The Mutant Proteins of a Neurodegenerative Disorder

J. Stine Elam[1], A.B. Taylor[1], R. Strange[2], S. Antonyuk[2], P.A. Doucette[3], J.A. Rodriguez[3], S.S. Hasnain[2], L.J. Hayward[4], J. Selverstone Valentine[3], T.O. Yeates[3], and P.J. Hart[1]

[1]Department of Biochemistry and the X-ray Crystallography Core Laboratory, The University of Texas, Health Science Center at San Antonio; [2]CCLRC Daresbury Laboratory; [3]Department of Chemistry and Biochemistry, University of California; [4]Department of Neurology, University of Massachusetts Medical School

Mutations in copper-zinc superoxide dismutase (SOD1) proteins cause the neurodegenerative disorder familial amyotrophic lateral sclerosis (FALS). In human spinal cord neurons and in transgenic mice expressing these proteins, proteinaceous inclusions (aggregates) containing pathogenic SOD1 are observed. These aggregates are believed to interfere with a variety of cellular processes, eventually leading to motor neuron death. We discovered that several metal-deficient, pathogenic SOD1 proteins can undergo conformational changes that do not occur in non-pathogenic (native) SOD1. This promotes a "gain-of-interaction" between the molecules, causing them to "stick" to each other. In turn, this leads to the formation of extensive helical and linear, or "amyloid-like," arrays of SOD1. Such non-native protein-protein interactions, leading to larger, or "higher order," SOD1 assemblies, could thus represent a toxic property that is common to mutants of SOD1 linked to FALS.

Amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease or motor neuron disease) is a neurodegenerative condition characterized by the loss of motor neurons in the spinal cord and brain. The disorder results in paralysis, leading to the death of the afflicted individual within two to five years of diagnosis. A subset of ALS cases are familial (FALS), and most of these are associated with dominantly inherited mutations in copper-zinc superoxide dismutase (SOD1). SOD1 is an antioxidant enzyme that is 32 kilodaltons (kDa) in size (where one Da is equal to one atomic mass unit), and is homodimeric, meaning it consists of two identical molecules. Initial hypotheses suggested that SOD1-linked FALS comes from extensive oxidative damage in the neurons, arising from diminished SOD1 activity. However, mice without SOD1 appear normal and live to adulthood without developing motor neuron disease. In contrast, transgenic mice expressing human FALS SOD1 mutants become paralyzed, even though they possess normal levels of SOD1 activity from their own endogenous SOD1. Together, these observations strongly suggest that pathogenic SOD1 molecules act by gaining the ability to kill other cells (becoming "cytotoxic") and not by losing their enzymatic function.

There has been increasing support for the concept that this "toxic gain of function" of pathogenic SOD1 is due to the propensity of these proteins to misfold and aggregate. This is supported by the manifestation of aggregates containing pathogenic SOD1 in human spinal cord neurons and in transgenic mice expressing these proteins. These pathogenic SOD1 aggregates (or their soluble precursors) could play a role in pathogenesis, either by occupying the neurons' heat

Authors (left to right)
Nick Criscimagna, Stephen Holloway, P. John Hart, Jennifer Stine Elam, Lisa Whitson, Alex Taylor, and Ahmad Galaleldeen

BEAMLINE X8C

Funding
National Institutes of Health's National Institute of Neurological Disorders and Stroke (NIH-NINDS); Robert A. Welch Foundation; Amyotrophic Lateral Sclerosis (ALS) Association; the Council for the Central Laboratory of the Research Councils (CCLRC) Daresbury Laboratory

Publication
J.S. Elam, A.B. Taylor, R. Strange, S. Antonyuk, P.A. Doucette, J.A. Rodriguez, S.S. Hasnain, L.J. Hayward, J.S. Valentine, T.O. Yeates, and P.J. Hart, "Amyloid-Like Filaments and Water-Filled Nanotubes Formed by SOD1 Mutants Linked to Familial ALS," *Nat. Struct. Biol.*, **10**, 461-467 (2003).

Contact information
P. John Hart, Ph.D.
Associate Professor of Biochemistry, Director of the X-ray Crystallography Core Laboratory ,The University of Texas Health Science Center at San Antonio

Email: pjhart@biochem.uthscsa.edu

shock proteins and preventing them from doing their jobs, which leaves the neuron vulnerable, and/or interfering with the way the neurons transport materials along their length (or along their axons) and get rid of unwanted proteins. However, the molecular basis underlying the formation of pathogenic SOD1 oligomers has remained undefined.

To partially address this issue, we determined the crystal structures of the pathogenic SOD1 mutants H46R and S134N, which are members of a larger class of "metal-binding region mutants." As the name suggests, members of this class of pathogenic SOD1 cannot properly bind either copper or zinc, or both. In the absence of the proper complement of these metal ions, structural features normally involved in metal binding, called loop elements, undergo conformational changes that allow H46R and S134N to polymerize into two types of higher-order filamentous arrays. **Figure 1** shows that both metal-free H46R and metal-deficient S134N (which crystallize in different crystal systems) form nearly identical linear, amyloid-like fibers. A variation on this theme can be seen in **Figure 2(D)**, which shows that copper-free, but zinc-loaded H46R engages in slightly different pathogenic SOD1-SOD1 interactions, leading to the formation of helical, rather than linear, filamentous arrays. From the perspective in **Figure 2(D)**, the interactions (which occur in the red patches) form a donut shape. Thus, propagating them leads to hollow, water-filled nanotubes. Importantly, when native SOD1 dimers are structurally aligned with the pathogenic SOD1 dimers in these linear and helical arrays, it is obvious that the native variety cannot participate in filamentous assembly. This is due to clashes and unfavorable electrostatic interactions between the loop elements described above.

In summary, the filamentous arrangement of mutant, metal-deficient SOD1 proteins provides justification for a specific and testable hypothesis that links various pathogenic SOD1 mutations to protein aggregation, thereby making it possible to draw a parallel between ALS and other established amyloid diseases.

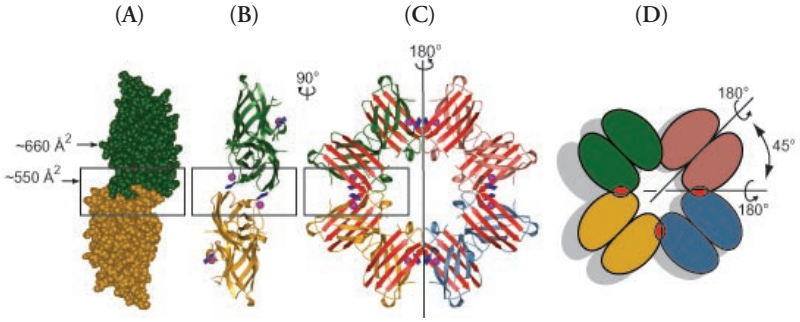

Figure 2. The gain-of-interaction in zinc-loaded H46R (Zn-H46R) SOD1, giving rise to water-filled helical nanotubes. The GOI interface between Zn-H46R dimers is boxed, and buries approximately 550 Å² of solvent-accessible surface area per polypeptide. (A) and (B) One-half of the helical Zn-H46R filament is represented by two dimers, shown from top to bottom in green and gold, respectively. Image B is related to the left half of image (C) by a rotation of 90 degrees. (C) The approximate location of the crystallographic two-fold axis, which runs along the diagonal in the tetragonal unit cell, is indicated by a black line without arrows and a 180-degree rotation symbol. Applying this two-fold operator generates one complete turn of the helical filament. One-half of each SOD1 β-barrel is shown in red. Residues of the zinc loop undergo a conformational change and form a short β-strand (shown in blue) that reciprocally adds to this sheet in neighboring Zn-H46R dimers, stabilizing the GOI interface. Zinc ions are shown as magenta spheres. (D) A schematic representation of SOD1 dimers in the helical filaments in the same orientation as in image (C). The GOI interfaces are represented by red patches.

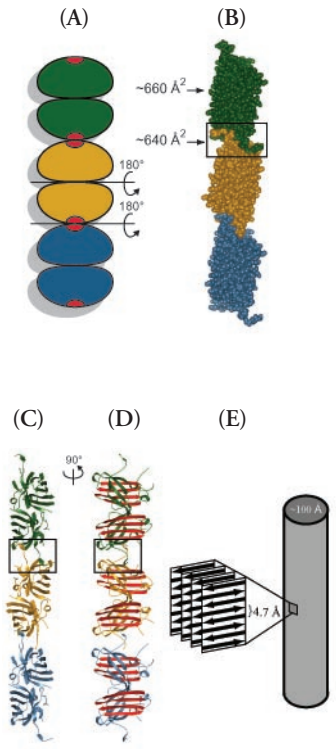

Figure 1. The gain-of-interaction (GOI) interfaces in pathogenic SOD1 give rise to cross-β fibers (or filaments) in two different crystal systems. Two orthogonal views of the linear, amyloid-like apo-H46R filament are represented by three dimers, shown from top to bottom in green, gold, and blue, respectively. Because the overall architecture and GOI interfaces in the S134N filament and the metal-free H46R filament are nearly identical, both filaments are represented by the single filament shown in this panel. The GOI interface is boxed, and buries approximately 640 square angstroms (Å²) of solvent-accessible surface area per polypeptide. (A) A schematic representation of SOD1 dimers in the amyloid-like filaments (in the same orientation as in image (D). The GOI interfaces are represented by red patches. (B) and (C) Space filling and ribbon representations of the filaments, respectively. These are rotated 90° relative to the images in (A) and (D). In (B), the naturally occurring interface between SOD1 monomers is also indicated and buries approximately 660 Å² of solvent-accessible surface area per polypeptide. (D) One-half of each SOD1 β-barrel, shown in red. (E) The long axes of the red strands run perpendicular to the long axis of the filament, an architecture similar to the "cross-β" structure observed in amyloid fibrils.