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Biophysics

Enzyme's special bonds finally revealed

High-resolution crystallography uncovers the first view of bonds important for enzyme structure and function

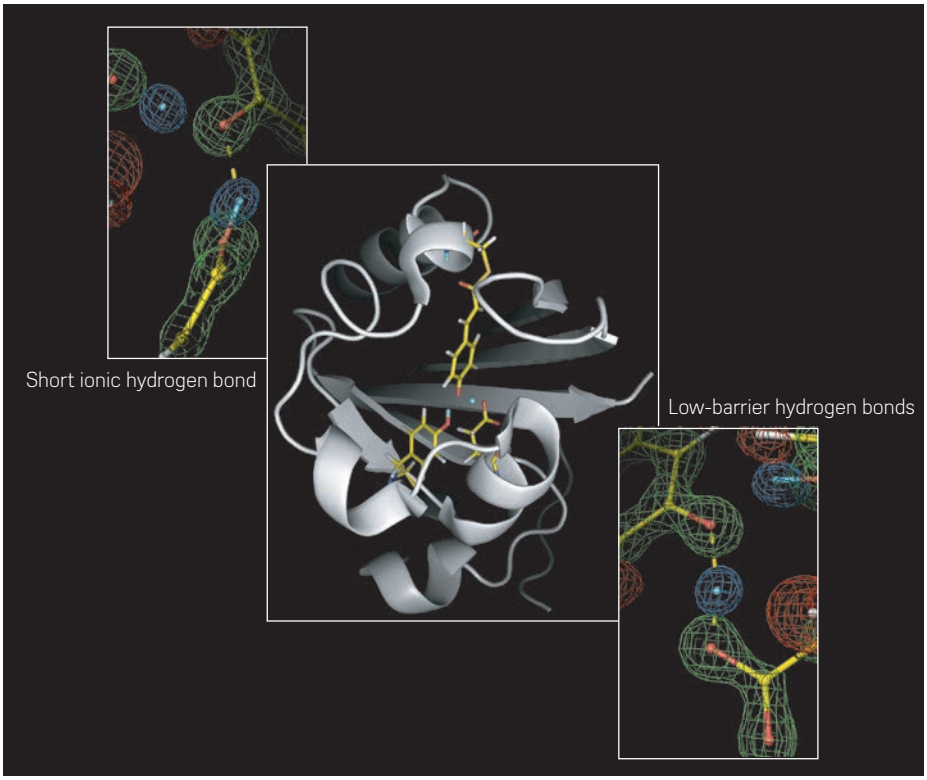
In 1994, a pair of papers published in *Science* suggested that a special class of intramolecular bonds known as low-barrier hydrogen bonds (LBHB) play an important role in enzymatic reactions. But it took until 2009 for a high-resolution crystallography technique developed by Japan's NAIST to allow scientists to finally observe these elusive bonds.

LBHBs are exceptionally short hydrogen bonds, resulting in the hydrogen ion, a proton, being more evenly shared between the partners. This confers additional flexibility to the molecule, enabling it to change shape more easily during reactions. However, the short length of LBHBs also makes them difficult to detect directly, leading to some debate about their existence. Observation of an LBHB calls for very high-resolution crystallography to determine the position of the proton and the donor and acceptor atoms.

> **"The resolution we attained was the highest in the world. Nobody had ever conducted such high-resolution neutron crystallography."**

Mikio Kataoka's team at NAIST was able to overcome these challenges in collaboration with researchers at other Japanese institutes. They developed a method to grow large crystals of the light-sensitive photoactive yellow protein (PYP) in heavy water, enabling them to determine its structure at an unprecedented resolution with neutron diffraction[1]. "The resolution we attained was the highest in the world. Nobody had ever conducted such high-resolution neutron crystallography," says Kataoka, adding that the team had to develop a new analytical method to handle their data.

Work on the research spanned five years, including two years to develop the crystallization conditions. "A talented and ambitious student selected the project as his doctoral research," observes Kataoka. "NAIST allows us to tackle such time-consuming projects."



Visualization of a portion of the molecular structure of photoactive yellow protein, with insets showing two different kinds of hydrogen bond.

The team was able to detect the position of 87% of the hydrogen ions in PYP, and determine that one of them — buried within the protein's interior — forms an LBHB. In addition to easing the protein's conformation change in response to light, the LBHB serves to stabilize a negative charge, which would otherwise be energetically unfavourable in the interior environment of the protein.

Kataoka is now using time-resolved crystallography to follow changes in the LBHB during PYP's photoreaction. "Before practical applications, we must clarify the properties of LBHB and the principle of LBHB formation. Basic research will be still required," he says.

A better understanding of LBHBs may significantly improve our ability to engineer molecules. "The formation of LBHBs is a key element for efficient proton transfer," explains Kataoka. "If we can control the formation of LBHBs, it will allow us to design artificial enzymes with high efficiency and artificial proton pumps, as well as [more] efficient medicine."

Reference

1. Yamaguchi, S., Kamikubo, H., Kurihara, K., Kuroki, R., Niimura, N. *et al.* Low-barrier hydrogen bond in photoactive yellow protein. *Proceedings of the National Academy of Sciences USA* **106,** 440–444 (2009).

More information about the group's research can be found at Mikio Kataoka's webpage:
http://www.naist.jp/en/about_naist/offices/administration_bureau/mikio_kataoka/index.html