

## FINDING ANSWERS TO THE PROTEIN MISFOLDING RIDDLE

In a study entitled, "Folding and Aggregation of ALS-Associated Mutant Superoxide dismutases," conducted by ALS researcher Dr. Elizabeth Meiring in the department of chemistry at University of Waterloo, it has been shown that the mutations in human copper zinc superoxide dismutase 1 (SOD1) associated with the familial form of ALS (fALS), lead to decreased stability in the protein, which may result in increased formation of aggregated proteins toxic to motor neurons. The study is funded by the Neuromuscular Research Partnership (NRP) for three years (2003 - 2006).

Mutations in the SOD1 gene were linked to fALS in 1993, and to date, more than 110 SOD1 mutations have been associated with fALS. Between five and 10 per cent of people with ALS suffer from the familial form of the disease, and of those cases, approximately 20 per cent have been traced to SOD1 gene mutation, making it the most commonly known cause of fALS.

The toxic mechanism of the mutant SOD1 remains unknown, but there is evidence that fALS, along with other diseases such as Alzheimer's, Parkinson's, and transmissible spongiform encephalopathy, are caused by the misfolding properties of the mutated protein. Since increased mis-

folding has been correlated with decreased thermodynamic stability of the protein, a better understanding of the thermodynamic effects of fALS may lead to valuable insights into the disease process.

Normal proteins fold and unfold themselves as they bind with other molecules to form functional structures, but SOD proteins fold and unfold at an altered rate, and this gives them a tendency to form toxic clumps, or plaques, in the motor neurons that cannot bind with other molecules in the normal way. Until now, insights into this process have been hampered by the denaturation of the mutated proteins in the lab, which are irreversible and alter the effects of the fALS mutations. Denaturation is a process by which protein is unfolded, rendering the protein biologically inactive. Meiring and her research team are using an innovative method of denaturation that is generally highly reversible and allows them to study the effects of the mutations on protein stability and the process of folding and unfolding of different mutants, both fully metallated and metal-free.

With these methods, Meiring can



measure the rate of unfolding in real time by placing the protein into unfolding conditions and monitoring changes. Meiring's team is also measuring structural fluctuations in the folded protein under folding conditions that may cause the mutant proteins to aggregate (when proteins stick together, or bind together, in an abnormal way).

There is controversy regarding which form of the protein may misfold and aggregate, in particular unmetallated versus metallated protein.

"We are investigating both forms of the protein, to obtain a reliable database of mutant properties from which we can investigate correlations with disease and ultimately disease interventions," says Meiring.

One possibility that has emerged from the study is that the SOD1 mutations can change the rate of unfolding. A prevalent form of fALS that involves a mutation of the A4V segment of the SOD1 gene has a brief disease duration. Since it also has faster unfolding and aggregation rates than other mutant genes, there is a strong possibility that the rapid process of unfolding is linked with the rapid progress of the disease. Meiering's goal is to find answers to the riddle of protein misfolding so the specific parts of the molecules that become destabilized are identified.

The next stage would be to develop targeted drug therapies that will act as inhibitors in this process.

In addition to her post as an associate professor of chemistry and biology, Meiering is a University Research Chair at the University of Waterloo. She earned her B.Sc. at Waterloo, went on to get a PhD at Cambridge University, and was a Jane Coffin Childs Postdoctoral Fellow in the department of biological chemistry and molecular pharmacology at Harvard Medical School.

Meiering describes her research as cross-disciplinary, using both biophysical and biochemical techniques. Her work is specifically focused on ALS-related mutant SOD1.

"General correlations between properties of the mutant enzymes and disease properties have not yet been identified," says Meiering.

"Identifying such correlations has been hampered by limited patient

data and limited/inaccurate (in vitro) data on properties of the mutant proteins. We are systematically studying the stability and misfolding of the purified proteins."

Meiering's team has developed methods to prepare large quantities of mutant proteins, and is pursuing collaborations to use this protein to determine biological effects of the mutants. The next step in her current research will include obtaining additional data on folding and misfolding of additional SOD mutants, and determining the biological effects of the mutant proteins, in particular, possible modes of toxicity of misfolded proteins.

When Meiering first started doing research on protein folding as a graduate student, her work was considered "basic" research, with no clear practical consequences. Since then, it's become clear that protein misfolding is associated with many different diseases, especially neurodegenerative ones.

"I want to use my expertise in studying proteins to understand and contribute to curing ALS," says Meiering. "My hope is that the research will also contribute to curing other misfolding diseases."

*By: Elaine MacNeill*