Biochemical mass spectrometry: worth the weight?

Michael C Fitzgerald and Gary Siuzdak

The utility of mass spectrometry for the analysis of biological molecules has been enhanced by the development of two techniques that generate gas-phase ions via nondestructive vaporization and ionization. These techniques can be used not only to determine the primary structure of biological molecules with unprecedented accuracy, but also to map noncovalent biomolecular interactions.

Addresses: Department of Chemistry, The Scripps Research Institute, BCC-157, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA; e-mail: siuzdak@scripps.edu

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Introduction

Mass spectrometry is fast becoming an integral part of biological research, principally due to the development of two ionization techniques: matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). Both MALDI and ESI have greatly advanced our ability to characterize large, thermally labile molecules by mass spectrometry, because, unlike other mass spectrometry techniques, they provide an efficient and non destructive means of generating intact gas phase ions. MALDI and ESI have significantly extended the applicability of mass spectrometry to a wide variety of biologically relevant molecules including peptides, proteins, carbohydrates, and oligonucleotides [1–9].

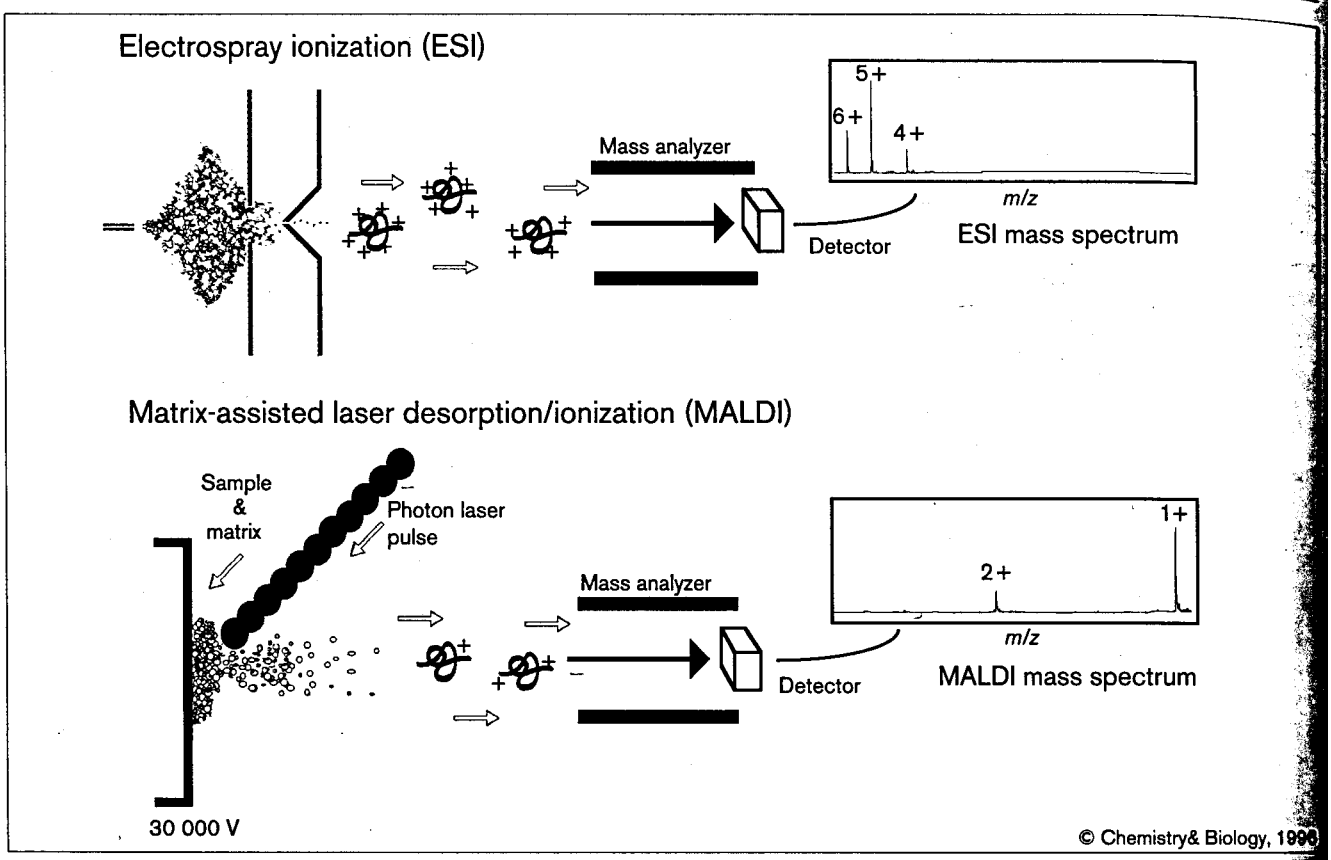
MALDI and ESI have been used to gain important information on the molecular weight of biological samples with unprecedented speed, accuracy, sensitivity and mass range. Recent developments in instrumentation and new sampling methods have not only made these advances possible, but also led to an increasing number of mass-spectrometry-based applications for the study of both the covalent and noncovalent structure of biopolymers. Here we summarize these technological advances and discuss their implications for biochemical research.

ESI and MALDI ionization

ESI and MALDI are fundamentally different ionization techniques, yet they achieve essentially the same end result — the generation of gas-phase ions via nondestructive vaporization and ionization [1]. In both techniques, ionization typically occurs through proton addition or proton abstraction to produce either $[M+H]^+$ or $[M-H]^-$ ions (where M is the molecule of interest). In the presence of salt, ionization can also occur by the addition of cations or anions to analyte molecules, producing, for example, $[M+Na]^+$ or $[M+Cl]^-$ ions.

In ESI, ions are formed directly from solution (usually an aqueous or aqueous/organic solvent system) by creating a fine spray of highly charged droplets in the presence of a strong electric field [10]. Vaporization of these charged droplets produces multiply charged gaseous ions. The number of charges retained by an analyte depends on several factors, such as the composition and pH of the electrosprayed solvent and the chemical nature of the sample [2–4]. For large molecules, the ESI process typically produces a series of multiply charged species for a given analyte. Because mass spectrometers measure the mass-to-charge (m/z) ratio, the ESI mass spectrum contains multiple peaks corresponding to the different charged states (Fig. 1).

Figure 1



Schematic representation of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. ESI occurs through charged droplet evaporation and MALDI via pulsed laser desorption/ionization of a sample from an absorbing matrix. In each case the ionized species are directed through a mass analyzer which allows for differentiation and detection of the ions according to their mass-to-charge ratio (*m/z*).

The multiple-charging phenomenom in ESI is a unique characteristic of the technique, which allows the mass of an analyte to be determined with great precision, because masses can be independently calculated from several different charged states [5]. The multiple-charging characteristics of ESI also permit the analysis of high molecular weight analytes, using conventional mass analyzers that are normally limited to the detection of ions with relatively low *m/z* ratios. For example, a 50 kDa protein will typically retain ~30–50 charges in ESI, yielding multiply charged species with *m/z* ratios of 1000–2000 that are easily detected with quadrupole mass analyzers.

In MALDI, gas-phase ions are generated by the laser vaporization of a solid matrix/analyte mixture in which the matrix (usually a small crystalline organic compound) strongly absorbs the laser radiation and acts as a receptacle for energy deposition (Fig. 1) [11]. This concentrated energy deposition results in the vaporization and ionization of both matrix and analyte ions containing very few charges [11,12]. Low molecular weight analytes (< 20 kDa) are typically ionized with only one or two charges, whereas larger analytes (> 20 kDa) can accumulate as many as three to five charges depending on the specific desorption conditions, in particular, the matrix and the laser power used. The relatively low number of charge states observed in MALDI makes the technique especially well suited for the analysis of multicomponent mixtures, as individual components can be easily identified by the mass-spectral signal generated from their 1+ charge state.

Because ESI and MALDI are different ionization techniques they offer different capabilities that in many cases are complementary. These capabilities are summarized in Table 1.

Primary structure determination

Peptides and proteins

ESI and MALDI are useful for analysis of primary structure because they provide very accurate molecular weight

information for intact compounds. Mass accuracies of ~0.01 % and 0.1 % are routine for ESI and MALDI, respectively, and are far superior to those attainable with conventional gel-based techniques. Such accurate information can be extremely useful for protein identification. For example, an unknown protein can often be unambiguously identified by mass spectral analysis of its constituent peptides, produced by either chemical or enzymatic treatment of the sample [6–13]. MALDI is especially well suited for such analyses as complex mixtures of peptides are directly amenable to MALDI analysis. The molecular weights of individual peptides in a protein digest are also easily determined by using a combination of liquid chromatography and ESI mass spectrometry (Fig. 2).

Peptide and protein analysis can also be facilitated by initiating fragmentation in the gas phase [1]. Fragment ions generated inside the mass spectrometer via collision-induced dissociation (CID) can often yield information about the primary structure of a sample. This tandem mass analysis technique involves selecting an ion of interest with the mass analyzer and isolating it in a collision cell. There, the selected ion undergoes collisions with an inert gas such as argon, creating fragments that can be mass analyzed, providing information about their sequence [14]. This multiple mass analysis approach is often referred to as tandem mass spectrometry or MS^2. Because the CID behavior of peptides is already well characterized, tandem mass spectrometry with CID can be used to acquire direct sequence information on small peptides (< 3 kDa).

Oligonucleotides and carbohydrates

Mass-based analysis of primary structure is not limited to peptides and proteins. Oligonucleotides, carbohydrates and a variety of other biomolecules can also be routinely examined using mass spectrometry [1–15] . MALDI and ESI have both proven useful for the analysis of nucleic acids of 50–100 bases in length [4–16]. In combination with partial exonuclease digestion, the MALDI technique can be used to obtain sequence information (Fig. 3) on oligonucleotides of up to 50 bases in length. Mass-spectrometry-based sequencing strategies have proven especially useful for obtaining sequence information on modified oligonucleotides [17,18].

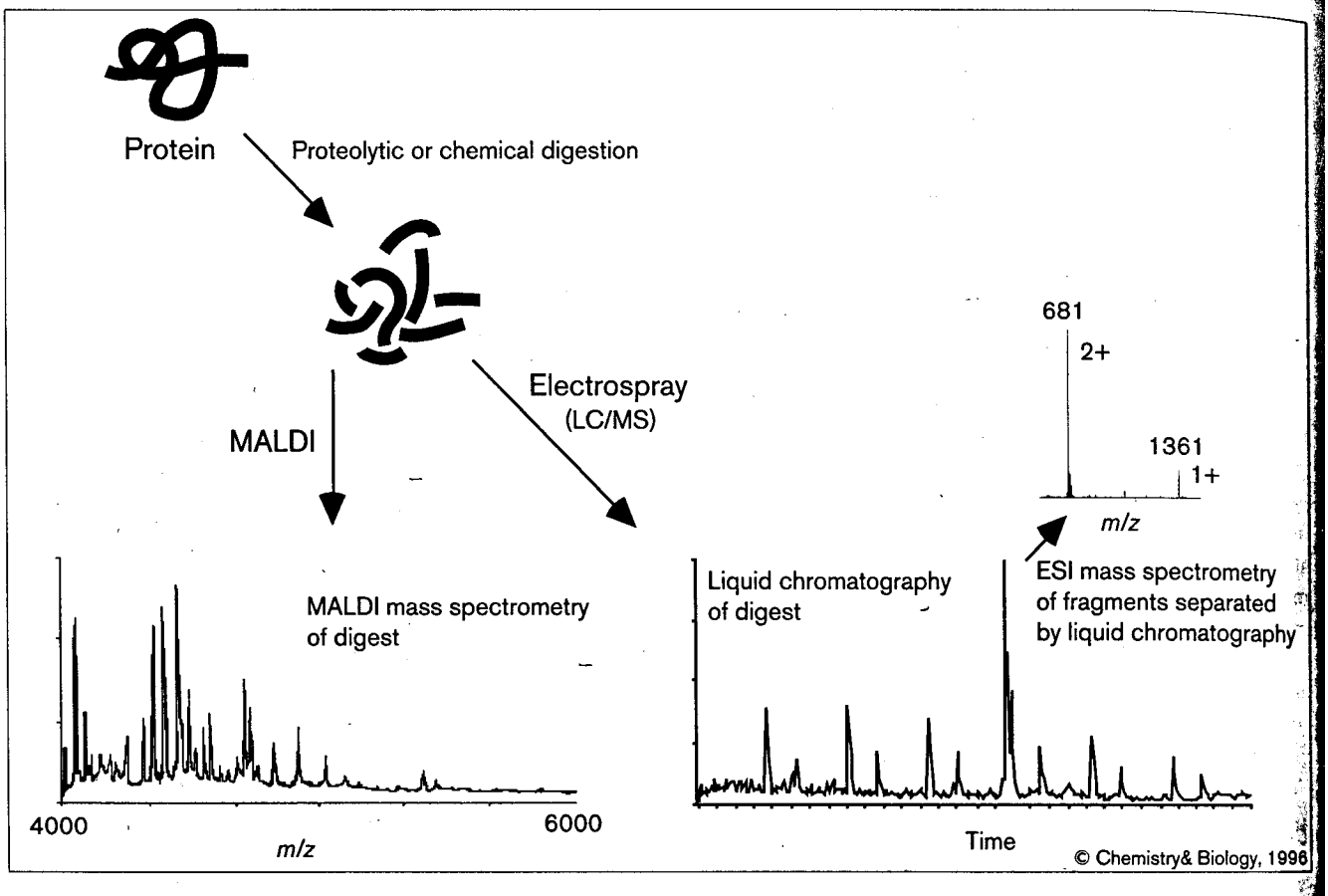
ESI and MALDI have also been used to study carbohydrates and other small molecules [15–19]. For instance, ESI combined with tandem mass spectrometry can yield important information about the structure of small biomolecules;

Table 1

Capabilities, limitations and recent improvements of ESI and MALDI mass spectrometry.

	Mass limit, Daltons (practical)	Routine capabilities and limitations	Recent improvements
ESI mass spectrometry	Essentially unlimited (70 000)	Femtomole to picomole sensitivity	Attomole sensitivity possible with microelectrospray
		Capable of observing noncovalent complexes directly from aqueous solutions	Amenable to structural studies using MS^n capabilities of ion trap mass analyzers
		Amenable to structural studies using tandem mass analysis	Adaptation to time-of-flight mass analyzers offers unlimited mass range for large biomolecules and biomolecular complexes
		Capable of HPLC mass spectrometry	
		Analysis of oligonucleotides up to ~100 bases in length	
		Accurate to ~±0.01 % or better	
MALDI mass spectrometry	~1 000 000 (300 000)	Attomole to picomole sensitivity	Attomole sensitivity possible with submicroliter sample preparations
		Accurate to ± 0.1 % to 0.01 %	Accuracy and resolution improved with implementation of delayed extraction technology on the ion source
		Tolerant of mM salt concentrations	
		Analysis of oligonucleotides up to ~100 bases in length.	Potential as a tool for DNA and peptide sequence analysis
		Analysis of proteins up to ~300 000 Da in size	
		Tolerant of mixtures	Potential as a tool for studying noncovalent biomolecular interactions.
		Not routinely amenable to liquid chromatography mass spectrometry	
		Not routinely amenable to observing noncovalent interactions	

Figure 2



Analysis of a proteolytic fragments using MALDI mass spectrometry or liquid chromatography with ESI mass spectrometry detection (LC/MS). Complex mixtures can be analyzed in a single step using MALDI mass spectrometry. The molecular weight of individual fragments can also be accurately determined using a combination of liquid chromatography and ESI mass spectrometry.

this was demonstrated in a study on the calcium-binding properties of the cell-surface carbohydrate sialyl Lewis x (sLe^x) (Fig. 4). The results of this study provided structural evidence that the calcium atom in the compound is coordinated to the trisaccharide of sLe^x [19].

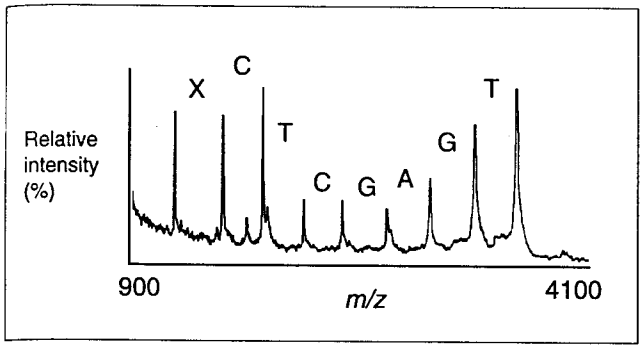
Probing higher order biomolecular interactions

'Native' ESI mass spectrometry

As shown in Figure 4, the gentle nature of electrospray ionization permits the analysis of noncovalent complexes. Because ESI can be used to analyze samples under solution conditions that preserve their native conformation, the technique has been useful for studying both noncovalent and covalent associations of biomolecules, including protein–protein [20,21], protein–ligand [22], enzyme–substrate [23], enzyme–inhibitor [24], and duplex DNA complexes [25,26]. Accurate mass analysis can be very useful in determining the stoichiometry of a particular noncovalent complex. For example, 'native' ESI mass spectrometry has been used to assess the oligomeric state of several different multimeric complexes, such as the 4-oxalocrotonate enzyme complex [27,28] (Fig. 5). In some cases, the ESI technique has also yielded important information about the relative strength of noncovalent, multimeric, complexes. For example, investigations with complementary oligonucleotides have demonstrated that intact, duplex DNA is amenable to analysis by 'native' ESI, and have shown that it is possible to assess the relative stability of different DNA duplexes by studying their propensity to dissociate into single-stranded oligonucleotides upon CID [29,30].

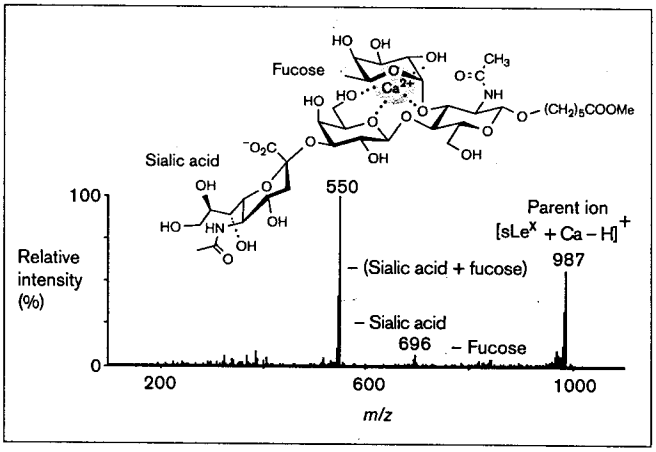
There is mounting evidence that the gas-phase complexes detected in 'native' ESI experiments are similar to those found in solution. Many of the 'native' ESI results obtained to date have been consistent with those from conventional solution-phase techniques. In addition, we have recently shown that a virus can remain viable after mass-spectral analysis [31], and the mass of an intact virus has been measured [32]. These results provide evidence that gas-phase noncovalent complexes can reflect their solution

Figure 3



MALDI mass spectral analysis of a small DNA fragment of sequence 5'-d(GCTTXCTCGAGT), carrying a modified nucleoside in the 5 position (X = 2'O-methyl adenosine), partially digested with an exonuclease to obtain sequence information. The amount of digestion can be controlled by varying exonuclease concentration and exposure. In this case, fragments from full length (12 nucleotides) to 5 nucleotides were obtained. Commercial oligonucleotide sequencing kits have been developed that allow for partial exonuclease digestion from either end of the molecule and can provide full sequence information with MALDI-MS on a single-stranded DNA up to 50 bases in length. Reproduced with permission from [18].

Figure 4



Electrospray tandem mass analysis of the cell-surface carbohydrate sialyl Lewis x (sLe^x) bound to calcium. The fragmentation data shown here, combined with other mass-spectral and modeling data, indicated that calcium is bound to sLe^x through the trisaccharide portion of the molecule and not the sialic acid [19].

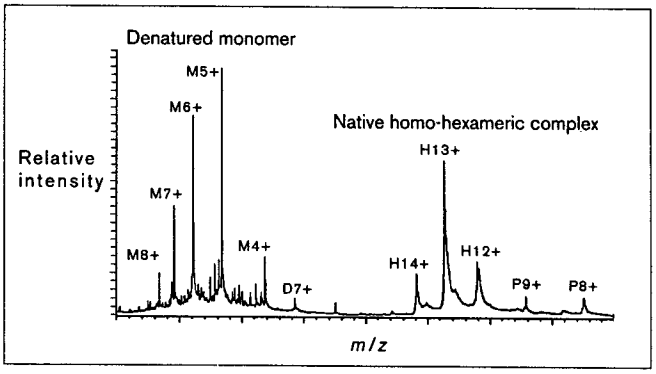
structure and demonstrate the utility of ESI for analysis of supramolecular complexes with molecular weights of over 40 million Daltons.

Mapping noncovalent biomolecular interactions using MALDI

The strongly acidic conditions commonly used for sample preparation in MALDI tend to denature biological samples and often preclude the direct characterization of higher order structure by this technique. Several methods have been developed, however, that use MALDI as a readout for defining specific noncovalent biomolecular interactions. One approach relies on the MALDI analysis of protein digest products. Since proteolysis is limited by solvent accessibility and protein flexibility, sites that are involved in binding or are buried inside a protein may not be digested; thus, structural information can be acquired by determining which cleavage sites in a molecule remain protected from digestion. The feasibility of this technique was first demonstrated by Chait and coworkers [33] in probing the solution structure of DNA-binding proteins. In this study a basic helix-loop-helix transcription factor, Max, was digested in the presence or absence of its cognate DNA. The Max–DNA complex was highly resistant to proteolysis compared to the uncomplexed protein. MALDI analysis of the products of these proteolytic digests made it possible to identify those regions of the Max polypeptide chain that were protected from proteolysis upon DNA binding, and provided insight into the location of the DNA interaction sites on the Max protein.

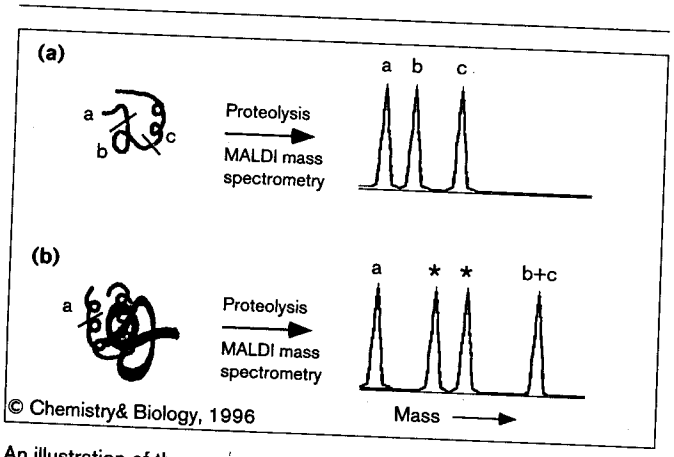
The approach outlined above has also been applied to the study of protein–protein interactions (Fig. 6). For example, MALDI analysis of the tryptic fragments of p21-B (the kinase inhibitory domain of the cell-cycle regulatory protein, p21) in the presence or absence of cyclin-dependent kinase (Cdk2) revealed a segment of 24 amino acids in p21-B that is protected from trypsin cleavage, thus identifying the segment as the Cdk2 binding site on p21-B (Fig. 7). In these experiments, MALDI analysis of the tryptic fragments produced by enzymatic digestion of the Cdk2–p21-B complex was simplified by using both p21-B containing the natural abundance of isotopes and ^{15}N-labeled p21-B [34]. The use of ^{15}N-labeled p21-B simplified interpretation of the mass spectra for the p21-B–Cdk2 complex; these spectra were complicated by the appearance of peaks due to tryptic cleavage of both p21-B and Cdk2.

Figure 5



The ESI-TOF mass spectrum of a multimeric enzyme, 4-oxalocrotonate tautomerase (4OT), showing that the noncovalent homohexameric enzyme complex can be detected by mass spectrometry [27].

Figure 6



An illustration of the mass-spectrometric proteolytic assay for the detection of protein–protein interactions [34], similar to the assay previously described for protein–DNA interactions [33]. The figure compares peptide maps obtained after enzymatic proteolysis of **(a)** a hypothetical protein and **(b)** the same protein in a complex with a ligand. Peaks marked with a * represent proteolytic fragments from the ligand.

A combination of proteolytic digestion and affinity-directed MALDI mass spectrometry has also been used to accurately map specific interaction sites in proteins [35]. In this approach, the approximate location of a continuous binding epitope within a protein ligand was determined by subjecting the protein ligand to proteolysis and then selecting for constituent peptide fragments that bind a specific, immobilized antibody using an immunoprecipitation step. The binding peptides were identified by direct MALDI analysis of the immune complex.

Other methods for examining protein–protein interactions, which rely on the chemical crosslinking of protein complexes prior to MALDI analysis, have also been developed. To determine the stoichiometry of multimeric proteins, they can be subjected to MALDI analysis after reaction with a crosslinking agent such as glutaraldeyde. In this case, glutaraldeyde is used to covalently link the protein subunits in solution [36]. The oligomeric state of the protein can be accurately assessed by MALDI mass spectrometry of the covalently linked complex.

Clearly, ESI and MALDI are suitable for tasks beyond molecular-weight determination. As illustrated above, their suitability for gathering structural information offers a unique approach to solving biomolecular problems. At just a decade old, however, their full potential is still being realized; several recent advances are described below.

Recent improvements to ESI and MALDI

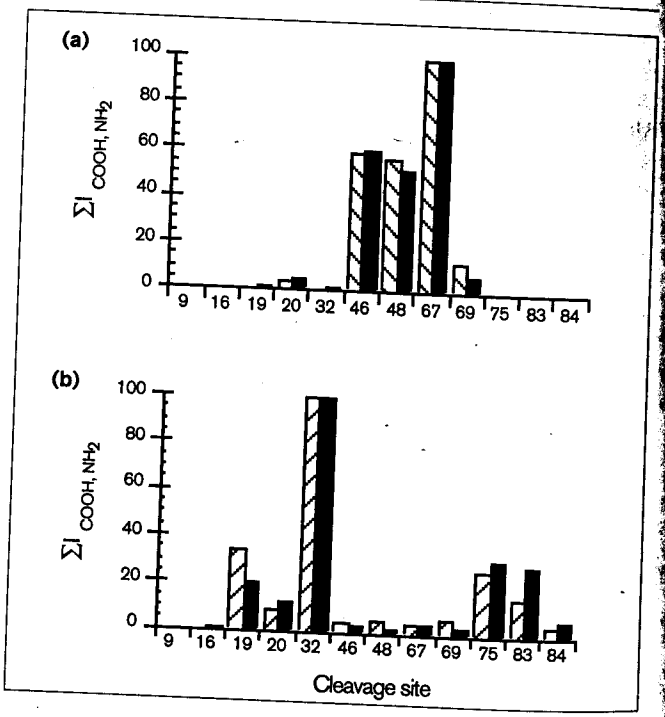
The continued development of both ESI and MALDI has led to an increasing number of applications. Among the improvements are the coupling of ESI and MALDI to new mass analyzers and the development of new sampling procedures.

ESI instrumentation

The ESI technique has been most commonly used in combination with quadrupole mass spectrometers. In most cases, conventional quadrupole mass analyzers are capable of scanning up to m/z 3000, which has been sufficient for the ESI analysis of large biomolecules under denaturing solution conditions. Under native solution conditions, however, the relatively compact structure of folded proteins retains only a limited number of charges during ESI, producing ions with m/z values > 3000. Thus, the application of 'native' ESI mass spectrometry to the analysis of high-molecular-weight, noncovalent complexes has required the use of mass analyzers with a greater m/z range. To this end, quadrupole mass filters with an extended m/z range of up to 5000 have been developed through improvements in electronics.

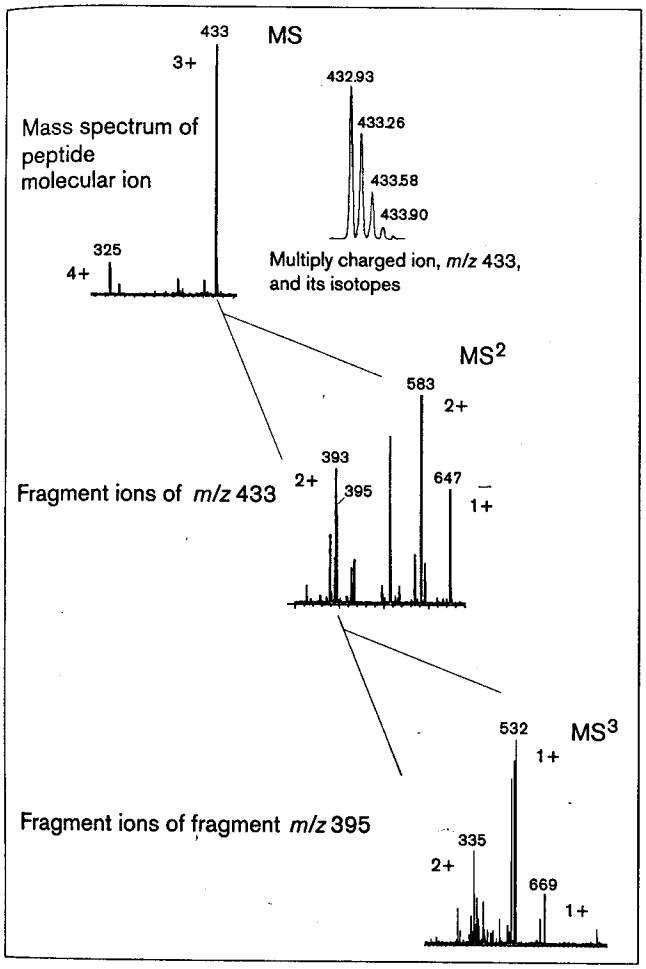
Time-of-flight (TOF) mass analyzers have also been successfully interfaced with ESI ion sources (Fig. 5). The

Figure 7



Protein–protein interactions can be detected using MALDI mass spectrometry [34]. The histograms represent **(a)** tryptic digest fragments of free protein (p21-B) labeled with ^{15}N (striped) or unlabeled (black), and **(b)** the tryptic digest of the complex of p21-B and Cdk2, with p21-B labeled with ^{15}N (striped) or unlabeled (black). ΣI represents the ion intensity of the amino- and carboxy-terminal fragments generated at each cleavage site. These results demonstrate the reduced accessibility of trypsin at particular cleavage sites protected in the complex.

Figure 8



Data obtained from an electrospray ion trap mass spectrometer (Finnigan LCQ) showing MS, MS^2, and MS^3 data acquired from angiotensin I. The *m/z* 433 ion inset represents the high resolving power possible with the ion trap, which allows the charge state to be determined. In this case the isotope spacing is 1/3, therefore the charge state is 3. The MS/MS data from the ion trap also complements database searching algorithms, providing a very powerful peptide mapping and protein identification system. The higher-order mass spectra can be used to obtain additional structural information on fragment ions leading to total or partial structural information on the compound of interest.

TOF analyzer separates ions according to the time it takes them to traverse a given distance; as they are given the same kinetic energy, ions of different mass reach the detector at different times. These analyzers have, in principle, an unlimited *m/z* range.

The recent commercial introduction of an ESI source combined with an ion-trap mass analyzer also promises to make the acquisition of fragmentation data routine. The ion-trap analyzer offers a mass range to ~*m/z* 6000 and the ability to perform multiple fragmentation (CID) experiments. These MS^n experiments are performed by repeatedly isolating fragment ions and further inducing fragmentation of these ions [37,38]. This information can be especially useful for structure determination of peptides, carbohydrates, and drug metabolites, providing a wealth of information on a molecule's structure (see Fig. 8 for an example using angiotensin I).

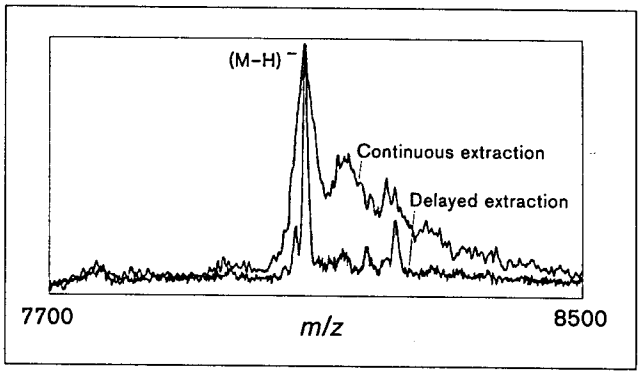
MALDI instrumentation

The MALDI technique has primarily been used in combination with TOF mass analyzers. These instruments are especially well suited for use with MALDI since the laser desorption/ionization event takes place at a specific point in space and time. Unfortunately, a major limitation of conventional TOF mass analyzers has been their relatively poor resolving power. Recently, however, the development of MALDI TOF instruments with delayed ion extraction has significantly enhanced their performance [39–41]. The data shown in Figure 9 illustrate the improvement this innovation has made in spectral quality. Whereas conventional instruments rely on a constant electric field to transfer ions into the TOF analyzer, the delayed extraction technique uses a submicrosecond delay between the time ions are produced and the time they are introduced into the analyzer. This short delay narrows the velocity distribution of the ions that are formed in the MALDI process and serves to improve mass resolution up to five-fold with an associated increase in mass accuracy.

Ion detectors

As shown in Figure 1, the mass spectrometer is the combination of an ion source, a mass analyzer, and a detector. Conventional detectors such as electron multipliers generate secondary electrons once an ion strikes its surface; with a resulting amplification of 10^6 electrons for each striking ion, these detectors offer high sensitivity with a lifetime of one to three years.

Figure 9



Improved resolution obtained with delayed-extraction MALDI, compared to continuous-extraction MALDI, on a 26-base phosphorothioate oligonucleotide [40]. Reproduced with permission from [40].

A recent innovation in detector technology, which could have broad-reaching implications, is charge-detection mass spectrometry. This technology, which relies on detecting the presence of a passing charge by the current it induces on the surface of a conducting plate, could potentially change the way we analyze high molecular weight biomolecules and possibly allow us to measure the mass of viruses and phage [42,43]. Charge detection has several important advantages over traditional detection methods, including a theoretically unlimited lifetime for the detector and the ability to measure the charge state directly, as the intensity of the signal is directly proportional to the charge. Charge detection also has the unique ability to detect ionized species independently of their mass or ion velocity. Large ions often yield weak signals with conventional ion detectors, because they strike the detector with insufficient velocity to generate enough secondary electrons for signal production. Charge detectors obviate this problem, because they rely on the accumulation of charge and not on the production of secondary electrons.

Sampling techniques

Several new sampling techniques have also added to the increased utility of ESI and MALDI. The ability to perform MALDI on proteins directly from two-dimensional gels [44] and on enzymatically activated surfaces [45] or from solid-phase resins [46] has greatly simplified sample preparation for MALDI mass spectrometry. The ESI technique has been modified to allow for nanoliter per minute sampling rates [47]. Using this new approach, designated microelectrospray, microliter sample quantities can be analyzed over the course of hours, consuming only small (subfemtomole) sample quantities.

Summary and conclusion

The increase in the use of mass spectrometry in the biotechnological field can be attributed in large part to the development of ESI and MALDI. Mass-spectral analysis of a variety of biologically important compounds has been carried out using these two relatively new ionization techniques, establishing mass spectrometry as a valuable tool for studying both the covalent and noncovalent structure of biopolymers.

In addition to improvements in the technology (Table 1), the availability of instrumentation has also been important in the successful development of mass spectrometry. In the last ten years both the price and size of mass spectrometers have decreased to a point where it is possible for both chemists and biologists to have access to these instruments. As more scientists are able to perform mass analysis, their appreciation of and reliance on its applications grows.

Acknowledgements

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References

1. Siuzdak, G. (1996). *Mass Spectrometry for Biotechnology*. Academic Press, San Diego.
2. Chait, B.T. & Kent, S.B.H. (1992). Weighing naked proteins – practical, high-accuracy mass measurement of peptides and proteins. *Science* **257**, 1885–1894.
3. Arnott, D., Shabanowitz, J. & Hunt, D.F. (1993). Mass spectrometry of proteins and peptides: sensitive and accurate mass measurement and sequence analysis. *Clin. Chem.* **39**, 2005–2010.
4. Smith, R.D., Loo, J.A., Edmonds, C.G., Barinaga, C.J. & Udseth, H.R. (1990). New developments in biochemical mass spectrometry – electrospray ionization. *Anal. Chem.* **62**, 882–899.
5. McCloskey, J.A. (1990). *Mass spectrometry*. Academic Press, San Diego.
6. Yates, J.R., Speicher, S., Griffin, P.R. & Hunkapiller, T. (1993). Peptide mass maps: a highly informative approach to protein identification. *Anal. Biochem.* **214**, 397–408.
7. Burlingame, A.L., Boyd, R.K. & Gaskell, S.J. (1996). Mass spectrometry. *Anal. Chem.* **68**, 599R–651R.
8. Stults, J.T. (1995). Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). *Curr. Opin. Struct. Biol.* **5**, 691–698.
9. Mann, M. & Wilm, M. (1995). Electrospray mass spectrometry for protein characterization. *Trends Biochem. Sci.* **20**, 219–224.
10. Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F. & Whitehouse, C.M. (1990). Electrospray ionization – principles and practice. *Mass Spectrom. Rev.* **9**, 37–70.
11. Hillenkamp, F., Karas, M., Beavis, R.C. & Chait, B.T. (1991). Matrix-assisted laser desorption ionization mass spectrometry of biopolymers. *Anal. Chem.* **63**, A1193–A1202.
12. Knochenmuss, R., Dubois, F., Dale, M.J. & Zenobi, R. (1996). The matrix suppression effect and ionization mechanisms in matrix-assisted laser desorption/ionization. *Rapid Comm. Mass Spectrom.* **10**, 871–877.
13. Mann, M., Hojrup, P. & Roepstorff, P. (1993). Use of mass spectrometric molecular weight information to identify proteins in sequence databases. *Biol. Mass Spectrom.* **22**, 338–345.
14. Hunt, D.F., *et al.*, & Englehard, V.H. (1992). Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. *Science* **255**, 1261–1263.
15. Harvey, D.J. (1996). Matrix-assisted laser desorption/ionization mass spectrometry of oligosaccharides and glycoconjugates. *J. Chromatogr. A* **720**, 429–446.
16. Fitzgerald, M.C. & Smith, L.M. (1995). Mass spectrometry of nucleic acids: the promise of matrix-assisted laser desorption-ionization (MALDI) mass spectrometry. *Annu. Rev. Biophys. Biomolec. Struct.* **24**, 117–140.
17. Baker, T.R., Keough, T., Dobson, R.L., Riley, T.A., Hasselfield, J.A. & Hesselberth, P.E. (1993). Antisense DNA oligonucleotides I: the use of ionspray tandem mass spectrometry for the sequence verification of methylphosphonate oligodeoxyribonucleotides. *Rapid Comm. Mass Spectrom.* **7**, 190–194.
18. Pieles, U., Zurcher, W., Schar, M. & Moser, H.E. (1993). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a powerful tool for the mass and sequence analysis of natural and modified oligonucleotides. *Nucleic Acids Res.* **21**, 3191–3196.
19. Siuzdak, G., *et al.*, & Wong, C.-H. (1994). Examination of the sialyl Lewis X calcium complex by electrospray mass spectrometry. *Bioorg. Med. Chem. Lett.* **4**, 2863–2866.
20. Light-Wahl, K.J., Schwartz, B.L. & Smith, R.D. (1994). Observation of the noncovalent quaternary association of proteins by electrospray ionization mass spectrometry. *J. Am. Chem. Soc.* **116**, 5271–5278.
21. Loo, J.A. (1995). Observation of large subunit protein complexes by electrospray ionization mass spectrometry. *J. Mass Spectrom.* **30**, 180–183.
22. Siuzdak, G., Krebs, J.F., Benkovic, S.J. & Dyson, H.J. (1994). Binding of hapten to a single-chain catalytic antibody demonstrated by electrospray mass spectrometry. *J. Am. Chem. Soc.* **117**, 7937–7938.
23. Krebs, J.F., Siuzdak, G., Dyson, H.J., Stewart, J.D. & Benkovic, S.J. (1995). Detection of a catalytic antibody species acylated at the active site by electrospray mass spectrometry. *Biochemistry* **34**, 720–723.
24. Baca, M. & Kent, S.B.H. (1992). Direct observation of a ternary complex between the dimeric enzyme HIV-1 protease and a substrate

based inhibitor. *J. Am. Chem. Soc.* **114**, 3992–3993.
25. Ganem, B., Li, Y.T. & Henion, J.D. (1993). Detection of oligonucleotide duplex forms by ion-spray mass spectrometry. *Tetrahedron Lett.* **34**, 1445–1448.
26. Light-Wahl, K.J., *et al.*, & Smith, R.D. (1993). Observation of a small oligonucleotide duplex by electrospray ionization mass spectrometry. *J. Am. Chem. Soc.* **115**, 803–804.
27. Fitzgerald, M.C., Chernushevich, I., Standing, K.G., Whitman, C.P. & Kent, S.B.H. (1996). Probing the oligomeric structure of an enzyme by electrospray ionization time-of-flight mass spectrometry. *Proc. Natl. Acad. Sci. USA* **93**, 6851–6856.
28. Cheng, X., Harms, A.C., Goudreau, P.N., Terwilliger, T.C. & Smith, R.D. (1996). Direct measurement of oligonucleotide binding stoichiometry of gene V protein by mass spectrometry. *Proc. Natl. Acad. Sci. USA* **93**, 7022–7027.
29. Greig, M.J., Gaus,H.J. & Griffey, R.H. (1996). Negative ionization microelectrospray mass spectrometry of oligonucleotides and their complexes. *Rapid Commun. Mass Spectrom.* **10**, 47–50.
30. Cheng, X., *et al.*, & Switzer, C. (1996). Comparison of 3',5'- and 2',5'-linked DNA duplex stabilities by electrospray ionization mass spectrometry. *Chem. Commun.* **6**, 747–748.
31. Siuzdak, G., *et al.*, & Chang, C.-M. (1996). Mass spectrometry and viral analysis. *Chemistry & Biology* **3**, 45–48.
32. Fuerstenau, S. & Benner, W.H. (1996). Charge detection mass spectrometry of macromolecules above 1 million AMU. In *The 44th Conference on Mass Spectrometry and Allied Topics*. The American Society for Mass Spectrometry, Portland, Oregon.
33. Cohen, S.L., Ferre-D'Amare, A.R., Burley, S.K. & Chait, B.T. (1995). Probing the solution structure of the DNA-binding protein Max by a combination of proteolysis and mass spectrometry. *Protein Sci.* **4**, 1088–1099.
34. Kriwacki, R., Wu, J., Siuzdak, G. & Wright, P.E. (1996). Characterizing protein–protein interactions by mass spectrometry: analysis of the p21/Cdk2 complex. *J. Am. Chem. Soc.* **118**, 5320–5321.
35. Zhao, Y., *et al.*, & Chait, B.T. (1996). Mapping protein–protein interactions by affinity-directed mass spectrometry. *Proc. Natl. Acad. Sci. USA* **93**, 4020–4024.
36. Farmer, T.B. & Caprioli, R.M. (1995). Mass discrimination in matrix-assisted laser desorption ionization time-of-flight mass spectrometry – a study using cross-linked oligomeric complexes. *J. Mass Spectrom.* **30**, 1245–1254.
37. McLuckey, S.A., Van Berkel, G.J., Goeringer, D.E. & Glish, G.L. (1994). Ion trap mass spectrometry. Using high-pressure ionization. *Anal. Chem.* **66**, 737A–743A.
38. McLuckey, S.A., VanBerkel, G.J., Goeringer, D.E. & Glish, G.L. (1994). Ion trap mass spectrometry of externally generated ions. *Anal. Chem.* **66**, A689–A696.
39. Whittal, R.M. & Li, L. (1995). High resolution matrix-assisted laser desorption ionization in a linear time-of-flight mass spectrometer. *Anal. Chem.* **67**, 1950–1954.
40. Juhasz, P., *et al.*, & Martin, S.A. (1996). Applications of delayed extraction matrix-assisted laser desorption ionization time-of-flight mass spectrometry to oligonucleotide analysis. *Anal. Chem.* **68**, 941–946.
41. Vestal, M.L., Juhasz, P. & Martin, S.A. (1995). Delayed extraction matrix-assisted laser desorption time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **9**, 1044–1050.
42. Park, M.A. & Callahan, J.H. (1994). An inductive detector for time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **8**, 317–322.
43. Fuerstenau, S.D. & Benner, W.H. (1995). Molecular weight determination of megaDalton DNA electrospray ions using charge detection time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **9**, 1528–1538.
44. Wilm, M., *et al.*, & Mann, M. (1996). Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. *Nature* **379**, 466–469.
45. Dogruel, D., Williams, P. & Nelson, R.W. (1995). Rapid tryptic mapping using enzymatically active mass spectrometer probe tips. *Anal. Chem.* **67**, 4343–4348.
46. Fitzgerald, M.C., Harris, K., Shevlin, C.G. & Siuzdak, G. (1996). Direct characterization of solid phase resin-bound molecules by mass spectrometry. *Bioorg. Med. Chem. Lett.* **6**, 979–982.
47. Wilm, M.S. & Mann, M. (1994). Electrospray and Taylor-cone theory, Doles beam of macromolecules at last. *Internatl. J. Mass Spectrom. Ion Process.* **136**, 167–180.