Blunting Nature's Swiss Army Knife

Researchers are trying to develop inhibitors of a wide variety of proteases, the protein-cleaving enzymes that play a role in everything from the common cold to cancer

A class of drugs with an unusual mechanism for fighting disease has breathed new hope into AIDS research during the past year. At least for some patients, these compounds, which block the activity of a protein-snipping enzyme that the AIDS virus needs to complete its life cycle, are turning what was once a death sentence into a chronic condition. But AIDS isn't the only disease that may be treatable by blocking the activity of these enzymes, called proteases. Researchers are now exploring the use of protease inhibitors against everything from infections, including the common cold and the parasitic disease schistosomiasis, to inflammatory conditions like asthma and rheumatoid arthritis, and even cancer.

Proteases are such an inviting target for therapies because they play key roles in disease development. James McKerrow, a molecular biochemist at the University of California, San Francisco (UCSF), describes them as "Mother Nature's Swiss army knife. They have many different functions, even though they often have nearly identical structures."

When the AIDS virus infects cells, for example, the proteins it needs to reproduce itself are synthesized as one large precursor molecule. It's then the job of the HIV protease to split out the individual proteins. If a drug blocks the protease, the replication of the virus is also blocked. Proteases play similar roles in the life cycles of other viruses, including the coronaviruses, which cause about one-third of all cases of the common cold. Proteases are also important contributors to inflammatory damage, and by breaking down proteins that normally hold cells in place, they apparently help cancer cells escape from the primary tumor and spread to new sites in the body.

These findings, combined with the encouraging precedent set by the AIDS antiprotease drugs, have spurred companies ranging from small start-ups like San Francisco's Arris Pharmaceutical to industry giants like Merck to try to develop therapies based on protease inhibitors. "A whole host ... of protease inhibitors is beginning to appear," says Les Hudson, Pharmacia & Upjohn's vice president for research.

None of these has yet hit the market, and information on drugs in development can be hard to come by because of proprietary concerns, but preliminary clinical trials of at least a few protease inhibitors are under way. The hope is that these will provide therapies for currently untreatable conditions such as colds, or drugs with fewer side effects for conditions such as rheumatoid arthritis that are now treated but with potent, but unspecific, drugs.

One of the protease inhibitors that has entered clinical trials is a drug called APC366, being developed by Arris as a possible treatment for asthma. It's directed against a protease called tryptase, released when immune cells known as mast cells are activated by an allergen, such as dust mite feces or pollen. Tryptase contributes to asthma symptoms both directly and indirectly.

Tryptase's protein-splitting action accounts for its direct effects, among them increasing the permeability of the blood vessels. The fluids that consequently ooze into the tissue lead to swelling in the narrow passages of the lungs. Tryptase also promotes scarring in the lungs by cleaving and activating enzymes that foster the deposition of collagen, a protein found in scar tissue. In addition, the enzyme can indirectly cause lung damage by helping recruit eosinophils, white blood cells that are the bane of asthmatics. "Eosinophils damage the epithelium of the lung, ... and tryptase is a potent chemoattractant for human eosinophils," says Stephen Holgate, an immunopharmacologist at the University of Southampton in the United Kingdom.

To block these effects, Arris chemists looked for a compound—preferably a small molecule that could be taken by mouth—that could fit into, and block, tryptase's active site, the relatively small part of an enzyme that binds its target molecules and performs the catalytic work. A strategy for fine-tuning candidate molecules to improve their protease binding sped up Arris's effort, says Heinz Gschwend, the company's executive vice president for research and preclinical development. He declined to divulge the details of the procedure, but it is apparently a variant of one of the new combinatorial methods that enable drug designers to generate and test many thousands of related compounds simultaneously (*Science*, 31 May 1996, p. 1266). Gschwend does say, however, that this "Delta technology," as the company calls it, "is phenomenally simple, and it really shortens the time it takes to develop [protease] inhibitors."

APC366, one of the products of that procedure, proved to be a potent tryptase inhibitor. Once animal testing had indicated that the drug would be sufficiently safe to test in humans, the company began clinical trials, which have already produced some promising results. In one small study of 16 asthma patients that was completed last September, the drug reduced by 32% the patients' "early airway response"—the swelling caused by mast cells, which sets in right after exposure to an allergen. At several hours after administration, it increased lung capacity by 42%.

At the same time, the treated patients apparently showed few side effects. That may give APC366 and other tryptase inhibitors an advantage over the steroids now commonly used to treat asthma, even though the inhibitors may be less effective at reducing inflammation, says Lawrence Schwartz, a microbiologist at Virginia Commonwealth University in Richmond. Still, the encouraging results of the APC366 trial, with its limited number of patients, will need to be

A SAMPLING OF PROTEASE INHIBITORS UNDER INVESTIGATION

Drug (Company)	Protease Target	Disease	Status
APC366 (Arris Pharmaceutical)	Tryptase	Asthma	Early clinical trials
E-64 (SmithKline) APC3328 (Arris)	Cathepsin K	Osteoporosis	Lab testing
TIMPS (British Biotech)	Metalloproteinases	Pancreatic and other cancers	Clinical trials beginning
(Pharmacia & Upjohn)	Factor Xa	Blood clotting	Lab testing
Cystatins	Coronavirus protease	Common cold	Lab testing
(Arris)	Schistosome protease	Schistosomiasis	Animal testing

confirmed in larger studies before the drug can be approved by the U.S. Food and Drug Administration.

A host of targets

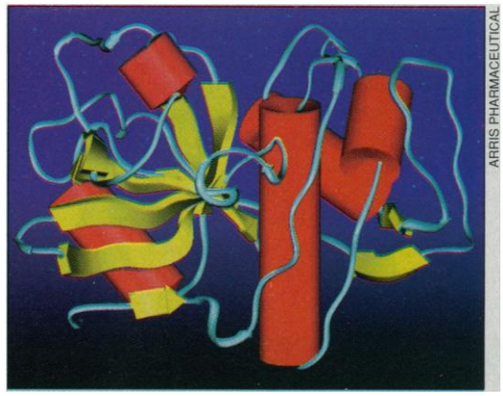
Tryptase is not the only cellular protease being targeted by Arris and other companies. Another is the enzyme cathepsin K, which is made in large quantities by human osteoclasts, cells that are engaged in the remodeling that constantly goes on in normal bones. The osteoclasts destroy bone, presumably aided by cathepsin K's protein-digesting abilities, while another type of cells, called osteoblasts, build it up. In healthy adults, the two are equally matched, so bone density remains roughly unchanged. But if the osteoclasts get the upper hand, the bones weaken, as may happen, for example, in osteoporosis, a disease that afflicts more than 25 million people, most of them postmenopausal women, greatly increasing their risk of bone fractures.

At least two companies, SmithKline Beecham and Arris, are attempting to develop drugs that inhibit cathepsin K, in hopes that they will be able to combat the bone weakening of osteoporosis. As a step toward that goal, both have determined the three-dimensional structure of the protease when it is complexed with smaller molecules—a compound SmithKline calls E-64 and one from Arris designated APC3328. (The results appear in the February issue of *Nature Structural Biology*.) By helping researchers tailor molecules to the geometry of the protease's active site, such structural information could aid in designing effective inhibitors.

Proteases may also play a crucial role in the deadly activity of metastasizing cancer cells. By digesting away the proteins that hold cells together, the enzymes apparently help cancer cells burrow through tissue so that they can spread to new sites throughout the body. "There's overwhelming evidence that [proteases] are a major mechanism in invading normal tissue," says Marc Shuman, an oncologist at UCSF. "If we can inhibit the proteolytic activity, we can prevent [metastasis] from happening." For example, Shuman's group has identified a new protease that is expressed on the membranes of prostate cancer cells and may contribute to the cells' ability to metastasize. The team has also found a naturally occurring protein that inhibits the protease and can, at least in lab cultures, block the spread of prostate cancer cells, Shuman says.

Shuman has no plans to attempt clinical trials with his inhibitor, but Lance Liotta's team at the National Cancer Institute in Bethesda, Maryland, has developed a family of protease inhibitors that is moving toward the clinic. These so-called "TIMPs"—tissue inhibitors of metalloproteinases—block the protease enzymes that several different types of cancer cells use to snip the collagen fibers in the extracellular matrix. This activity not only helps the cancer cells to spread, but also fosters the growth of the new blood vessels needed to supply blood and oxygen to growing metastatic tumors. British Biotech, plc., a company based in Oxford, United Kingdom, is beginning clinical trials of TIMPs in a number of cancers, including those of the pancreas, lung, and brain.

Besides trying to stop specific cells, like osteoclasts or cancer cells, drug companies have also set their sights on members of a series of proteases—a so-called protease cascade—that act successively to bring about blood-clot formation. These companies hope that inhibitors of individual proteases could act as anticoagulant drugs more selective than the ones now given to heart attack, stroke, and other patients in danger of suffering life-threatening blood clots. Heparin, for example, inhibits many "factors" in the clotting cascade. A protease inhibitor might be able to break the cascade at only a single point, making it less prone to cause unwanted side effects.



Under fire. Drug developers want to target proteases such as this one, cathepsin K.

One promising candidate for such an inhibitor comes from biochemist Joanna Chmielewska of Pharmacia & Upjohn in Stockholm, Sweden, and her colleagues. It is directed against an early participant in the clotting cascade: factor Xa, which splits prothrombin, releasing another active protease, thrombin. Thrombin in turn cuts fibrinogen, ultimately producing fibrin, the raw material of clots.

Chmielewska, like many of the other researchers designing protease inhibitors, built on a knowledge of her target protein's three-dimensional structure that had previously been determined by other investigators. "The combining site [of factor Xa] is quite well understood; you can predict the structure of a protein or chemical that will sit in the site," says Pharmacia & Upjohn's Hudson. That enabled Chmielewska to come up with a small-molecule inhibitor that gums up factor Xa's active site. She says, however, that the inhibitor is still in an early phase of research.

Parasites have proteases, too

While many researchers are looking for inhibitors that can block the activity of proteases indigenous to the body, others have as their targets the proteases that invading pathogens deploy to help them establish infections. HIV is only one example. The cold-causing coronaviruses also rely on proteases to release their component proteins from a large precursor protein.

The body makes its own inhibitors of these viral proteases. Neuroimmunologist Arlene Collins of the State University of New York, Buffalo, is investigating proteins called cystatins, found in saliva and tears, that jam the coronavirus protease. Unfortunately, the cystatins themselves can't be used to treat colds, for they are digested if given orally, making it difficult to get the drug to where it is needed. An inhibitor "won't work if you can't get it to the protease," says Collins.

Larger invaders, including the parasites that cause diseases such as schistosomiasis, also need proteases. The mature schistosome, which lives in the bladder, intestines, and other organs, uses a protease to break down hemoglobin in the blood for food and also to process proteins needed for egg production. It is a good target for antischistosomiasis drugs, McKerrow says, because blocking it will interrupt the parasite's life cycle even after it is established in the body.

Arris has designed several low-molecular-weight compounds that resemble the particular linked amino acids split by the adult schistosomiasis protease and would thus be expected to bind to the enzyme's active site. In test tube studies, McKerrow's group has shown that these compounds inhibit the protease. What's more, McKerrow says, the compounds may work on related proteases from other parasites, like the one that causes Chagas' disease, which is a major cause of heart disease in Latin America. The inhibitors are about to be tested in dogs.

Even protease inhibitors that are showing promise in early human trials won't make it to market anytime soon, however. They won't benefit from the streamlined drug approval that allowed the HIV protease inhibitors to be marketed quickly. But the research is unlikely to slacken. Says Arris's Gschwend, "There are plenty of targets in proteases to keep us busy for a long time."

–Charles Seife

Charles Seife is a science writer in Riverdale, New York.