Electrospray-Assisted Laser Desorption/Ionization Mass Spectrometry for Continuously Monitoring the States of Ongoing Chemical Reactions in Organic or Aqueous Solution under Ambient Conditions

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Electrospray-assisted laser desorption/ionization (ELDI) combined with mass spectrometry allows chemical and biochemical compounds to be characterized directly from hydrophilic and hydrophobic organic solutions mixed with carbon powders under ambient conditions. Organic and inorganic compounds dissolved in polar or nonpolar solvent such as methanol, tetrahydrofuran, ethyl acetate, toluene, dichloromethane, or hexane can be detected using this ambient ionization technique without prior pretreatment. We have used this technique to monitor the progress in several ongoing reactions: the epoxidation of chalcone in ethanol, the chelation of ethylenediaminetetraacetic acid with copper and nickel ions in aqueous solution, the chelation of 1,10-phenanthroline with iron(II) in methanol, and the tryptic digestion of cytochrome c in aqueous solution. Liquid-ELDI analyses simply require irradiation of the surface of the sample solution with a pulsed ultraviolet laser; the laser energy is adsorbed by the carbon powder presuspended in the sample solution; the absorbed laser energy is then transferred to the surrounding solvent and to the analyte molecules in the solution, leading to their desorption; the desorbed gaseous analyte molecules are then postionized within an electrospray (ESI) plume to generate ESI-like analyte ions.

The development of analytical techniques for continuous monitoring of the states of ongoing chemical and biochemical reactions occurring in various solvents has been a formidable challenge for analytical chemists. Because most reactions occur in liquid phases (aqueous solutions for most biochemical reactions,

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10.1021/ac800952e CCC: $40.75 \odot$ 2008 American Chemical Society Published on Web 09/20/2008

organic solvents for most abiotic reactions), it is necessary for any developed technique to respond rapidly to changes in the states of the chemicals (including reactants, intermediates, and products) present in solution. Other preferable requirements for such an analytical technique include (1) operation under ambient conditions, (2) simultaneous response to multiple components, (3) no sample pretreatment, and (4) no response to solid materials, such as catalysts, in the sample solution. Most previously existing techniques are based on measuring changes in the spectroscopic or electrochemical characteristics of the chemical compounds involved in the reaction.¹⁻⁴ One of the disadvantages of using these techniques is that structural characterization of the individual compounds involved in the reaction is nearly impossible, especially when the solution comprises mixtures of organic or biological compounds.

Although mass spectrometry is an extremely useful technique for characterizing small organic and large biochemical compounds, several sample pretreatment steps are usually required to effectively ionize them: heating or drying a sample solution for the analyte to be ionized in the gaseous state under vacuum (e.g., electron impact ionization and chemical ionization (;^{5–8} dissolving the analyte in a suitable organic solvent or acidic solution to generate ions under a high electric field or through heating [e.g., electrospray ionization (ESI) and atmospheric pressure chemical ionization];^{9–12} or mixing the analyte with a matrix to increase

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Analytical Chemistry, Vol. 80, No. 20, October 15, 2008 7699

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the efficiency of desorption and ionization (e.g., fast atom bombardment and matrix-assisted laser desorption/ionization).^{13–16} These pretreatment processes make it extremely difficult to use mass spectrometry to continuously monitor the ongoing state of a chemical reaction under ambient conditions.

Recently, the development of ambient ionization methods [e.g., electrospray laser desorption/ionization (ELDI) and desorption electrospray ionization (DESI)] has made it unnecessary to heat the sample solution or mix it with a matrix; nevertheless, only solid samples are suitable for such analysis, meaning that drying of the sample remains inevitable prior to ionization.^{17–22} An ideal approach toward continuously monitoring the stages of ongoing reaction would be to desorb and ionize the analyte molecules directly from the solutions without drying the sample. Herein, we report a modified ELDI technique—liquid ELDI—that allows analyte ions to be generated directly from organic solvents and aqueous solutions.^{21–26}

In essence, the liquid ELDI technique presented herein is a combination of two ionization methods: ELDI and surface-assisted laser desorption/ionization (SALDI) operated under ambient conditions.^{20–22,27–30} The possible mechanisms of ELDI and SALDI have been discussed previously.^{20,21,27} Recently, the principle of ELDI has been adapted in several techniques, including matrix-assisted laser desorption/ionization electrospray ionization and laser ablation electrospray ionization.^{31–33} In SALDI, a sample solution is mixed with an "inorganic liquid matrix" comprising a suspension of micrometer-sized carbon powder in a mixture of glycerol, sucrose, and methanol.^{27,28} Because SALDI

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is performed under vacuum, the sample solution must be mixed with a viscous liquid matrix, such as these glycerol/sucrose solutions, to prevent it from drying in the source. It has been proposed that, in SALDI, the carbon powder is the energy-transfer medium coupling the laser UV energy into the molecules in the viscous liquid solution under vacuum.^{27,28} For liquid ELDI, the laser energy is first adsorbed by the fine carbon particles suspended in the sample solution; the energy is subsequently transferred to the solvent and the analyte molecules in the sample solution; the desorbed analyte molecules are then postionized through ESI processes. In this study, we applied liquid ELDI combined with mass spectrometry to monitor the progress of several ongoing chemical and biochemical reactions in different solution.

EXPERIMENTAL SECTION

Hemin chloride, cytochrome c, trypsin, 1,10-phenanthroline (PA), nickel chloride, copper sulfate, iron(II) diammonium disulfate, and ethylenediaminetetraacetic acid (EDTA), and the organic solvents (HPLC grade) were purchased from Sigma or Aldrich (Milkaukee, WI) and used without further purification. Carbon graphite powder was purchased from Merck (Darmstadt, Germany). The synthesis of magnetic nanoparticles coated with trypsin has been described previously.^{34,35}

The sample preparation process for liquid ELDI-MS analysis is extremely simple: merely suspend a small amount of the fine carbon powder (4 mg/mL) in the sample solution and then subject it to analysis without any further sample pretreatment. Commonly, a small amount of the sample solution $(5-10 \ \mu L)$ deposited on an acrylic sample plate [5 (L) \times 2 (W) cm] using a micropipet was sufficient for complete liquid ELDI analysis. The sample plate was positioned on an acrylic plate placed on a XYZ-stage, which was set in front of the sampling capillary (inlet) of an ion trap mass spectrometer. The surface of the sample solution was then exposed to a pulsed nitrogen laser operated at a wavelength of 337 nm (Physical Science), a frequency of 10 Hz [controlled by a sweep function generator (model FG-101A, Kowa, Taiwan)], a pulsed energy of $\sim 150 \mu$ J, and length of 4 ns. The laser beam (spot size: $\sim 100 \ \mu m \times 150 \ \mu m$) was focused through an objective lens. The strongest ion signal (using cytochrome c as the standard for evaluation) was obtained at an incident laser angle of $\sim 45^{\circ}$ and a focal length of ~ 25 cm.

The configuration of the ESI source for liquid ELDI was similar to those used in solid ELDI, pyrolysis/ESI, and fused-droplet ESI sources.^{20–22,36–39} The capillary electrosprayer was aligned parallel to, and ~3.5 mm above, the acrylic sample plate. The ESI plume was directed toward the ion sampling orifice of the mass analyzer (i.e., parallel to the sample plate). The electrospray needle and the sample plate potentials were both held at 0 V (grounded);

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the sampling cone voltage in the ion trap mass spectrometer was maintained at -4.5 kV. The electrospray solutions comprised methanol/water mixtures with or without acetic acid; a typical composition of the ESI solution used in the ELDI analysis was 80% MeOH/H₂O. The ESI solution was delivered through a capillary by a syringe pump at a flow rate of 2 μ L/min. A nebulizing gas, commonly used in conventional ESI sources, was not used during ELDI. The ions generated in this manner were further analyzed using a quadrupole/time-of-flight (Bruker Daltonic, Bio-TOF-q, Billerica, MA) or an ion trap (Bruker Daltonics, Esquire 3000 plus) mass analyzer. The mass spectra were recorded at a scan rate of ~2 s/scan.

In this study, four chemical reactions were performed in a range of organic solvents in glass beakers, with ELDI-MS used to continuously monitor the extent of each chemical's ongoing transformation. To continuously monitor the ongoing state of the reactions 1-3 (see the Experimental Section below), the surface of the solution containing reactants and carbon powder in a beaker was continuously irradiated with an UV laser beam. The laser-ablated molecules subsequently entered into an electrospray plume and were ionized there. For reaction 4, every minute, a drop of the solution was withdrawn (through a dropper), deposited on the acrylic sample plate, and rapidly analyzed using liquid ELDI-MS.

Reaction 1. Epoxidation of 1,3-Diphenyl-1-propen-3-one (Chalcone) in Ethanol.^{40,41} An ethanol solution (3 mL) containing chalcone (10^{-3} M) and carbon powder (4 mg/mL) was stirred for 1.7 min and then the catalyst, H₂O₂ (30% aqueous solution; 200 μ L) was added. Because this catalyst functions only under basic conditions, 1 M NaOH (200 μ L) was added to catalyze the peroxidation reaction. The solution was continuously stirred with a magnetic bar while adding the reagent and catalyst solution.

Reaction 2. Chelation of EDTA with Copper and Nickel Ions in Aqueous Solution.⁴² An aqueous solution (3 mL) of EDTA (1.5×10^{-3} M) and carbon powder (4 mg/mL) in a beaker was stirred continuously with a magnetic bar while CuSO₄ solution (1.5×10^{-2} M, 100 μ L) was added. After 20 s, a NiCl₂ solution (1.5×10^{-2} M, 100 μ L) was added to the mixture.

Reaction 3. Chelation of 1,10-Phenanthroline with Iron(II) in Methanol.⁴³ A methanol solution (3 mL) containing PA (1.5×10^{-3} M) and carbon powder (4 mg/mL) was stirred continuously with a magnetic bar while aqueous (NH₄)₂Fe(SO₄)₂ solution (2.5×10^{-2} M, 100 μ L) was added.

Reaction 4. Tryptic Digestion of Cytochrome *c* **in Aqueous Solution.** A solution of cytochrome *c* (10^{-4} M) and fine carbon powder (4 mg/mL) was mixed with a solution of magnetic nanoparticles coated with trypsin ($2.5 \ \mu g/\mu L$), and the solution was continuously stirred with a magnetic bar.

RESULTS AND DISCUSSION

Figure 1a illustrates the possible desorption/ionization processes occurring during liquid ELDI. Unlike SALDI, the liquid

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Figure 1. Liquid ELDI system. (a) Schematic representation of the desorption and ionization of molecules dissolved in liquid during liquid ELDI. a, ESI capillary; b, Taylor cone; c, charged ESI droplet; d, laser beam; e, analyte ions; f, desorbed analyte; g, glass tube; h, sample plate; i, carbon powder; j, stirring bar; k, magnetic stirrer. (b, c) Photographic images of the liquid ELDI system displaying (b) the electrospray plume and (c) the mixing of the analyte droplets (generated through laser beam irradiation of a methanol solution containing carbon powder) with the electrospray plume.

ELDI technique is performed under ambient conditions; no viscous liquid matrix is necessary: the aqueous sample solution itself is used as the medium for transferring the adsorbed laser energy from the carbon particles to the analyte molecules. This feature greatly simplifies the sample pretreatment process.

Panels b and c in Figure 1 display photographic images of the electrospray plume only (laser off) and the mixing of the analyte droplets (generated by irradiating the sample solution containing carbon powder with an UV laser beam) with the electrospray plume, respectively, in the liquid ELDI system. We found that, as the surface of the sample solution was continuously irradiated with the UV laser beam, a stable fine mist was generated from the surface of the sample solution. The mist moved upward initially and then turned 90° toward the inlet of the mass analyzer as it joined the ESI plume.

Direct analysis of a hemin solution (in methanol) without carbon powder revealed that the solution was transparent to the UV laser light: no hemin signal (m/z 616) was detected (Figure 2a). Only after we had added 4 mg/mL or more of the carbon powder into the sample solution did we observe the molecular ion of hemin (Figure 2b). The intensity of the analyte ion signals increased upon increasing the amount of carbon powder added to the sample solution, reaching a plateau at concentrations of carbon powder of 8 mg/mL and higher (Figure 2c).

The detection of the hemin ion signal directly from the methanol solution when carbon powder was present indicates that the laser energy was adsorbed by the carbon powder and transferred to the surrounding methanol and hemin molecules, leading to their desorption. It seems that the liquid that dissolves the analyte plays no role in desorption or ionization of the analyte—apart from being used as the energy-transfer medium. Therefore, chemical compounds dissolved in the liquid medium,



Figure 2. Positive liquid ELDI mass spectra of hemin (m/z 616, M⁺) dissolved in methanol (MeOH) (10⁻⁴ M, 10 μ L) containing carbon powder at concentrations of (a) 0, (b) 4, and (c) 8 mg/mL.



Figure 3. Liquid ELDI spectra of small molecules. Positive-ion liquid ELDI mass spectra of (a) hemin (m/z 616, M⁺) dissolved in methanol (MeOH), (b) ¹⁸crown-6 ether (m/z 287 [MNa⁺] and m/z 303 [MK⁺]) dissolved in THF, (c) 1-hexadecylamine (m/z 242 [MH⁺]) dissolved in EA, (d) methyl(triphenylphosphoranylidene) acetate (m/z 335 [MH⁺]) dissolved in dichloromethane, (e) cinnamic acid benzyl ester (m/z 261 [MNa⁺]) dissolved in toluene, (f) cetylpyridium chloride (m/z 304, [M - Cl]⁺) dissolved in hexane, (g) C₄₀H₂₈IrN₄·PF₆ (m/z 757 [M - PF₆]⁺) dissolved in dichloromethane. The insets to (g) and (h) display the isotope patterns of the molecular ions containing Ir.

which may include hydrophilic or hydrophobic and volatile or nonvolatile organic solvents, can be characterized using ELDI.

To test this hypothesis, we performed liquid ELDI analyses of six organic compounds dissolved in a range of polar and nonpolar organic solvents, namely, MeOH, tetrahydrofuran (THF), ethyl acetate (EA), dichloromethane, toluene, and hexane. Pure methanol was used for the electrospray to ionize the molecules desorbed by the UV laser. As expected, we detected the molecular ions of hemin (M^+ , m/z 616), ¹⁸crown-6 (MNa^+ , m/z 287; MK^+ , m/z 303), 1-hexadecylamine (MH^+ , m/z 242), methyl(triphenylphosphora-



Figure 4. Using liquid ELDI to continuously monitor the state of the epoxidation of chalcone in ethanol. Positive-ion liquid ELDI mass spectra were recorded at three different stages: (a) immediately after H_2O_2 had been added into the sample solution and (b) immediately and (c) 1 min after the sample solution had been changed to basic through the addition of 1 M NaOH. (d) Extract ion chromatograms of the ions at m/z 209 ($[M_1H]^+$ for the reactant chalcone) and 247 ($[M_2Na]^+$ for the product).

nylidene) acetate (MH⁺, m/z 335), cinnamic acid benzyl ester (MNa⁺, m/z 261), and cetylpyridium chloride ([M - Cl]⁺, m/z304) (Figure 3a-f). To test whether compounds having higher molecular weights could also be detected, we synthesized two inorganic compounds containing iridium—C₄₀H₂₈IrN₄·PF₆ ([M -PF₆]⁺, m/z 757) and C₃₃H₂₆F₄IrN₆·PF₆ ([M - PF₆]⁺, m/z775)—and subjected them to liquid ELDI analysis. Again, we detected the molecular ions of both compounds from their CH₂Cl₂/carbon powder solutions. The insets to Figure 3g and h clearly display the isotope patterns expected for these two molecular ions.

We expected, therefore, that liquid ELDI could be used to continuously monitor many of the chemical reactions that occur in organic solvents under ambient conditions. Figure 4 displays the results obtained when using liquid ELDI-MS to monitor the epoxidation of chalcone (1,3-diphenyl-1-propen-3-one).^{34,35} The reaction was performed in a small glass beaker using an ethanol solution containing carbon powder. We found that, even through the chalcone solution was stirred with a magnetic bar, a stable total ion current from the reactant ions (chalcone; m/z 209 for M₁H⁺; m/z 231 for M₁Na⁺, Figure 4d) was obtained when the surface was irradiated continuously with the UV laser pulse. The catalyst, H₂O₂, was added into the solution 1.7 min after the onset of stirring, resulting in the generation of gas bubbles and an abrupt

increase in the intensity of the reactant ion $(m/z \ 209)$; Figure 4a and d). Because the catalyst functions only under basic conditions, we did not detect any ion signal from product-1,3-diphenyl-1,2epoxy- propan-3-one (M₂Na⁺: m/z 249; see Figure 4a and d; M₂, epoxided chalcone molecule) at this stage. Only after making the solution basic, by adding NaOH solution (1 M, 200 μ L) 2.4 min after the onset of stirring, did we detect the ion of the reactant (Figure 4c and d). The decrease in intensity of the ion signal of the reactant coincided with the increased intensity of the product. Figure 4d indicates clearly that the state of an ongoing reaction can be evaluated continuously by monitoring the changes in the intensities of the ion signals of the reactant (M_1) and product (M_2) over time. However, due to certain mixing and diffusion time is required to bring the reactant, reagent, and catalyst together, the ion signal of reactant did not fall abruptly as the product signal rises.

Figure 5 displays the results obtained when using ELDI-MS to monitor the chelation of EDTA with copper and nickel ions in aqueous solution. A glass beaker was charged with a solution (3 mL) of EDTA (1.5×10^{-3} M) and carbon powder (4 mg/mL). For ELDI analysis, the solution was stirred while the surface was irradiated continuously with the UV laser pulse; a stable extract ion current was obtained from the negatively charged reactant ions (EDTA; m/z 291 for [M – H]⁻; Figure



Figure 5. Continuously monitoring the state of the chelation reactions of EDTA with copper and nickel ions in aqueous solution. (b) Extract ion chromatograms of the reactant at m/z 291 ([EDTA - H]⁻) and the product at m/z 352 ([EDTA + Cu - H]⁻) and 347 ([EDTA + Ni - H]⁻). Negative-ion liquid ELDI mass spectra were recorded at three different stages: (c) EDTA solution only, (d) 10 s after adding CuSO₄ (1.5 × 10⁻² M, 100 μ L) solution, and (e) 20 s after adding NiCl₂ (1.5 × 10⁻² M, 100 μ L) solution.



Figure 6. Continuously monitoring the state of the chelation reaction of PA with iron(II) in methanol. (a) Extract ion chromatograms of the reactant at m/z 181 ([PA)H]⁺) and product at m/z 298 ([(PA)₃ + Fe]²⁺). Positive-ion liquid ELDI mass spectra were recorded at three different stages: (d) PA solution only, (e) 6 s after adding (NH₄)₂Fe(SO₄)₂ (2.4 × 10⁻² M, 100 μ L) solution, and (f) 30 s after adding (NH₄)₂Fe(SO₄)₂ solution. The reaction was performed using a buffer solution at pH 10. The inset to (f) displays the isotope pattern of the molecular ion of [(PA)₃ + Fe]²⁺.

5b). The ELDI mass spectrum recorded from the aqueous solution featured the $[M - H]^-$ ion of EDTA predominantly (Figure 5c). Immediately after CuSO₄ solution was added into the EDTA solution, a stable signal for the EDTA·Cu complex ion (*m*/*z* 352) was detected (Figure 5b). The ELDI mass spectrum featured the [EDTA]⁻ (*m*/*z* 291) and (EDTA·Cu)⁻ (*m*/*z* 352) ions predominantly (Figure 5d). A NiCl₂ solution was subsequently added into the solution now containing EDTA, EDTA·Cu, and Cu²⁺. We observed a stable signal for the [EDTA·Ni]⁻ ion (*m*/*z* 347). The ELDI mass spectra featured the [EDTA·Cu]⁻ and [EDTA·Ni]⁻ ions predominantly

(Figure 5e). The results in Figure 5 indicate clearly that the states of ongoing EDTA-copper and EDTA-nickel chelation reactions can be evaluated continuously by monitoring the changes in the intensities of the signals of the $[EDTA\cdotCu]^-$ and $[EDTA\cdotNi]^-$ ions over the time.

We also used liquid ELDI/MS to monitor the reaction of PA with iron(II) in methanol. We recorded positive-ion ELDI mass spectra as the chelating reaction proceeded in a methanol/carbon powder solution. Panels b and c in Figure 6 present extract ion chromatograms of the reactant at m/z 181 [(PA)H]⁺ and the product at m/z 298 [(PA)₃ + Fe]²⁺. The mass spectra in Figure



Figure 7. Using liquid ELDI to monitor the state of a tryptic digestion. Positive-ion liquid ELDI mass spectra recorded from sample droplets collected (a) 0, (b) 15, and (c) 30 min after mixing a solution containing trypsin-coated magnetic nanoparticles with a cytochrome *c*/carbon powder solution. \bullet , Multiply charged cytochrome *c* ions; \bigcirc , peptide ions from cytochrome *c*.

6d-f reveal three different stages of the reaction: (d) the PA solution only and (e, f) the mixtures obtained (e) 6 s and (f) 20 s after adding (NH₄)₂Fe(SO₄)₂ into the PA solution. The inset to Figure 6f displays the isotope pattern of the molecular ion of [(PA)₃ + Fe]²⁺.

The progress of ongoing biochemical reactions-in this case, the tryptic digestion of cytochrome *c* in aqueous solution–can also be monitored using liquid ELDI. In this example, we mixed an aqueous solution containing a cytochrome c standard (10^{-4} M) with the fine carbon powder. Magnetic nanoparticles coated with trypsin were then added into the solution to digest the protein in the solution.^{36,37} Every minute, a drop of the solution was withdrawn (through a dropper), deposited on the acrylic sample plate, and rapidly analyzed using ELDI. Figure 7 displays representative ELDI mass spectra obtained from sample droplets collected after 0, 15, and 30 min, respectively. We observe a gradual increase in the peptide ion signals upon increasing the digestion time. Interestingly, as the reaction proceeded, the charge state of cytochrome c ion was changed to lower number (from +9 to +7). It seems that the presence of the peptides originated from cytochrome c in the sample solution will deprotonate the cytochrome c ion during ionization. The ELDI mass spectra display no interference from any ions derived from trypsin; this phenomenon is probably due to the magnetic nanoparticle-bound trypsin not being desorbed by the laser pulse or ionized in the ESI plume during ELDI.

CONCLUSION

We have demonstrated that ELDI can be used to characterize organic, inorganic, and biological compounds directly from liquids (including aqueous solution and polar and nonpolar volatile organic solvents) under ambient conditions. The presence of fine carbon particles in the sample solution assists the desorption of the analyte molecules during irradiation of the solution surface with a pulsed laser beam. The states of ongoing biochemical and organic reactions occurring in the solution can, therefore, be monitored continuously. Because this liquid ELDI technique utilizes an extremely simple sample pretreatment procedure and provides rapid analyses under ambient conditions, we hope that it will allow physical organic chemists and biochemists to monitor a wide variety of reactions through continuous monitoring of the changing ion intensities of their reactants, intermediates, and products. Although it is not demonstrated in this paper, obtaining useful kinetic information about a typical chemical reaction is possible if suitable internal standards are used for quantification of the reactants and products and the lifetime of the chemicals (especially for intermediates) involved in the reaction is long enough to be detected.

Received for review May 8, 2008. Accepted August 13, 2008.

AC800952E