Surface Chemistries Enabling Photoinduced Uncoupling/Desorption of Covalently Tethered Biomolecules

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The development of new chemistries for the covalent but reversible attachment of macromolecules to well-defined inorganic surface structures is being driven by the expanded realization of opportunities to investigate and exploit molecular recognition[1,2] of the surface-bound molecules. Molecules of interest that are "permanently" tethered to a probe surface may be exposed to a wide variety of environments or subjected to physical/chemical perturbations that would dissociate molecules simply adsorbed through noncovalent interactions.

The overall goal of this investigation was to better define the chemistries needed to build nanoscale probe surface architectures with molecular devices functioning to bind and release covalently tethered molecules in defined areas on demand. We have focused on the use of coherent light to control the molecular release function. We also desired a mechanism to investigate directly the number, type, and chemical nature of surface residues involved in the covalent immobilization of proteins and other macromolecules to these surfaces.

An objective was to explore the photolytic efficiency and photochemical reaction homogeneity of bonds involving azobenzene derivatives[3] for the purpose of designing photolabile tethers that would enable simultaneous uncoupling/desorption upon laser irradiation.

The photolytic component chosen for the construction of this device, 4,4′-azodianiline (ADA), was incorporated at the end of a relatively short spacer arm that was terminated with carbonylimidazole as a suitable leaving group for covalent attachment of molecules (e.g., peptides) with reactive nucleophile(s) (Figure 1). The model peptide chosen represents an interesting portion of the metal-binding domain in a protein referred to as histidine-rich glycoprotein (HRG).[4] The peptide sequence is defined by a five-residue histidine-rich arrangement (GHHPH) repeated four times.

The peptide was introduced to an array of ADA tether-probe devices on a silica-based probe surface for evaluation of laser-induced uncoupling. An extensive surface wash procedure followed covalent coupling to remove all noncovalently bound material. Subsequent laser irradiation revealed several different photolytic events. In each case, uncoupling of the covalently tethered biomolecule was achieved, together with desorption, by irradiation with a single laser pulse (3 ns). The light-dependent

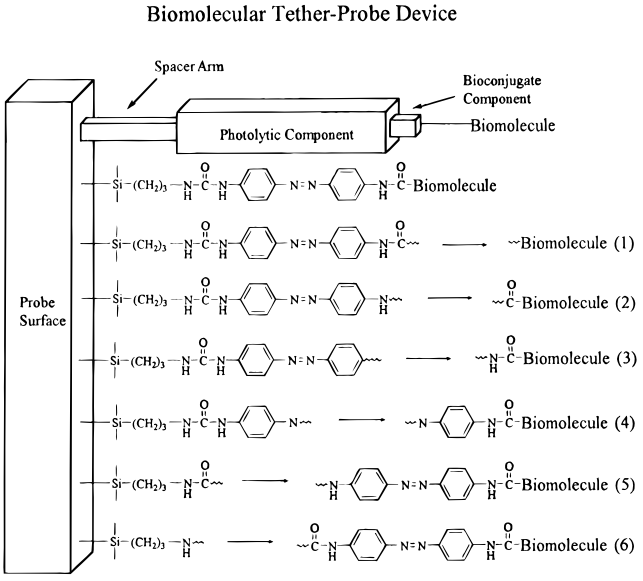

Figure 1. Schematic design of photolabile tether-probe device for covalent attachment of target molecules to silica-based probe surfaces. ADA was incorporated as the photolytic component within a spacer arm terminated with carbonyldiimidazole as the bioconjugation component for covalent attachment of molecules (e.g., peptides). The detailed chemical structure of the intact ADA tether-probe device is shown along with each of the six (6) different photochemical reaction products and dissociated molecules observed as a result of photolytic cleavage.

release of the tethered biomolecules was characterized, in most cases, by an increase in mass of the uncoupled/desorbed biomolecule that allowed direct verification of the photolytic cleavage site within the tether-probe device (Figure 2). Results such as those shown in Figure 2A were typical; photolytic reaction products represented by peaks labeled (1) and (5) were most predominant (n = 12). In some experiments, the total number and predominant species of photolytic cleavage products appeared to vary, with peak (6) becoming the predominant species (Figure 2B, top). Although only two major photolytic reactions products were typically observed (i.e., peak 1 together with either peak 5 or 6), in some cases (n = 5), only a single photolytic cleavage product was observed (Figure 2B, bottom). Factors most likely to affect the type of desorbed product observed upon photon-induced uncoupling include laser power, irradiation wavelength, and type of matrix preparation used to promote desorption/ ionization. A detailed investigation of these factors is underway.

The chemical remnants of tether left covalently attached to the dissociated biomolecule provide direct evidence of covalent linkage to the probe surface. The detailed chemical structure of the intact tether-probe device is shown along with each of the photochemical reaction products and dissociated molecules observed as a result of photolytic cleavage (Figure 1); calculated and observed mass values for these are presented in Table 1.

The mass values observed for each of the photolytic cleavage products identified were approximately equal (within 2 Da) to the mass values predicted based on the photochemical reactions illustrated in Figure 1. Note that the calculated mass values did not take into consideration the possible addition of one to two hydrogen atoms or protons at the site of photochemical cleavage. We believe that the difference in the observed and

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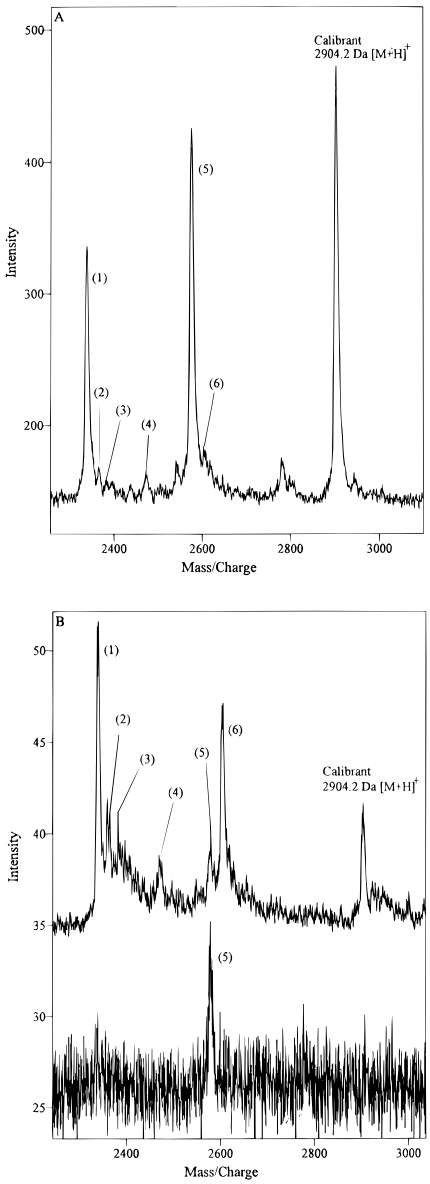

Figure 2. Molecular products generated by laser-induced uncoupling and desorption of covalently bound HRG 4-mer from the ADA tether-probe device. Observed mass values for the protonated pseudomolecular ions $[M + H]^+$ are shown; peaks are numbered to correspond to chemical structures shown in Figure 1. See the supporting information for additional details.

calculated mass values reflects such an event. These differences are not large enough to be explained by alternative photolytic cleavage events, but they may represent a time delay or some other physical event (e.g., altered initial kinetic energy) occurring within the high voltage source region during desorption, ionization, and acceleration of the uncoupled biomolecule.

Thus, the surface-enhanced photolabile attachment and release (SEPAR) procedure introduced here seems to be an excellent tool to evaluate surface-based photochemical reaction mechanisms and products. Observed differences in the number and type of individual species of photodissociated peptide can provide important information for the design and implementation of new photolabile linkage chemistries. Several other types of photolabile tethers are also under investigation.

Table 1. Mass Analysis of Covalently Bound Model Peptides after Laser-Induced Uncoupling/Desorption from Silica-Based Surfaces

ref no. (peak)	photodissociated biomolecule $[M + H]^+$ calcd[a] (Da)	Figure 2A $[M + H]^+$ obsd (Da)	Figure 2B $[M + H]^+$ obsd (Da)
1	2337.4	2338.2	2338.9
2	2365.4	2365.1	2365.0
3	2380.4	2382.6	2382.7
4	2470.4	2473.1	2471.1
5	2575.4	2576.8	2576.3
6	2603.4	2605.7	2605.1

[a] Calculated as a free radical (i.e., without hydrogen or proton addition) after photodissociation from the surface.

All of the molecules dissociated with a remnant of the tether ("tails") were covalently attached. In the present case, we observed evidence for a maximum of only one covalent linkage site per tethered molecule. This suggests to us the involvement of only the amino group in the N-terminus. If there were imidazole nitrogens (12 His residues) involved in covalent attachment and stable under the conditions imposed, we would have expected to see evidence for more than one attachment. If there was covalent attachment through one or more of the His residues, it was either unstable or transparent to us. Even if stable, the formation of a covalent bond involving the His residue followed by a photolytic cleavage event at the same site would likely produce a dissociated molecule without an increase in mass.

We believe the molecules dissociated as a "0-tail" species were indeed covalently bound to the probe surface. First, the existence of dissociated molecules with 1 tail is often observed without evidence for a 0-tail species. Second, we have conducted an exhaustive evaluation of wash/extraction conditions necessary to remove all but covalently bound biomolecules from the probe surface. The surface wash procedures described here effectively eliminate any detectable form of biomolecular attachment other than covalent binding. Specifically, we were the first laboratory to covalently attach biomolecules as affinity capture devices directly to mass spectrometer probe surfaces through *nonphotolabile* bonds;[5] this practice is now widespread, and considerable experience has been acquired. Removal of noncovalently adsorbed reagents and biomolecules from these types of probe surfaces typically requires less vigorous wash procedures than those employed here.

This is the first demonstration of surfaces enabling the covalent docking and laser-induced uncoupling/desorption of intact macromolecules through the use of photolytic tethers. This type of surface-based tether-probe device, operated by coherent light, will aid our ability to explore both molecular recognition and covalent modifications in biopolymer structure through chemical/enzymatic manipulations performed directly *in situ*.

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Supporting Information Available: Experimental procedures and additional details for Figure 2 (3 pages).

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