New "Insiders" View of Anti-Aids Drug Captured by UCSD Chemists Huguette Pelletier and Joseph Kraut

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NEW "INSIDERS" VIEW OF ANTI-AIDS DRUG CAPTURED BY UCSD CHEMISTS

The first view of an anti-AIDS drug bound to a key enzyme responsible for DNA replication and repair in the body has been revealed in atomic detail by a research team led by chemists at the University of California, San Diego.

With the help of this molecular snapshot, the researchers say, it may now be possible to understand why certain anti-AIDS drugs cause toxic side effects, or how resistance to such medications may build up over time.

It also could offer insights into the design of more potent and less toxic drugs to combat HIV, the virus that causes AIDS.

"This is the best grounds we have so far for rational drug design to improve anti-HIV drugs," said Huguette Pelletier, a UCSD postdoctoral research chemist at UCSD and co-author of two studies about the finding appearing in today's issue of the journal Science.

Pelletier said the studies also might alter previous views of how the enzyme binds to DNA and anti-HIV drugs.

"We're proposing a different model for how these kinds of enzymes work which could be very important when trying to inhibit the enzyme from the AIDS virus that these drugs attack," she said.

The two studies, which took six years to complete, revolve around beta polymerase, one member of a family of widely distributed enzymes that are essential for the replication, repair and recombination of genetic material--DNA and RNA. Polymerases are believed to be the first enzymes to appear on earth.

Among the better-known members of this family is HIV reverse transcriptase, the highly complex enzyme that permits the AIDS virus to replicate. Anti-HIV drugs--including AZT, ddI and ddC-- bear a close resemblance to DNA building blocks called nucleotides. When these chemical mimics are taken up instead by reverse transcriptase, the enzyme is prevented from doing its job.

Because of its complexity, however, the precise manner in which HIV reverse transcriptase works has proven difficult to study. For this reason, the UCSD-led research team turned to a smaller and less complicated relative: beta polymerase, which is believed to play a central role in repairing damaged DNA, a role that could have implications for cancer and aging.

"Repairing DNA damage may be the main function in life for beta polymerase," said Joseph Kraut, a UCSD professor of chemist who initiated the studies. "When it makes mistakes, you end up with a mutation that in almost every case is deleterious."

Both studies reported in Science required X-ray crystallography, a technique in which X-rays are fired through a crystalline sample of protein. The pattern of deflection of the X-rays off the atoms is captured by detectors, giving clues to the shape of the molecule. This information is stored as numerical values and entered into a supercomputer, which then analyzes and translates that information into a visual image of the molecule.

In their first study, Pelletier and Kraut--along with UCSD graduate student Michael R. Sawaya--described the structure of rat beta polymerase in atomic detail, the first polymerase viewed in this manner for a higher organism.

Among the key features disclosed by the study was an area resembling the architecture of a cupped right hand, with regions best characterized as fingers, palm and thumb. It's here, in this cupped hand, where DNA docks, binds and ultimately replicates. A similar structure can be found in other members of the polymerase family, suggesting they all share a common mechanism for carrying out their catalytic functions.

In the second study, the researchers went one step further by capturing in atomic detail the enzyme linked to a DNA molecule in the process of taking up ddCTP, a biologically active version of the anti-HIV drug ddC.

Capturing an enzyme in the act, in the so-called transition state, is considered one of the "grand prizes of crystallography."

"It's very rare, because by the time you get the real complex together, it's going to react," said Pelletier. "That was the trick, to get the enzyme to bind to something that looked similar to the real thing, but instead of causing a reaction, the enzyme gets stuck."

Because anti-HIV drugs also are believed to work in this manner when attacking the AIDS virus, Pellitier added, it's not surprising that patients suffer side effects when the same medication attacks polymerases needed by the body, such as beta polymerase.

"So now we have a detailed view, the atomic structure, of how anti-HIV drugs like AZT, ddI and ddC work, how they fool the enzyme into getting stuck and inhibit it," said Pelletier.

The work also might set in motion other studies to find out why patients build up resistance to anti-HIV drugs over time.

"This may help other researchers now," she said. "They can use my technique in the near future to make other complexes, for instance, with HIV reverse transcriptase, to get more detailed views of what's going on."

One surprising result, Pelletier added, was the fact that beta polymerase seemed to bind to DNA in an opposite direction from what researchers had proposed for other polymerases, including reverse transcriptase.

This could mean that all previous models for how this family of enzymes works might be incorrect, she said.

"What I'm proposing now is when it (reverse transcriptase) performs one certain task it binds (to the genetic material) in one direction, and then later on, in a different cycle of the virus, it binds in another direction.

"This is the first solid proposal describing in detail how HIV transcriptase may work. The fact that we know something new and fresh, which offers a different approach to how this enzyme actually works, could be very important to finding a new way of inhibiting HIV reverse transcriptase."

Also participating in the studies were Amalendra Kumar and Samuel Wilson--both from the Sealy Center for Molecular Science, University of Texas Medical Branch--who provided the UCSD scientists with purified rat beta polymerase used in the experiments.

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