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Combination of solid-phase affinity capture on magnetic beads and mass spectrometry to study non-covalent interactions: example of minor groove binding drugs

Gitta Schlosser[1,2†], Károly Vékey[2], Antonio Malorni[1] and Gabriella Pocsfalvi[1]*

[1]Proteomic and Biomolecular Mass Spectrometry Center (CeSMa-ProBio), Institute of Food Science and Technology, C.N.R., via Roma 52 a/c, 83100 Avellino, Italy
[2]Institute of Structural Chemistry, Hungarian Academy of Sciences, Pusztaszeri út 59/67, H-1025 Budapest, Hungary

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A simple and novel approach was developed to detect non-covalent interactions. It is based on combination of solid-phase affinity capture with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS). One of the interacting molecules is bound to magnetic beads and is incubated with the target molecules in solution. The complex bound on the solid support is removed from the solution and transferred for MALDI analysis. Mass spectrometry is used only to detect the target compound, which is far more straightforward than detecting the intact non-covalent complex. To demonstrate the applicability of the method, an AT-rich oligonucleotide (5′-CCCCCAATTCCCCC-3′) and its complementary biotinylated sequence (5′-biotin-GGGGGAATTGGGGG-3′) were hybridized and immobilized to paramagnetic particles by streptavidin-biotin interaction. The immobilized duplex oligonucleotide was reacted with minor groove binding drugs, Netropsin, Distamycin A, Hoechst 33258 and 4′,6-diamidino-2-phenylindole. The resulting DNA-drug complex bound to the particles was separated and analyzed by linear MALDI-TOFMS after washing. Drugs were selectively detected in the spectra. Relative binding strengths were also estimated using competitive complexation. Copyright © 2005 John Wiley & Sons, Ltd.

The study of specific non-covalent interactions is developing quickly, with diverse applications in chemistry, biology, pharmaceutical research, etc. Mass spectrometry (MS) is becoming a widely used tool in this field due to its sensitivity, selectivity and versatility. There are several strategies and experimental approaches to study non-covalent complexes by MS. Generally, the monomer/complex equilibrium is established in solution and the mass spectrometer is used to analyze its composition. Conventionally in MS studies of this kind a non-covalent complex is isolated in the gas phase and its composition, conformation, stability, etc., are studied. This gives information on gas-phase characteristics, which can be a significant disadvantage in biochemical applications as gas-phase and solution-phase properties may differ. This approach requires that the complex must survive ionization and mass analysis, while the monomers must not associate. It is not trivial to satisfy all criteria, so, although such studies are in high demand, they are fraught with diverse methodological problems.

**Correspondence to*: G. Pocsfalvi, Proteomic and Biomolecular Mass Spectrometry Center, Institute of Food Science and Technology, C.N.R., via Roma 52 a/c, 83100 Avellino, Italy.
E-mail: gpocsfalvi@isa.cnr.it
†Present address: Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös L. University, P.O. Box 32, H-1518 Budapest 112, Hungary
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In the study of non-covalent interactions by MS, the ionization method of choice is generally electrospray ionization (ESI),[1] due to its capability to release intact non-covalent complexes from solution into the gas phase as singly or multiply charged ions. Since the first publications by Ganem *et al.*,[2–4] several excellent reviews and a large number of papers have been published on the application of ESI-MS for the study of non-covalent complexes.[5–8]

Observation of intact complexes in ESI mass spectra clearly shows that non-covalent interactions may survive the ESI process. The success of the experiments (i.e. detection of the complex), however, depends on various instrumental and experimental parameters, many of them determining the internal energy of electrosprayed ions. Application of 'too soft' conditions can result in the detection of non-specific aggregation (e.g., clusters with the solvent, oligonucleotide complexes of non-complementary strands, peptide dimers) while 'not sufficiently soft' conditions may lead to the dissociation of specific complexes. Thus, an important disadvantage of this method is the lack of fine control of the amount of internal energy deposited in the molecular ions of the complex, and therefore distinction between specific and non-specific interactions is often a serious problem. Experiments on non-covalently bound complexes with biological importance should be performed under physiological conditions (i.e., close to neutral pH, high salt concentration, etc.). However, traditional ESI generally has low ionization

efficiency from aqueous solution mainly because of difficulties in generating a stable spray from water, and in addition has relatively low tolerance for impurities, salts, detergents, etc. In addition to the molecular mass and the composition, information on the stability of the complex (binding energy, stability constant) is also of interest. An important advantage of MS compared to other molecular spectroscopic techniques in this regard is that relative binding affinities of different ligands can be predicted using competitive reactions (usually performed between two molecules) by the measurement of relative peak intensities or by energy-dependent fragmentation of the complexes. This requires that both monomers and complexes should be transferred from solution into the gas phase without changing relative abundances (i.e., not changing the solution-phase equilibrium, neither dissociating the complex nor associating the monomers during solvent evaporation and ion formation), and that all components involved in the complexation should be detected with equal sensitivity by the mass spectrometer. In spite of significant successes, it is not a trivial task to satisfy all criteria, and many groups are still working on understanding, controlling and optimizing conditions for studying complexation phenomena by MS in this way.

Matrix-assisted laser desorption/ionization (MALDI)-MS[9] is also applied to detection of non-covalent interactions,[10–12] although less frequently than ESI. For example, observation by MALDI-MS of highly specific antibody-antigen immunocomplexes has been recently reported.[13] Direct application of MALDI for efficient detection of non-covalent complexes is hampered by the low pH of most matrix/analyte mixtures, by involvement of the solid phase in the analyte/matrix mixture, and by the high matrix concentration. The ionization process may also hinder successful detection of intact complexes, since energy deposition by the laser is difficult to control and can easily break up weak non-covalent bonds.

Here we present an alternative approach to analysis of non-covalent interactions that combines MALDI-MS and the use of paramagnetic beads (Dynabeads). One of the interacting molecules is bound to the solid-phase support and is incubated in solution with the target molecules. After equilibrium is established, the immobilized complex can be readily removed from the solution and analyzed by MS. Instead of the intact complex, only one of the interacting molecules needs to be detected. This significantly simplifies current techniques based on MS for studying molecular interactions, making analysis more robust and more reliable.

Affinity techniques are becoming increasingly used in MS.[14] In the last few years, bio-affinity MS has become a promising tool in proteomics, in biomarker research, as well as in biomolecular interaction analysis. Affinity-based separations have been interfaced off-line with both ESI-MS[15,16] and MALDI-MS.[17] Alternatively, affinity separation can be performed on-line using a chromatographic system directly coupled to the electrospray interface[18] or on a solid surface (chip or beads) which can be analyzed subsequently by MALDI-MS.[19] Surface plasmon resonance biomolecular interaction analysis mass spectrometry (SPR-BIA-MS) combines real-time BIA analysis with MALDI-MS[20] to identify molecules retained on the surface of a sensor-chip. In surface-enhanced laser desorption/ionization (SELDI)-MS,[21] the surface-modified sample probe plays the role of the chromatographic or affinity support, and the captured analyte can be detected directly. The applicability of chip-based bio-affinity methods has been demonstrated in various research fields,[14,22–29] e.g., epitope mapping, affinity selection from complex biological samples ('ligand fishing'), and proteomic applications. Availability of various chip surfaces with different chemical and physical properties makes the technique suitable for diverse biochemical and medical applications. However, chip-based bio-affinity applications are hampered by limited instrumentation and expensive accessories.

Application of small magnetic particles as a solid support is an emerging technology in biology and biochemistry. The use of magnetic force may be combined with conventional separation or identification methods to purify proteins, nucleic acids, cell organelles, and cells, directly from crude samples.[30–32] Magnetic beads functionalized by different reactive groups, such as amine, carboxy, epoxy, tosyl, streptavidin, protein A, IgG, etc., are commercially available. These are frequently used in molecular biology for the selective purification of nucleic acids and proteins. The separation process for the purification of a target molecule using magnetic particles and magnetic separators usually consists of three fundamental steps. The suspension containing the biomolecule of interest is mixed with the specially coated magnetic particles, and interaction occurs during the incubation step. Then the affinity-captured molecule is separated from the other molecules in the solution using an appropriate magnetic separator, and the supernatant is discarded or used for other applications. The magnetic particles are washed several times to remove contaminants. Purified molecules are eluted from particles using an appropriate method, and are then detected and/or used for subsequent applications. To detect the eluted molecules, conventional immunoassay, Western blotting, one-dimensional (1D) gel electrophoresis, polymerase chain reaction (PCR), MS, etc., are used. Combined application of affinity capture using magnetic beads and MS was reported for genetic mutation analysis.[33–35] In these works, peptide nucleic acids[36] (PNAs) were used as sequence-specific molecular probes to detect single and multiple nucleotide polymorphism on appropriately amplified DNA fragments captured to magnetic particles. PNAs were selectively detected by MALDI-MS directly from the beads. Another application of beads placed directly on the MALDI target for analysis was reported by Raska *et al.*[37] for the characterization of protein phosphorylation sites using immobilized metal ion affinity beads.

EXPERIMENTAL

Materials

Minor groove binding drugs: Netropsin ($C_{18}H_{26}N_{10}O_3$, MW (Da, average) = 430.5, Distamycin A ($C_{22}H_{27}N_9O_4$, MW (average) = 481.5, abbreviated as Distamycin), Hoechst 33258 ($C_{25}H_{24}N_6O$, MW (average) = 424.5 abbreviated as Hoechst), 4′,6-diamidino-2-phenylindole) ($C_{16}H_{15}N_5$, MW

(average) = 277.3 abbreviated as DAPI), and angiotensin I (human, sequence Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu, MW (average) = 1294.7) were obtained from Sigma-Aldrich (St. Louis, USA). A 14-mer oligonucleotide 5′-CCCCCAATTCCCCC-3′ (MW (average) = 4064.7) and its complementary biotinylated sequence 5′-biotin-GGGG-GAATTGGGGG-3′ (MW (average) = 4870.6) were custom synthesized by MWG-Biotech (Ebersberg, Germany). Biotin was incorporated directly at the 5′ end of the oligonucleotide during DNA synthesis. Oligonucleotides were purified by reverse-phase high-performance liquid chromatography (HPLC), and their stock solutions were prepared in water (MilliQ) at 2.5 nmol/μL concentration. Streptavidin-functionalized paramagnetic particles (Dynabeads, M-280) were obtained from Dynal Biotech. (Oslo, Norway); they were used at 10 mg/mL concentration corresponding to $6–7 \times 10^8$ beads/mL. The binding capacity of the beads is dependent on DNA fragment length. According to the product specification, 1 mg of Dynabeads M-280 Streptavidin typically binds 700–1000 pmol of free biotin and 200 pmol of single-stranded biotinylated oligonucleotides. MALDI matrices, α-cyano-4-hydroxycinnamic acid (CCA) and 2,4,6-trihydroxyacetophenone (THAP), and other chemicals, were purchased from Sigma-Aldrich. Gradient grade solvents were purchased from Merck (Darmstadt, Germany).

Mass spectrometry

MALDI mass spectra were acquired using a Voyager DE-Pro MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA, USA), equipped with a 337 nm nitrogen laser, in positive ion and linear acceleration modes. Spectra were acquired using 20 kV acceleration voltage, 95% grid voltage, 0.05% guide wire potential, and 100 ns delay time, in the range *m/z* 150–2500. Mass-to-charge (*m/z*) calibration was performed externally using a standard peptide mixture (Perseptive Biosystems). CCA matrix was prepared at 10 mg/mL concentration in acetonitrile/water (1:1, v/v) containing 0.1% trifluoroacetic acid (TFA). MALDI mass spectra of oligonucleotides were acquired using THAP matrix (3 mg/mL in 25 mM ammonium citrate solution). Both oligonucleotides yielded a simple mass spectrum containing intense singly and doubly charged protonated molecule peaks, measured at *m/z* 4067.7 and 2034.7 and *m/z* 4873.5 and 2437.6 for the non-biotinylated and biotinylated sequences, respectively. The peaks for the protonated non-biotinylated oligonucleotide were detectable at quantities on-probe as low as 10 fmol. MALDI measurements of affinity-captured Dynabeads were performed using 0.5 μL of the magnetic particles mixed with 0.5 μL matrix solution (CCA or THAP), placed onto the target, and allowed to dry in air.

ESI mass spectra were obtained using a Micromass Q-Tof-Micro (Manchester, UK) hybrid quadrupole-orthogonal acceleration time-of-flight mass spectrometer. For the analysis of oligonucleotides the instrument was used in negative ion mode, with 2800 V capillary voltage and 30 V sample cone voltage. No heating was applied in the ion source or for desolvation. The instrument was calibrated in the range *m/z* 900–2000 by using cluster peaks obtained by spraying 0.1% H_3PO_4 solution (acetonitrile/water 1:1, v/v).

Procedure for solid-phase affinity capture combined with MALDI-TOF analysis

Oligonucleotide annealing

For annealing, an equimolar mixture of complementary oligonucleotides was dissolved in 50 mM ammonium acetate (NH_4OAc) buffer at 100 pmol/μL. Hybridization was performed by heating the solution to 80°C and keeping it at this temperature for 10 min, followed by very slow cooling (several hours) to room temperature. Formation of the duplex was checked using ESI-MS by diluting the annealed solution in 50 mM NH_4OAc to a concentration of 10 pmol/μL. In the ESI negative ion mass spectrum (not shown, available on request from the authors), intense multiply charged ion peaks were observed at *m/z* 1488.1 and 1786.1 (deduced MW = 8935.4 ± 0.32) indicating successful duplex formation. Less abundant peaks at *m/z* 1014.9, 1354.5 (MW 4063.7 ± 0.08) and *m/z* 973.2, 1216.5, 1622.3 (MW 4870 ± 0.42) corresponded to the single-stranded non-biotinylated and biotinylated oligonucleotides, respectively. The presence of the monomer peaks could be explained in terms of incomplete formation of the duplex, and/or its in-source dissociation.

Immobilization of duplex oligonucleotide

Immobilization on streptavidin-functionalized paramagnetic particles was achieved as follows. For the coating procedure, 10 μL of the Dynabeads suspension were washed three times with 200 μL of 5 mM EDTA containing 0.5 M NaCl and with 200 μL 50 mM NH_4OAc, followed by washing once with 200 μL 0.1% bovine serum albumin (BSA) (in 50 mM NH_4OAc) and twice with 200 μL 50 mM NH_4OAc, using a magnetic separator device (MPC®-S 120.20, Dynal, Oslo, Norway). The beads were re-suspended in 10 μL 50 mM NH_4OAc, and then 2 μL (200 pmol) of the oligonucleotide duplex solution were added. The mixture was then incubated at room temperature for 30 min, resulting in immobilization by the strong non-covalent binding between streptavidin and biotin. Then the resulting molecular probes were washed with 200 μL of 50 mM NH_4OAc, and re-suspended in the same solution.

Affinity capture and washing

First, 2 μL of a solution containing a minor groove binding drug (100 pmol/μL in 50 mM NH_4OAc unless otherwise mentioned) were added to the molecular probes (beads functionalized with duplex oligonucleotide), and the particles were then incubated at room temperature for 30 min. After incubation the beads were washed three times with 200 μL 50 mM NH_4OAc, three times with 200 μL water to wash off non-binding molecules, and re-suspended in 5 μL water. Then 0.5 μL of this Dynabeads suspension was taken for MALDI-TOFMS analysis.

MALDI-TOFMS analysis of affinity-captured Dynabeads

This was simply performed by placing 1 μL of a 1:1 matrix/analyte mixture directly on the MALDI target plate, as described above.

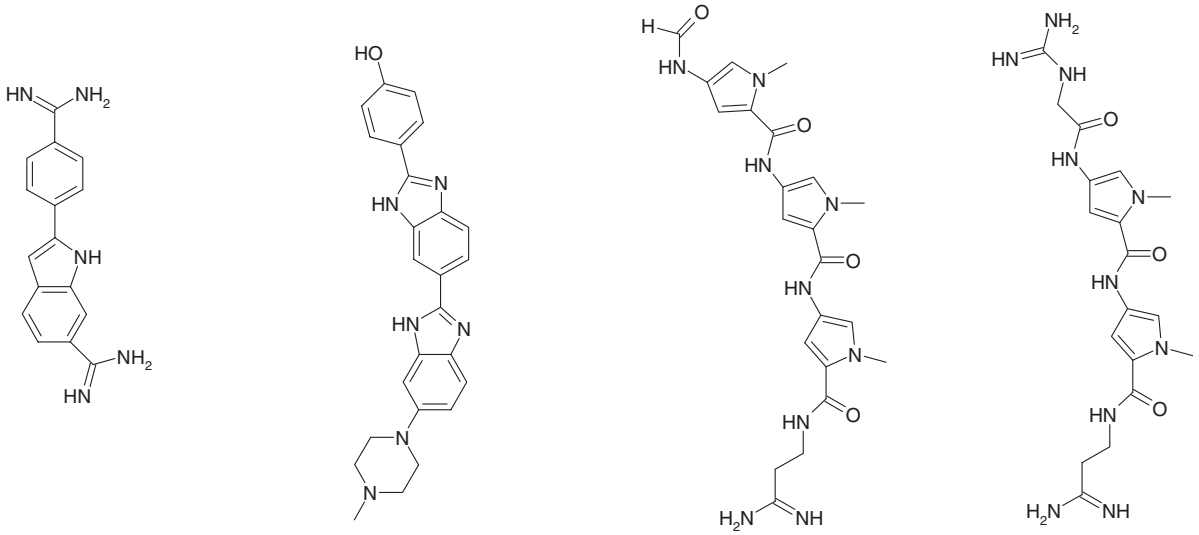

Figure 1. Molecular structures of minor groove binding drugs.

RESULTS

In this work, interactions between a duplex DNA and minor groove binding drugs (Fig. 1) were studied as a model system. Small molecules that interact with DNA have potential therapeutic applications, e.g., as antitumor or antiviral agents. Minor groove binders constitute an important group of DNA-binding drugs that are able to fit tightly in the narrow minor groove of the duplex B-DNA, with a high binding preference for short AT-rich sequences while their binding at CG pairs is sterically hindered. These compounds are usually polyamide molecules with a flat, crescent-shaped structure and carrying positive charges. ESI-MS has been used previously to characterize the binding affinities and stoichiometry of various complexes of minor groove binding drugs and duplex oligonucleotides.[38–44] Here, these interactions were investigated using a combination of solid-phase affinity capture and MALDI-MS, as outlined above.

Solid-phase affinity capture mass spectrometry is a simple method consisting of four basic steps for the study of non-covalent interactions (Fig. 2). (1) Immobilization of one of the interacting molecules on the solid phase, which permits easy manipulation and repeated use of the molecular probe. In this work a biotinylated duplex oligonucleotide containing an AT-rich motif was bound to streptavidin-coated paramagnetic particles with high capacity (ca. 20 pmol oligonucleotides immobilized on 10 μL beads). Note that only one of the strands is biotinylated, and that annealing was performed prior to immobilization. (2) The immobilized molecular probe is incubated with a solution containing the ligand molecules to allow equilibration between complexes and the free ligand. This step can be performed in any chosen solution conditions (e.g., those favoring complex formation and/or those corresponding to physiological conditions); here we used a 50 mM NH_4OAc buffer. (3) The next step is washing the beads with buffer or water to remove non-binding molecules. (4) The last step is detection of the non-covalently bound ligand (or ligand mixture) by MALDI-MS. To do this the beads are mixed with a suitable matrix (CCA and THAP in the present case), placed directly on the MALDI target plate, and analyzed.

To study and control various steps in the outlined analytical procedure, two kinds of blank beads were prepared and several tests were performed. To check the background signals due to the duplex oligonucleotide immobilized on the beads, the affinity capture procedure was performed in the absence of drug. MALDI mass spectra were obtained from these beads using two different matrices (CCA and THAP). No peaks other than those arising from the corresponding matrices were observed. To test the effect of

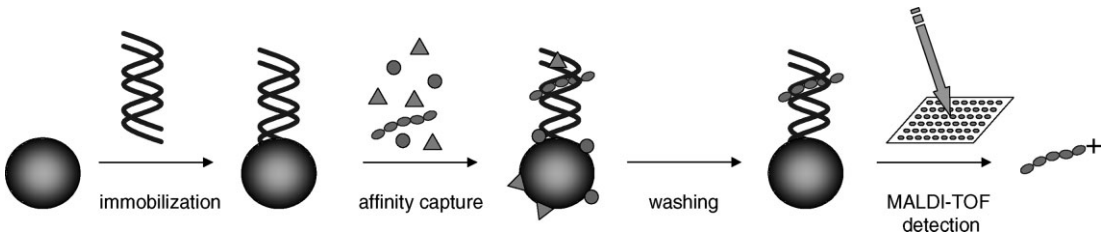

Figure 2. Schematic of basic steps of solid-phase affinity capture mass spectrometry applied for the detection of non-covalent interactions.

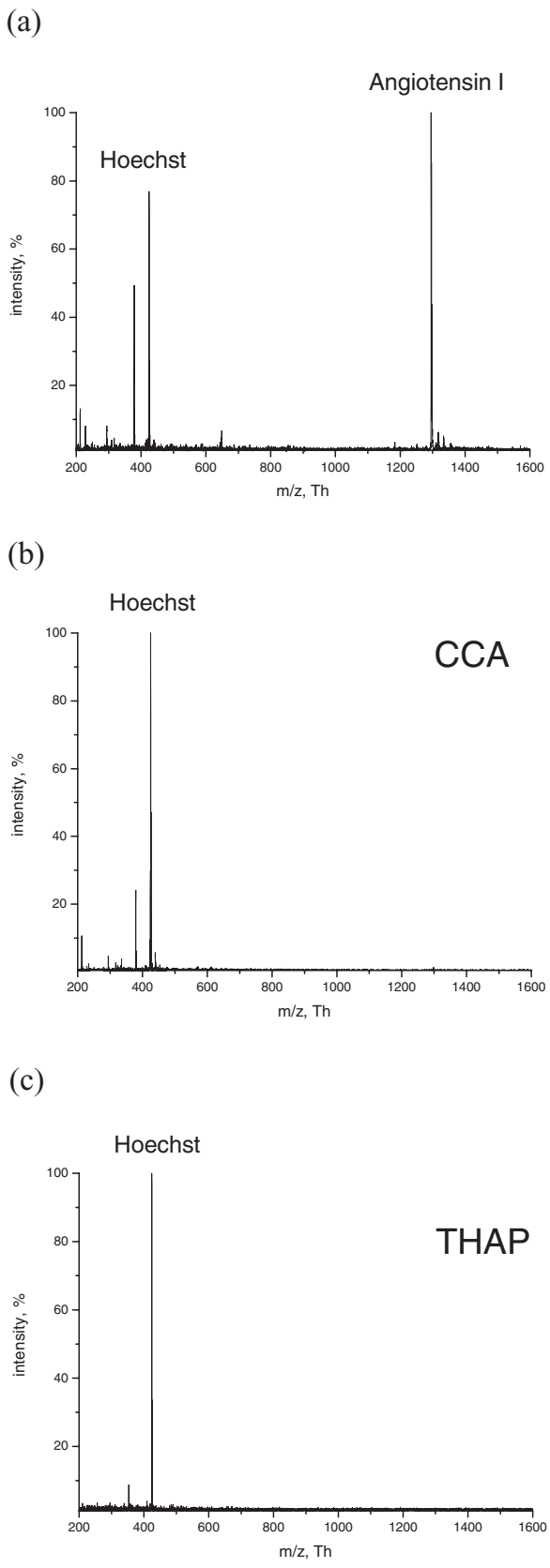

Figure 3. MALDI-TOF mass spectra of a 1:10 mixture of Hoechst 33258 and angiotensin I peptide. (a) Spectrum of the original mixed solution. (b, c) Spectra of the affinity-captured mixture using CCA and THAP as matrix, respectively.

the presence of the beads on the ionization of the drugs, the specificity of the molecular probe and the efficiency of the washing procedure, beads were prepared without addition of the duplex oligonucleotide during the immobilization step. It was observed that relative abundances of the protonated molecules of the drugs did not change in the presence of the beads. After applying 200 pmol of a drug or drug mixture to these beads, followed by washing three times with NH_4OAc solution, peaks corresponding to the drugs were still observed in the MALDI mass spectra. This could be explained by non-specific binding of the drugs to the particles, or by an inefficient washing procedure after affinity capture. To overcome this problem, an additional washing step with BSA solution (0.1%) was implemented prior to the affinity capture step, in order to block the non-specific binding sites on the surface of the beads. In addition, between the affinity capture and the MALDI measurements, a highly stringent washing using water was also introduced into the procedure in order to efficiently remove non-binding or non-specifically bound molecules. The volume of the washing solution and the number of washing steps were adjusted in order that no peaks due to the drug molecules could be observed in the mass spectra recorded for the blank beads. The adjusted washing procedure was further tested in a competitive reaction between one of the drugs (Hoechst 33258) and angiotensin I peptide; this peptide should not interact specifically with the duplex oligonucleotide studied. Figure 3(a) shows the MALDI mass spectrum of the 1:10 mixture of Hoechst and angiotensin I before affinity capture, and Figs. 3(b) and 3(c) after the washing procedure. Even though the peptide is in a large excess compared to the drug, complexation only with the minor groove binding drug is observed, indicating the specificity of the approach.

Detection of affinity-captured minor groove binding drugs

A biotinylated duplex oligonucleotide, immobilized on streptavidin-coated magnetic beads, was used as molecular probe for the affinity capture experiments. Based on the characteristics of Dynabeads and the coating procedure, approximately 20 pmol duplex oligonucleotide should have been immobilized on the surface of 10 μL of the Dynabeads suspension. For the competitive reactions a large excess of ligand molecules was used in order to ensure that the competitive equilibrium condition was reached. Four well-studied minor groove binding drugs, Netropsin, Distamycin A, Hoechst 33258 and 4′,6-diamidino-2-phenylindole (Fig. 1), were used to test the analytical method.

MALDI-MS is seldom used for the analysis of low molecular weight compounds; however, it was found that the four minor groove binding drugs studied here could be detected down to the low-pmol level. Abundant peaks due to the matrix, like the dimeric ion of CCA at *m/z* 379.1, were also observed in this mass range but did not coincide with analyte peaks. When drugs were simply mixed with the beads (without oligonucleotides attached and without washing) they also could be detected with a similar sensitivity. MALDI-TOF mass spectra of the four affinity-captured compounds, acquired directly from the beads (using 20 pmol drug) mixed with matrix, are shown in Figs. 3 and 4.

Abundant protonated molecules of the drugs were detected in all cases. Similar experiments using different quantities of ligands (in the range 200–2 pmol) in the affinity capture step were also performed, and the ligands could be detected at the lowest concentration used. In the case of 2 pmol initial quantity, 0.5 μL of the bead suspension subjected to MALDI analysis should contain not more than 200 fmol drug, demonstrating the high sensitivity of the method.

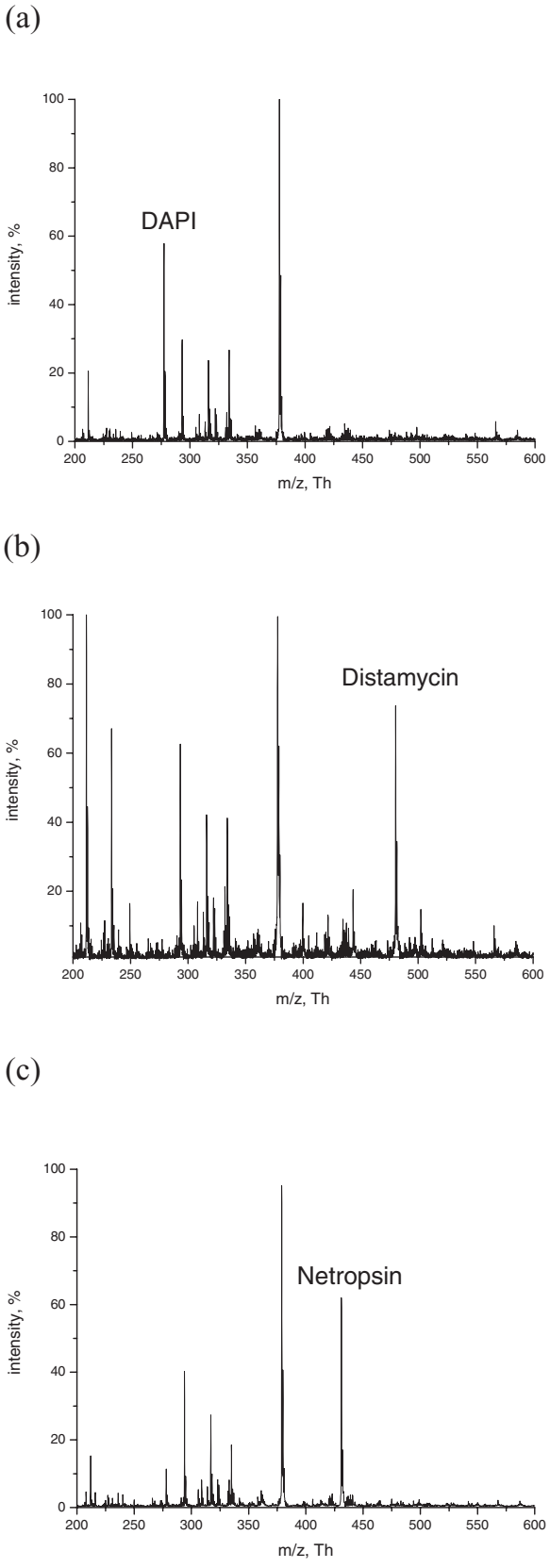

Figure 4. MALDI-TOF mass spectra of affinity-captured (a) DAPI, (b) Distamycin A, and (c) Netropsin.

Competitive binding of minor groove binding drugs

Competitive reactions were performed using two or more drugs during the affinity capture. All six of the possible pairwise combinations (equimolar) of the four drugs were measured by MALDI-MS, and then incubated with the immobilized duplex oligonucleotide. In these experiments ligands were applied in 10:1 excess relative to the bound oligonucleotide. Relative intensities of MH^+ ion peaks for the drugs before incubation (in solution) and after affinity capture were compared. Figures 5 and 6 show the mass spectra of the drug pairs DAPI-Netropsin and Distamycin-Netropsin. In the first case there is no significant change between the relative intensities after the complexation step, suggesting similar binding affinities for these two molecules. Mass spectra acquired for the drug pairs Hoechst-Netropsin

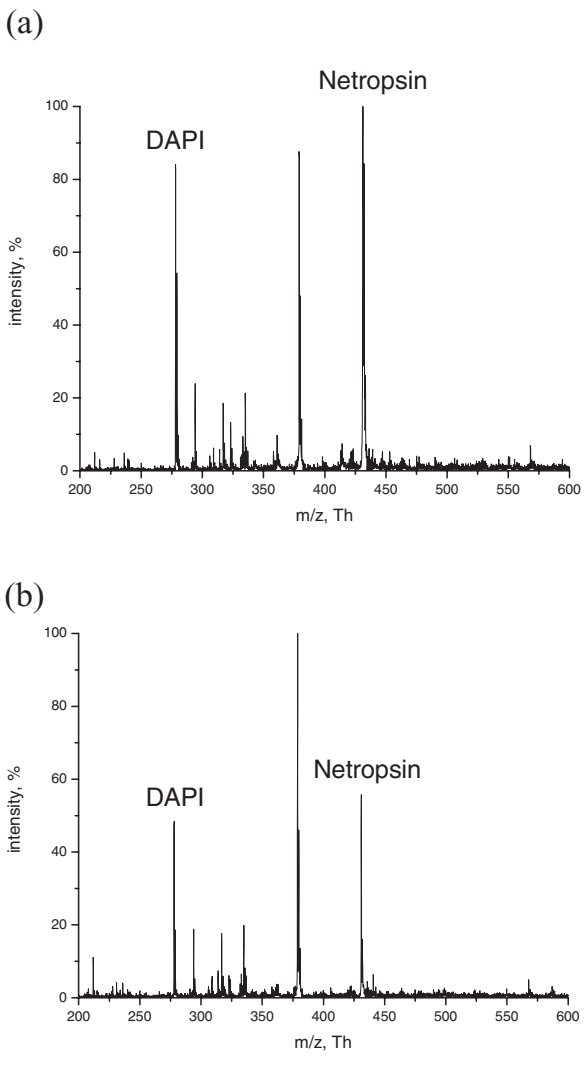

Figure 5. MALDI-TOF mass spectra of an equimolar mixture of DAPI and Netropsin (a) before incubation and (b) after affinity capture.

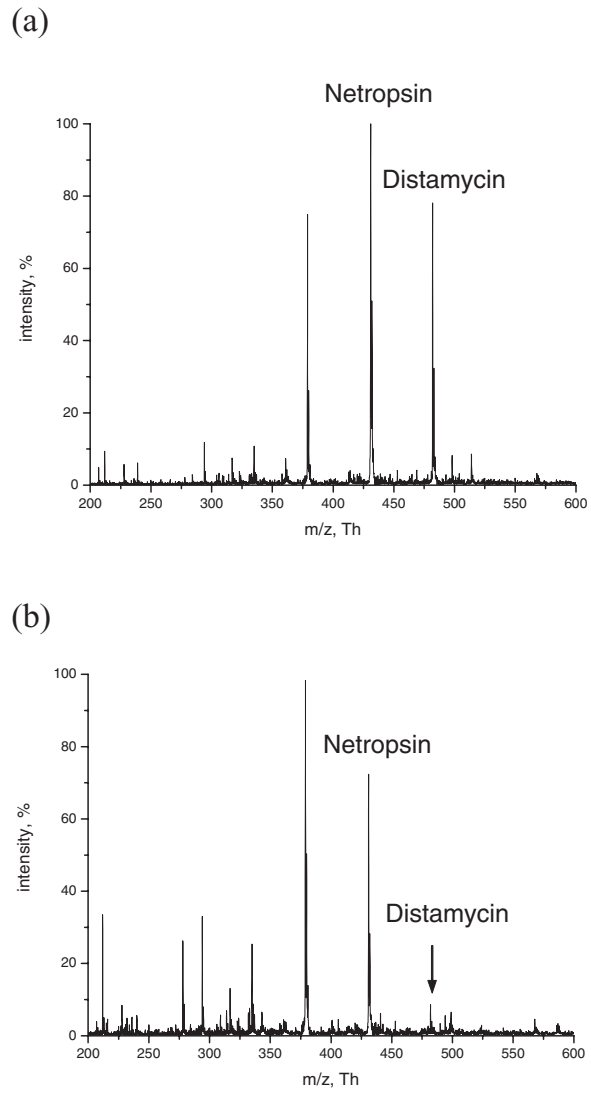

Figure 6. MALDI-TOF mass spectra of an equimolar mixture of Distamycin A and Netropsin (a) before incubation and (b) after affinity capture.

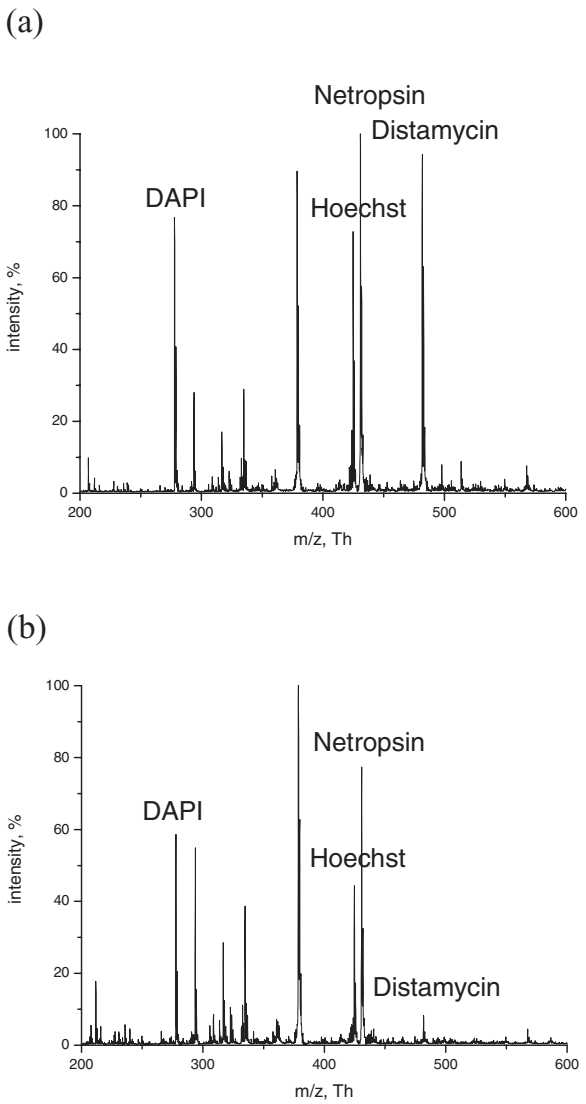

Figure 7. Competitive experiment using an equimolar mixture of the four drugs Netropsin, Distamycin A, Hoechst 33258 and DAPI. MALDI mass spectra of the mixture (a) before incubation and (b) after affinity-capture.

and Hoechst-DAPI showed similar behavior (not shown). In contrast, as shown in Fig. 6, the ratio of the relative intensities of the MH^+ ion peaks for Distamycin and Netropsin was reduced considerably after complexation compared with the initial ratio (from solution). Distamycin showed similar behavior when it was mixed with DAPI and Hoechst, indicating its lower binding affinity compared with the other three drugs studied.

In a fashion similarly to that used for these competitive experiments between pairs of ligands, an equimolar mixture of all four ligands was used in the affinity capture experiment, and the resulting mass spectrum was compared to that acquired for the non-complexed mixture (Fig. 7). In the latter, the intensities of peaks for the four MH^+ ions were quite similar (Fig. 7(a)). However, after the affinity reaction, the intensity of the peak corresponding to Distamycin was reduced considerably while those of the other three peaks did not change. These results are in agreement with the results obtained with ligand pairs, confirming very similar binding affinities for Netropsin, DAPI and Hoechst. Thus the order of relative binding affinities determined by this method is: Distamycin $\ll$ Netropsin $\sim$DAPI $\sim$Hoechst. Boger and co-workers have studied the binding selectivity of these drugs as a function of the length and sequence of the AT-rich motif, and concluded that Distamycin needs the longest AT site.[45] For optimal binding, five base pairs are required for Distamycin, four for Netropsin, while the smaller size of DAPI makes its binding possible also to smaller AT sequence motifs. These findings are consistent with the present results since binding of Distamycin could be hampered by the small groove size of the four base pairs of the oligonucleotide used in this study.

DISCUSSION

This paper has presented a new on-line technique for studying non-covalent binding using solid-phase affinity capture in combination with mass spectrometry. As an example, com-

plexations between duplex oligonucleotides containing an AT-rich motif and minor groove binding drugs were studied. The duplex oligonucleotide is first immobilized to magnetic particles through streptavidin-biotin binding. Subsequently, this molecular probe is immersed in a solution containing the drug, and equilibrium is established. Then the magnetic beads are washed to remove any non-binding compounds present. Finally, the beads containing the oligonucleotide-bound drug are directly analyzed by MALDI-MS. Affinity capture of the molecules in the solid phase provides more control of the complex formation, and makes sample manipulation easy. This approach is somewhat analogous to the SELDI concept, but offers more flexibility and better control over the sample preparation and complexation process. Commercially available magnetic particles are useful not only for immobilization of biomolecules with high capacity, but also because they can be directly analyzed by MALDI-MS. The quantities of both the duplex oligonucleotide and the drug used in one experiment are much less than in a traditional ESI-MS approach, and at the same time the sensitivity appears to be higher. MALDI is not suitable for the detection of all molecules, but the present method is only usable with MALDI. In the case of the ESI-MS procedure already established for various compounds, the complex (on the magnetic beads) must first be decomposed in solution and analyzed in a subsequent step.

The advantage of the present approach is that non-covalent complexation and mass spectrometric analysis are completely separated. Non-covalent binding may be studied in any solution, with no need for a compromise that this solution should be well adapted to mass spectrometry. (Note that here the sol support, and not the electrospray interface, is used to transfer the complex into the mass spectrometer.) The solutions used may be not only those approximating physiological conditions, but also very dilute solutions for which conventional electrospray would not be able to detect complexes. Mass spectrometry is used here only to detect the drug and not the intact oligonucleotide-drug complex, which is much simpler and presents far fewer methodological problems. Drug binding may be best studied in experiments in which two or more compounds are in competition for the binding site; in such cases relative binding constants may also be determined. Another advantage of the direct MS approach is the future potential to measure absolute binding strengths.

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