ferredoxin at pH 10.0. Analyzed at pH 7.0.

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