Protein Chemists Gain a New Analytical Tool

Mass spectrometry bursts onto the protein scene, providing more accurate molecular weights and a new way of getting sequences

WHO SAYS YOU CAN'T teach an old machine new tricks? In the past year, mass spectrometers, long the workhorses of analytical chemistry labs, have leaped through a new hoop, pleasing biochemists to no end by determining the molecular weights of large proteins and nucleic acids. "It's a revolution not that far different from the revolution of DNA sequencing in molecular biology," says Donald Hunt, a mass spectrometrist at the University of Virginia.

With the new methods biochemists can find the masses of proteins with an accuracy of 0.01% or better. That's a mind-boggling improvement over the next best method, gel electrophoresis, which can be off by 5% or more.

Such accurate masses allow researchers to compare a protein's molecular weight with that predicted by the DNA sequence of its gene to determine more easily than ever before whether a protein has been chemically modified during its synthesis, as many are. And mass spectrometry has also been adapted for determining the amino acid sequences of proteins (see box on p. 33).

The new methods are a boon for biotechnology quality control as well: They can be used to verify the structure of commercial protein products, eliminating the need for laborious chemical confirmations.

Mass spectrometers have been used for decades to analyze the chemical compositions of samples ranging from moon rocks to waste water. The principle on which they operate is simple enough: Sample molecules are ionized and launched into the instrument, which determines their mass-to-charge ratio by their behavior in an electrostatic or magnetic field.

For years, mass spectrometry only worked for relatively small molecules. Proteins and nucleic acids were just too big and too fragile to withstand the high temperatures required for ionization.

That all began to change 10 years ago, with the development of an ionization method that uses a stream of ions or neutral atoms to blast peptides or small proteins out of a drop of glycerol. Known as fast atom bombardment (FAB) or secondary ion mass spectrometry (SIMS), depending on whether the particle beam is made up of neutral atoms or ions, the technique can ionize proteins and other molecules without dangerously overheating them.

But the new bombardment methods were still limited to proteins under about 35,000 daltons. So mass spectrometrists continued to search for even better ionization tricks.

In the past year, two new techniques have burst onto the scene: one, called laser desorbtion, ionizes proteins with pulses of laser light; and another, dubbed electrospray, squirts a protein solution through a highly charged needle, creating tiny drops that burst to release multiply charged proteins.

Protein Analysis by Mass Spectrometry

Spectrometer	Applications	Price	Mass Determination
Magnetic sector	Molecular weights Protein sequencing (with two machines)	$1.5 million for tandem instrument	Accelerated ions are subjected to a magnetic field, which bends their paths based on their mass-to-charge ratio
Quadropole (or triple-quad—three spectrometers in one)	Molecular weights Protein sequencing?	$400,000 for triple-quad	Alternating electrical fields separate ions of different mass-to-charge ratios based on their oscillation patterns
Time-of-flight	Molecular weights	About $300,000 for instrument and laser desorbtion sampler	Determine the mass-to-charge ratios of accelerated ions based on the time they take to reach the instrument's detector

Franz Hillenkamp of the University of Munster, Germany, used laser desorbtion to achieve the current record for a molecular weight determination—172,000 daltons. "There is no real high mass limit to ion generation [by laser]," he boasted, when describing his method at the Second International Symposium on Mass Spectrometry in the Health and Life Sciences, held in San Francisco last August.

The ability to pulse a laser makes the method perfectly suited to time-of-flight mass spectrometers. They require precise timing of ionization because they determine mass-to-charge ratios based on how long it takes accelerated ions to reach the instrument's detector. But the time-of-flight machines are not the most versatile of mass spectrometers. They cannot be easily adapted to break up accelerated ions and analyze their parts, a method essential for protein sequencing and other forms of analysis.

And it is not clear that laser desorbtion can be readily adapted to the more versatile magnetic sector and quadropole machines, because they require larger amounts of ionized sample molecules than lasers can presently provide.

That is where electrospray has an advantage. Developed for use with quadropole spectrometers, it nicely overcomes a major weakness of those instruments: they require a mass-to-charge ratio no greater than about 1500, a problem for proteins. But electrospray can place up to 100 or more charges on a large protein, bringing it down to the quadropole's mass range.

John Fenn and co-workers at Yale University review electrospray techniques in this issue of *Science* (see page 64).

"Electrospray has hit the field like a ton of bricks in the last 3 months," says Alma Burlingame, who directs the mass spectrometry facility at the University of California, San Francisco. It already has proven its usefulness. Burlingame's group used the technique on several fatty acid–binding proteins with masses of about 15,000 daltons and found their masses to be within 1 dalton of those predicted by sequence determination. In one case, the mass did not agree with that predicted by the sequence, alerting the group that it had missed a two–amino acid fragment from one end of the protein.

Electrospray and laser desorbtion have also made it possible for the first time to do mass spectrometry on nucleic acids. Previous ionization methods were unable to strip the solvent molecules that cling to the nucleic acids' negatively charged phosphate groups. But the new ones can.

"It's staggering, in a chemical sense, to see that it's been done," says James McCloskey

Mass Specs Move in on Protein Sequencers

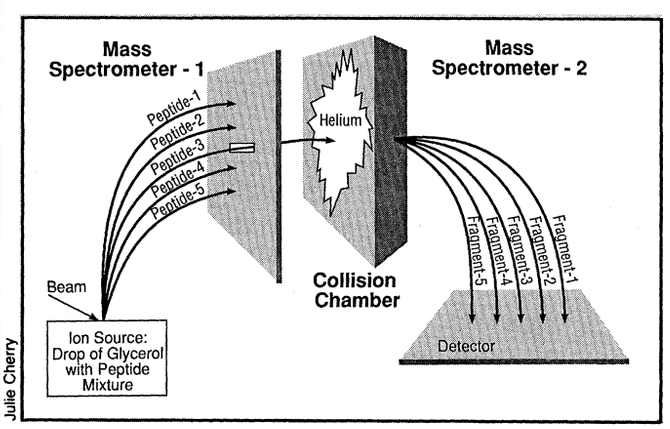
In the arena of protein sequencing, mass spectrometers have taken on the tried-and-true automated sequencing machines and are about to give them a run for their money. What mass spectrometers have going for them is speed—they are hundreds of times faster than the conventional machines, known as "sequenators." They also have the ability to identify chemical modifications to proteins, which often confound the older methods.

And recently developed ion detectors make them as sensitive as sequenators. But there's a downside to mass spectrometers: cost. A state-of-the-art set-up, which requires two magnetic sector spectrometers, costs $1.5 million, compared to $200,000 for a sequenator. So the choice, in many cases, isn't obvious.

Only a couple of years ago there wasn't a real choice. Although ionization with particle beams (see main story) made protein sequencing with magnetic sector mass spectrometers possible in the early 1980s, the method required 100 times more material than the more sensitive sequenators.

But several years ago, researchers at the Massachusetts Institute of Technology and Kratos Analytical, Inc., in Manchester, England, began adapting a new ion detector to the system and succeeded in closing the sensitivity gap. By increasing the range of masses that can be scanned at one time, the detector cut the amount of sample needed to 5 to 50 picomoles, a sensitivity competitive with sequenators.

"The thing that has made the major impact in sequencing is the availability of the [new] detector," says Alma Burlingame of the University of California, San Francisco.



Julie Cherry

Protein sequencing by mass spectrometry. *The first magnetic sector mass spectrometer selects a peptide, which is then fragmented by collisions with helium. The second instrument determines the mass of the fragments thus produced.*

Sequencing a protein by mass spectrometry requires two magnetic sector instruments connected in tandem. After a protein is enzymatically digested, producing a mixture of peptide fragments, the first machine separates by mass one species of peptide at a time and sends it to a collision chamber. There the peptide molecules are smashed into atoms of an inert gas, such as helium, breaking each molecule at a bond somewhere along its backbone.

This produces a new set of fragments, the masses of which are determined by the second machine. By means of mathematical analysis, the amino acid sequence of the peptide that produced the fragments can then be determined. If this is repeated for all of the peptides in the original mixture, the complete protein can be reconstructed.

The complexity of the mathematical analysis is presently a drawback, says John Stults of Genentech. "You can get the data within a couple of minutes, but [that doesn't count the] time needed for interpretation," he warns. But computer programs under development promise to convert the computation to a user-friendly black box.

How big an inroad mass spectrometers will make depends on whether their cost comes down. Donald Hunt, of the University of Virginia, believes proteins can be sequenced with quadropole spectrometers, versatile instruments with a relatively modest $400,000 price tag.

In Hunt's vision of the future, "protein chemistry [labs] will have two mass spectrometers for every sequenator, where now, you're lucky if you find a mass spectrometer for every six or seven." But his critics argue that no one but Hunt has yet been able to extract sequence data from a quadropole.

Many researchers think mass spectrometers will complement rather than supplant sequenators. They may take on the heavy sequencing workload in large academic or commercial laboratories and be used elsewhere to fill in the gaps left by sequenators—sequencing proteins whose chemical modifications stall the sequenators and helping to identify the types of modifications as well.

While the budget-bound may wait to see if others can repeat Hunt's successes with the quadropole, those who can afford it favor the $1.5 million magnetic sector system. But they have an additional problem to face: about 20% of the peptides generated for sequencing are beyond reach, too large to be fragmented by collisions with helium. Burlingame hopes that hurdle will be overcome by an electron gun under development at UCSF, designed to fire at and fragment those large peptides. ■ **M.B.**

of the University of Utah in response to a mass determination from Hillenkamp's group for a 35,000-dalton RNA. McCloskey, who studies unusual nucleotides in transfer RNAs, says the technique will be invaluable to pinpoint the positions of the nucleotides within nucleic acid structures.

Those caught up in the excitement of the past year's developments argue endlessly about the most promising directions to follow. According to Ian Jardine of Finnigan Corporation, which manufactures mass spectrometers, electrospray may make the $400,000 quadropole capable of both molecular weight and sequence analysis and therefore a "reasonable intermediate" between the slightly less expensive but relatively limited time-of-flight machines and the pricey magnetic sector instruments.

Others are waiting for electrospray or laser desorbtion to be adapted to magnetic sector machines, which have greater resolution and a more proven record in protein sequencing. When that happens, "the sky is the limit," predicts Steven Carr, a mass spectrometrist with Smith, Kline and French Laboratories.

Regardless of which avenue they favor, researchers in the field are brimming with expectancy. "If you look at where we are . . . and compare that with where we were even 2 years ago, there has been a tremendous increase in our capabilities," says John Stults, an analytical chemist with Genentech. "Who knows where we'll be 2 or 3 years from now?" ■ **MARCIA BARINAGA**