Protein adaptation shows that life on early earth lived in a hot, acidic environment

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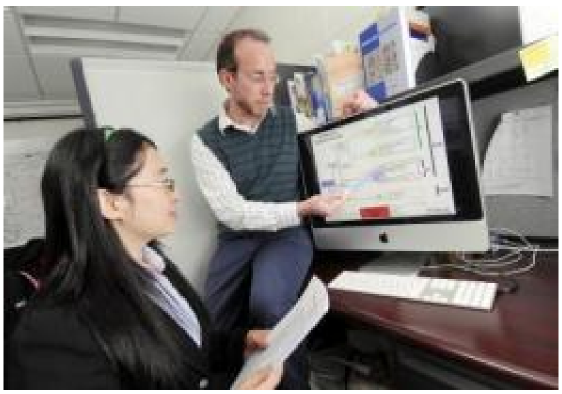

Georgia Tech School of Biology associate professor Eric Gaucher and graduate student Ziming Zhao discuss the evolutionary history of the thioredoxin gene family. Ancestral proteins of this gene family were resurrected in the laboratory and provide clues about the environmental conditions that hosted early life on Earth. Credit: Georgia Tech/Gary Meek

A new study reveals that a group of ancient enzymes adapted to substantial changes in ocean temperature and acidity during the last four billion years, providing evidence that life on Early Earth evolved from a much hotter, more acidic environment to the cooler, less acidic global environment that exists today.

The study found that a group of ancient enzymes known as thioredoxin were chemically stable at temperatures up to 32 degrees Celsius (58 degrees Fahrenheit) higher than their modern counterparts. The enzymes, which were several billion years old, also showed increased activity at lower pH levels -- which correspond to greater acidity.

"This study shows that a group of ubiquitous proteins operated in a hot, acidic environment during early life, which supports the view that the environment progressively cooled and became more alkaline between four billion and 500 million years ago," said Eric Gaucher, an associate professor in the School of Biology at the Georgia Institute of Technology.

The study, which was published April 3 in the advance online edition of the journal *Nature Structural & Molecular Biology*, was conducted by an international team of researchers from Georgia Tech, Columbia University and the Universidad de Granada in Spain.

Major funding for this study was provided by two grants from the National Aeronautics and Space Administration to Georgia Tech, a grant from the National Institutes of Health to Columbia University, and a grant from the Spanish Ministry of Science and Innovation to the Universidad de Granada.

Using a technique called ancestral sequence reconstruction, Gaucher and Georgia Tech biology graduate student Zi-Ming Zhao reconstructed seven ancient thioredoxin enzymes from the three domains of life -- archaea, bacteria and eukaryote -- that date back between one and four billion years old.

To resurrect these enzymes, which are found in nearly all known modern organisms and are essential for life in mammals, the researchers first constructed a family tree of the more than 200 thioredoxin sequences available from the three domains of life. Then they reconstructed the sequences of the ancestral thioredoxin enzymes using statistical methods based on maximum likelihood. Finally, they synthesized the genes that encoded these sequences, expressed the ancient proteins in the cells of modern Escherichia coli bacteria and then purified the proteins.

"By resurrecting proteins, we are able to gather valuable information about the adaptation of extinct forms of life to climatic, ecological and physiological alterations that cannot be uncovered through fossil record examinations," said Gaucher.

The reconstructed enzymes from the Precambrian period -- which ended about 542 million years ago -- were used to examine how environmental conditions, including pH and temperature, affected the evolution of the enzymes and their chemical mechanisms.

"Given the ancient origin of the reconstructed thioredoxin enzymes, with some of them predating the buildup of atmospheric oxygen, we thought their catalytic chemistry would be simple, but we found that thioredoxin enzymes use a complex mixture of chemical mechanisms that increases their efficiency over the simpler compounds that were available in early geochemistry," said Julio Fernández, a professor in the Department of Biological Sciences professor at Columbia University.

Fernández led a team that included Columbia University postdoctoral researchers Raul Perez-Jimenez, Jorge Alegre-Cebollada and Sergi Garcia-Manyes, and graduate student Pallav Kosuri in using an assay based on single molecule force spectroscopy to measure the activity level of the thioredoxin enzymes under different pH levels.

For their experiments, the researchers used an atomic force microscope to pick up and stretch an engineered protein in a solution containing thioredoxin. They first applied a constant force to the protein, causing it to rapidly unfold and expose its disulfide bonds to the thioredoxin enzymes. The rate at which a thioredoxin enzyme snipped the disulfide bonds determined the enzyme's level of efficiency.

The study results showed that the three oldest thioredoxin enzymes -- those thought to have inhabited Earth 4.2 to 3.5 billion years ago -- were able to operate in lower pH environments than the modern thioredoxin enzymes.

"Our analysis indicates that ancient thioredoxin enzymes were well adapted to function under acidic conditions and that they maintained their high level of activity as they evolved in more alkaline environments," said Fernández.

To measure the temperature range in which the enzymes operated, professor Jose Sanchez-Ruiz and graduate student Alvaro Inglés-Prieto from the Departamento de Química-Física at the Universidad de Granada in Spain used a technique called differential scanning calorimetry. This method measures the stability of enzymes by heating the enzymes at a constant rate and measuring the heat change associated with their unfolding.

The researchers found that the ancient proteins were stable at temperatures up to 32 degrees Celsius higher than the modern thioredoxins. The experiments showed that the enzymes exhibited higher temperature stability the older they were. The results provide evidence that ancestral thioredoxins adapted to the cooling trend of ancient oceans, as inferred from geological records.

"Our results confirm that life has the remarkable ability to adapt to a wide range of historical environmental conditions; and by extension, life will undoubtedly adapt to future environmental changes, albeit at some cost to many species," said Gaucher.

This study also showed that the experimental resurrection of ancient proteins together with the sensitivity of single-molecule techniques can be a powerful tool for understanding the origin and evolution of life on

Earth.

The researchers are currently using this strategy to assess other enzymes to get a clearer picture of what life was like on Early Earth. They are also applying these tools to the field of biotechnology, where enzymes play important roles in many industrial processes.

"The functions and characteristics we observed in the ancestral enzymes show that our techniques can be implemented to generate improved enzymes for a wide range of applications," added Perez-Jimenez.

Provided by Georgia Institute of Technology

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