Characterization of Cu/Zn-superoxide dismutase mutant C57S lacking intrasubunit disulfide bond

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The biophysical and biochemical characteristics of the Cys57Ser (C57S) mutant of human copper-zinc superoxide dismutase (Cu/ZnSOD or SOD1), which lacks the intramolecular disulfide bond, were examined. The thermostability and H/D exchange behavior of C57S apo-SOD1 and disulfide-reduced wild type (WT) apo-SOD1, prepared using the reducing reagents, tris(2-carboxyethyl)phosphine (TCEP) or dithiothreitol (DTT), were found to be identical, indicating that C57S SOD1 is a good model system for reduced wild type SOD1. *In vitro* metal ion titration studies of C57S SOD1 demonstrated that the absence of disulfide bond caused a change in metal binding properties, namely, only one subunit of C57S SOD1 was reconstituted with metals using the same conditions that result in complete and proper remetallation of WT apo-SOD1. Moreover, the copper site geometry of C57S SOD1 was shown to be different from the copper geometry in WT SOD1 by electron paramagnetic resonance spectroscopy. Growth studies of yeast cells expressing human C57S SOD1 as well as the gel-based assay for SOD activity showed no evidence that this protein has SOD activity *in vivo*. As-isolated C57S SOD1 purified from yeast was found to contain four equivalents of zinc and no copper, presumably accounting for the absence of SOD activity *in vivo*. Addition of cupric ions to as-isolated C57S SOD1 *in vitro* resulted in replacement of two zinc ions with copper ions to give SOD-active enzyme. These results suggest that the presence of the intrasubunit disulfide bond of human SOD1 is not necessary for SOD activity, if copper is properly bound, but that it may be necessary to establish and/or maintain proper metallation by copper *in vivo*.