Improving on Nature: James Travis’ Work on Recombinant Human $\alpha_1$-Proteinase Inhibitor

Isolation and Properties of Recombinant DNA Produced Variants of Human $\alpha_1$-Proteinase Inhibitor

In 1985, when recombinant DNA technology was still in its infancy, James Travis at the University of Georgia and colleagues developed a nonoxidizable form of a protein critical for preventing emphysema. Emphysema is an obstructive lung disease caused by progressive destruction of the lung tissue. The researchers synthesized the natural and variant forms of the protein through a partnership with the biotechnology firm Chiron and then tested it in rabbits to see if it could be used to supplement the protein in patients. They described their results in a *Journal of Biological Chemistry* (JBC) paper in 1985, which is now considered to be a JBC Classic.

“This paper showed that you could express this protein in very high yields in yeast—higher than had ever been shown before,” says Steven Olson, a vascular biologist at the University of Illinois at Chicago. “It was one of the first demonstrations of the ability to tailor-make therapeutics.”

Neutrophil elastase is a protein-degrading enzyme that is important for tissue remodeling in the lung. A protein known as human $\alpha_1$-proteinase inhibitor prevents the elastase from destroying everything in sight. “If the proteinase inhibitor is not sitting in the jaws of the elastase,” says Fred Guengerich, a biochemist at Vanderbilt University and the JBC Deputy Editor, “the elastase will just go around chewing holes in your lung.”

But some people are born with a double genetic mutation. Their $\alpha_1$-proteinase inhibitor won’t be properly folded, which is essential for the proper functioning of the inhibitor. As a result, only about 10% of the normal amount of inhibitor makes it into the blood flow. Furthermore, repeated exposure to oxidants—such as cigarette smoke, smog, and certain other chemicals—causes the inhibitor to malfunction, allowing the elastase to destroy lung tissue unchecked. Over time, one or both of these factors can lead to emphysema.

When Travis and his colleagues had purified the inhibitor in previous work, he had been surprised and excited to find a methionine residue in its active site. They suspected the oxidation of this methionine was primarily to blame for the inhibitor becoming dysfunctional. “It immediately suggested to me that nature had provided a mechanism for regulating its activity,” Travis says. “For me, this finding was the hallmark for understanding why smokers with normal inhibitor concentrations in their plasma develop pulmonary emphysema.”

By altering the structure of the inhibitor, the researchers set out to demonstrate in the JBC paper that this methionine residue made the inhibitor inefficient. Travis partnered with researchers at Chiron, who used yeast to synthesize the natural inhibitor and one with the active-site methionine replaced with a valine.

Measurement of the half-life of $\alpha_1$-proteinase inhibitor variants in rabbits.
The recombinant inhibitor, according to Travis, worked perfectly. By manipulating just one residue in the protein, Guengerich says, they had created “a better protein than nature has produced, which is not susceptible to oxidation.” For people who have only 10% of the normal amount of inhibitor, protecting the functional inhibitor they do have is essential.

The next step was to test the synthesized molecules’ half-lives in rabbits. Travis brought natural and altered recombinant molecules with him to the University of Canterbury in Christchurch, New Zealand, during a 6-month sabbatical. He and his colleagues in Christchurch labeled the inhibitors using iodine-125 and then took aliquots at various times to see how much remained in the rabbit plasma. They found that the natural molecule had a half-life of 2.2 days, whereas the half-life of the recombinant molecule was under 7 h.

Because yeast do not glycosylate or label recombinant molecules in the normal way, the investigators found that this protein is turned over too rapidly to be of therapeutic use. “To infuse it into the blood plasma would not work at all because the half-life is so short,” says Travis. “Very little of it would get to its target before it was removed from circulation.”

Because of the glycosylation problem, Chiron abandoned the project. As an alternative, some drug companies currently purify the inhibitor from plasma and administer it to people with advanced emphysema; the purified inhibitor has been shown to improve symptoms and prolong lives. But Olson notes that the plasma treatment currently available is very expensive, approximately $100,000 per year.

Travis is hopeful the valine-modified inhibitor might be used in treatment one day. He believes it could be administered as an aerosol, which would deliver it directly to the lungs where the half-life should not matter. Recombinant proteins such as the one Travis helped to develop could provide an alternative to plasma derivatives. Olson says, “I think it’s potentially a way of reducing the price of this therapy in the future.”

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