Pergamon

Tetrahedron Letters, Vol. 37, No. 32, pp. 5669-5672, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01201-4

Surface Arrays of Energy Absorbing Polymers Enabling Covalent Attachment of Biomolecules for Subsequent Laser-Induced Uncoupling/Desorption

Kamen I. Voivodov[1,2], Jesus Ching[2], and T. William Hutchens[2]*

[1]Molecular Analytical Systems, 2121 Sage Road, Suite 280, Houston, TX 77056
and, [2]University of California, 110 FSTB, Davis, CA 95616

Abstract: Synthetic polymers with desirable film-forming characteristics have been chemically modified to incorporate, covalently, UV energy-absorbing molecules (EAM) of a type used for matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry. The polymer-bound EAM were activated to covalently bind macromolecules *through the carboxyl group of the bound EAM.* The EAM was shown to act as a photolabile macromolecular tether. Biomolecules covalently bound to surface arrays of EAM-polymer were uncoupled and desorbed/ionized with single pulses of laser irradiation. Copyright © 1996 Elsevier Science Ltd

New strategies for biopolymer detection and structure evaluation continue to evolve with enabling technologies that are surface based. We report here the *laser-induced* desorption of biomolecules after covalent attachment to surface arrays of polymeric energy absorbing molecules.

Pulsed laser irradiation is an energy-depositing mechanism that has already been used to induce the desorption of structurally intact biopolymers from probes with chemically defined molecular adsorption/desorption surface properties[1]. The process introduced in 1993, known as surface-enhanced laser desorption/ionization (SELDI)[1] or affinity mass spectrometry[2], was based on the use of new probe material compositions and/or defined surface chemistries. In one category of process identified as surface-enhanced affinity capture (SEAC), chemically-defined and biologically-defined affinity capture devices are covalently bound directly to the modified probe surfaces[1]. Only the captured target biomolecules, docked on the probe surface through *noncovalent* molecular recognition events, are selectively desorbed by laser irradiation. Recently, our laboratory was able to accomplish, for the first time, the covalent attachment of biopolymers to a probe surface with tether chemistries enabling single pulses of laser irradiation to initiate subsequent *uncoupling and desorption/ionization* of the bound biomolecules[3]. This advance in laser-induced desorption capabilities revealed several new possibilities for the development of solid-phase bioconjugate chemistries for the investigation of biopolymer structure.

We have now explored an entirely different approach to the design and synthesis of surfaces with the desired energy absorbing and photolytic tethering capabilities. Polymeric probe surface constructed with energy absorbing molecules (EAM), developed to enable laser-induced desorption of macromolecules presented alone (i.e., neat) to the surface, have been modified to involve the covalent bound EAM as tethers for the covalent coupling and subsequent photon-induced release of target biomolecules.

EXPERIMENTAL METHODS

Covalent incorporation of energy-absorbing molecules as the photolytic component of a polymeric tether-probe device. Poly(1-octadecene-*co*-maleic anhydride) (POMA; M_n 30,000-50,000, Aldrich) was incubated in

methyl ether with the UV energy absorbing molecule α-cyano-4-hydroxycinnamic acid (CHCA) in the presence of triethylamine (20 h at 25°C). Copolymer modified with CHCA (POMA-CHCA) was purified and analyzed by ^{1}H NMR, UV/VIS spectroscopy, and evaluated by laser desorption/ionization time-of-flight mass spectrometry. The covalent attachment of CHCA to POMA shifts the absorbance maximum of CHCA from 327 to 343 nm in contrast to CHCA simply adsorbed to POMA.

Photolabile tether construction: activation of polymer-bound CHCA with a bioconjugate component for covalent attachment of the target biomolecule. POMA-CHCA was mixed with 1,3-diisopropylcarbodiimide (Aldrich) in THF and continuously stirred for 30 min at 25°C. The activated product was separated by centrifugation, washed several times with excess THF and dispersed into small particles. Peptide (21-residue histidine-rich glycoprotein fragment) was dissolved in THF and added to the polymer suspension. After mixing for 48 h at 25°C, the final product was pelleted, washed with THF, and refrigerated.

Photo-induced uncoupling/desorption and time-of-flight mass analysis. Probe platform preparation and mass analyses were performed as described previously[3]. Mass values was calculated based on the use of internal calibrants (e.g., 1954.9 Da) and by comparison with external calibrants after desorption in the SEND (surface-enhanced neat desorption) mode (i.e., without matrix)[1]. The polymer film probe surface with covalently coupled target peptide was formed from a methanol solution. Internal mass calibration standards and matrix (either 4 or 100 mM 3,5-dimethoxy-4-hydroxycinnamic acid in 50% aqueous acetonitrile) were added to the polymer-based probe surface. The probe surface was air-dried before laser irradiation *in vacuo* (10^{-7} torr). Spectra shown (ave 5-20 pulses) were collected with a N_2 laser (337 nm) operating with 3-ns pulses attenuated to 5-15 μJ output.

RESULTS AND DISCUSSION

A wide variety of surface-based chemical array technologies would be improved if it were possible to covalently tether target molecules so that they may be subsequently *uncoupled and desorbed simultaneously* by laser irradiation alone[3]. The optimal biomolecular-surface tether for these purposes should employ a photolytic reaction mechanism that is highly efficient and homogeneous. We chose to construct photolabile tethers derived from chemically modified energy absorbing molecules of the type reported to assist laser-induced macromolecular desorption/ionization, either in the MALDI mode or bound to the probe surface for operation in the SEND mode[1]. We have synthesized probe surfaces from film-forming polymers with covalently bound energy absorbing molecules using derivatives of α-cyano-4-hydroxycinnamic acid, introduced originally as a "matrix" molecule[4] for MALDI. We have found the covalently bound EAM to undergo laser-induced photolytic cleavage/release events that are both predictable and reproducible. This new polymer, developed for SEND applications, was evaluated as a foundation to prepare photoresponsive arrays of surface docking sites for target biomolecules.

We determined whether the utility of polymer-bound EAM could be expanded to include a functional role as covalent tethers for biomolecular docking by chemical modification of the polymeric EAM (Fig. 1A) to introduce suitable leaving groups to enable covalent biomolecule attachment. The intent was to develop the polymer-bound EAM as a tethering device without loss of the energy absorption/photolytic characteristics necessary to accomplish a subsequent photon-induced uncoupling reaction that would occur simultaneously with desorption/ionization. A separate goal was to use the covalent polymer-EAM-biomolecule assembly to present bound target biopolymers in predetermined arrays (Fig. 1B) on a variety of different probe surfaces, without prior chemical derivatization.

The biomolecule chosen as a model was covalently attached to the polymer directly through the carboxyl group of the EAM (α-cyano-4-hydroxycinnamic acid) after the EAM was covalently bound to the polymer by reaction of its phenoxy group with maleic anhydride in POMA. We have found the ester bond formed upon reaction of the EAM phenoxy group with POMA to be photolabile and to enable the surface bound biomolecule to undergo laser-induced uncoupling and desorption/ionization.

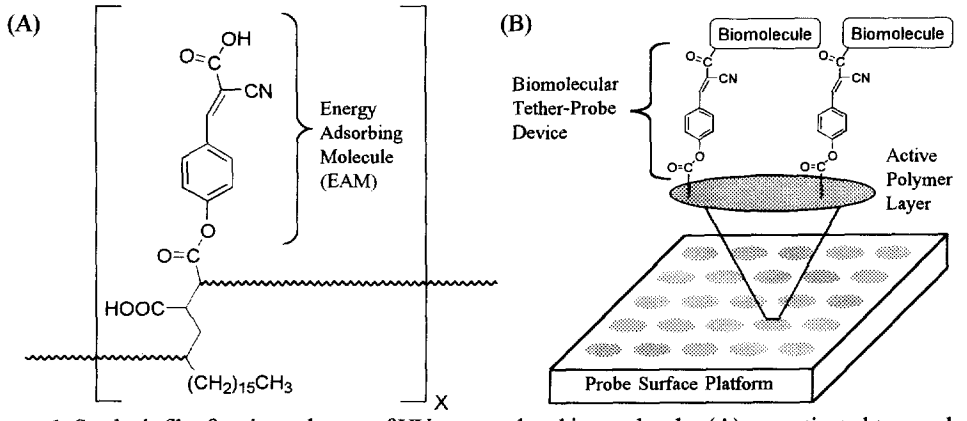

Figure 1: Synthetic film-forming polymers of UV energy absorbing molecules (A) are activated to covalently bind biomolecules for presentation in defined arrays (B) for laser-induced uncoupling/desorption.

Figure 2 presents direct evidence for the covalent attachment of target peptides through the carboxyl group of the polymer-bound EAM. Acid cleavage (2% TFA) of the purified, covalent POMA-EAM-peptide complex was used to investigate the covalent binding chemistries involved in biomolecule attachment. The model peptide alone, before covalent attachment to the EAM-polymer, has a molecular mass of 2337.4 Da. Acid treatment of the covalently tethered polymer-EAM-peptide complex resulted in cleavage of the ester bond linking the polymer to the EAM-peptide (Fig. 2A). The increase in peptide mass observed after acid cleavage of the covalent polymer-EAM-peptide complex was within 1 to 2 Da of the mass increase predicted (171 Da) by the reaction of the primary amino group at the N-terminus of the peptide with the activated carboxyl group of the α-cyano-4-hydroxycinnamic acid bound to the polymer surface through an ester linkage at the phenoxy group (see Fig. 1). Smaller quantities of peptide were detected without a bound EAM (2338.6 Da) or with two bound EAM (2679.4 Da).

The polymer-EAM-peptide was next evaluated to determine whether the photolabile nature of the phenoxy ester bond linking the EAM to the polymer on the probe surface was preserved after covalent attachment of the peptide. Figure 2B shows the mass spectra obtained by laser irradiation of the covalent polymer-EAM-peptide complex *without prior chemical cleavage*. The desorbed/ionized peptides were detected primarily as covalent EAM-peptide conjugates. Although some peptide was uncoupled/desorbed without any bound EAM (2338.7 Da), the major peptide species typically observed had a mass (2508.6 Da) consistent with its covalent attachment through one EAM followed by photon-induced cleavage of the phenoxy ester linkage used to bind the EAM to the polymer (see Fig. 1). Some peptide was observed to be uncoupled/desorbed with two bound EAM (2679.4 Da).

One way to exploit the benefits of laser desorption/ionization methods for biological mass spectrometry and to eliminate interference from matrix and other contaminants during interim analyte modification/recognition events

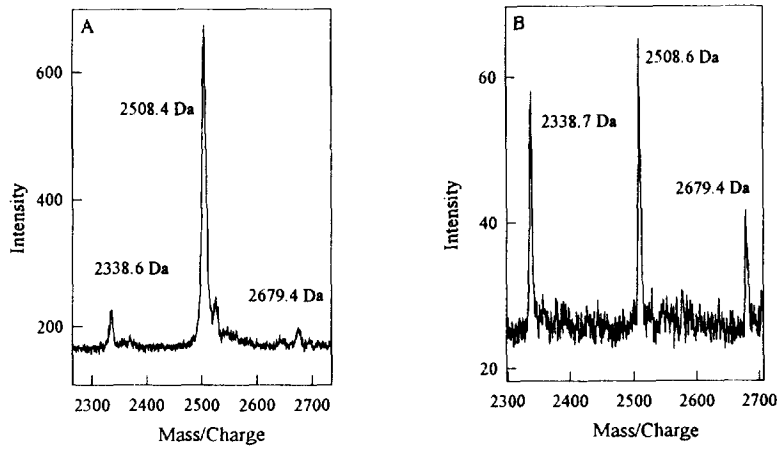

Figure 2: Laser-induced desorption/ionization time-of-flight mass spectra showing EAM-modified peptide released from the covalent polymer-EAM-peptide conjugate by chemical cleavage with TFA (A) or by photon-induced uncoupling only (B), *without prior treatment with TFA*. Values shown represent $[M+H]^+$.

conducted *in situ* is to covalently couple biopolymer analytes directly to probe surfaces. We have accomplished this by converting bound energy absorbing molecules on a SEND polymer into covalent tethers for biomolecules. Although surface-enhanced photolabile attachment and release (SEPAR) has been developed using a variety of photolytic bonds incorporated within the covalent tethering device[3], before now, the use of an operational EAM to achieve the covalent attachment of biopolymers to the probe surface has not been reported. Furthermore, the generation of "signature peaks" (i.e., reporter tail) broadens the application potential of SEPAR and affinity mass spectrometry beyond that possible with uncoupling procedures not resulting in direct verification of covalent attachment[5]. We are now exploring the ability of probe surfaces defined by the polymer-EAM-peptide described here to promote laser-induced desorption of the tethered biomolecules without added matrix (in preparation).

These results, involving defined chemistries for presentation of covalently bound analyte can be useful not only to explore ways to eliminate the ambiguities and uncertainties associated with the random process of matrix-dependent distribution of analytes on a probe surface but also to improve our understanding of the chemistry and physics of successful photon-induced desorption/ionization events involving large non-volatile macromolecules.

Acknowledgment: This work was supported, in part, by the NIH (R41GMS1658-01) and by Hewlett-Packard.

REFERENCES

1. Hutchens, T. W.; Yip, T. T. *Rapid Commun. Mass Spectrom.* **1993**, 7, 576-580.
2. Hutchens, T. W.; Yip, T. T. *Protein Science* **1994**, 3; Suppl 1; 81 (abstr 170-S), 1994.
3. Ching, J.; Voivodov, K. I.; Hutchens, T. W. *J. Org. Chem.* **1996**, 61, 3582-3583.
4. Beavis, R.C.; Chaudhary, T.; Chait, B.T. *Org. Mass Spectrom.* **1992**, 27, 156-158.
5. Fitzgerald, M.C.; Harris, K.; Shelvin, C.G.; Suizdak, G. *Bioorg. Med. Chem. Lett.* **1996**, 6, 979-982.

(*Received in USA* 2 *May* 1996; *accepted* 13 *June* 1996)