Unequivocal Determination of Site-Specific Protein Disulfide Bond Reduction Potentials by Top-down FT-ICR MS: Characterization of the N- and C-Terminal Redox-Active Sites in Human Thioredoxin 1

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Results and Discussion

We report the reliable determination of equilibrium protein disulfide bond reduction potentials ($E°'$) by isotope-coded cysteine alkylation coupled with top-down Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). This technique enables multiple redox-active sites to be characterized simultaneously and unambiguously without the need for proteolysis or site-directed mutagenesis. Our model system was *E. coli* thioredoxin and we determined $E°'$ for its CGPC active-site disulfide as −280 mV in accord with literature values (See Figure 1). $E°'$ for the homologous disulfide in human thioredoxin 1 (Trx1) was determined as −281 mV; a value considerably more negative than the previously reported −230 mV. We also observed *S*-glutathionylation of Trx1 and localized that redox modification to Cys72; $E°'$ for the intermolecular disulfide was determined as –186 mV. Intriguingly, that value corresponds to the intracellular glutathione/glutathione disulfide (GSH/GSSG) potential at the redox boundary between cellular differentiation and apoptosis.

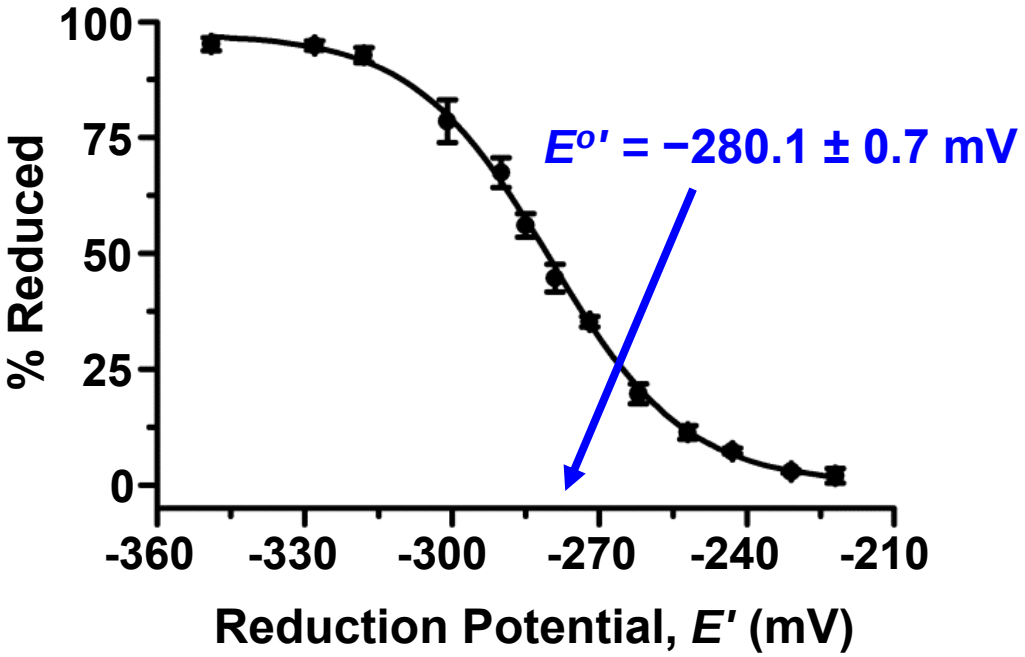

Figure 1. Plot of the percentage of reduced bacterial Trx (dithiol form) versus E'. Error bars show the standard deviation for three experiments. $E°'$ is calculated as −280.1 ± 0.7 mV from nonlinear regression, in excellent agreement with literature values.

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References

[1] Scotcher, J., et al., Anal. Chem., **85**, 9164-9172 (2013).