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Review

The emergence of mass spectrometry in biochemical research

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ABSTRACT **The initial steps toward routinely applying mass spectrometry in the biochemical laboratory have been achieved. In the past, mass spectrometry was confined to the realm of small, relatively stable molecules; large or thermally labile molecules did not survive the desorption and ionization processes intact. Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry allow for the analysis of both small and large biomolecules through "mild" desorption and ionization methods. The use of ESI and MALDI mass spectrometry extends beyond simple characterization. Noncovalent interactions, protein and peptide sequencing, DNA sequencing, protein folding, *in vitro* drug analysis, and drug discovery are among the areas to which ESI and MALDI mass spectrometry have been applied. This review summarizes recent developments and major contributions in mass spectrometry, focusing on the applications of MALDI and ESI mass spectrometry.**

Advances in chemical technology have been the engine powering the biotechnology industry. Analytical chemists have added fresh impetus to bioresearch with two new mass spectrometry ionization tools: electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). Commercial availability of these instruments has made routine the analysis of compounds including proteins, peptides, carbohydrates, oligonucleotides, natural products, and drug metabolites. ESI and MALDI mass spectrometry offer picomole-to-femtomole sensitivity, enabling the direct analysis of biological fluids with a minimum amount of sample preparation. These techniques can be used to measure the mass of biomolecules >200,000 Da, to provide structural information, and to detect noncovalent complexes with molecular weight accuracy on the order of ±0.01%. They signify another dimension in molecular characterization through a new level of sensitivity, accuracy, and mass range.

Mass spectrometry is based on producing, differentiating, and detecting ions in the gas phase. The transfer of small molecules into the gas phase has traditionally been accomplished by thermal vaporization; however, thermal vaporization for biopolymers and other nonvolatile or thermally unstable molecules has little use. In fact, the search for ionization sources that would transfer large, thermally labile molecules into the gas phase without degradation has occupied mass spectrometrists for many years. It has largely been the development of ESI and MALDI that have made routine what was, until recently, impossible.

The trick has been forming ions in the gas phase; once formed, the ions can be directed electrostatically into a mass analyzer that differentiates the ions according to their mass-to-charge ratio (m/z). Although the analyzers have not changed significantly in the past two decades, the changes that have been made have been largely stimulated by the development of the ESI and MALDI ionization sources.

Both ESI and MALDI-MS support biochemical research, each having unique capabilities, as well as some fundamental similarities. Although there are many exciting examples of how they are being used as bioanalytical tools, their basic utility in molecular characterization should not be underestimated. ESI and MALDI-MS offer a rapid, simple, and accurate means of obtaining molecular weight information on a wide range of compounds. Their utility for mass measurement meets the needs of chemists and biologists alike, facilitating routine characterization in small molecule synthesis, protein synthesis, and compounds obtained directly from biological matrices.

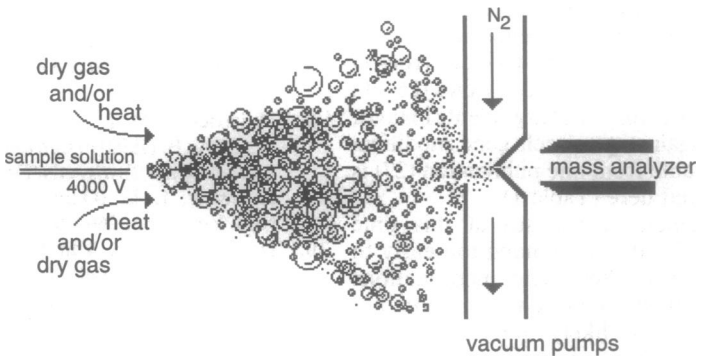

FIG. 1. ESI source.

ESI

The utility of ESI (1–9) lies in its ability to produce singly or multiply charged gaseous ions directly from an aqueous or aqueous/organic solvent system by creating a fine spray of highly charged droplets in the presence of a strong electric field (Fig. 1) (8). The sample solution is typically sprayed from the tip of a metal syringe maintained at ≈4000 V. Dry gas, heat, or both are applied to the highly charged droplets, causing the solvent to evaporate. Evaporation causes the droplet size to decrease, while surface charge density increases. Ions are transferred to the gas phase as a result of their expulsion from the droplet and then directed into a mass analyzer through a series of lenses.

Typically, ESI is interfaced with quadrupole mass analyzers because quadrupoles tolerate high pressures (10^{-5} torr; 1 torr = 133.3 Pa) and have good resolving power (≈2000). An intrinsic property of all mass analyzers (including quadrupole mass analyzers) is that they separate ions according to their mass-to-charge ratio (m/z), *not* the mass. This is often a point of confusion, especially with the ESI techniques, which typically generate multiply charged ions (Fig. 2). An advantage of multiple charging is that a mass analyzer with a relatively small m/z range (quadrupole instruments) can be used to observe very large molecules. The data generated via multiple charging can then be used to determine molecular weight. Another advantage of multiple charging is that more accurate molecular weight can be obtained from the distribution of multiply charged peaks. The mass spectrum of egg white lysozyme (in Fig. 2) illustrates the ability of the ESI technique to generate multiply charged ions. It is especially in-

Abbreviations: ESI, electrospray ionization; MALDI, matrix-assisted laser desorption/ionization; FTMS, Fourier-transform ion cyclotron resonance mass spectrometry.

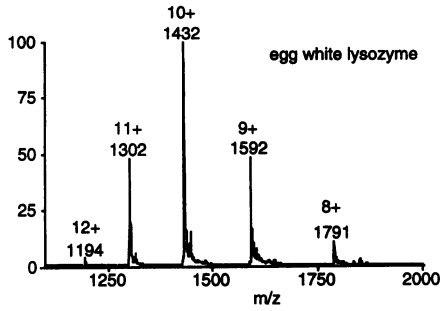

FIG. 2. ESI mass spectrum of egg white lysozyme. The molecular weight can be calculated from the equation: M_r = (mass-to-charge ratio × total charge) − total mass of charging species or $M_r = (m/z \times z) - z$ (if charging species are protons). Sample calculations for egg white lysozyme are as follows: $m/z = 1302.5$, $z = 11$ (+), $M_r = (1302.5 \times 11) - 11 = 14{,}317$; $m/z = 1432.4$, $z = 10$ (+), $M_r = (1432.4 \times 10) - 10 = 14{,}317$; $m/z = 1591.7$, $z = 9$ (+), $M_r = (1591.7 \times 9) - 9 = 14{,}317$.

teresting to note that an instrument with a mass range of $<m/z$ 2000 was used to observe a protein of mass 14,317 Da. The equation and sample calculations shown with Fig. 2 illustrate how molecular weight information can be calculated from the multiply charged peaks. If the charge states are not known and adjacent peaks differ by a charge of one, the molecular weight can easily be calculated from the m/z values (9). Fortunately, all of the manufactured ESI-MS instrumentation come with programs that rapidly perform these calculations.

HPLC Mass Spectrometry and Tandem Mass Spectrometry. In the past, attempts were made to couple HPLC analysis with a variety of mass spectrometry techniques. These attempts resulted in some success; however, until the advent of ESI, HPLC-MS applications could not have been considered routine. The ability of ESI-MS to directly analyze compounds from aqueous or aqueous/organic solutions has established the technique as a convenient mass detector for HPLC. ESI also allows for MS analysis at relatively high HPLC flow rates (1.0 ml/min) and high mass accuracy (±0.01%), adding a new dimension to the capabilities of HPLC. In fact, using ESI-MS as a detector for HPLC was one of its first obvious applications. Numerous reports (10–19) have been published on this application; special emphasis has been placed on peptide and protein analysis. HPLC combined with ESI-MS is excellent for routine and reproducible molecular weight determinations on a wide variety of compounds, whether they are positively (i.e., peptides and proteins) or negatively (i.e., oligonucleotides) charged. One such example is shown in Fig. 3. Besides its use for HPLC analysis, ESI-MS detection has also been successfully applied to capillary zone electrophoresis (20).

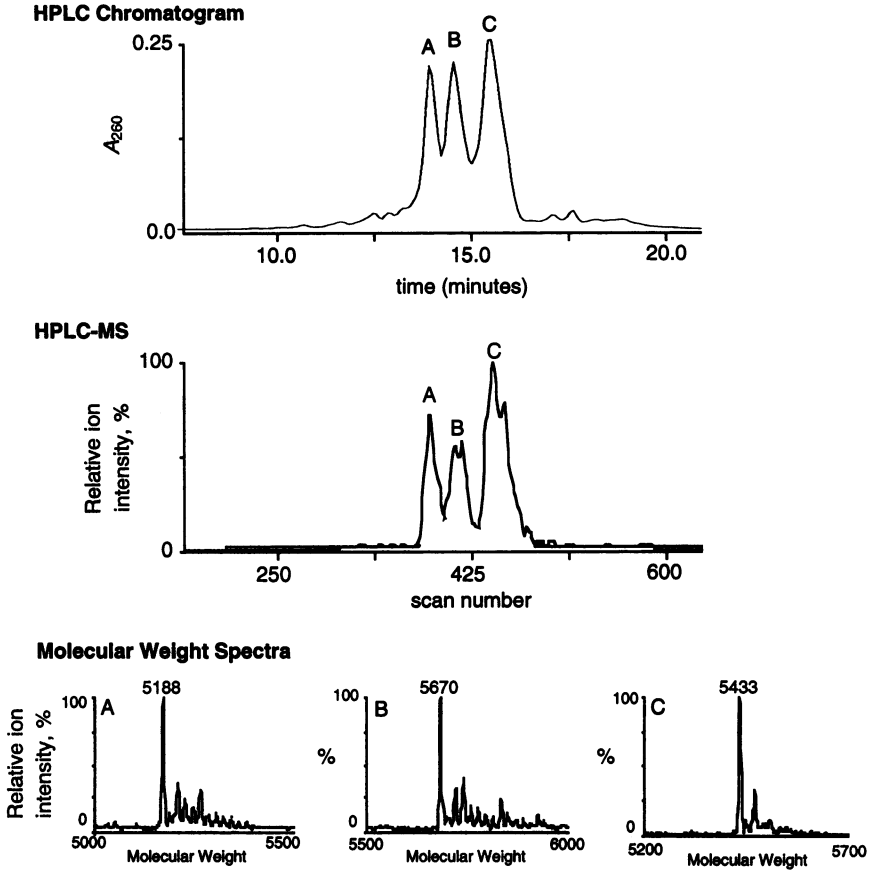

FIG. 3. HPLC chromatogram, HPLC-MS, and MS data acquired from a single run of a mixture of three oligonucleotides. The HPLC-MS data show the extracted ion current corresponding to a 17-mer, and two 18-mer oligonucleotides, respectively. The molecular weight spectra were reconstructed from multiply charged ions observed from each of the three peaks shown (unpublished data, Brian Bothner, Kelly S. Chatman, Molly Sarkisian, and G.S., The Scripps Research Institute).

In addition to characterizing compounds by molecular weight, it is also important to gain structural information. Most chemists are familiar with the fragmentation observed with electron ionization mass spectrometry; however, because ESI often produces only a minimal amount of fragmentation, it has been necessary to induce fragmentation on the ions formed by ESI. In general, the ability to induce fragmentation and perform successive mass spectrometry experiments on these fragment ions is known as tandem mass spectrometry (abbreviated MS^n; where n refers to the number of generations of fragment ions being analyzed) and is illustrated in Fig. 4.

Fragmentation is usually achieved by inducing ion/molecule collisions. This process, known as collision-induced dis-

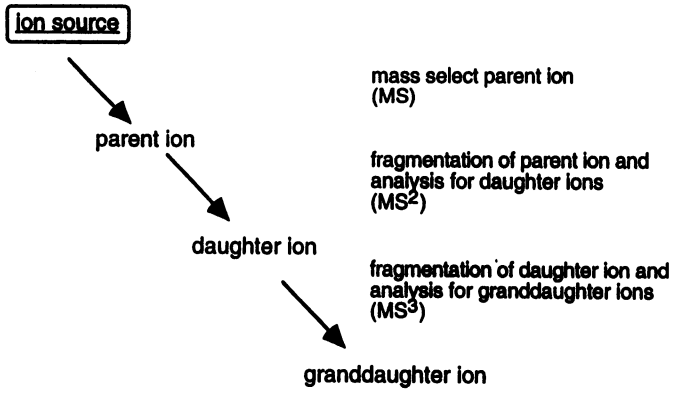

FIG. 4. Generation of fragment ions via collision-induced dissociation (CID) and the mass analysis (MS^n) of the progeny fragment ions. The terms parent, daughter and granddaughter ions (21) were used throughout this review to facilitate understanding, especially with the MS^3 discussion. However, precursor, product, and second-generation product ions are also commonly used (21).

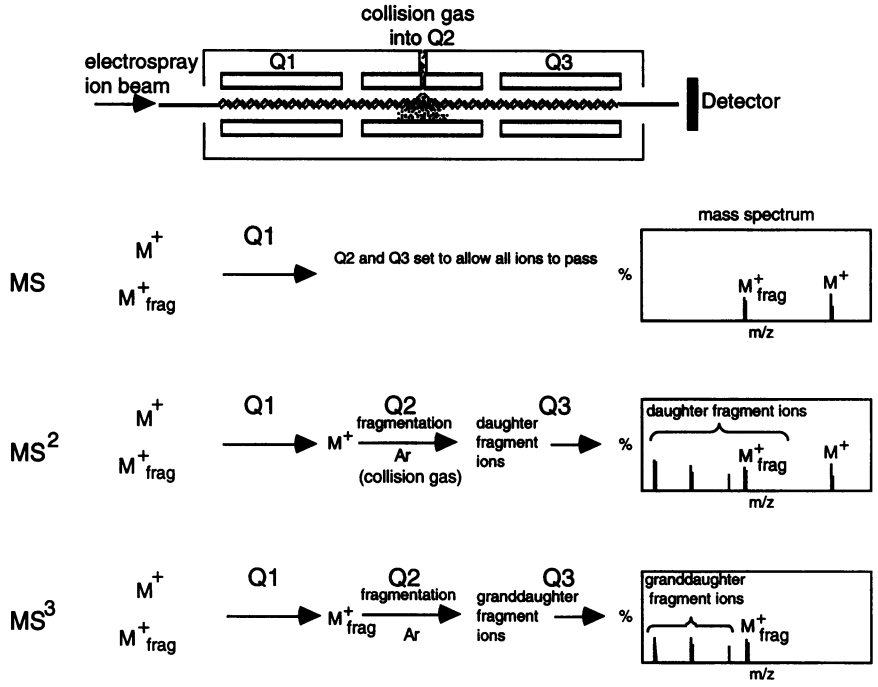

FIG. 5. A triple-quadrupole ESI mass spectrometer with ion selection and fragmentation capabilities. Each quadrupole has a separate function: The first quadrupole (Q1) is used to scan across a preset m/z range or to select an ion of interest, the second quadrupole (Q2), also known as the collision cell, transmits the ions while introducing a collision gas (argon) into the flight path of the ion selected by Q1, and the third quadrupole (Q3) serves to analyze the fragment ions generated in the collision cell (Q2). For an MS experiment Q1 scans a over a selected m/z range, and all the ions are observed. In the MS^2 experiment the molecular ion M^+ can be selected by Q1, which results in its fragmentation at Q2. Analysis of the fragments occurs at Q3. An MS^3 experiment can be performed if a daughter fragment ion is generated in ESI—i.e., M^+_{frag}. Q1 can be used to select the daughter ion, Q2 will generate granddaughter fragment ions, and Q3 will mass analyze for the granddaughter fragment ions.

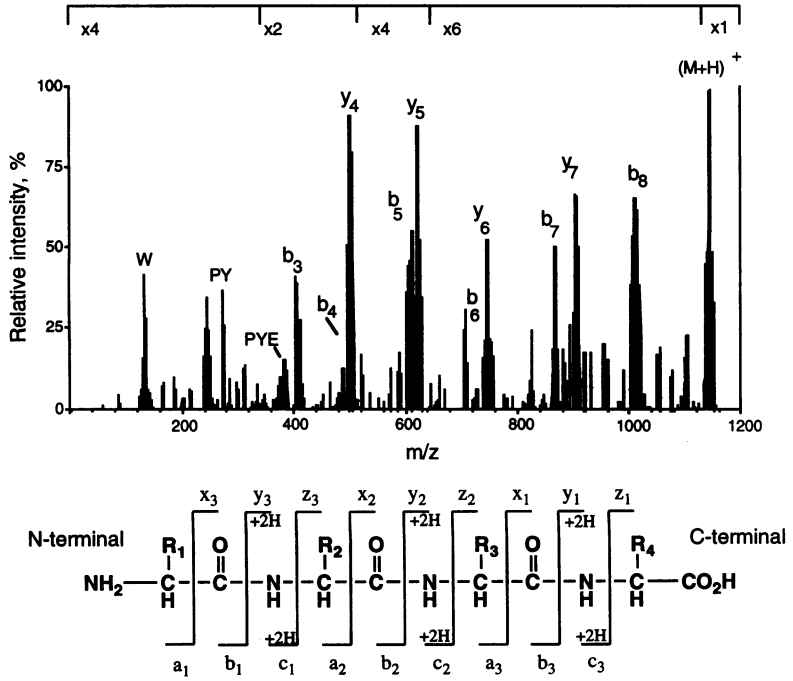

FIG. 6. An MS^2 experiment (*Upper*) done on peptide $(M+H)^+$ ions at m/z 1121 using 100–300 fmol of material derived from 2×10^8 cells. The fragmentation processes (*Lower*) of protonated peptides occur through bond cleavages resulting in different classes of fragment ions. Fragment ions of type a_n, b_n, and c_n are generated if the charge is retained on the N terminus of the peptide. The x_n, y_n, and z_n ions are formed when the charge is retained on the C terminus. The figure is reproduced with permission from ref. 25 (copyright American Association for the Advancement of Science, Washington, DC) and ref. 4 (copyright Annual Reviews, Inc., Palo Alto, CA).

sociation (CID) and/or collision-activated dissociation (CAD), is accomplished by selecting an ion of interest with the mass analyzer and introducing that ion into a collision cell. The selected ion will collide with a collision gas such as argon, and the collision may result in fragmentation. The fragments can then be analyzed to obtain a daughter ion spectrum. The information obtained from tandem mass analysis is primarily used to obtain structural information. ESI typically uses a triple-quadrupole mass spectrometer (22) with collision-induced dissociation to perform these analyses. A brief description of MS^2 and MS^3 experiments with a triple-quadrupole mass analyzer is given in Fig. 5. ESI with a single-quadrupole mass analyzer is also capable of generating and analyzing fragment ions; however, this is generally applicable to only very pure samples.

Perhaps the most well-known application of ESI tandem mass analysis (with a triple-quadrupole mass analyzer) is the work done by Don Hunt and colleagues (23–26). In these studies ESI tandem mass spectrometry was used in conjunction with HPLC separation techniques to identify major histocompatibility complex-bound peptides (Fig. 6). The quantity of peptide used to obtain structural information was on the order of tens of femtomoles, ultimately resulting in the identification of a peptide with a high affinity for cytotoxic T lymphocytes (killer T cells) (23).

Many biological problems are now being addressed at the molecular level; therefore, the ability to characterize a compound or compounds from biological media has taken on additional importance. Don Hunt and colleagues have demonstrated the ability to characterize 9-residue peptides at very low quantities, thus addressing the characterization of major histocompatibility complex-bound peptides. Three laboratories at The Scripps Research Institute have also utilized ESI capabilities to investigate sleep (27) and the compounds associated with this event. Lerner and colleagues (27) have performed studies on cerebrospinal fluid, extensively using HPLC-MS, MS^2, and the MS^3 (Fig. 7) capabilities of ESI triple-quadrupole mass analysis (Fig. 6). In these studies two novel lipid molecules have been isolated.

ESI-MS is generally thought of as a tool for macromolecule analysis; however, as Hunt and Lerner have demonstrated, it is also useful in the characterization of small molecules.

Noncovalent Interactions. Another application that has generated a great deal of excitement involves the ability of ESI to produce and mass-analyze biological noncovalent complexes in the gas phase. Since the first two papers (28, 29) describing ESI as a tool for the observation of noncovalent complexes, the

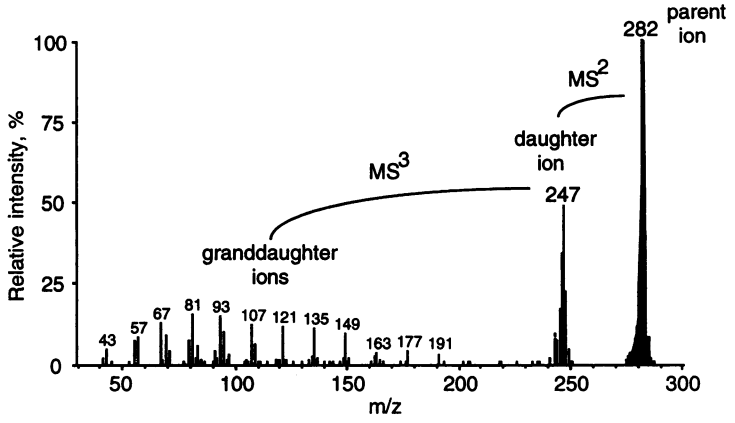

FIG. 7. Electrospray MS^3 data on the daughter ion (m/z 247) of a cerebrodiene (27). The parent ion, m/z 282, is added to the spectrum for clarity. The mass data, in conjunction with NMR and independent synthesis, have aided in the characterization of this compound.

possibility of correlating condensed-phase intermolecular interactions with mass spectrometry has captured the attention of many researchers. For the first time, mass spectrometry can be used as a tool to observe complexes in the gas phase taken from an aqueous environment, thereby providing insights into specific noncovalent associations in solution. The selectivity of mass spectrometry may eventually help avoid impurity problems associated with immunoaffinity procedures and also facilitate drug screening. Examples include the observation of the heme globin complex (29), the ternary complex of dimeric human immunodeficiency virus type 1 protease and an inhibitor (30), oligonucleotide duplex (31, 32), calcium-mediated cell-surface carbohydrate association (33), catalytic antibody–hapten interactions (34) (Fig. 8), and leucine zipper peptides (35).

Recent work demonstrates that the gas-phase intermolecular interactions of biomolecular ions observed with ESI-MS may be related to the interactions of these compounds in solution. Smith and Light-Wahl (36) have raised some interesting questions concerning noncovalent complexes observed with ESI and the relevance of these observations with respect to the solution-phase interactions. Recent data have suggested that, at least for some biopolymers, the gas-phase ions may indeed reflect their solution-phase properties (37–41), with answers to these questions being obtained, in part, from experiments on protein folding.

Protein Folding. Protein folding represents another example of a noncovalent interaction: an intramolecular interaction. Brian Chait, one of the innovators in applying ESI mass spectrometry (37), quickly recognized ESI as a tool for observing noncovalent intramolecular interactions. One of Chait's and Katta's initial experiments (37) qualified ESI as an effective new method for probing conformational changes of proteins in solution. The method is based on the mass spectrometric measurement of hydrogen–deuterium exchange that occurs in different protein conformers over time; as a protein's tertiary structure changes, so does the number of its exchangeable protons. Specifically, the method was used to probe bovine ubiquitin conformational changes induced by the addition of methanol to aqueous acidic solutions of the protein. The information obtained from hydrogen–deuterium exchange measurements has also been used as a complement to nuclear magnetic resonance (NMR) spectroscopy experiments (41). A combination of ESI-MS and NMR spectroscopy was used in experiments with hen lysozyme to distinguish between alternative mechanisms of hydrogen exchange, providing insight into the nature and populations of transient folding intermediates (Fig. 9). Further study (40) of other gaseous protein ions suggests their compactness *in vacuo* corresponds directly to known conformer structures in solution. These studies suggest the nature of proteins in the gas phase is, to some extent, a reflection of the solution-phase structure.

The ability to observe a basic protein characteristic (native structure versus denatured) has also been addressed by using ESI-MS. ESI-MS has been identified as a simple and effective device for investigating the denaturation of proteins (37, 39) where denaturation has been observed as a function of temperature, solution pH, and solvent.

The utility of ESI-MS in molecular characterization, as a detector for HPLC and capillary zone electrophoresis, in the study of noncovalent complexes, and in obtaining structural information, all illus-

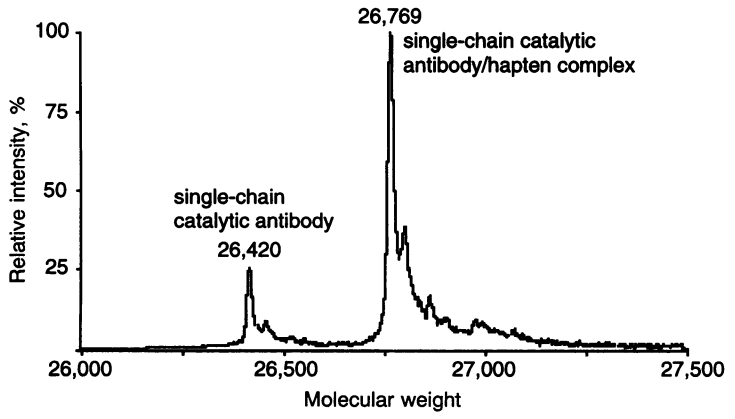

FIG. 8. Noncovalent single-chain catalytic antibody–hapten complex as observed with pneumatically assisted electrospray (ion spray) mass analysis. Reproduced with permission from ref. 34 (copyright American Chemical Society, Washington, DC).

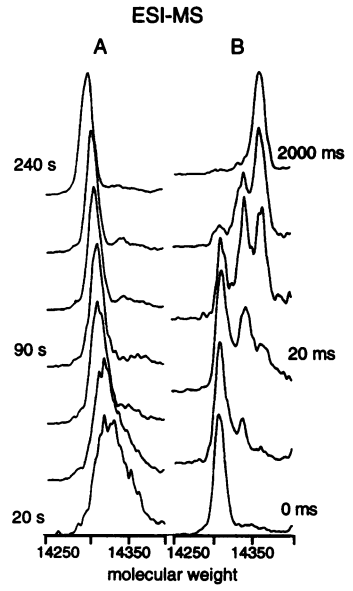

FIG. 9. Time evolution of the ESI-MS spectra of lysozyme monitoring hydrogen–deuterium exchange (*A*) and from pulse-labeling studies of protein refolding (*B*). For *A*, deuterated lysozyme was dissolved in ^2H_2O and equilibrated. After specified lengths of time, hydrogen exchange was quenched by the rapid cooling. For *B*, the samples were prepared such that buffer exchange was made into H_2O (as in *A*), and exchange was performed before the samples were mass analyzed. Reproduced with permission from ref. 41 (copyright American Association for the Advancement of Science, Washington, DC).

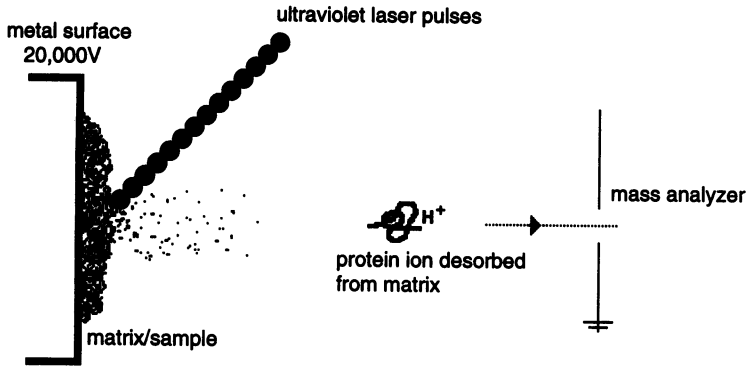

FIG. 10. MALDI source.

trate its capacity toward probing and solving biological problems. ESI does have limitations in that it is not very tolerant of the presence of salts (>1.0 mM) or of the analysis of multicomponent samples. Fortunately, in several aspects of mass analysis where ESI is not very useful, MALDI-MS has proven very effective. The combination of these two techniques provides great versatility in sensitivity, mass range, and applicability.

MALDI-MS

MALDI-MS has also emerged as an effective bioanalytical tool (4, 5, 42–48). The MALDI ionization technique (Fig. 10) typically uses a pulsed UV laser beam to desorb and ionize cocrystallized sample/matrix from a metal surface. The matrix (e.g., 2,5-dihydroxybenzoic acid) serves to minimize sample damage from the laser beam by absorbing the incident laser energy, resulting in the sample and matrix molecules being ejected into the gas phase. The sample ion may be preformed in the condensed phase or result from molecules that have reacted with the matrix or other molecules to form either singly or multiply charged ions (the singly charged species are typically the dominant ions in the MALDI mass spectrum). Once ions are formed in the gas phase, they can be electrostatically directed to a mass analyzer.

MALDI is typically used in conjunction with time-of-flight mass analyzers. Time-of-flight analysis is well-suited to the pulsed nature of laser desorption in MALDI (49). In addition, because time-of-flight analysis has virtually no upper mass range, it is compatible with MALDI, which can produce very high m/z ions. However, MALDI-MS is not limited to large biopolymers. It allows for the analysis of both small and large molecules on the femtomole level and has proven useful for both qualitative and, recently, quantitative analysis (50–54). In addition, the utility of MALDI-MS for heterogeneous samples makes it very attractive for biological samples (43).

MALDI is unique in its capacity to analyze complex mixtures (55), producing spectra uncomplicated by multiple charging or significant fragmentation. For example, MALDI provides a routine means of analyzing glycoproteins (45, 56) and tryptic digests (57), which typically have a high degree of heterogeneity. ESI-MS, on the other hand, is useful for analyzing mixtures after they have been separated by HPLC; yet its ability to directly analyze very heterogeneous samples is limited.

A dramatic demonstration of the ability of MALDI to analyze heterogeneous samples is shown with "protein ladder sequencing" (58). Protein ladder sequencing involves the simultaneous analysis of a mixture of peptides/proteins that have undergone a stepwise Edman degradation. Ladder-generating chemistry is initially required, whereby a family of sequence-defining peptide fragments are generated by using wet chemistry techniques. Each of these fragments differs from the next by one amino acid. Once the mixture of peptides is obtained, analysis by MALDI-MS can be done, thus generating a sequence ladder (Fig. 11). Each amino acid in this sequence is identified from the mass difference between successive peaks. The protein ladder sequencing method has also been shown to be useful in locating posttranslation modifications—e.g., phosphoserine residues in a phosphopeptide (see Fig. 11 *Upper*).

DNA Sequencing. The analysis of oligonucleotides by MALDI has enjoyed a reasonable degree of success (59–63), especially with the development of new

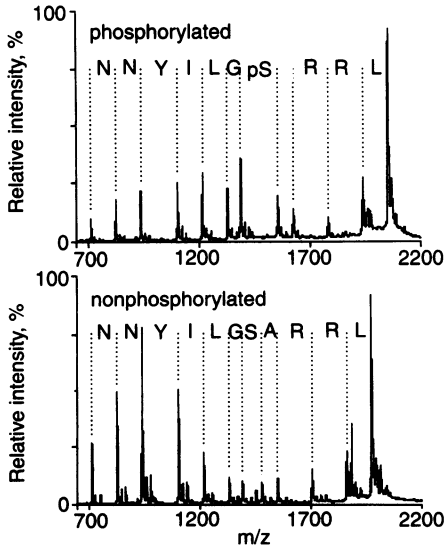

FIG. 11. Protein ladder sequencing of a 16-residue synthetic peptide that is phosphorylated (*Upper*) and not phosphorylated. A partial amino acid sequence is noted above the mass spectra. Reproduced with permission from ref. 58 (copyright American Association for the Advancement of Science, Washington, DC).

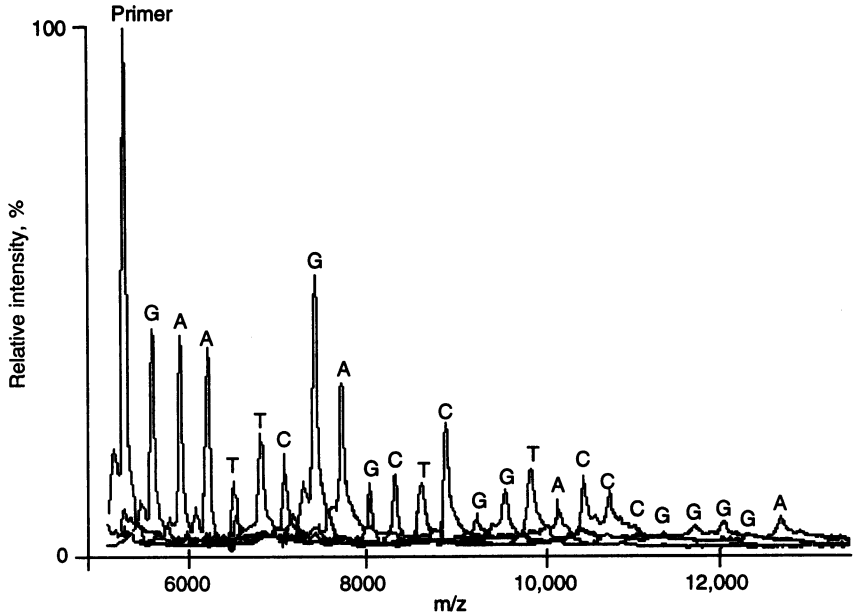

FIG. 12. Negative-ion MALDI mass spectra of synthetic oligonucleotides corresponding to mock A, C, G, and T sequencing reactions. The order of the peaks corresponds to the sequence. Reproduced with permission from ref. 64 (copyright Wiley, Sussex, U.K.).

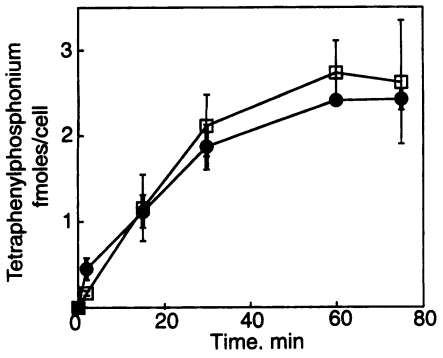

FIG. 13. Uptake of tetraphenylphosphonium (TPP) by carcinoma cells as a function of exposure time, as observed for unlabeled TPP with MALDI-MS (●) and for tritiated TPP with scintillation counting (□) (52). Note the significantly smaller error bars (representing 95% confidence limits) observed for MALDI-MS. In these experiments methyltriphenylphosphonium was used as an internal standard.

matrices. Several groups (59, 64) have further demonstrated that MALDI may also be useful in DNA sequencing. One approach is similar to protein ladder sequencing and uses the time-dependence of exonucleases (59) to generate a sequence ladder. MALDI-MS has also shown some utility in the analysis of DNA fragments generated in conventional dideoxynucleotide chain-termination sequencing reactions (see Fig. 12) (64). These experiments have evaluated MALDI-MS for oligonucleotide analysis in terms of resolution, sensitivity, multiple charging, and adduct formation, suggesting that MALDI-MS may have potential as a DNA sequence analysis tool.

Combinatorial Libraries. In addition to the use of MALDI as a sequencing tool, its capability toward the analysis of combinatorial libraries has also been explored (65). Specifically, Thomas Keough and colleagues (65) have used MALDI-MS in an interesting set of experiments for the rapid sequence determination of biologically active peptides. These peptides have been isolated from support-bound combinatorial peptide libraries. Sequence determination is accomplished by encoding each resin bead with the information needed to establish the sequence of the products also on the beads. MALDI-MS could then be used to read the sequences. In an example of the use of this technique, an anti-gp120 monoclonal antibody was screened against a hexapeptide library, and six of the eight peptides isolated were shown to possess the exact recognition sequence for the antibody.

The applicability of MALDI-MS to proteins, peptides, complex samples, and combinatorial libraries is evidence that this technique has achieved the primary function of an analytical device, to ". . . derive a measurable signal which is characteristic of the identity and/or amount of the species" (Fred W. McLafferty). The hundreds of papers that have been published on MALDI typify its utility for characterization.

Quantitative Aspects of MALDI-MS. In addition to producing a "characteristic signal," quantitative information is often necessitated from analytical equipment. Recent papers have demonstrated the ability of MALDI to yield quantitative data. The use of MALDI-MS as a technique for molecular weight determinations on biopolymers has been demonstrated repeatedly, yet until recently only a limited amount of work has been presented on its applicability to quantitation and the characterization of low-molecular-weight compounds. Recent research on the quantitative aspects of MALDI has included both small and large molecules. These initial studies have been done with carbohydrates (50, 66, 67), peptides (51, 68–70), proteins (53, 68), small molecules (51), and drugs (52, 66). In general these results suggest that the use of an internal standard, similar to the molecule of interest, will enable quantitative information to be obtained. Fig. 13 illustrates the applicability of MALDI-MS to quantifying cell drug uptake using an internal standard as compared with scintillation counting.

MALDI-MS has an inherent problem in obtaining quantitative information simply as a function of signal intensity; the nonuniform nature of the matrix/analyte solid mixture and variations in detector response often result in a nonreproducible signal response. When MALDI-MS is used for qualitative work, this is not important, as the laser fluence is simply adjusted for each sample to provide an acceptable signal. For MALDI to be used for quantitative analysis, it is essential to compensate for this variation. The incorporation of an internal standard allows for controlled shot-to-shot and sample-to-sample variability. A prerequisite for a suitable internal standard is that it should mimic the behavior of the analyte. In most studies referenced, this has been accomplished by the addition of structural analogues or stable-isotope-labeled analogues as internal standards.

Mark Duncan and colleagues (51) have pointed out several reasons why MALDI-MS is an attractive method for quantitative analysis in the low-molecular-weight range. These reasons include rapid analysis time, high sensitivity, specificity, low cost, and versatility. These advantages also are true for biopolymers, as demonstrated by Randy Nelson and colleagues (53), who have also illustrated the ability of MALDI to be used as a tool for quantitative analysis of proteins.

These examples further demonstrate the capabilities of MALDI-MS as a tool for characterization and quantitation. However, because MALDI is usually combined with time-of-flight mass analysis, it typically suffers from low resolution, which, in turn, adds difficulty to interpreting the mass spectra accurately. Increasing accuracy has been a primary motivating factor for MALDI-MS in the development of other higher-resolution analyzers, including the time-of-flight reflectron and Fourier-transform ion cyclotron resonance mass analyzers. For instance, the time-of-flight reflection analyzer can provide good resolution and has recently been demonstrated as a tool for tandem mass analysis (71, 72). The combination of time-of-flight and reflectron mass analyzers has significantly increased the resolving power of these instruments for $m/z \leq 6000$. However, Fourier-transform ion cyclotron resonance mass spectrometry (FTMS), combined with both ESI and MALDI, are now offering the greatest potential in high-resolution mass analysis.

High-Resolution ESI and MALDI-MS. An important characteristic of any mass spectrometer is its resolving power. Resolution is the ability of a mass spectrometer to distinguish between ions of different mass-to-charge ratios. Greater resolving power corresponds directly to the increased ability to differentiate ions. For instance, a mass spectrometer with a resolution of 500 can distinguish between ions of m/z = 500 and 501, whereas a

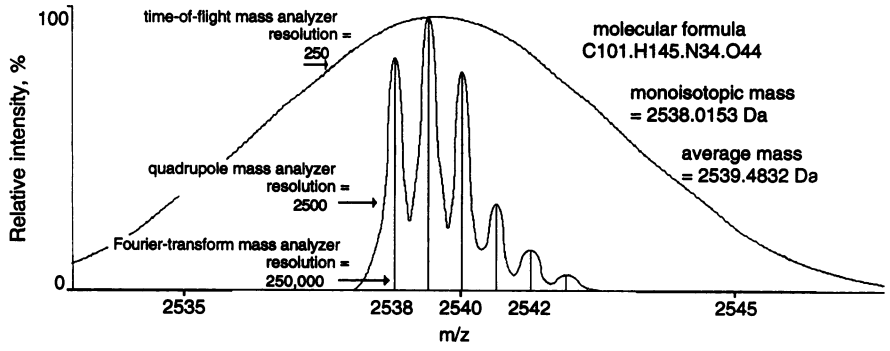

FIG. 14. The mass spectra of a singly protonated peptide calculated at a resolution of 250, 2500, and 250,000. The respective resolving powers correspond to the resolution typically obtained for time-of-flight, quadrupole, and Fourier-transform mass analyzers. Notice that the individual isotope peaks can be distinguished at the higher resolution.

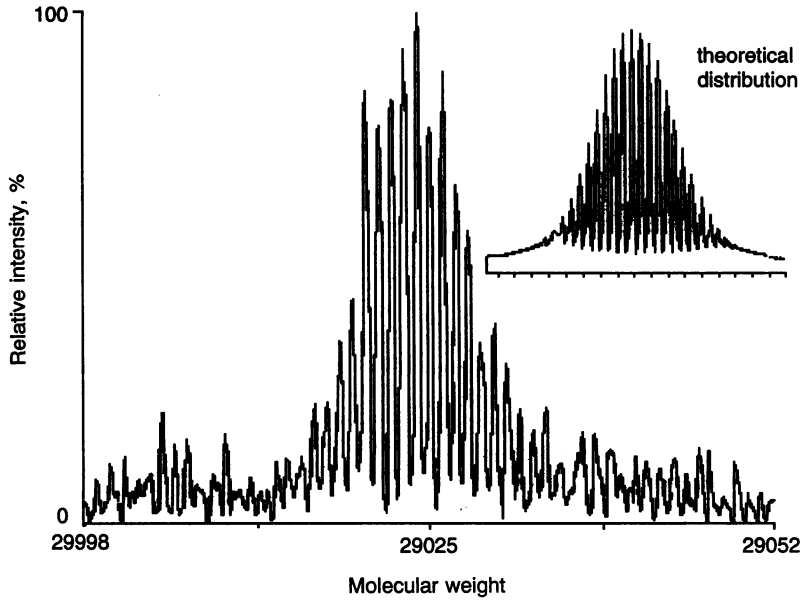

FIG. 15. Single-scan ESI spectrum of carbonic anhydrase with a FTMS analyzer. Reproduced with permission from ref. 75 (copyright Elsevier Science, Inc., New York).

mass spectrometer with a resolution of 2500 can distinguish between ions of m/z = 2500 and 2501. The most common definition of resolution is given by the equation: Resolution = $M/\Delta M$.

This equation can be described in a number of different ways (73). Perhaps the most common is where M is the mass of a peak of interest and ΔM can be defined as the width of the peak at 50% of the height or "full-width at half maximum" (FWHM).

High resolution is important for biopolymer analysis because it allows for high accuracy. Fig. 14 demonstrates how resolution affects the peak shape in the mass spectrometer for a compound having the molecular formula $C_{101}H_{145}N_{34}O_{44}$.

ESI and MALDI-MS commonly use quadrupole and time-of-flight mass analyzers, respectively. The limited resolution offered by time-of-flight mass analyzers, combined with adduct formation observed with MALDI-MS, results in accuracy on the order of 0.1% to a high of 0.01%, whereas ESI typically has an accuracy on the order of 0.01%. Both ESI and MALDI are now being coupled to higher-resolution mass analyzers, such as the ultra-high resolution ($>10^5$) FTMS. The result of increasing the resolving power of ESI and MALDI mass spectrometers is an increase in accuracy for biopolymer analysis.

FTMS, first introduced in 1974 by Comisarow and Marshall (74), is based on the principle of a charged particle orbiting in the presence of a magnetic field. While the ions are orbiting, a radio frequency signal is used to excite the ions. As a result of radio frequency excitation, the ions produce a detectable image current. The time-dependent image current can then be Fourier-transformed to obtain the component frequencies of the different ions. The component frequencies of the ions correlate to their corresponding m/z. FTMS offers two distinct advantages, high resolution and the ability to perform multiple collision events (MS^n, where typically $n \leq 4$) to gain structural information.

Coupled with FTMS, ESI (75) (Fig. 15) and MALDI (76, 77) promise to be important research tools for biotechnology. Although both techniques are still in their infancy, their application provides valuable molecular weight information on a wide variety of samples. ESI produces many multiply charged species of the same mass, and isotopic peak resolution provides unequivocal charge-state assignment from the spacing of the isotopes. This capability is of special value when the spectrum also has many ions, such as from fragmentation. Mass measuring errors not only are lower (<0.1 Da or < ±0.001% error) than when the isotopic peaks are unresolved but also are independent of variations in $^{13}C/^{12}C$ natural isotopic abundances (the ability to distinguish the individual isotopes of a protein is shown in Fig. 15). Furthermore, larger errors are avoided that occur when the measured peak envelope includes impurity or adduct ions. This attribute also benefits tandem mass spectrometry of peptides, in that the dissociation of these ions yields fragment masses consistent (to <0.1 Da) with their amino acid sequences (78). This technology has also been proven useful for the rapid sequencing of oligonucleotides as large as 25 bases (79).

CONCLUDING REMARKS AND FUTURE PROSPECTS

The development of new ionization sources has been the platform for routine

Table 1. Comparison of ESI-MS and MALDI-MS

	Mass limit, Da (practical)	Advantages	Disadvantages	Suitable compounds
ESI-MS	≈200,000 (70,000)	HPLC/MS capable Multiple charging Resolution ≈2000 Capable of observing noncovalent complexes directly from water Femtomole-to-picomole sensitivity Good accuracy ≈±0.01%	Multiple charging can be confusing with mixtures Typically need ≤ mM salt concentrations for good signal Not tolerant of mixtures (if sample too heterogeneous often little or no signal)	Peptides Proteins Carbohydrates Nucleotides Oligonucleotides Phosphoproteins Small chargeable molecules
MALDI-MS	>300,000 (150,000)	Tolerant of mM concentrations of salts Highest mass capability Tolerant of mixtures Femtomole sensitivity Being developed as a tool for sequence analysis	Typically low resolution (≤500) Accuracy ±0.1%–0.01% Not amenable to LC/MS	Peptides Proteins Glycoproteins Carbohydrates Nucleotides Oligonucleotides Phosphoproteins Small chargeable molecules Heterogeneous samples

LC, liquid chromatography.

biomass analysis, resulting in the application of ESI and MALDI-MS toward a wide range of biochemical problems. The complementary nature of ESI and MALDI-MS (Table 1) has made it increasingly important that researchers have access to both types of instrumentation. Independently, ESI and MALDI-MS can help answer many questions; yet together they represent a formidable research tool with new levels of sensitivity, accuracy, and mass range. Fortunately, because this instrumentation is becoming more commercially available, chemists and biologists may soon see these mass spectrometers as companions to their NMR spectrometers and electron microscopes.

Significant design changes in the ion source have redefined the applicability of mass spectrometry for biotechnology research. Perhaps it is time to consider a device that hasn't changed significantly over the past 30 years: the ion detector. If larger or more complex systems are to be observed, changes in the ion detector will likely be the next step in the development of mass spectrometry.

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1. Fenn, J. B., Mann, M., Meng, C. K., Wong, S. F. & Whitehouse, C. M. (1990) *Mass Spectrom. Rev.* **9**, 37–70.
2. Carr, S. A., Hemling, M. E., Bean, M. F. & Roberts, G. D. (1991) *Anal. Chem.* **63**, 2802–2824.
3. Ashton, D. S., Beddell, C. R., Green, B. N. & Oliver, R. W. A. (1994) *FEBS Lett.* **342**, 1–6.
4. Biemann, K. (1992) *Annu. Rev. Biochem.* **61**, 977–1010.
5. Chait, B. T. & Kent, S. B. H. (1992) *Science* **257**, 1885–1894.
6. Arnott, D., Shabanowitz, J. & Hunt, D. F. (1993) *Clin. Chem.* **39**, 2005–2010.
7. Smith, R. D., Loo, J. A., Edmonds, C. G., Barinaga, C. J. & Udseth, H. R. (1990) *Anal. Chem.* **62**, 882–899.
8. Kebarle, P. & Tang, L. (1993) *Anal. Chem.* **65**, A972–A986.
9. McCloskey, J. A., ed. (1990) *Methods Enzymol.* **193**.
10. Schnolzer, M., Jones, A., Alewood, P. F. & Kent, S. B. H. (1992) *Anal. Biochem.* **204**, 335–343.
11. Heath, T. G. & Giordani, A. B. (1993) *J. Chromatogr.* **638**, 9–19.
12. Lawrence, J. F., Lau, B. P. Y., Cleroux, C. & Lewis, D. (1994) *J. Chromatogr.* **659**, 119–126.
13. Eshraghi, J. & Chowdhury, S. K. (1993) *Anal. Chem.* **65**, 3528–3533.
14. Schneider, R. P., Ericson, J. F., Lynch, M. J. & Fouda, H. G. (1993) *Biol. Mass Spectrom.* **22**, 595–599.
15. Straub, R. F. & Voyksner, R. D. (1993) *J. Chromatogr.* **647**, 167–181.
16. Hess, D., Covey, T. C., Winz, R., Brownsey, R. W. & Aebersold, R. (1993) *Protein Sci.* **2**, 1342–1351.
17. Glassgen, W. E., Seitz, H. U. & Metzger, J. W. (1992) *Biol. Mass Spectrom.* **21**, 271–277.
18. Ling, V., Guzzetta, A. W., Canova-Davis, E., Stults, J. T., Hancock, W. S., Covey, T. R. & Shushan, B. I. (1991) *Anal. Chem.* **63**, 2909–2915.
19. Hemling, M. E., Roberts, G. D., Johnson, W., Carr, S. A. & Covey, T. R. (1990) *Biomed. Environ. Mass Spectrom.* **19**, 677–691.
20. Smith, R. D., Wahl, J. H., Goodlett, D. R. & Hofstadler, S. A. (1993) *Anal. Chem.* **65**, A574–A584.
21. Price, P. (1991) *J. Am. Soc. Mass Spectrom.* **2**, 336–348.
22. Yost, R. A. & Enke, C. G. (1978) *J. Am. Chem. Soc.* **100**, 2274.
23. Cox, A. L., Skipper, J., Chen, Y., Henderson, R. A., Darrow, T. L., Shabanowitz, J., Engelhard, V. H., Hunt, D. F. & Slingluff, C. L., Jr. (1994) *Science* **264**, 716–719.
24. Hunt, D. F., Michel, H., Dickinson, T. A., Shabanowitz, J., Cox, A. L., Sakaguchi, K., Appella, E., Grey, H. M. & Sette, A. (1992) *Science* **256**, 1817–1820.
25. Hunt, D. F., Henderson, R. A., Shabanowitz, J., Sakaguchi, K., Michel, H., Sevilir, N., Cox, A. L., Appella, E. & Engelhard, V. H. (1992) *Science* **255**, 1261–1263.
26. Henderson, R. A., Michel, H., Sakaguchi, K., Shabanowitz, J., Appella, E., Hunt, D. F. & Engelhard, V. H. (1992) *Science* **255**, 1264–1266.
27. Lerner, R. A., Siuzdak, G., Prospero-Garcia, O., Henriksen, S. J., Boger, D. L. & Cravatt, B. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 9505–9508.
28. Ganem, B., Li, Y. T. & Henion, J. D. (1991) *J. Am. Chem. Soc.* **113**, 6294–6296.
29. Katta, V. & Chait, B. T. (1991) *J. Am. Chem. Soc.* **113**, 8534–8535.
30. Baca, M. & Kent, S. B. H. (1992) *J. Am. Chem. Soc.* **114**, 3992–3993.
31. Light-Wahl, K. J., Springer, D. L., Winger, B. E., Edmonds, C. G., Camp, D. G., II, Thrall, B. D. & Smith, R. D. (1993) *J. Am. Chem. Soc.* **115**, 803–804.
32. Ganem, B., Li, Y. T. & Henion, J. D. (1993) *Tetrahedron Lett.* **34**, 1445–1448.
33. Siuzdak, G., Ichikawa, Y., Munoz, B., Caulfield, T. J., Wong, C.-H. & Nicolaou, K. C. (1993) *J. Am. Chem. Soc.* **115**, 2877–2881.
34. Siuzdak, G., Krebs, J., Benkovic, S. J. & Dyson, J. D. (1994) *J. Am. Chem. Soc.* **116**, 7937–7938.
35. Li, Y. T., Hsieh, Y. L., Henion, J. D., Senko, M. W., McLafferty, F. W. & Ganem, B. (1993) *J. Am. Chem. Soc.* **115**, 8409–8413.
36. Smith, R. D. & Light-Wahl, K. J. (1993) *Biol. Mass Spectrom.* **22**, 493–501.
37. Katta, V. & Chait, B. T. (1991) *Rapid Commun. Mass Spectrom.* **5**, 214–217.
38. Katta, V. & Chait, B. T. (1993) *J. Am. Chem. Soc.* **115**, 6317–6321.
39. Mirza, U. A., Cohen, S. L. & Chait, B. T. (1993) *Anal. Chem.* **65**, 1–6.
40. Suckau, D., Shi, Y., Beu, S. C., Senko, M. W., Quinn, J. P., Wampler, F. M., III, & McLafferty, F. W. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 790–793.
41. Miranker, A., Robinson, C. V., Radford, S. E., Aplin, R. T. & Dobson, C. M. (1993) *Science* **262**, 896–900.
42. Burlingame, A. L., Boyd, R. K. & Gaskell, S. J. (1994) *Anal. Chem.* **66**, 634R–683R.
43. Bahr, U., Karas, M. & Hillenkamp, F. (1994) *Fresenius Z. Anal. Chem.* **348**, 783–791.
44. Spengler, B. & Kaufmann, R. (1992) *Analusis* **120**, 91–101.
45. Hillenkamp, F., Karas, M., Beavis, R. C. & Chait, B. T. (1991) *Anal. Chem.* **63**, A1193–A1202.
46. Karas, M., Bahr, U. & Giessmann, U. (1991) *Mass Spectrom. Rev.* **10**, 335–357.
47. Matsuo, T. (1991) *Org. Mass Spectrom.* **26**, 374–388.
48. Karas, M., Bachmann, D., Bahr, U. & Hillenkamp, F. (1987) *Int. J. Mass Spectrom. Ion Proc.* **78**, 53–68.
49. Hillenkamp, F., Unsöld, E., Kaufmann, R. & Nitsche, R. (1975) *Appl. Phys.* **8**, 341–348.
50. Harvey, D. J. (1993) *Rapid Commun. Mass Spectrom.* **7**, 614–619.
51. Duncan, M. W., Matanovic, G. & Cerpa-Poljak, A. (1993) *Rapid Commun. Mass Spectrom.* **7**, 1090–1094.
52. Rideout, D., Bustamante, A. & Siuzdak, G. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 10226–10229.
53. Nelson, R. W., McLean, M. A. & Hutchens, T. W. (1994) *Anal. Chem.* **66**, 1408–1415.
54. Tang, K., Allman, S. L., Jones, R. B. & Chen, C. H. (1993) *Anal. Chem.* **65**, 2164–2166.
55. Beavis, R. C. (1992) *Fresenius Z. Anal. Chem.* **343**, 25-25.
56. Wada, Y., Gu, J. G., Okamoto, N. & Inui, K. (1994) *Biol. Mass Spectrom.* **23**, 108–109.
57. Billeci, T. M. & Stults, J. T. (1993) *Anal. Chem.* **65**, 1709–1716.
58. Chait, B. T., Wang, R., Beavis, R. C. & Kent, S. B. (1993) *Science* **262**, 89–92.
59. Pieles, U., Zurcher, W., Schar, M. & Moser, H. E. (1993) *Nucleic Acids Res.* **21**, 3191–3196.
60. Currie, G. J. & Yates, J. R. (1993) *J. Am. Soc. Mass Spectrom.* **4**, 955–963.
61. Wang, B. H. & Biemann, K. (1994) *Anal. Chem.* **66**, 1918–1924.
62. Wu, K. J., Shaler, T. A. & Becker, C. H. (1994) *Anal. Chem.* **66**, 1637–1645.
63. Schneider, K. & Chait, B. T. (1993) *Org. Mass Spectrom.* **28**, 1353–1361.
64. Fitzgerald, M. C., Zhu, L. & Smith, L. M. (1993) *Rapid Commun. Mass Spectrom.* **7**, 895–897.
65. Youngquist, R. S., Fuentes, G. R., Lacey, M. P. & Keough, T. (1994) *Rapid Commun. Mass Spectrom.* **8**, 77–81.
66. Siegel, M. M., Hollander, I. J., Hamann, P. R., James, J. P., Hinman, L., Smith, B. J., Farnsworth, A. P. H., Phipps, A., King, D. J., Karas, M., Ingendoh, A. & Hillenkamp, F. (1991) *Anal. Chem.* **63**, 2470–2481.
67. Garozzo, D., Spina, E., Sturiale, L., Montaudo, G. & Rizzo, R. (1994) *Rapid Commun. Mass Spectrom.* **8**, 358–360.
68. Zaluzec, E. J., Gage, D. A. & Watson, J. T. (1994) *J. Am. Soc. Mass Spectrom.* **5**, 359–366.
69. Preston, L. M., Murray, K. K. & Russell, D. H. (1993) *Biol. Mass Spectrom.* **22**, 544–550.
70. Gusev, A. I., Wilkinson, W. R., Proctor, A. & Hercules, D. M. (1993) *Appl. Spectrosc.* **47**, 1091–1092.
71. Spengler, B., Kirsch, D. & Kaufmann, R. (1991) *Rapid Commun. Mass Spectrom.* **5**, 198–202.
72. Huberty, M. C., Vath, J. E., Yu, W. & Martin, S. A. (1993) *Anal. Chem.* **65**, 2791–2800.
73. VG Analytical (1993) *Back to Basics* (Fisons Instruments, Manchester, U.K.).
74. Comisarow, M. B. & Marshall, A. G. (1974) *Chem. Phys. Lett.* **25**, 282–283.
75. Beu, S. C., Senko, M. W., Quinn, J. P. & McLafferty, F. W. (1993) *J. Am. Soc. Mass Spectrom.* **4**, 190–192.
76. Castoro, J. A. & Wilkins, C. L. (1993) *Anal. Chem.* **65**, 2621–2627.
77. McIver, R. T., Jr., Li, Y. & Hunter, R. L. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 4801–4805.
78. Loo, J. A., Quinn, J. P., Ryu, S. I., Henry, K. D., Senko, M. W. & McLafferty, F. W. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 286–289.
79. Little, D. P., Chorush, R. A., Speir, J. P., Senko, M. W., Kelleher, N. L. & McLafferty, F. W. (1994) *J. Am. Soc. Mass Spectrom.* **116**, 4893–4897.

Review. In the article "The emergence of mass spectrometry in biochemical research" by Gary Siuzdak, which appeared in number 24, November 22, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 11290–11297), Fig. 11 was incompletely relettered at the printers. The corrected Fig. 11 appears as follows.

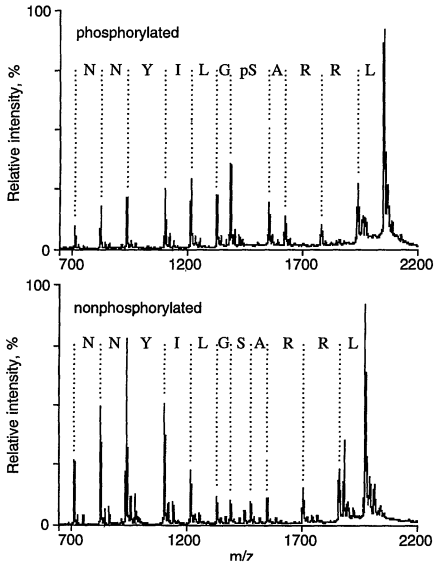

FIG. 11. Protein ladder sequencing of a 16-residue synthetic peptide that is phosphorylated (*Upper*) and not phosphorylated (*Lower*). A partial amino acid sequence is noted above the mass spectra. Reproduced with permission from ref. 58 (copyright American Association for the Advancement of Science, Washington, DC).

Biochemistry. In the article "Toward a code for the interactions of zinc fingers with DNA: Selection of randomized fingers displayed on phage" by Yen Choo and Aaron Klug, which appeared in number 23, November 8, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 11163–11167), the following note should be added.

Since this paper was submitted, Jamieson *et al.* (36) have reported the use of random mutagenesis and phage display to alter the DNA-binding specificity of Zif268, in order to investigate DNA recognition properties.

36. Jamieson, A. C., Kim, S.-H & Wells, J. A. (1994) *Biochemistry* **33,** 5689–5695.

Immunology. In the article "Idiotypic mimicry and the assembly of a supramolecular structure: An anti-idiotypic antibody that mimics taxol in its tubulin–microtubule interactions" by Jyh-Gang Leu, Bi-Xing Chen, Andrew W. Diamanduros, and Bernard F. Erlanger, which appeared in number 22, October 25, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 10690–10694), the authors request that the following be noted. The GenBank accession numbers for the light and heavy chain sequences for 82H are U17477 and U17476, respectively. With respect to the light chain sequence, it differs from the sequence of the mouse subgroup V light chain, no. 65, as given in Kabat *et al.* [Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S. & Foeller, C. (1991) *Sequences of Proteins of Immunological Interest* (Natl. Inst. Health, Bethesda, MD), NIH Publ. No. 91-3242, 5th Ed., p. 211], only in having a Ser in position 51 (TCA) instead of a Thr (ACA).

Evolution. In the article "Evidence for intron capture: An unusual path for the evolution of proteins" by G. Brian Golding, Nora Tsao, and Ronald E. Pearlman, which appeared in number 16, August 2, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 7506–7509), the following correction should be noted. Upon automated sequencing of the *Paramecium primaurelia* phosphoglycerate kinase intron, an error was found that went undetected in manual sequencing. The correct sequence is lengthened by 1 nucleotide to GTAATATAAGATATTA-ATTTTATAG. It was suggested that an in-frame intron could be easily "captured" and incorporated into protein as found in trypanosomes. This was indicated by its small size and its in-frame character. But there has been ample evolutionary time between *Paramecium* and *Trypanosoma* to permit insertions and deletions. The possibility of intron capture is still jointly indicated by the small size of the introns, the conserved location of the intron, and the rapid evolutionary rate of the trypanosome inserts. Further sequence results (both automated and manual) of *Paramecium tetraurelia* shows a small 24-bp intron in the same location with the sequence GTGTC-CTATATGAATATATTTTAG. Like the *P. primaurelia* intron, this is a small intron that could be easily captured into a new protein sequence.

Genetics. In the article "Identification of *comS*, a gene of the *srfA* operon that regulates the establishment of genetic competence in *Bacillus subtilis*" by Cletus D'Souza, Michiko M. Nakano, and Peter Zuber, which appeared in number 20, September 27, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 9397–9401), the authors request that the following correction be noted. In Fig. 3, the Lac phenotype of the pMMN174 plasmid should be indicated as + and not −. The correct figure and its legend are shown below.

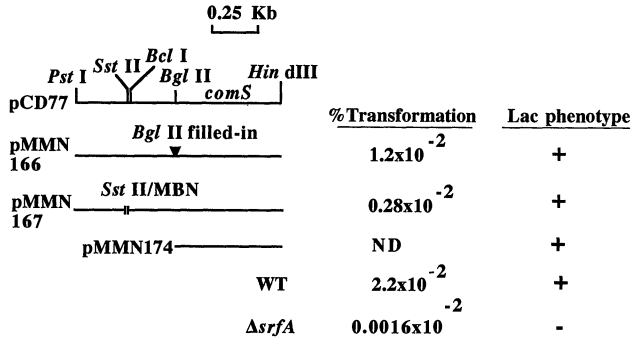

FIG. 3. Complementing activity of pCD77 and mutated derivatives. Line labeled pCD77 shows the plasmid insert and its restriction map. Lines below indicate the mutant derivatives of pCD77; pMMN166 was made by cleaving at the *Bgl* II site followed by fill-in synthesis and ligation; pMMN167 was made by cleaving at the *Sst* II site followed by mung bean nuclease (MBN) treatment and ligation. pMMN174 contains a 569-bp *Bgl* II/*Hin*dIII fragment. The transformation efficiency (number of transformants/total viable cell count) is shown for Δ*srfA comG*::*lacZ* cells with and without the plasmid constructs together with a wild-type (WT) control. Lac⁻ phenotype of plasmid-bearing Δ*srfA comG*::*lacZ* cells is also indicated.

Genetics. In the article "Recombinant adeno-associated virus (rAAV)-mediated expression of a human γ-globin gene in human progenitor-derived erythroid cells" by Jeffery L. Miller, Robert E. Donahue, Stephanie E. Sellers, Richard Jude Samulski, Neal S. Young, and Arthur W. Nienhuis, which appeared in number 21, October 11, 1994, of *Proc. Natl. Acad. Sci. USA* (**91,** 10183–10187), the authors request that the following correction be noted. The article should have acknowledged support from National Institutes of Health Grant HL 48347-03.