

## **Global disorder of disulfide-reduced ALS mutant SOD1s measured by H/D exchange.**

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Misfolding and aggregation of mutant forms of the cytosolic metalloenzyme copper-zinc superoxide dismutase (CuZnSOD or SOD1) are strongly implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS). Currently, there are over 100 known mutations in this enzyme that are known to cause ALS – including point, deletion, and truncation mutations. Wild-type (WT) holo-SOD1 is an extremely stable ( $T_m > 90^\circ\text{C}$ ) 32 kDa homodimeric enzyme that gains its high stability due to metal coordination, intrasubunit disulfide bond formation, and dimerization. Acidic dialysis against EDTA removes the metal ions from this holo-enzyme; subsequent treatment of the apo enzyme with DTT yields the disulfide-reduced apo form. Although aggregation of mutant SOD1 enzymes is implicated in the pathogenesis of ALS, not all of the oxidized ALS-mutant apo SOD1s are dramatically less stable than WT. With the ALS-mutant SOD1s that are less stable than WT in their oxidized form, reduction of their disulfide bond destabilizes them even more dramatically relative to reduced WT. Given the high potential of unstable or partially unfolded proteins to aggregate, the apo reduced state of the ALS mutants may be involved in causing SOD1-linked ALS.

The rate at which backbone amide hydrogens in a protein undergo isotopic exchange in deuterated buffer provides valuable information about the dynamics, structure, and degree of order in the protein. The rate and extent of deuterium incorporation is readily measured using ESI-MS. We have used this method to investigate these properties for WT SOD1 and four ALS-mutant SOD1s, in both the disulfide-oxidized and disulfide-reduced forms. At 20 °C, the reduced forms of A4V and G93R undergo complete isotopic exchange at a rate consistent with a completely disordered, unfolded polypeptide. In contrast, the reduced forms of WT and the ALS mutants H48Q and D101N exhibited partial protection from isotopic exchange characteristic of structured proteins. The disorder of the reduced form of A4V may be partially responsible for the aggressive progression of its SOD1-linked ALS.

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