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RESEARCH NEWS

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Researchers Discover Hemoglobin's Enzymatic Nature

The nematode worm *Ascaris lumbricoides* , an intestinal parasite that infects one billion people worldwide, has uncommonly strong hemoglobin that binds to oxygen 25,000-times more tightly than does human hemoglobin. The affinity of *Ascaris* hemoglobin for oxygen is so powerful that many researchers believed the molecule could not possibly play a role in respiration.

Learning the true function of *Ascaris* hemoglobin and understanding how and why the worm's hemoglobin embraces oxygen molecules so tightly have been great challenges, said Howard Hughes Medical Institute (HHMI) investigator Jonathan Stamler. "Why would a molecule that functions as an oxygen carrier bind oxygen so tightly that the oxygen molecules would never come off?"

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Two collaborating groups of HHMI scientists have ferreted out the secrets of this oxygen-hungry molecule by using a variety of biochemical techniques and by taking physical measurements of the hemoglobin inside the worm. In so doing, they have identified a biochemical link between the hemoglobins used by primordial bacteria to detoxify themselves of atmospheric gases and the modern mammalian hemoglobins that underpin respiration.

The findings not only open a window to understanding how a wide variety of organisms evolved to utilize or guard against atmospheric gases such as oxygen and nitric oxide, but they may also provide new ideas for ways to starve cancerous tumors of oxygen.

In the September 30, 1999, issue of the journal *Nature* , research groups led by Stamler, an HHMI investigator at Duke University, and Daniel Goldberg , an HHMI investigator at Washington University in St. Louis, describe how *Ascaris* hemoglobin acts as an enzyme to neutralize oxygen, which in high doses is toxic to the worm.

"In humans, hemoglobin delivers oxygen and nitric oxide to tissues," said Stamler. According to the new data, however, the nematode hemoglobin uses nitric oxide to trigger an enzymatic reaction that actually consumes oxygen. These data suggest that the primary role of the nematode's hemoglobin is to destroy oxygen. This would make sense, Stamler explained, because the *Ascaris* parasite has a low tolerance for oxygen. "The worms can deal with oxygen, but they don't really like it," he said.

Even in low-oxygen environments such as the human intestine, where the worms live, oxygen molecules do seep in. These must be neutralized, which the worm's hemoglobin does by grabbing and consuming oxygen molecules in a unique enzymatic reaction driven by nitric oxide.

Using spectroscopic techniques, the scientists studied how the nematode hemoglobin acts in the presence of different concentrations of oxygen and hemoglobin. The results of these experiments led the researchers to propose that *Ascaris* destroys oxygen via a 10-step chemical reaction.

The key to this process, says Stamler, lies with the exact positioning of a single sulfur-containing amino acid within the oxygen-binding pocket of the nematode's hemoglobin. "If this amino acid is on one side of the pocket, the hemoglobin uses nitric oxide to destroy oxygen. If it's on the other side of the pocket, as it is in mammalian hemoglobin, nitric oxide acts as a regulator of oxygen delivery," explained Stamler.

The discovery places *Ascaris* hemoglobin at a unique evolutionary juncture between primitive hemoglobins that evolved in the first living creatures on the planetwhen the Earth's atmosphere was composed mostly of nitric oxideand modern hemoglobin found in mammals and birds.

"In the primordial atmosphere, nitric oxide came before oxygen," Stamler said. "It was probably there before any life form, and the first bacteria needed a way to protect themselves from nitric oxide." Nitric oxide, like oxygen, can destroy many biologically important molecules if allowed to roam freely throughout an organism.

These first hemoglobins were likely enzymes that consumed nitric oxide and had nothing to do with oxygen, Stamler noted. About 450 million years ago, as Earth's atmosphere began to contain more oxygen, mammalian hemoglobin evolved the ability to carry oxygen molecules to tissues and cart away the waste gas carbon dioxide.

In recent years, studies from a number of laboratories have identified a variety of roles for hemoglobin. Stamler, for example, has demonstrated that when hemoglobin binds to nitric oxide it causes blood vessels to dilate. But demonstrating that the nematode hemoglobin can function as an enzyme that catalyzes a series of chemical reactions is a first.

Moreover, the researchers' discovery of the worm's ability to use nitric oxide to kick start an enzymatic reaction helps explain the complicated interplay of hemoglobin and the three gases involved in respiration, oxygen, carbon dioxide and nitric oxide.

"The worm is evolving the first indications of a respiratory function involving oxygen concentrations," Stamler said. "The worm is controlling oxygen concentrations by using nitric oxide just as we do — only with a different outcome. Instead of regulating its delivery to tissues, the worm's hemoglobin destroys unneeded oxygen."

Looking beyond the evolutionary implications of the findings, Stamler is excited by the prospect of using *Ascaris* hemoglobin as a molecular scaffolding upon which to design new therapeutic agents that can starve cancerous tumors of the oxygen they need to survive. "This is the first enzyme that eliminates oxygen," he said. "The hope is it could be used therapeutically," much like the so-called antiangiogenesis agents now being tested for their ability to shut off the blood supply to tumors.

Other authors of the *Nature* paper include Dena M. Minning of HHMI at Washington University; and Andrew J. Grow, Joseph Bonaventura, Rod Braun and Mark Dewhirst of Duke University.