William R. Wiley EMSL In Brief

Environmental Molecular Sciences Laboratory

Researchers Use EMSL NMR to Uncover Unusual Protein Structure

A user of the Environmental Molecular Sciences Laboratory (EMSL) and her team have determined an unusual protein three-dimensional structure from nuclear magnetic resonance (NMR) spectra generated in EMSL's High-Field Magnetic Resonance Facility.

Montana State University (MSU) researcher Valérie Copié and her team of researchers from MSU, the University of Montana, and EMSL, used MSU's NMR capabilities as well as EMSL's 800-MHz NMR spectrometer to gather several spectra. Study of the spectra led to discovery of the first detailed structure of NosL—an accessory protein from the nitrous oxide reductase (N_2OR) gene cluster, and a protein necessary for assembly of functional N_2OR, the enzyme that plays a role in denitrification-conversion of nitrous oxide (N_2O), a major greenhouse gas, to dinitrogen (N_2).

Above: EMSL's 800-MHz NMR system used in the research. Right: Valérie Copié.

Denitrification is the anaerobic process of converting nitrate and nitrite to N_2 gas and is catalyzed by a series of bacterial enzymes, including N_2OR. NosL is a copper (I) binding protein crucial to the assembly of the catalytic copper site (Cu^Z) of N_2OR. The fact that NosL binds copper (I) in the relative oxidizing environment of the periplasm and releases copper upon oxidation to Cu(II) has suggested that the protein might function as a novel copper transporter (i.e., metallochaperone). In an effort to provide structural clues about the biological function of NosL, the research team undertook the high-resolution three-dimensional structure determination of the apo form of NosL [i.e., without Cu(I) bound] originating from *Achromobacter cycloclastes*.

The team discovered that the three-dimensional fold of apo NosL is atypical for a copper chaperone and that NosL possesses structural homology to only one other protein in the protein data bank—MerB, an enzyme responsible for broad mercury resistance. The unexpected structural similarity between apo NosL and MerB provides unanticipated insights into the potential role NosL serves during the biogenesis of N_2OR into catalytically active enzyme. Their work suggests that NosL may be multifunctional and well adapted for a role in copper or sulfur transfer and delivery or redox sensing.

The study, funded in part by the National Science Foundation, will help researchers understand in detail the molecular mechanism of metal cluster assembly in metalloenzymes such as N_2OR. The long-term goal is to better understand the biochemistry of N_2O reduction by N_2OR. Results will provide useful insight to aid in the design of efficient biological strategies to limit N_2O emission in the atmosphere and to remove excess nitrogen in groundwater, soils, and wetlands contaminated with fertilizer run-off.

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