Study uncovers a unique, efficient method of copper delivery in cells

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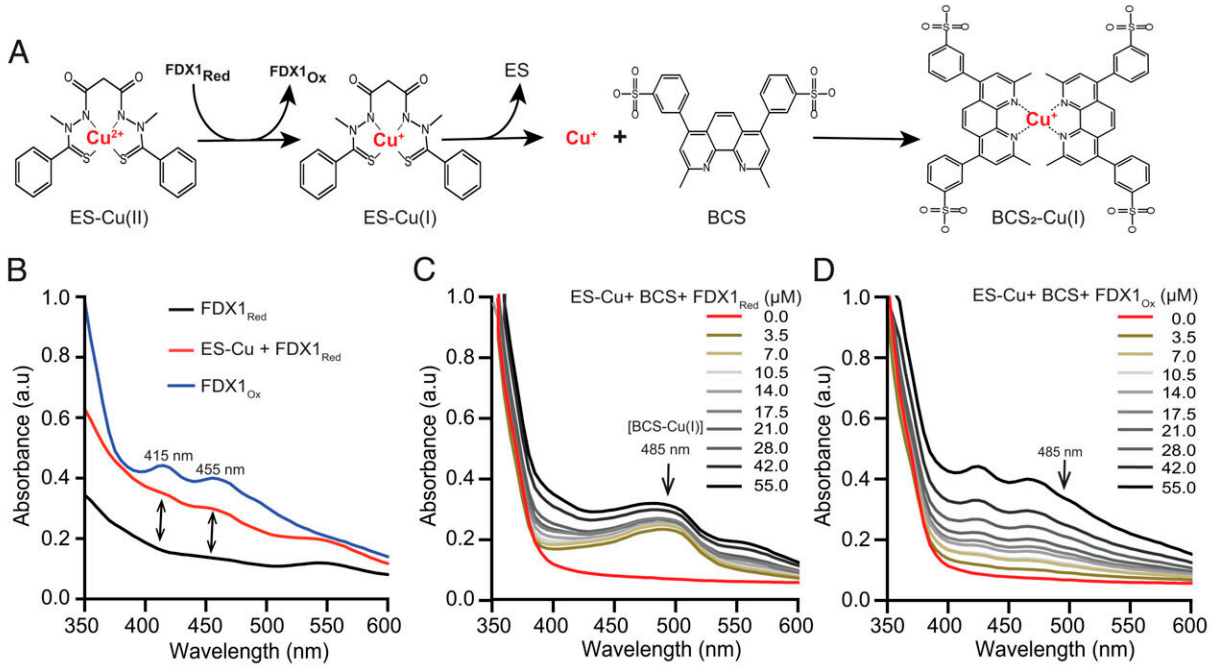

Reduced FDX1 releases ES-bound Cu in vitro. (*A*) A schematic representation of in vitro assay to determine FDX1-mediated release of Cu from ES. (*B*) UV/Vis spectra of 50 μM human $FDX1_{Red}$ /$FDX1_{Ox}$ ± 50 μM ES–Cu(II) in Tris buffer (10 mM Tris, 50 mM NaCl, pH 7.5). (*C*) UV/Vis spectra of the BCS–Cu(I) complex in the presence of increasing concentrations (0 to 55 μM) of $FDX1_{Red}$ or (*D*) $FDX1_{Ox}$ protein. $FDX1_{Ox/Red}$ proteins were mixed with 1 mM BCS in Tris buffer, and 20 μM ES–Cu(II) was added to start the reaction. The final dimethyl sulfoxide (DMSO) concentration in the solution was 20% after injection. Credit: *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2216722120

A new study has uncovered a unique way in which the anti-cancer drug elesclomol enables copper delivery in cells, aiding in the search for treatments for copper deficiency disorders such as Menkes disease.

Menkes disease is an extremely rare hereditary copper-deficiency disorder in infants. It is characterized by progressive neurological injury culminating in death, typically by the age of three.

A Texas A&M AgriLife Research team led by Vishal Gohil, Ph.D., associate professor in the Department of Biochemistry and Biophysics in Texas A&M's College of Agriculture and Life Sciences, Bryan-College Station, first discovered the therapeutic potential of elesclomol for treating copper deficiency disorders. Additionally, previous research by Gohil's team showed that elesclomol could be used effectively in a mouse model to treat Menkes disease.

The new study, "FDX1-dependent and independent mechanisms of elesclomol-mediated intracellular copper delivery," was recently published in the *Proceedings of the National Academy of Sciences*, a peer-reviewed journal of the National Academy of Sciences. The research was led by Gohil, with Mohammed Zulkifli, Ph.D., a research scientist in the same department, as the first author of the study.

The study was conducted in collaboration with scientists from the University of Houston, Oregon Health and Science University, University of Missouri and the Advanced Photon Source at Argonne National Laboratory, a U.S. Department of Energy multidisciplinary science and engineering research center.

Elesclomol and copper deficiency

Genetic defects in copper transport to copper-containing enzymes, referred to as "cuproenzymes," result in fatal disorders such as Menkes

disease. No effective treatment is currently available for these copper deficiency disorders.

"To realize the full potential of elesclomol, it was necessary to gain a mechanistic understanding of how this drug makes copper available to different cellular cuproenzymes," Gohil said. "We needed to look at the mechanism by which copper brought into cells by elesclomol is released and delivered to cuproenzymes present in different subcellular compartments."

He said the study used a combination of biochemistry, cell biology and genetics to demonstrate that the release of copper from elesclomol occurs both inside and outside mitochondria.

Vishal Gohil, Ph.D., left, and Mohammad Zulkifli, Ph.D., right, in the Gohil Laboratory at Texas A&M University. Credit: Texas A&M AgriLife photo by Michael Miller

Copper and human health

Copper is an essential trace element required for the activity and stability of several cuproenzymes involved in a wide array of physiological processes.

"Copper is an essential micronutrient, and genetic mutations that prevent copper transport across cellular membranes or its delivery to cuproenzymes can result in lethal human disorders such as Menkes disease," Gohil said.

Currently, no Food and Drug Administration-approved therapies are available for treating Menkes disease. Additionally, direct administration of hydrophilic copper salts has shown limited efficacy in clinical trials.

"We hypothesized that this limited efficacy was likely due to inefficient copper delivery across cellular membranes, so there was an unmet need to identify compounds that can safely and effectively transport copper across biological membranes and restore cellular copper balance," Gohil said.

The study

Previous research had shown that ferredoxin 1, FDX1, a mitochondrial enzyme, was the protein target of elesclomol. In the current study, Gohil and his team showed that FDX1 releases copper bound to elesclomol by reducing it to a form of copper cells can use. The study also showed that

even when FDX1 was absent, elesclomol could still bring some copper into cells in other unknown ways.

Zulkifli said FDX1 can also help release copper from other clinically used copper-transporting drugs, but compared with elesclomol, these drugs are much less dependent on FDX1 to make the copper bioavailable to cuproenzymes.

"These modes of copper release by elesclomol are distinct from those of other currently used copper-transporting drugs," Zulkifli said. "This may explain the high potency of elesclomol in rectifying copper deficiency."

Building on past research

Previous studies by Gohil and his team have highlighted the therapeutic potential of elesclomol in treating diseases of copper deficiency. Some of this previous research also showed that elesclomol can restore the levels of cytochrome c oxidase protein complex, a critical copper-dependent enzyme required for mitochondrial energy production.

The Gohil lab also demonstrated that elesclomol improves copper deficiency in yeast, zebrafish and mouse models by delivering copper to mitochondria and restoring the function of the cytochrome c oxidase.

Additionally, the use of elesclomol to treat copper deficiency disorders is at the center of a licensing agreement between The Texas A&M University System, managed through the Intellectual Property and Commercialization office of Texas A&M AgriLife Research, and California-based Engrail Therapeutics.

More information: Mohammad Zulkifli et al, FDX1-dependent and independent mechanisms of elesclomol-mediated intracellular copper delivery, *Proceedings of the National Academy of Sciences* (2023). DOI:

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