Report: Nobel Prize in Chemistry, 2002

Using Mass Spectrometry for Proteins

by Martha M. Vestling

The 2002 Chemistry Nobel Prize has mass spectrometrists everywhere celebrating. It recognizes work that put large proteins—10,000 Da and larger—into mass spectrometers. In order to obtain a mass spectrum of a protein, the protein must go through an ion source and an analyzer to reach the detector (see Figure 1). Half of the 2002 Nobel Prize was shared by Koichi Tanaka and John B. Fenn for obtaining mass spectra of large biomolecules. To do this, they used two different innovations in ion source design that were developed in the 1980s. For their award winning experiments, Tanaka used laser desorption ionization while Fenn used electrospray ionization. These two techniques became commercially available in the early 1990s and have *revolutionized* the way mass spectrometry is done.

Both laser desorption ionization and electrospray ionization can be used with all sorts and sizes of molecules, most of which could *not* be analyzed by mass spectrometry fifteen years ago. For example, small peptides char when heated and need derivatization for analysis with gas chromatography/mass spectrometry (GCMS). Both laser desorption and electrospray easily produce protonated peptide ions. Sugars like sucrose caramelize when heated and without derivatization are not amenable to GCMS. However, both laser desorption and electrospray easily give carbohydrate ions adducted to sodium ions. Figure 2 lists the common types of ions that mass spectrometers can detect. Both techniques can and have been used with a wide variety of mass analyzers. Commercial instruments with electrospray ionization sources and time-of-flight analyzers and instruments with laser desorption ionization sources and quadrupole ion trap analyzers have been quite successful. *The revolution the 2002 Prize highlights is the mass spectrometry of whole molecules.* When successful, the oldest commercially available and still used ionization method, electron impact ionization, gives spectra containing fragment ions.

Laser Desorption Ionization

In Tanaka's experiments, a protein solution (10 μM in water) was mixed with a fine cobalt metal powder (300 Å diameter) in glycerol, ethanol, and acetone and deposited on the sample holder. After vacuum drying, the holder was inserted into a time-of-flight mass spectrometer where a nitrogen laser (337 nm) was fired at the sample spot. The cobalt powder absorbed the laser energy and exploded off the sample holder, entraining the protein, into the instrument vacuum. The instrument was set up so that any positive ions present would be accelerated out of the ion source into the analyzer. Figure 3 illustrates the desorption entrainment process. The beauty of the system is that charged intact proteins were put into the gas phase. Tanaka and coworkers successfully desorbed chicken lysozyme (see Figure 4, ref *1*) and bovine chymotrypsinogen.

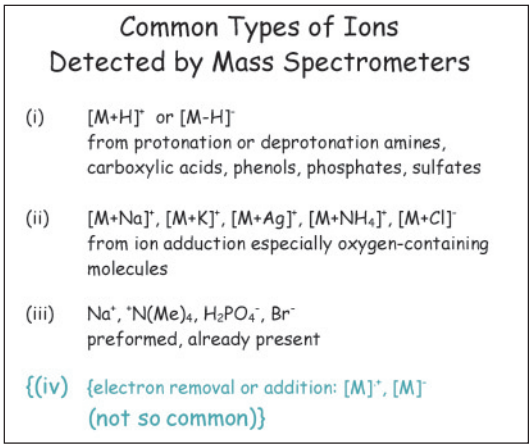

Figure 2. Common ion types for mass spectrometry.

Electrospray Ionization

In Fenn's experiments a protein solution (~10 μM in acidified methanol/water) was delivered to a metal capillary with a voltage applied to it and surrounded by flowing

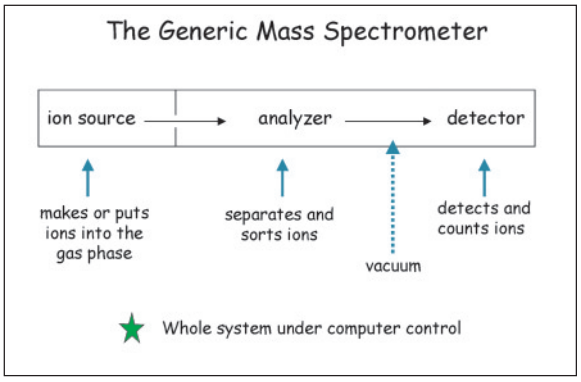

Figure 1. Diagram of a generic mass spectrometer.

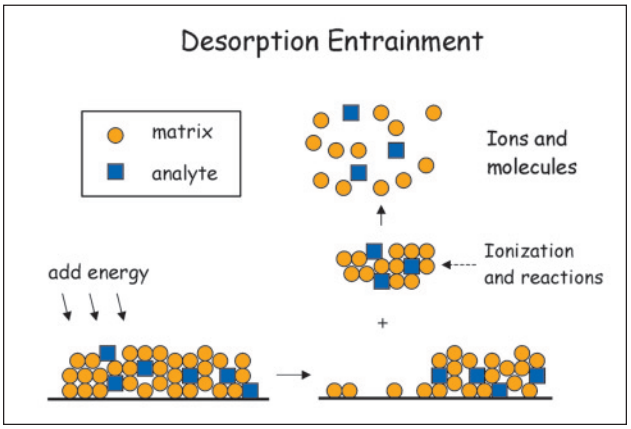

Figure 3. Desorption entrainment: the laser desorption process.

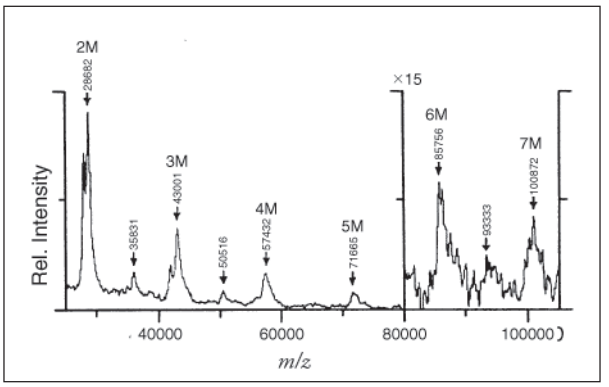

Figure 4. Laser desorption mass spectrum of chicken lysozyme. Reproduced with permission from ref *1*. © 1988 John Wiley & Sons, Ltd.

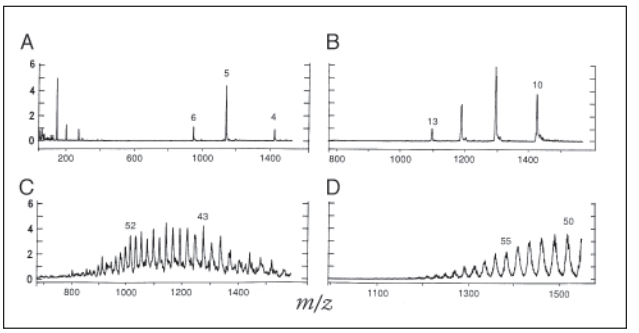

Figure 6. Electrospray ionization mass spectra: (A) insulin [m/z = 1145 when z = 5]; (B) chicken lysozyme [m/z = 1429 when z = 10]; (C) α-amylase [m/z = 1271.1 when z = 43]; (D) conalbumin [m/z = 1519 when z = 50]. Reprinted with permission from ref *2*. © 1988 American Association for the Advancement of Science.

nitrogen gas (drying gas). If a liquid is just pushed through a capillary, a series of drops form; but if a voltage (~3–5 kV) is applied to the capillary, the drops disappear and a fine spray (mist) with ions appears. After traveling through the drying gas, ions from the mist enter the analyzer region of a mass spectrometer (see Figure 5a and 5b). The beauty of this system is that it also puts charged intact proteins into the gas phase. Figure 6 is a reproduction of a figure in Fenn's 1988 paper *(2)*. In order to observe ion signals from proteins with a quadrupole analyzer, the protein ions had to have enough charges *(z)* so that *m/z* (mass-to-charge) values were below 2000. An example of the arithmetic used in getting mass data from an electrospray ionization mass spectrum is shown in Figure 7. Proteins usually have enough arginine, lysine, and histidine residues to carry the charge needed for quadrupole analysis. Fenn and coworkers successfully analyzed chicken lysozyme, bacterial α-amylase, and chicken egg conalbumin.

While Tanaka and Fenn are being awarded the Nobel Prize, they were only two of many researchers during the 1980s trying to make mass spectrometry analyze biological molecules. For example, Tanaka was certainly aware of R. D. Macfarlane group's californium-252 plasma desorption mass spectrometer *(3)*, and M. Barber group's fast atom bombardment ion source *(4)*. This work made it possible to analyze peptides without first derivatizing them. And Tanaka knew many researchers who were desorbing small molecules off of surfaces using lasers, including Wilkins *(5)*, and Hillenkamp and Karas *(6)*. In 1985, Franz Hillenkamp and Michael Karas, whose group had been looking at desorbing mixtures of amino acids off of surfaces with a laser, coined the term matrix-assisted laser desorption/ionization (MALDI). A figure from 1985 showing that an amino acid absorbing the laser light promoted desorption of an amino acid that did not absorb the light is shown in Figure 8. So when Tanaka published his Prize-winning paper in 1988 showing the generation of protein ions in the gas phase, Hillenkamp and Karas *(7)* were also able to publish protein spectra, albeit with a different sample preparation method from the one Tanaka developed. Instead of a fine metal powder, the German group used organic acids such as 2,5-dihydroxybenzoic acid and nicotinic acid to absorb the laser's energy. It is the use of organic matrices (MALDI) that made laser desorption/ionization a commercial success.

Meanwhile, Fenn's lab was trying to figure out how to interface liquid chromatography with mass spectrometry just as Ryhage *(8)*, Watson and Biemann *(9)*, and others had connected gas chromatography to mass spectrometry. The challenge was to remove the chromatographic solvent and generate analyte ions. Fenn was certainly aware of Beckey's field ionization and field desorption work *(10)* from liquids,

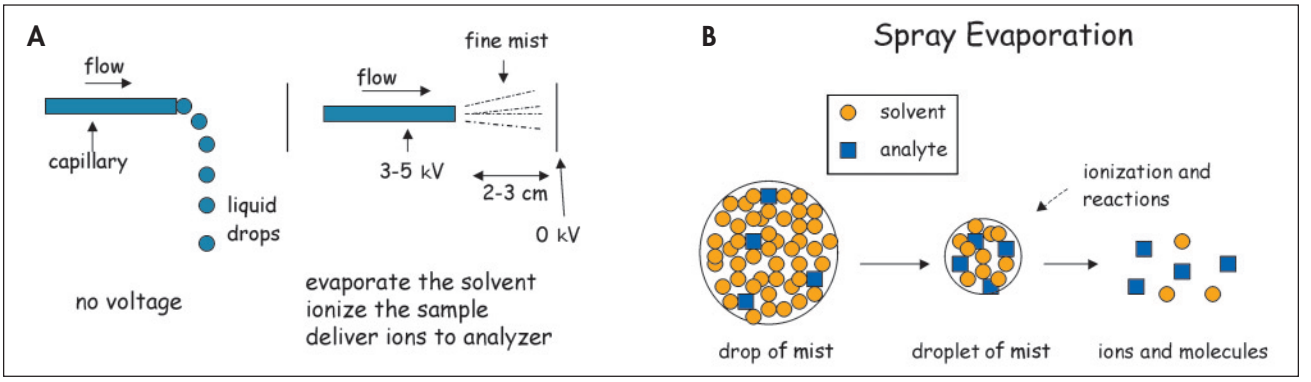

Figure 5. The electrospray process: (A) electrospraying, (B) spray evaporation.

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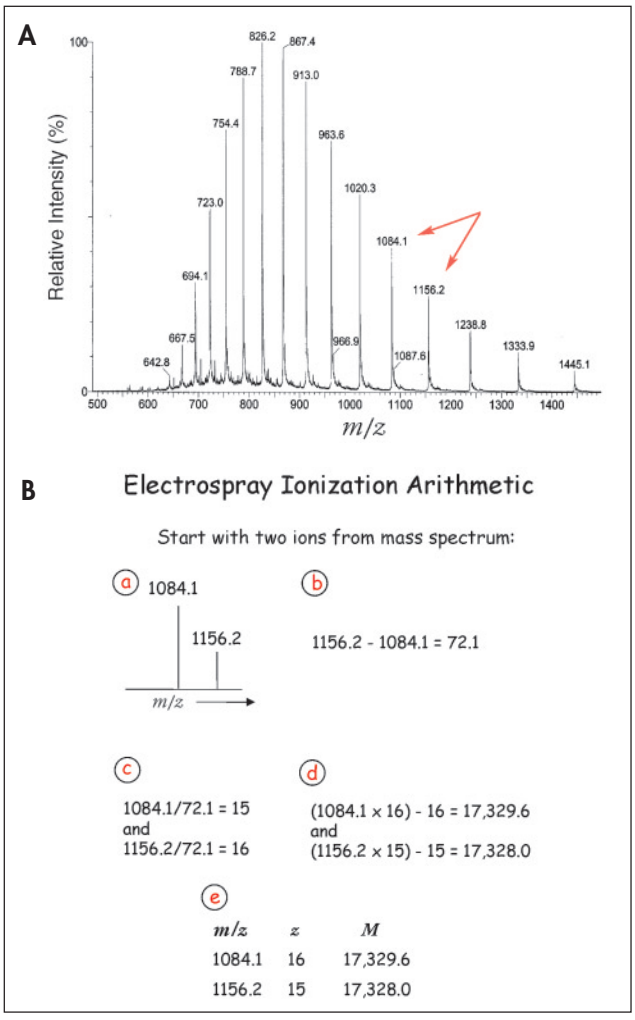

Figure 7. (A) Electrospray ionization spectrum of a protein. (B) Electrospray ionization arithmetic using two peaks from the electrospray ionization spectrum.

Evans and coworkers' electrohydrodynamic source *(11)*, atmospheric pressure ion evaporation source of Iribarne and Thomson *(12)*, and Vestal and coworker's thermospray source *(13)*. In 1985 Fenn's group described an electrospray interface for liquid chromatographs and mass spectrometers *(14)*, and in 1988 they showed Prize-winning spectra from intact proteins generated by their electrospray device.

A Revolution in Mass Spectrometry

The 2002 Nobel Prize in Chemistry has mass spectrometrists celebrating. The Prize is recognition of the revolution that has occurred our field, a revolution that has yet to appear in current chemistry textbooks, whatever the level. Only the much older and very size limited electron impact ionization technique is mentioned in most 2002 textbooks. Yet today both laser desorption ionization (MALDI) and electrospray ionization mass spectrometry are significant tools in genomics and proteomics, drug discovery, forensics, polymer characterization, and combinatorial chemistry research.

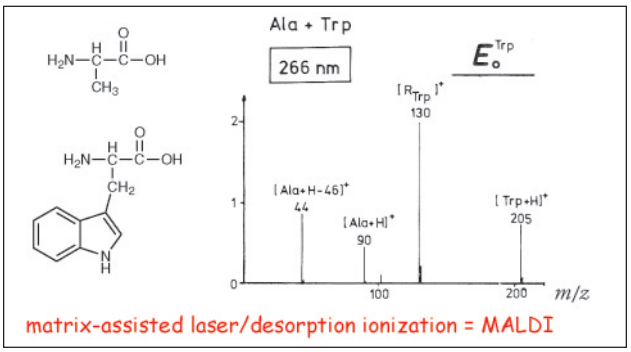

Figure 8. Laser desorption mass spectrum of a mixture of alanine that does not absorb the laser light and tryptophan that does absorb the laser light. Reprinted with permission from ref 6. © 1988 American Chemical Society.

The Prize will certainly bring even more compounds and mixtures into our laboratories, as awareness of what mass spectrometry can do will be accelerated. The Prize also demonstrates that high quality experiments can be done by people of all ages. In 1988, Tanaka was 29 years old while Fenn was 71 years old. And finally, the Prize reassures our nonscientific friends and families that we are working in an exciting and important area at a very interesting time.

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Martha M. Vestling is Director of the Mass Spectrometry Laboratory, Department of Chemistry, University of Wisconsin–Madison, Madison, WI 53706-1396; vestling@chem.wisc.edu.