Nitric oxide (NO) is an enigma. Chemists know it as a colorless gas which, when pure, is stable despite being a free radical, a fact that should make it react almost instantaneously with any molecule it encounters. All the more puzzling then to find that it is constantly generated within living organisms and utilized to carry messages between cells and within cells. Salvador Moncada, then at the Wellcome Research Laboratories, Beckenham, England, first realized that this unlikely molecule had a biological role when, in 1987, he showed that it relaxed muscle cells. Today, it is known to be involved in the regulation of the cardiovascular system, the immune system, the central nervous system, and the peripheral nervous system. It even achieved popular notoriety as the molecule that triggers erection of the penis. However, because it is a free radical, its lifetime in the body is brief, making it almost impossible to observe it in action. Methods have been developed for detecting NO at low concentrations, but these are not suitable for studying it in vivo.

All that changed with the publication of paper #6. This ground-breaking communication is the work of Tetsuo Nagano and a group of analytical-chemistry colleagues at the Graduate School of Medicine of the University of Tokyo, Japan. They solved the problem of tracking it by designing compounds that fluoresce when they react with NO. These compounds are diaminofluorescein dyes, which the authors refer to as DAFs, and the molecules have two amino (NH₂) groups adjacent to each other on a component benzene ring. It is these which react with NO, in the presence of oxygen, to form a three-nitrogen atom ring, and the DAF then glows with a characteristic green light, 100 times more intense than that of the dye. The reaction with NO is highly specific, so the reagent does not generate spurious signals by reacting with other molecules in biological tissue, and it is extremely sensitive, able to detect NO at concentrations as low as 5 nanomolar (5X10⁻⁹M).

Nagano’s group made several DAFs with the two amino groups positioned in various ways, plus attaching chlorine atoms and acetyl...
groups to the molecule. The DAF that turned out to work the best was one with two acetyl groups, and this they coded DAF-2 DA. It was able to penetrate membranes, where its acetyl groups are there removed, leaving DAF-2 to react with NO and emit an easily detected signal. They injected DAF-2 DA into smooth muscle cells of a rat aorta and were able to prove that the fluorescence in the rat cells increased in a manner that was dependent on the concentration of NO.

Commenting on the popularity of paper #6, Nagano says: “This kind of molecular visualization in living cells is a trendy topic scientifically. The clarification of biologically significant molecules has been recognized as an important challenge in the coming post genome era, and NO is a ubiquitous molecule. Scientists in fields such as neurology, cardiology, and immunology are interested in NO imaging. Our research interest is now directed at other biological applications of chemical techniques which I am certain will play a central role in the future.”

Another reason why Nagano’s work is being highly cited is that DAF-2 DA is now commercially available as a standard reagent, and it is easy to use. (It is sold by Sigma and CalbioChem in the USA, Alexis in Europe, and Daiichi Pure Chemicals in Japan.)

Last year Nagano’s team published a second paper on their method for analyzing NO, in Angewandte Chemie - International Edition (see H. Kojima, et al., 38[21]:3209-12, 1999), which describes a more sensitive DAF molecule with increased pH stability. More recently, they have developed a fluorescing dye capable of detecting the trace element zinc in biological systems (see T. Hirano, et al., Angew. Chem. Int. Ed., 39[6]:1052, 2000).

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