Sensitive sequencing: Instrumentation led to biological revolution

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Today, if you want to know the sequence of a protein, you can spend a few minutes searching in a genome browser. But things were different in 1981; sequencing was challenging and slow. However, that year, a team of researchers at Caltech started a sequencing revolution in their report of a machine that could sequence more amino acids using less starting material than ever before (1).

Before the advent of the sequencer, the Edman degradation method, developed by Swedish scientist Pehr Edman in the 1950s, required researchers to specifically cleave one amino acid at a time from the end terminus of a protein, a laborious process. "I remember as an undergraduate working in a laboratory where one graduate student’s Ph.D. thesis was [to determine] the sequence of a small protein," says Joel Gottesfeld, a JBC associate editor and a professor in the molecular medicine department at Scripps Research Institute California. Gottesfeld earned his doctorate from Caltech in 1975.

Edman later developed the spinning-cup sequencer in the 1970s. The machine used liquid reagents, which meant that a lot of protein was lost during washing. As a result, it required micrograms worth of starting material, an amount that was usually prohibitive for anything other than an abundant protein. The method could sequence only a few amino acids at once. "By the time you got to 30 amino acids, you were done for," says Gottesfeld. "You couldn’t resolve the next amino acid from the noise of all the previous amino acids."

William Dreyer had had the idea of using the gas phase to do the coupling and cleavage of Edman sequencing chemistry, which Rodney Hewick, Mike Hunkapiller, and Leroy Hood helped to develop into a more efficient protein sequencer (Fig. 1). Hood, who is now president of the Institute of Systems Biology in Seattle, says a turning point came when Hunkapiller met with a lab group in Germany that had expertise in valves. There, scientist Brigitte Wittmann–Liebold gave Mike the absolutely critical suggestions for the valve that he came back and constructed that really made this sequencer work effectively." The group’s methods paper appeared in the Journal of Biological Chemistry in 1981 (1); Hood and Hunkapiller had also published on a version of the machine in the journal Science the prior year (2).

The new machine’s miniaturized reaction cell required 200 times less material than the spinning-cup sequencer, as the protein of interest was absorbed by a cationic polymer dried onto a porous glass fiber disc. This absorption step also made the machine more reliable with hydrophobic sequences that are not readily solvated by water, expanding the range of proteins that could be sequenced. Previous machines had used liquid-phase reagents, which required a lot of washing and thus decreased the yield. The introduction of the gas-phase reagents made each step more than 98% efficient, allowing shortening of individual reaction times and saving on reagents and solvents.

The machine’s increased sensitivity meant that a researcher could get up to 90 amino acids from as few as hundreds of nanograms of protein. “This was a major, major advance,” says Gottesfeld. “For the first time, you could then reverse-translate the amino acid sequence into a nucleotide sequence and clone the gene for the protein.”

This work, Gottesfeld says, enabled a revolution in biotechnology and molecular biology. Around the same time, Hunkapiller and colleagues were developing methods for DNA sequencing and peptide and DNA synthesis. “We realized that we could actually create a microchemical facility where you could take a small amount of protein, and you could sequence it, and you could from that sequence construct degenerate DNA probes that could be used to screen a cDNA library to get the corresponding gene out,” Hood says. They used this method to clone a series of genes.

“One you’d cloned the gene, a DNA sequencer could sequence it and the surrounding territory,” Hood says. “If you started at the other end with the gene, the peptide synthesizer allowed you to make large peptides.” Hood says they used the synthesizer to make functional peptides that were sometimes more than a hundred residues in length. “It was kind of a mission of integrating instruments at this very high level to be able to do much more sophisticated tasks.”

The sequencer made it possible to look at proteins that were previously out of reach, such as human platelet-derived growth factor. “We opened up a whole series of new fields by just sequencing proteins, cloning the genes, and putting them out there for biologists to start exploring things,” Hood says. “The power of this instrument was transformational.”

Hood and his colleagues had an eye toward commercializing the instruments. “My deep conviction is that anything you do in science, if it’s useful, should be given to society in some way,” he says. “And one of the most effective ways of doing that is by starting a company that actually enables the technologies that you’ve created.” But, he recalls, they met resistance from Caltech’s administration and were turned down many times before they were able to find an investor.

Eventually, Hood helped to found a company called Applied Biosystems, which focused on the production of the DNA and peptide sequencers and synthesizers. The company “was in the black the first year because Hunkapiller had so well engineered the gas-phase protein sequencer,” Hood says. Hunkapiller

Joel Gottesfeld at Scripps Research Institute California nominated this paper as a Classic.

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eventually became CEO of Applied Biosystems, which today is part of Thermo Fisher Scientific.

When Applied Biosystems started selling the instruments, Gottesfeld says, many universities bought them for their core facilities. “The cost of sequencing fell to the realm where any laboratory could do it,” he says. “Everybody was off sequencing their favorite protein.” Researchers could then clone genes from cDNA libraries, leading to widespread insights and a greater understanding of biology at the molecular level.

References
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