"Radical" Means

Researchers solve century-old chemistry conundrum

BY SAREN JOHNSTON

FOR MORE THAN 100 YEARS, CHEMISTS debated the nature of the mechanism that triggers the Fenton reaction, one of the most powerful oxidizing reactions available for breaking apart organic compounds. Back and forth the controversy persisted, generating a profusion of professional articles that supported either one or the other of the likely candidates – short-lived, difficult-to-measure hydroxyl radicals or the extremely rare iron(IV). Which one was the highly reactive Fenton intermediate that could initiate the oxidations of countless substances, from biomolecules, such as proteins, sugars, fatty acids and nucleic acids to the pollutants found in smog and industrial wastes?

Decades passed, and the identity of the elusive Fenton intermediate remained a mystery. Now, however, Ames Lab senior chemist Andreja Bakac and assistant chemist Oleg Pestovsky have generated, characterized and ruled out iron(IV) as the Fenton intermediate. Their irrefutable research results tip the balance heavily toward hydroxyl radicals, or OH radicals, as the crucial intermediate by which the Fenton reaction proceeds to completion.

Fenton facts

Discovered in 1894 by H. J. H. Fenton, the Fenton reaction is the oxidation of aqueous iron(II), or Fe(II), with hydrogen peroxide, a versatile, safe and effective oxidant. (An oxidant is a substance containing oxygen that reacts chemically with other materials to produce new substances.)

The pervasive nature of the Fenton reaction accounts for scientists' longstanding efforts to unravel the century-old mystery surrounding the famous Fenton intermediate that allows the reaction to proceed to completion. And no wonder – the reaction operates or is employed almost everywhere, with both good and not-so-good effects. It's critical in the treatment of organic pollutants that are introduced into the environment by the uncontrolled use of such things as pesticides and herbicides, among many other man-made contaminants. It's vital within the industrial chemistry arena, where researchers investigate catalysts with the goal of making various chemical processes go faster and in a more selective and efficient manner. In contrast, the Fenton reaction plays not-so-benign roles in the biology of aging and disease, contributing to certain types of DNA damage that may not self-repair and, so, accumulate with age.

(left) Bakac and Pestovsky bubble ozone through a solution and use it to generate Fe(IV).

Closing in on the chemistry

"Knowing the nature of the intermediate is crucial to understanding the role of Fenton chemistry in issues related to environmental and atmospheric chemistry, as well as human health and aging," says Bakac, who is also an Iowa State University adjunct professor of chemistry. "The fact that we have now eliminated iron(IV) in Fenton reaction and confirmed it in ozone reaction, may provide a foundation for the development of new and useful catalytic reactions based on iron(IV)."

Ames Lab's basic research effort was done in collaboration with Carnegie Mellon University in Pittsburgh and the University of Minnesota in Minneapolis. An article by the collaborating scientists describing their research and its indisputable results was published in *Angewandte Chemie*. The journal gave the article a "Very Important Paper," or VIP, rating, which less than 5 percent of their manuscripts receive.

As with many scientific achievements, the work done by Bakac and Pestovsky that eventually ruled out iron(IV) as the Fenton intermediate did not initially have that goal. "We started out studying aqueous iron(IV) for several reasons," says Bakac. "It is a very unusual species, but in stabilizing biological environments, iron(IV) has been found to play a role, especially in enzymatic reactions. And as soon as you find something that is considered unusual to actually function in real life, you know it's not that uncommon – it just hasn't been recognized before."

Andreja's idea

"Iron(IV) was not a totally unknown species when we started looking at it," says Bakac. A research group in Denmark had done the reaction of iron(II) with ozone and proposed that iron(IV) was produced," she explains. "That was about a decade ago, and the work kind of went unnoticed."

While reviewing the literature relating to iron(IV), Bakac came across the Danish papers again. "As I read those papers, I figured if that was iron(IV), then there ought to be much more chemistry there, some of it potentially important in both catalytic and biological contexts," she says. "Of course, nobody knew whether iron(IV) was really involved. It was some sort of intermediate that hadn't been characterized. This is where Oleg and I got involved."

Bakac and Pestovsky set out to generate this species from iron(II) and ozone, and look at its chemistry. "All along we were hoping to find that this really was aqueous iron(IV), a simple, but probably extremely reactive species,"

(left) ***Preserving a life:*** *Andreja Bakac demonstrates the device that allows her and Oleg Pestovsky to generate and freeze iron(IV) in less than a second.*

(right) ***Very "cool" stuff:*** *A delicate spray of newly generated iron(IV) has frozen instantly to the inner walls of this copper cylinder that has been cooled to liquid-nitrogen temperature (77 Kelvin, or minus 321 degrees Fahrenheit).*

Bakac says. "The initial reactivity data were truly exciting and consistent with an iron(IV) species, but still we had no proof," she adds.

Mössbauer "magic"

"We saw some beautiful chemistry, but we definitely needed to identify this species," says Bakac, "so we got in touch with Eckard Münck at Carnegie Mellon. He's the world expert in Mössbauer spectroscopy, which is considered to be the most definite of spectroscopic methods when working with iron."

Bakac explains that iron(IV) is a short-lived species, lasting about 10 seconds at room temperature. Although that is orders of magnitude more than some intermediates she works with that live only milliseconds or microseconds, Bakac notes that the 10-second life of iron(IV) did present a problem. "It was a lot to deal with when we were producing the material here in Ames and the group that was analyzing it was in Pittsburgh," she says. Fortunately, the two research teams collaborated and found a way to get a sample from Ames to Pittsburgh before it "died."

"We designed and built a device that allows us to generate iron(IV) and immediately cool it down to liquid nitrogen temperature and freeze it in a fraction of a second," says Bakac. "We then packed the solid into a liquid-nitrogen-cooled dewar and shipped it overnight to Pittsburgh. There, the Carnegie Mellon team collected the Mössbauer spectrum at liquid helium temperature."

Mössbauer studies of the Ames samples done under Münck's direction by Carnegie Mellon research associate Emile Bominaar and graduate student Sebastian Stoian proved the intermediate generated by Bakac and Pestovsky was exactly what they were hoping for – the iron(IV) species.

In addition to the Carnegie Mellon work, contributions by Lawrence Que and his postdoctoral associate Xiaopeng Shan at the University of Minnesota further confirmed the iron(IV) species. "They took our sample to Stanford University to get an X-ray absorption spectrum, or XAS, and that spectrum was consistent with the oxidation state of iron(IV)," says Bakac.

The Mössbauer and XAS analyses, combined with all the chemistry carried out at Ames Lab, told Bakac and Pestovsky what iron(IV) looks like and what it does. "For the first time we knew what both iron(IV) and OH radicals would do and could figure out which one is involved in the Fenton reaction," says Bakac. "Nobody knew how to make iron(IV) or look at it before so that we could distinguish between the two."

Identical experiments, different products

At that point, knowing the nature of the intermediate, Bakac and Pestovsky decided to carry out some very specific experiments. In one set of experiments, they oxidized a substance with iron(IV), and in a parallel series of experiments, they oxidized the same substance using the Fenton reaction.

"The products were different," says Bakac. "And more than that, the products generated from the Fenton reaction were identical to those known to be formed from reactions involving OH radicals. So we both ruled out iron(IV) as the intermediate and indirectly confirmed OH radicals," she says.

Bakac and Pestovsky's work took about a year, and not every sample was good. "There were times when we thought our samples weren't surviving the trip, so on one occasion Oleg actually traveled to Pittsburgh with all of our equipment to make the sample there," recalls Bakac. "It's been rocky at times, but we knew we had something special, and that kept us going."

In addition to ruling out iron(IV) and indirectly establishing OH radicals as the Fenton intermediate, Bakac and Pestovsky's research shows iron(IV) to be a very useful chemical species. "The fact that iron(IV) is very short-lived doesn't matter because in catalytic reactions you make it in situ and use it immediately," says Bakac. "There are certainly situations in chemistry and biology where various iron(IV) complexes, including our aqueous iron(IV), may be involved," she suggests, then adds; "just don't go searching for iron(IV) anymore in Fenton chemistry."

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Research funded by:
DOE Office of Basic Energy Sciences, Chemical Sciences Division