Electronic Supporting Information

Construction of Highly Stable Artificial Glutathione Peroxidase on Protein Nanoring

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1. Analyses of MALDI-TOF Mass Spectrometry

As shown in Figure. S1a, the original gift W. T. SP1 displayed a mass of 12279.8, in agreement with the predicted mass. After overexpression in the cysteine auxotrophic expression system, Se-SP1-57Cys displayed a mass of 12390.7 as shown in Figure. S1b. The mass difference of 110.9 between Se-SP1-57Cys and W. T. SP1 almost corresponded to Ala57Cys plus the atomic weight difference between selenium and sulfur and the atomic weight of two oxygen atoms. The mass difference between W. T. SP1 and SP1 mutants (A57C) was 32.0, and the mass difference between Se-SP1-57Cys ($ESeO_2H$) and SP1 mutants (A57C, ESH) was 78.9, so the mass difference between Se-SP1-57Cys ($ESeO_2H$) and W. T. SP1 was 32.0+78.9 =110.9. This experiment indicated that the Se-SP1-57Cys existed in an oxided form of $ESeO_2H$ and clearly demonstrated that Sec residue was incorporated into the protein.

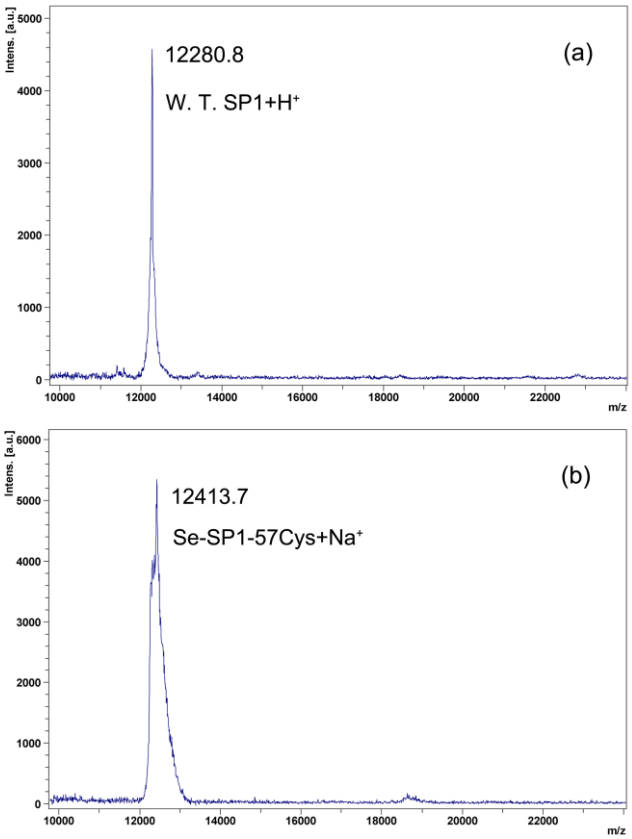

Figure. S1 MALDIA-TOF mass spectrometry analysis. (a) W. T. SP1. (b) Se-SP1-57Cys.

2. Overexpression and purification of W. T. SP1 and SP1-57Cys

The W. T. SP1 and SP1-57Cys domains were transformed into *E. coli* BL21 (DE3) respectively and grew to 1 L LB culture containing 50 mg/ml ampicillin with shaking at 37 ℃. The protein expression was induced by adding 1 mM IPTG when OD_{600} reached 0.8. The cells were harvested 4 hr after induction. The purification of W. T. SP1 and SP1-57Cys were similar to Se-ddSP1-57Cys. In SDS-PAGE sample preparation, 1 mL solution of 1L culture after IPTG induction of W. T. SP1, SP1-57Cys and Se-SP1-57Cys was obtained respectively. After centrifugation at 10000 rpm for 10 min, the cell pellets were resuspended in 10 μL loading buffer. The mixtures were boiled for 20 min and loaded into the SDS-PAGE. Finally, the homogeneous and unique protein bands of ~12kD were obtained after inducing by IPTG (lane 2-4, Figure. 2a). By contrast, the content of Se-SP1-57Cys after IPTG induction was lower than W. T. SP1 and SP1-57Cys because the selenium in auxtrophic expression system is toxic to *E.coli*.

3. Characterization of W. T. SP1

The ring-like SP1 dodecamer structure was shown in the TEM image of Figure. S1a. The nanoring structures were uniformly distributed in the field of vision. The diameter of the ring was approximately 11 nm and the central hole was 2.5 nm. 18% SDS-PAGE in Figure. S1b showed the molecular weight of the protein before and after boiling. While boiling for 30 min before loading onto the gel, SP1 could maintain its oligomeric structure as a complex on SDS-PAGE.

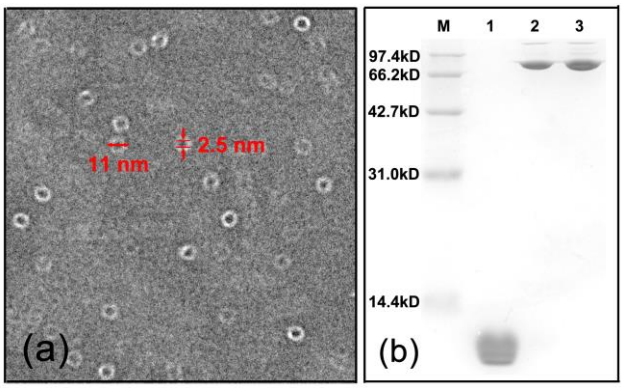

Figure. S2 W. T. SP1 protein structure and thermostability. (a) TEM image of ordered W. T. SP1 dodecamer. (b) SDS-PAGE analysis of SP1 protein. W. T. SP1 boiled in the presence of 2% SDS with (lane 1) and without (lane 2) boiling treatment; lane 3: W. T. SP1 boiled for 30 min without SDS presence.

4. Enzymatic Analysis of Se-ddSP1-57Cys for GPx Activity

The GPx activity of Se-ddSP1-57Cys was determined to be 390 U/μM at various concentrations as shown in Figure. S3.

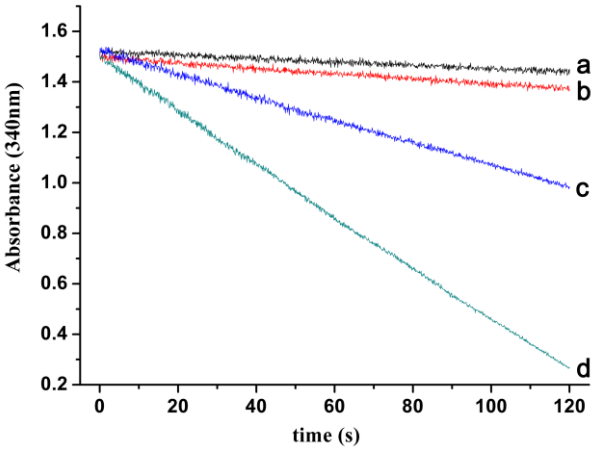

Figure. S3 Plots of absorbance vs time during the catalytic reduction of H_2O_2 (0.5 mM) by GSH (1 mM) at pH 7.0 and 37 ℃. The concentrations of the Se-ddSP1-57Cys were 0 μM (a), 0.1 μM (b), 0.8 μM (c), and 1.0 μM (d), respectively.