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Letters

Using MALDI-TOF Mass Spectrometry to Characterize Interfacial Reactions on Self-Assembled Monolayers

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This paper demonstrates that matrix-assisted laser desorption/ionization and time-of-flight mass spectrometry (MALDI-TOF MS) is very well suited for characterizing self-assembled monolayers (SAMs) of alkanethiolates on gold and, in particular, for analyzing chemical reactions on monolayers. Three examples establish the generality of MALDI-TOF for this purpose. The first example characterizes the Diels−Alder reaction of a cyclopentadiene to maleimide groups of a monolayer. The second example characterizes the immobilization of glutamine *tert*-butyl ester to a carboxylic acid terminated monolayer and subsequent removal of the *tert*-butyl group. The third example characterizes the exchange of alkanethiolates in a monolayer with alkanethiols in a contacting solution. The availability of commercial instruments for MALDI-TOF MS and the straightforward use of this technique for characterizing SAMs will make this an important analytical technique for programs that prepare and apply model substrates in chemistry and materials science.

Self-assembled monolayers (SAMs) of alkanethiolates on gold now provide a reliable and versatile synthetic methodology for tailoring the structures and properties of surfaces. SAMs have been instrumental in an exceedingly wide range of scientific studies and applications. These areas include wettability,[1] friction,[2] electron transfer,[3] interfacial reactivity,[4] molecular recognition,[5] cell adhesion,[6] and bioanalytical assays.[7] The widespread use of SAMs stems from the synthetic flexibility this surface chemistry permits in assembling well-defined yet complex surfaces. In practice, monolayers can be prepared either directly, by using terminally substituted alkanethiols, or alternatively by chemical elaboration of a preformed monolayer. For the latter cases, a battery of analytical methods are used to characterize these surface modifications, including ellipsometry, X-ray photoelectron spectroscopy, contact angle measurement, IR spectroscopy, and cyclic voltammetry, in part because no one method provides definitive information on surface structure.[8]

Mass spectrometry (MS) can provide direct information on the structure of a monolayer: the masses of the alkanethiolates.[9] Secondary ion mass spectrometry (SIMS)[10] and direct laser desorption/ionization mass

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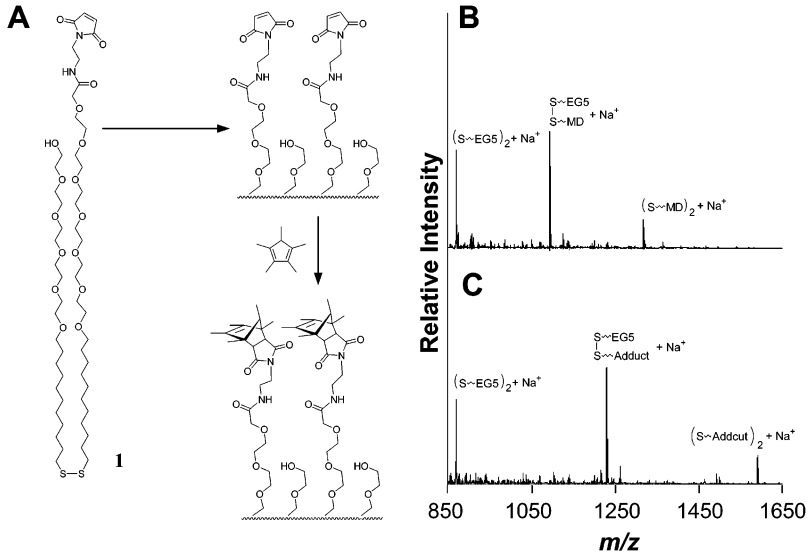

Figure 1. (A) A monolayer presenting maleimide (MD) and penta(ethylene glycol) (EG5) groups is prepared from disulfide **1**. The MD group reacts with pentamethylcyclopentadiene (pmCp) to give the Diels−Alder cycloadduct. (B) A MALDI MS spectrum of the maleimide-terminated monolayer shows peaks at *m*/*z* 870, 1094, and 1318. (C) A MALDI MS spectrum of the monolayer after treatment with pmCp shows mass peaks at *m*/*z* 1230 and 1590 resulting from the interfacial Diels−Alder reaction.

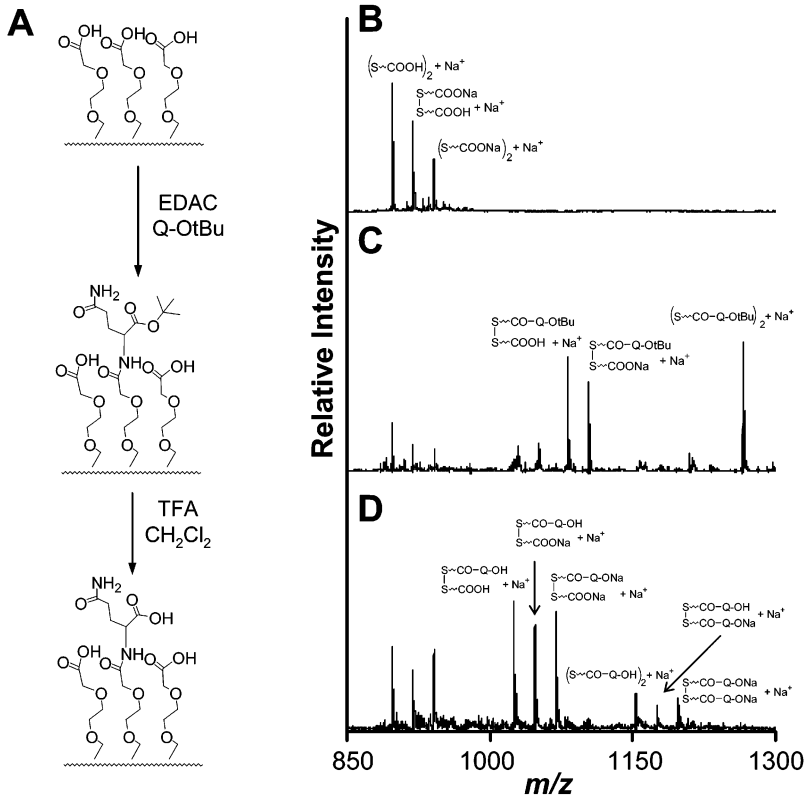

Figure 2. (A) A monolayer presenting carboxylic acid groups was treated with a solution containing 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) and L-glutamine *tert*-butyl ester (Q-OtBu) to generate the corresponding amides and then treated with TFA to remove the *tert*-butyl group to reveal the terminal carboxylate. (B) A MALDI MS spectrum of the monolayer presenting carboxylic acids shows a peak for the symmetrical disulfide at *m*/*z* 898 and its double and triple sodium adducts. (C) Following amidation of the carboxylate groups, MALDI-TOF MS reveals peaks at *m*/*z* 1082, 1104, and 1266, corresponding to the mixed disulfides and symmetrical disulfide terminating in glutamide *tert*-butyl ester resulting from the interfacial reaction. (D) Following the removal of the *tert*-butyl group, two new groups of peaks at *m*/*z* 1026 and 1154 appeared, corresponding to mixed and symmetrical disulfides presenting free carboxylate-terminated glutamide and their double and triple sodium adducts, respectively.

spectrometry (DLDI MS)[11] have been applied to characterize self-assembled monolayers on gold, yet these techniques often require instrumentation and data analysis protocols that are not commonly available. Recent work has shown that matrix-assisted laser desorption/ionization and time-of-flight mass spectroscopy (MALDI-TOF MS) can image molecules chemisorbed to a gold substrate.[12] In this paper, we show that MALDI-TOF MS is well suited for directly characterizing reactions on monolayers of

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alkanethiolates on gold and therefore represents a significant addition to the repertoire of techniques now in common use.

Experimental Protocols. The monolayers used in this work were prepared on gold-coated glass slides as described previously.[7] The substrates were cut into 1 cm^2 chips and treated with a solution containing 2,5-dihydroxyl benzoic acid (10 mg/mL, 1 μL) in tetrahydrofuran (THF) or acetonitrile, resulting in a matrix-to-analyte ratio of approximately 100:1. The substrates were allowed to dry and placed directly onto a MALDI sample plate and loaded into a Voyager-DE Biospectrometry mass spectrometer. MS analysis was operated in the delayed extraction mode. A 3-ns pulse nitrogen laser (337 nm) was used for desorption and ionization with an accelerating voltage of 20 kV. Ions were detected as positive on a time-of-flight mass detector in the reflector mode. Commercial peptides Des-Arg Bradykinin $[M + H]^+$ 904 and Neurotensin $[M + H]^+$ 1673 were used as external standards for mass calibration.

In a first example of the utility of MALDI-TOF MS for characterizing reactions on SAMs, we characterized an interfacial Diels−Alder reaction (Figure 1A). A monolayer was prepared from an unsymmetrical disulfide (**1**) substituted with a penta(ethylene glycol) group (EG5) and a maleimide group (MD).[13] Analysis of the monolayer with MALDI-TOF MS revealed peaks at *m*/*z* 870, 1094, and 1318 (Figure 1B), corresponding to sodium adducts of the disulfides derived from two EG5-terminated alkanethiolates, from one EG5- and one MD-terminated alkanethiolate, and from two MD-terminated alkanethiolates, respectively.[14] We find that disulfides are the predominant species observed in MALDI analysis of SAMs. The observation of disulfides is consistent with previous studies that used laser desorption MS to analyze SAMs in positive ion mode, and with thermal desorption of alkanethiols.[9] We next treated this MD-terminated monolayer with a solution of pentamethylcyclopentadiene (pmCp, 25 mM in THF) for 2 h at room temperature. A MALDI MS spectrum of the resulting monolayer revealed that the original peaks at *m*/*z* 1094 and 1318 were quantitatively converted to two new peaks at *m*/*z* 1230 and 1590 (Figure 1C). These new peaks represent the disulfides that resulted from Diels−Alder cycloaddition of the diene with the maleimide groups.

In a second example, we used MALDI MS to characterize a reaction that is frequently employed to functionalize monolayers: the formation of amide bonds between carboxylic acids and amines (Figure 2). Whitesides and co-workers have developed this method as a general route to modify surfaces with ligands and functional groups.[15] We prepared a monolayer from the alkanethiol $HS(CH_2)_{11}(OCH_2CH_2)_4OCH_2COOH$ (Figure 2A). A MALDI-TOF MS spectrum of this monolayer showed peaks at *m*/*z* 898, 920, and 942, corresponding to the single, double, and triple sodium adducts, respectively, of the symmetrical disulfide (Figure 2B). An amidation reaction was performed by treating this monolayer with a mixture of

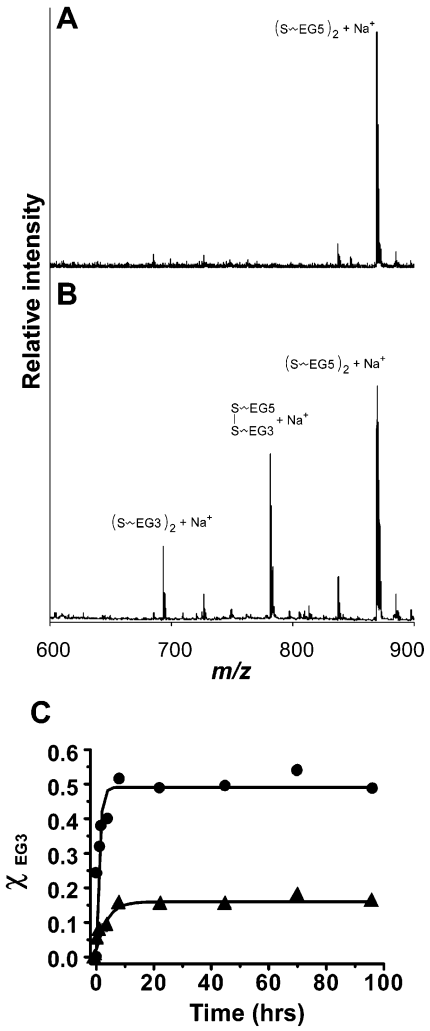

Figure 3. MALDI-TOF was used to characterize the exchange of alkanethiolates in monolayers and alkanethiols in a contacting solution. (A) A MALDI MS spectrum of a monolayer formed from $(S(CH_2)_{11}(OCH_2CH_2)_5OH)_2$ shows a single peak at *m*/*z* 870, corresponding to the symmetrical disulfide terminated with penta(ethylene glycol) groups (EG5). (B) A MALDI spectrum of the monolayer after immersion in a solution containing a tri(ethylene glycol)-terminated alkanethiol $HS(CH_2)_{11}(OCH_2CH_2)_3OH$ (EG3, 10 mM in EtOH) for 30 min shows new peaks at *m*/*z* 694 and 782, corresponding to the symmetrical disulfide containing EG3 groups and the mixed disulfide containing one EG5 and one EG3 group. (C) The time dependence of surface coverage of EG3-terminated alkanethiolates during exchange was shown by MALDI MS analysis. The line with filled circles shows time-dependent incorporation of EG3-terminated alkanethiol to the monolayer preformed from EG5-terminated disulfides. The line with triangles shows incorporation of EG3-terminated alkanethiol to the monolayer of EG5-terminated alkanethiols ($HS(CH_2)_{11}(OCH_2CH_2)_5OH$).

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, 0.1 M) and L-glutamine *tert*-butyl ester (Q-OtBu, 0.1 M) in phosphate-buffered saline (pH 7) for 2 h at room temperature. MALDI-TOF analysis of the resulting monolayer showed peaks at *m*/*z* 1082, 1104, and 1266. The first two peaks correspond to the single and double sodium adducts of the unsymmetrical disulfide wherein one of the carboxyl groups underwent amidation. The peak at *m*/*z* 1266 corresponds to the symmetrical disulfide wherein both carboxyl groups were converted to the amide product (Figure 2C). We next treated this monolayer with a 1:1 mixture of trifluoroacetic acid (TFA) and methylene chloride at room temperature for 2.5 h. MALDI-TOF MS analysis shows that the *t*-butyl esters were quantitatively

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removed to give carboxylic acids on monolayers (Figure 2D). Peaks at *m*/*z* 1026, 1048, and 1070 represent the single, double, and triple sodium adducts of the unsymmetrical disulfides after removal of the *tert*-butyl group. The peaks at *m*/*z* 1154, 1176, and 1198 represent the sodium adducts of the symmetrical disulfides presenting two carboxylic acids after removal of the *tert*-butyl groups. The multiple sodium adducts derive from exchange of carboxylic acid protons with sodium ions, and this has been observed previously.[16]

In a third example, we characterized the exchange between alkanethiolates in a monolayer and alkanethiols in a contacting solution. We prepared monolayers from the disulfide $(S(CH_2)_{11}(OCH_2CH_2)_5OH)_2$ (1 mM in EtOH, room temperature for 24 h).[13] MALDI-TOF MS analysis of this SAM showed a single peak at *m*/*z* 870 corresponding to the sodium adduct of the symmetrical disulfide (Figure 3A). A series of identical monolayers were immersed in solutions of the tri(ethylene glycol)-terminated alkanethiol $HS(CH_2)_{11}(OCH_2CH_2)_3OH$[17] (EG3, 10 mM in EtOH) for times ranging from 30 min to 4 days and then analyzed by MALDI-TOF MS to determine the fraction of alkanethiolates that were replaced by the soluble alkanethiol. Figure 3B shows the MS spectrum of the EG5-terminated SAM after a 30-min immersion in the solution containing EG3-terminated alkanethiols. The peak at *m*/*z* 694 corresponds to the sodium adduct of the disulfide presenting two EG3 groups, and the peak at *m*/*z* 782 corresponds to the sodium adduct of the mixed disulfide presenting one EG3 and one EG5 group. We quantitated the extent of exchange by integrating the peaks for each species and reporting the fraction of EG3 alkanethiolates in the monolayer based on the equation $\chi_{EG3} = (A_{694} + (A_{782}/2))/(A_{694} + A_{782} + A_{870})$. The curve in Figure 3C shows the time-dependent change in surface composition. The exchange of EG5-terminated alkanethiolates with the EG3-terminated alkanethiols increased with time and reached a maximum of 50% occupancy in the monolayer. We next repeated this experiment with SAMs that were assembled from the corresponding alkanethiol $HS(CH_2)_{11}(OCH_2CH_2)_5OH$.[17] A series of these monolayers was treated with the same solution of EG3-terminated alkanethiol. MALDI MS analysis shows significantly less exchange of alkanethiolates, with a maximum exchange of 15%. The higher level of exchange observed on SAMs prepared from the disulfide is not unexpected because these monolayers are believed to contain a larger density of defect sites than do monolayers prepared from thiols,[18] and exchange at the defect sites is faster than at crystalline domains where the molecules are tightly packed.[19] MALDI-TOF MS is an effective technique for addressing the stability of SAMs in this and related applications.

This paper demonstrates that MALDI-TOF MS is well suited for characterizing the products resulting from interfacial reactions on SAMs. This technique has the primary advantage that it gives direct information on the surface structure. Because it measures mass by way of the mass-to-charge ratio, an intrinsic property of any alkanethiolate, it is far more general than are analytical methods that require labels (i.e., isotopic labels or vibrationally active functional groups). The widespread availability of commercial instruments makes this technique straightforward to implement for analyzing SAMs. A current limitation of MALDI-TOF MS is that it is not well suited for characterizing alkanethiolates having masses below 300 because of the numerous and intense background signals that arise from the matrix molecules. We note that the use of SIMS and DLDI MS in the absence of matrix may have advantages in this mass domain. Efforts to develop new formulations of matrices for detecting small molecules and nonpolar species will also add to the capability of this technique in surface science.[20,21] We believe that the attributes of MALDI-TOF MS for characterizing SAMs will make this an essential technique for programs that develop and employ functionalized surfaces in basic and applied science.

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