A Dual Platform for Selective Analyte Enrichment and Ionization in Mass Spectrometry Using Aptamer-Conjugated Graphene Oxide

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A Dual Platform for Selective Analyte Enrichment and Ionization in Mass Spectrometry Using Aptamer-Conjugated Graphene Oxide

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Abstract: This study demonstrates the use of aptamer-conjugated graphene oxide as an affinity extraction and detection platform for analytes from complex biological media. We have shown that cocaine and adenosine can be selectively enriched from plasma samples and that direct mass spectrometric readouts can be obtained without a matrix and with greatly improved signal-to-noise ratios. Aptamer-conjugated graphene oxide has clear advantages in target enrichment and in generating highly efficient ionization of target molecules for mass spectrometry. These results demonstrate the utility of the approach for analysis of small molecules in real biological samples.

The analysis of biologically important small molecules and their subsequent detection by mass spectrometry (MS) in complex biological matrices (e.g., serum, plasma) is an area of great interest. Despite the great potential of MS based on its high sensitivity, speed, reproducibility, and label-free readout, signal suppression effects and the need for careful sample preparation still limit its overall use.[1,2] To address these problems, chromatographic extraction and fractionation methods are often applied before MS is performed.[3] However, since these techniques lack specificity, the next best option utilizes affinity reagents tethered on a substrate, from which direct MS readouts can be obtained. To achieve this goal, different kinds of affinity reagents have been applied in different formats, such as mass spectrometric immunoassay (MSIA), nanoprobe affinity mass spectrometry, and surface-enhanced laser desorption/ionization (SELDI).[4]

As a novel affinity reagent, aptamers could provide the specificity lacking in many extraction matrixes. Aptamers are single-stranded oligonucleotides that bind target molecules with very high affinity in a manner similar to antibodies. However, as a capturing reagent, the affinity of aptamers can be adjusted, depending on the application. Aptamers can be generated against a variety of targets, including metal ions, metabolites, proteins, and even whole cells.[5] Aptamers have very distinct advantages as capturing reagents, such as small size, nontoxicity, easy modification, and easy surface immobilization. Moreover, aptamers can be produced without the need of an animal source, and they can be chemically modified with various functional groups. All of these unique properties increase the likelihood that aptamers will outperform other affinity reagents.[6]

To date, very few studies have been carried out on developing a single platform whose function is based on simultaneous capture and ionization.[7] This particular area still requires new high-efficiency techniques and new materials. Recently, graphene oxide (GO) has attracted interest as a substrate for analyte detection because of its unique electronic, thermal, and mechanical properties.[8] Herein, we report aptamer-modified GO as a selective enrichment and matrix-free detection platform for MS detection of cocaine and adenosine from complex biological systems.

The synthesis of graphene oxide has been reported elsewhere.[9] Briefly, GO was obtained by oxidizing graphite using Hummer's method, which results in water-soluble GO having a carboxyl-rich structure. It has previously been demonstrated that graphene (G) can be used as a matrix for laser desorption/ionization.[10,11] To demonstrate that GO has very similar properties, a series of small molecules were analyzed using GO as an efficient energy-absorbing molecule (Figures S2−S5 in the Supporting Information). Next, we immobilized thiol-functionalized cocaine and adenosine aptamers onto GO by activating the carboxyl (−COOH)-rich groups with the use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide)/*N*-hydroxysuccinimide (EDC/NHS) chemistry and by introducing a bifunctional poly(ethylene glycol) (PEG) molecule (NH_2−PEG−SH) as a spacer unit, which provides stability in physiological media such as serum or blood.[11] Since aptamers intrinsically possess a secondary structure, the PEG linker enables them to attain their three-dimensional conformations, thus permitting target recognition. The bifunctional PEG linker carries amine ($-NH_2$) groups on one side to bind to the carboxyl groups on the GO surface and thiol (−SH) groups to help anchor the SH-functionalized aptamers through disulfide bond formation (Figure 1). Aptamer-modified GO was characterized using FTIR (Figure S6), and then aptamer-conjugated GO was applied in selective enrichment and detection of cocaine spiked in human plasma along with respective control experiments.

Figure 2A shows the MS analysis of cocaine-spiked plasma samples analyzed directly on GO without any enrichment step (no aptamer modification), during which GO served only as a matrix. The cocaine peaks can be detected among huge background ions with an average signal-to-noise (S/N) ratio of 15. Figure 2B shows the analysis of cocaine-spiked plasma extracted and ionized with unmodified GO. Even in the presence of unmodified GO, enrichment of the sample was observed to some extent, along with a reduction in background ions. This result can be explained by the fact that the structure of cocaine includes an aromatic ring, which aids in $\pi-\pi$ interactions with GO. In another control experiment, GO with physically adsorbed cocaine aptamer was used for target extraction. In this case, following washing steps, little or no

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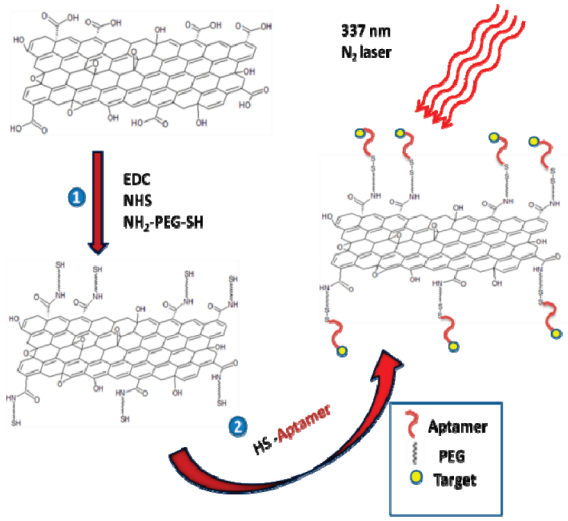

Figure 1. Scheme showing aptamer modification and GO-assisted target capture and analysis.

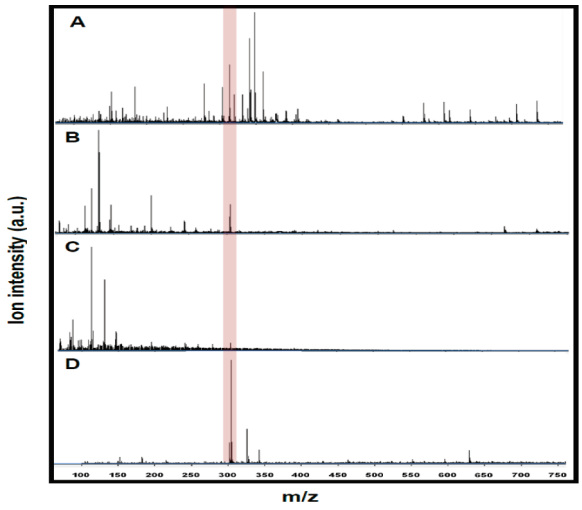

Figure 2. Analysis of cocaine (a) spiked into plasma, (b) extracted with GO, (c) extracted with noncovalently aptamer-modified GO (cocaine was washed away without chemical modification before MS) and (d) extracted with covalently aptamer-modified GO.

extraction of cocaine with an S/N ratio of 5 was obtained. On the contrary, GO has been shown to physically adsorb DNA in high yields.[9] We can explain this phenomenon by assuming that (i) surface coverage of GO by physically adsorbed aptamer prevents the loading of cocaine by means of nonspecific interactions (as is the case with $\pi-\pi$ interactions; Figure 2B) and (ii) upon target capture, aptamer is released from the GO surface following the washing steps as a result of the absence of any chemical functionalization. This result is very consistent with previous results for GO-based fluorescence sensors. In those biosensor experiments, DNA (either complementary DNA or aptamer) left the surface upon target addition, leading to the restoration of fluorescence that had previously been quenched by GO.[12] In our experiments, MS is used for detection, and the signal is generated from the GO surface. Consequently, we also see a decrease in signal upon washing steps. However, when chemical conjugation was applied to covalently modify GO with cocaine aptamer, an efficient capture of target analyte with an average S/N ratio of 52 was achieved (Figure 2D). Since we see an increase in S/N ratio, this result further proves our previous experiments and indicates that chemical conjugation

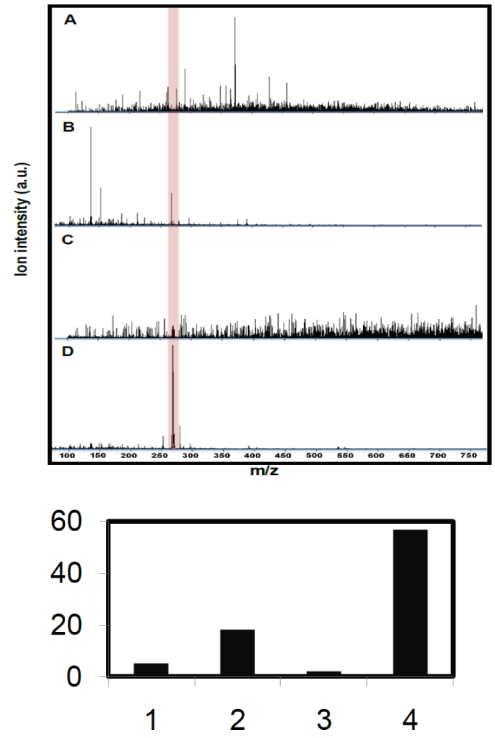

Figure 3. (top) Analysis of adenosine (a) spiked into plasma, (b) extracted with GO, (c) extracted with noncovalently aptamer-modified GO (adenosine was washed away without chemical modification before MS), and (d) extracted with covalently aptamer-modified GO. (bottom) S/N ratios of adenosine (1) spiked into plasma, (2) extracted with GO, (3) extracted with noncovalently aptamer-modified GO, and (4) extracted with covalently aptamer-modified GO.

of the aptamer is necessary for analyte capture. (A schematic representation of the whole series of experiments is given in Figure S1.) To further demonstrate the analytical utility of our approach, we applied the same concept to an adenosine aptamer.

As shown in the top panel of Figure 3, a trend similar to that for cocaine was clearly observed. Even if some extraction can be achieved with pristine GO, this result proves that aptamer conjugation yields significantly improved extraction efficiency and a significant increase in the S/N ratio, as shown in the bottom panel of Figure 3.

In conclusion, we have prepared aptamer-conjugated GO for selective enrichment and detection of cocaine and adenosine from plasma samples. This method has several advantages: first, it represents a conjugation chemistry that is very easy to perform; second, the use of GO eliminates (1) the need for any additional energy-absorbing matrix for ionization and (2) background interference, which is the biggest problem when conventional MALDI matrices are employed. When combined with aptamer-based affinity capture, our results show that GO provides an efficient platform for selective enrichment of target analytes and the attainment of direct mass spectrometric readouts, even from very complex media, which would not be possible using either GO or aptamers alone. Since a huge repertoire of aptamers exists for different targets, including proteins, metabolites, and cell-surface markers, we believe on the basis of our proof-of-concept study that graphene-based, aptamer-enhanced extraction mass spectrometry can be extended to a more complex system and be used when analyzing different biological samples.

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Supporting Information Available: Experimental details, additional MS analyses, and FTIR and TEM images. This material is available free of charge via the Internet at http://pubs.acs.org.

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