When the Good and the Bad Make the Ugly: The Discovery of Peroxynitrite

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Peroxynitrite Oxidation of Sulfhydryls. The Cytotoxic Potential of Superoxide and Nitric Oxide

At first, no one took them seriously. In 1991, Rafael Radi, Joseph Beckman, Kenneth Bush, and Bruce Freeman published a paper in the Journal of Biological Chemistry (JBC) demonstrating that a molecule called peroxynitrite, the product of a reaction between nitric oxide and superoxide radicals, selectively attacked sulfhydryls in proteins. “Nobody believed much of any of it,” recalls Beckman at Oregon State University. “It was considered an unproven theory. I was surprised we even got the paper accepted in JBC.”

Today, “Peroxynitrite Oxidation of Sulfhydryls” is recognized as a JBC Classic. The paper has been cited in the scientific literature more than 2100 times.

Until 1990, the chemistries of nitric oxide and oxygen radicals were thought to be unrelated. Nitric oxide was known to physiologists as the molecular radical that caused vasodilation, played a role in neurotransmission, and killed invasive pathogens. The chemistry of superoxide and other oxygen radicals fell under the purview of biochemists interested in the damage wreaked by these reactive entities. Neither group considered that their radical of interest had anything to do with the other.

That view was challenged in 1990 with a paper in the Proceedings of the National Academy of Sciences (PNAS), with Beckman as the first author and Freeman as the corresponding author. At that time, the group was at the University of Alabama in Birmingham. Freeman held a faculty position. Beckman was a tenure-track assistant professor who had done a postdoctoral fellowship with Freeman.

In the PNAS paper, the authors described how nitric oxide reacted with superoxide to form peroxynitrite. “We proposed that nitric oxide was toxic because it reacted with superoxide to form peroxynitrite,” explains Beckman.

Radi, who had joined the Freeman group as a postdoctoral fellow, had been working on oxygen radicals at the Universidad de la República in Uruguay (he later returned to the institution as a principal investigator). For the JBC paper, he and Beckman analyzed the reaction kinetics of peroxynitrite with bovine serum albumin and cysteine and discovered that peroxynitrite was capable of directly oxidizing sulfhydryls, much more so than hydrogen peroxide. “This completely opened a new paradigm of oxygen-radical-dependent toxicity by means of the cross-talk with the nitric oxide pathway,” says Radi. Bush, the third author on the paper, was a research technician who later became a lawyer.

Radi says he and Beckman found inspiration from Clint Eastwood’s movie “The Good, The Bad, and the Ugly.” It was unthinkable to physiologists that nitric oxide could “be converted in such a nasty molecule just because of the reaction with superoxide,” says Radi. “Nitric oxide was the good guy; superoxide, the bad; and peroxynitrite, the ugly.”

These days, peroxynitrite is recognized as an oxidant and nucleophile that can attack mitochondria and lead to cell death by a slew of oxidation and nitration reactions. Radi explains that peroxynitrite has a dual personality. It can be “liberated by our immune cells to...”
kill invading pathogens,” says Radi. However, he adds that the molecule has been implicated in atherosclerosis, hypertension, type 2 diabetes, and neurodegenerative conditions, such as amyotrophic lateral sclerosis.

Peroxynitrite can break down to form secondary molecules such as nitrogen dioxide and hydroxyl radicals. Freeman says nitrogen dioxide is capable of nitrating protein tyrosine and tryptophan residues and unsaturated fatty acids. The latter reaction leads to products with signaling capabilities that modulate metabolic and inflammatory responses. The fatty acid reaction with nitrogen dioxide is being scrutinized as a drug target.

However, back in the early 1990s, “it took a few years and redundant ways to show that these reactions were of any importance in biology,” says Radi. Beckman sees the silver lining in having naysayers: not too many others were interested in working on peroxynitrite. The field was left wide open for investigators like him, Radi, and Freeman to get a head start on peroxynitrite research. He says, “The moral here is don’t get discouraged if people don’t immediately jump to your ideas.”

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*Ruma Banerjee at the University of Michigan, Ann Arbor (JBC Associate Editor) nominated the paper as a Classic. Rajendrani Mukhopadhyay (ASBMB’s chief science correspondent) wrote the introduction.*