NCI Team Remodels Key AIDS Virus Enzyme

The original structure of the AIDS virus protease was not quite right. The new view may aid anti-AIDS drug development

CONSIDERABLE EXCITEMENT GREETED the report, in February of this year, that x-ray crystallographers from Merck Sharp & Dohme Research Laboratories had determined the three-dimensional structure of a critical AIDS virus protein. Not only was this the first time that the three-dimensional structure of any AIDS virus protein had been worked out, but here was a protein that might be an attractive target for potential anti-AIDS drugs. So if the structure of the protein—a protease—was accurate, the information could help researchers design agents that render the protein harmless.

But the structure was not completely accurate. On page 616 of this issue of *Science*, Alexander Wlodawer and his colleagues at the Frederick Cancer Research Facility of the National Cancer Institute report that the protease structure as originally proposed is wrong in some significant ways. And the NCI scientists are not the only ones who think that. A British group is coming to a similar conclusion, as are even the Merck researchers themselves.

"People who are not crystallographers think that these structures are gospel," Wlodawer says. "It's a cautionary tale that we have to be careful in interpreting these things. We're not infallible, unfortunately."

There were indications from the start that the Merck group's protease structure, which they described in the 16 February issue of *Nature*, might not be quite right. The AIDS virus and related viruses need proteases because their proteins are made hooked together in large "polyproteins." It is the protease's job to release the individual proteins.

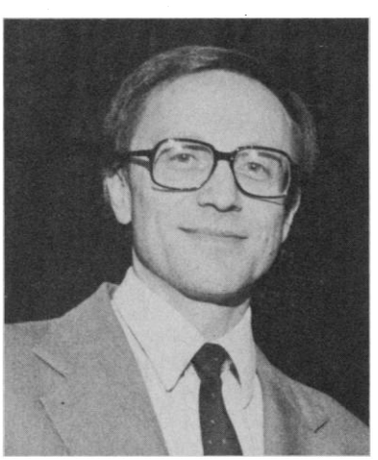
As it happened, at the time that the Merck results came out, the Frederick workers had just determined the three-dimensional structure of a comparable protease from another virus, namely the Rous sarcoma virus, and had used this structural information to predict (in the 17 February issue of *Science*, p. 928) what the enzyme from the AIDS virus might look like. The predicted structure and the Merck structure were significantly different.

Another model for the protease that was proposed by Laurence Pearl and William Taylor of Birkbeck College of the University of London also differed from the Merck structure, although it did resemble that constructed by the Frederick group.

Faced with these discordant structures for the AIDS virus protease, Wlodawer decided to do his own x-ray crystallographic analysis of the enzyme. Here the work received a big boost from Stephen Kent and Jens Schneider of the California Institute of Technology in Pasadena, who had achieved the complete chemical synthesis of the 99–amino acid protease. The synthesis provided the pure protein needed for the crystallographic analysis now completed by the NCI group, the first time this has been achieved with a completely synthetic protein.

The structure they have produced confirms that elements of the original Merck structure were wrong, as the Frederick and London models had predicted. So does an independent crystallographic analysis performed by Tom Blundell and his colleagues at Birkbeck College on material that they made in bacteria by recombinant DNA technology. Meanwhile, the Merck group's James Springer says, "We've reinterpreted our structure based on what Wlodawer found and also on higher resolution data that we collected." The upshot is that the Merck group concedes that their original structure needs to be corrected.

One correction affects the interface between the two 99–amino acid chains that come together in a dimer to form the active enzyme. The interface can now be seen to contain the amino and carboxyl terminal segments of both chains arranged in a four-stranded anti-parallel beta sheet. This contrasts with the original Merck structure, in which the amino end was found to be flexible and free. It is not. It is well ordered and tied down in the interface.



Changing shapes. *Alexander Wlodawer's group gave the AIDS virus protease a new look.*

In addition, the Wlodawer group finds that each protease chain contains an alpha helix near its carboxyl end that was not seen in the Merck structure.

These changes are relatively modest from the standpoint of structure alone—they affect about 15% of the protease molecule. But they may have an impact on efforts to design inhibitors to block the protease.

Both Wlodawer and Pearl suggest, for example, that one good way of blocking the enzyme's activity would be to prevent dimer formation. "It's very clear that you need to make a dimer for the enzyme to have any activity at all," Wlodawer points out. Having a clear view of the structure at the dimer interface should help to design agents that block its formation.

Researchers are also trying to make more conventional inhibitors that block the active site of the protease, and here, everyone agrees, the structural corrections will not make much difference, because they did not affect the enzyme's active site. The newer structures do, however, provide previously unavailable information about the positions of the amino acid side chains that should be helpful in designing conventional competitive inhibitors.

The structural corrections are also very significant, Pearl says, for understanding the biological function of the protease. The Merck group had based a proposal for controlling the enzyme's activity on the supposed flexibility of the amino terminal segment of the molecule.

They suggested that this segment can fold back over the active site of the enzyme, allowing one molecule to cut itself out of the polyprotein—like a dog biting its own tail, in Wlodawer's description. But the new structure shows that this is not possible. The amino terminal segment is held rigidly in place away from the active site, meaning that the cut must be made by another enzyme molecule.

According to Pearl, this may mean that the protease becomes active at a later stage of viral maturation than originally proposed by the Merck group. Even small corrections, then, in a protein's structure may generate new ideas about how it works, as well as pointing the way to designing drugs to counteract its action. ■ JEAN L. MARX