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Mass Spectrometry–based bioassay for the screening of soluble orphan receptors

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Abstract

The suitability of a novel mass spectrometric (MS) based flow-injection bioassay format to rapidly detect ligands for soluble orphan receptors in complex matrices was demonstrated, using digoxin and antidigoxigenin FAb as the model ligand and the model protein target, respectively. In addition, characteristic MS and MS^n ($n = 2$ or 3) data, that is, molecular mass as well as mass fingerprints, were obtained during a single run and provided a basis for the identification of the active compounds that were found. The flow-injection setup used is characterized by the integration of two restricted access columns (RA column) and a pH dissociation step. Bioactive compounds are separated both from unbound ligands as well as the targeted proteins, e.g., as described in the text. This approach allows the isolated bioactive molecules to be introduced in the ion trap MS detector in a clean and well-defined matrix, which minimizes background noise during sample analysis while simultaneously improving bioassay robustness. In this article, we demonstrate the potential of the bioassay format to detect ligands for soluble orphan receptors. Natural extracts, both spiked and unspiked with the model compound digoxin, were analyzed, which demonstrated that only those compounds possessing affinity for the model protein target were detected by MS. Using an antidigoxigenin FAb concentration of 2 μM, digoxin could be detected down to 250 nM in spiked plant extracts. In contrast, however, molecules that did not bond to the protein target were efficiently trapped by the RA column and did not show up in the MS spectra, thus illustrating the principle and potential of the bioassay format. (Int J Mass Spectrom 210/211 (2001) 625–636) © 2001 Elsevier Science B.V.

1. Introduction

It has long been recognized that the elucidation of the physiological role of orphan receptors, that is, receptors for which no endogenous signaling molecules have been identified to date, could provide additional insights into biochemical processes and possibly could reveal relationships to human diseases. Recent reports on the discovery of new regulatory molecules, and in particular the identification of a new class of antidiabetic drugs [1] and the linkage of orphan receptors to a variety of diseases including cancer [2], support this view and underline the huge pharmaceutical potential of orphan-receptor targets.

Currently, a considerable number of orphan targets have been discovered [3], a number that is believed to increase dramatically within a few years as a result of the human genome project.

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In contrast to the discovery of orphan targets, the detection and identification of ligands for orphan receptors, which represents one of the crucial steps in elucidating the orphan receptor's physiological role, has proven to be a major challenge, as screening of orphan receptors using conventional bioassay formats is not straightforward. Since the late 1980s, several approaches have been developed for detecting and identifying ligands for orphan receptors.

Cell-based screening methods, which convert the binding of a ligand to an orphan receptor into a measurable signal using an intracellular signaling pathway, have been reported as potential detection strategies and currently represent probably the most widely used bioassay strategies for orphan receptor screening [4–11]. However, as little information is known about the orphan receptors, the constructed signaling pathways do not guarantee that the binding of a ligand to the orphan receptor is actually converted in a detectable signal. Moreover, identification and isolation of bioactive compounds in complex matrices is not straightforward and requires extensive fractionation either before or after screening. Banner et al. [12] coupled tissue fractionation with a chimeric transactivation assay to screen human plasma for endogenous activators for PPAR-α. Fractionation of the active subfractions was repeated several times to characterize the bioactive compound by High Performance Liquid Chromatography (HPLC) or Gass Chromatography-mass spectrometry (GC-MS). Similar chemical fractionations in combination with cell-based assays were reported by Soontjens et al. [13] and Civelli et al. [14].

The use of receptor-binding assays, which solely exploit the affinity interaction between ligand and target protein, represents yet another way of detecting ligands for orphan receptors. Banner et al. [15] isolated progesterone from pregnancy plasma using the progesterone-receptor ligand-binding domain by incubating the target proteins with complex sample matrices. Subsequently, analytical chemistry was used to separate bound from unbound ligands, after which the bioactive molecules were derivatized and analyzed by GC-MS. This way, tedious fractionation of the sample matrices was circumvented, and the same approach could be used to screen a range of orphan targets belonging to the steroid hormone-receptor superfamily.

Similarly, fully automated systems capable of detecting and identifying ligands for orphan receptors using a LC-UV/MS have been reported by Hsieh et al. [16], Kaur et al. [17], and Lenz et al. [18]. In general, the protein targets are incubated with compound libraries before injection for a certain period of time, after which the bound and unbound ligands are separated by means of size-exclusion chromatography (SEC). The affinity complexes are subsequently trapped on a reversed phase column, after which dissociation and concentration takes place. The trapped molecules are then eluted and analyzed by MS. As a result of the separation time required, however, the use of SEC as a tool to separate affinity complexes from unbound molecules only allows those molecules to be detected that possess high affinity toward the protein target. In addition, analysis of complex matrices such as natural product extracts, which can contain macromolecules, could lead to erroneous results because of insufficient separation power provided by the SEC column.

In this article we describe a fully automated MS-based bioassay approach toward orphan target screening that is highly suitable for the rapid selection of ligands for soluble orphan targets in complex matrices, such as natural product extracts. Sample matrices and target proteins are mixed on-line, after which restricted-access columns rapidly separate bioactive compounds from unbound ligands as well as from the target proteins of interest. Finally, the isolated bioactive molecules are eluted toward a MS detector operating in data-dependent scan mode. This way, characteristic data of the bioactive molecules are obtained within a single run and can be used as a basis for compound identification.

To simulate the interaction between orphan target and ligand, digoxin, and antidigoxigenin FAb (M_R =

45 kDa) were chosen as, respectively, model ligand and model affinity protein.

2. Experimental

2.1. Chemicals

Antidigoxigenin FAb was purchased from Roche Diagnostics BV (Almere, The Netherlands). TRIS, trifluoric acetic acid (TFA), bovin serum albumine (BSA), and digoxin were bought at Sigma-Aldrich BV (Zwijndrecht, The Netherlands). Hydrocloric acid and sodium acetate were purchased at E. Merck BV (Amsterdam, The Netherlands). ALEXA Fluor 488 succinimidylic ester, dilithium salt (FLUOS) was bought at Molecular Probes Europe (Leiden, The Netherlands). Acetonitrile and methanol were obtained from Rathburn (Walkerburn, Scotland). Restricted access material, C18 ADS, was kindly donated by Merck (Darmstadt, Germany).

2.2. Apparatus

2.2.1. LC-system.

The MS-based bioassay suitable for orphan target screening was incorporated into ScreenTec's HRS340-B screening system and controlled by ScreenControl 3.0 software. In total, six Knauer (Berlin, Germany) Ministar LC pumps were used to deliver all reagents required for the MS-based bioassay. A Cavro autosampler (Karlsheim, Germany) was used for sample handling. A Valco stainless-steel six-port switching valve (Schenkon, Switzerland) equipped with a 20-μL injection loop was used as an injection valve. RA columns were mounted on Valco stainless-steel six-port switching valves. A Valco six-port solvent-select valve was used to enable proper RA-column regeneration.

The carrier phase pump delivered a solution of 10 mM TRIS/10% DMSO (pH 7.0) at a flow rate of 200 μL/min. The flow rate was temporarily decreased to 100 μL/min the moment a sample was injected. Simultaneously, a second LC pump, delivering a 2-μM antidigoxigenin FAb solution in MilliQ to the carrier solution, was started at a flow rate of 100 μL/min. After 60 s, the introduction of antidigoxigenin FAb solution was stopped, whereas the flow rate of the carrier solution was restored to the original setting.

A third LC pump was used to deliver an acidic solution consisting of 0.25% TFA/10% acetonitrile at a flow rate of 0.2 mL/min. A fourth LC pump was introduced to desorb trapped bioactive molecules from the second RA column using a 75%MeOH/2 mM ammonium acetate solution at a flow rate of 50 μL/min. The mobile phase was constantly introduced into a Finnigan DECA mass spectrometer (Breda, The Netherlands).

In addition, two LC pumps were used to regenerate and flush the RA columns. One LC pump was connected to a Valco six-port solvent-select valve and used deionized water (MilliQ) and 60% isopropanol/0.1%TFA to regenerate the first RA column. Nonspecific binding sites were blocked by flushing the RA column with a 0.1 g/L BSA solution. A second LC pump was used to flush the second RA column with a 1% acetic acid solution at a flow rate of 1 mL/min. The dimensions of the C18 ADS RA columns used were, 2 $\times$ 30 mm for the first column and 2 $\times$ 5 mm for the second column. Knitted 0.25-mm I.D. (internal diameter) PTFE reaction coils were used to perform the association as well as the dissociation reaction.

2.2.2. MS instrumentation

Mass spectrometric measurements were performed on a Finnigan DECA mass spectrometer equipped with an ESI probe. During measurements, in SIM, normal full-scan MS, or MS/MS data-dependent scanning mode, the ESI probe was operated in positive-ion mode. The capillary temperature was set at 170 °C. The 75%MeOH/2 mM ammonium acetate solution was constantly introduced in the ESI probe at a flow rate of 50 μL/min. The sheath gas and auxillary gas flow rate equaled 70 and 15 arbitrary units, respectively. The DECA mass spectrometer was not tuned to a specific *m/z* value but, instead, general settings were applied to cover a broad *m/z* range.

During MS/MS data-dependent scanning, the most intense ion present in the MS spectrum was automat-

ically designated as the parent mass for MS/MS measurements. Background ions were specified up front and were automatically ignored as potential parent masses. The activation amplitude was set at 50%, whereas the *q*-value equaled 0.25. The isolation width of the parent mass was adjusted to 10 a.m.u. (atomic mass units).

2.3. Regeneration of RA columns

Depending on the type of samples analyzed, the first RA column, used to separate nonbioactive molecules from affinity complexes, was regenerated at certain time intervals. In short, the RA column was rinsed with deionized water for 3 min at 1 mL/min. Subsequently, compounds trapped onto the RA column were eluted by flushing with a 60% isopropanol/0.1%TFA solution for 5 min at 1 mL/min. Before blocking the nonspecific binding sites, the RA column was flushed with deionized water for 3 min at 1 mL/min. Subsequently, the first RA column was flushed with a BSA solution (0.1 g/L in PBS) for 3 min at 1 mL/min. Excess BSA was removed by rinsing with deionized water for 3 min at 1 mL/min.

2.4. pH optimum dissociation of affinity complexes

Batch samples, containing 2 μM anti digoxigenin FAb and 1 μM digoxin, were incubated for 2 min at different pH values. The pH values used ranged from 1 to 7. The extent of affinity complex dissociation was evaluated by monitoring the concentration of unbound digoxin as a function of pH value. This was achieved by performing selective ion monitoring of *m/z* 798.2, which represents the ammonium ion of digoxin.

Batch incubate samples were injected into a carrier phase (0.4 mL/min), which was directly passed over a RA column (ADS C18, 2×5 mm). The buffer composition of the carrier phase was identical to that of the sample injected. Affinity complexes were eluted towards waste, whereas unbound digoxin was trapped. After flushing with acetic acid (1%) for 1 min at 1 ml/min, the RA-column was eluted with 75%MeOH/2 mM ammonium acetate at 50 μl/min, which was directly leaded towards the MS detector.

2.5. Labeling of anti digoxigenin FAb fragments

Fluorescent labeling of antidigoxigenin FAb fragments was performed according to the specifications included with the labeling kit, provided by Molecular Probes. In short, 0.5 mg of antidigoxigenin FAb was dissolved in 500 μL PBS to which 50 μL of 1 M carbonate buffer (pH 8.3) was added. The mixture was transferred to a vial containing the fluorescent dye FLUOS. The mixture was allowed to react for 2h at room temperature under constant magnetic stirring. Subsequently, the fluorescent antidigoxigenin-labeled FAb fragments were separated from excess fluorescent dyes by size-exclusion chromatography. Fluorescent-labeled antidigoxigenin FAb was monitored at excitation and emission wavelengths of 486 and 516 nm, respectively.

3. Results and discussion

3.1. Description MS-based bioassay format

The general principle of the MS-based bioassay suitable for orphan target screening approach is outlined in Fig. 1. After injecting the sample into the carrier phase, a plug of affinity protein is added during a period of 60 s. A time interval that ensures that, under the conditions described, the entire sample is able to react with the target proteins. The duration of the reaction period is determined by the volume of the reaction coil applied. By introducing a protein plug instead of constantly adding the target proteins to the carrier phase, a considerable reduction in affinity-protein consumption is achieved. Subsequently, by implementing a RA column, low–molecular mass molecules, which did not form an affinity complex during the reaction period, are trapped inside the small hydrophobic pores of the RA beads. In contrast, the high–molecular mass affinity proteins and complexes are allowed to pass the RA column unretained, as their size prohibits diffusion into the small hydrophobic pores. Nonspecific binding of these proteins is largely avoided because of the hydrophilic nature of the surface of the RA beads. As a result, a fast and

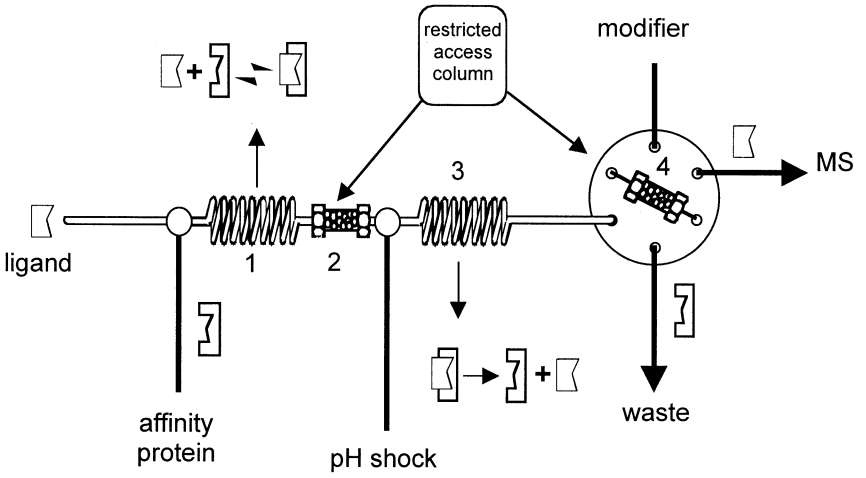

Fig. 1. Scheme of the MS-base bioassay. 1, reaction coil for association; 2, trapping of unbound ligands by RA-column; 3, reaction coil for dissociation; 4, trapping of bioactive molecules by RA-column.

efficient separation is achieved between those compounds either that, do not or that only weakly form an affinity complex with the target protein and those that do. Besides efficiently separating the bound molecular from the unbound molecules, implementation of RA columns expands the range of affinity interactions, which can be monitored using the current setup, to lower K_D values as a result of the short residence times onto the RA column. As the affinity complexes typically pass the RA columns within seconds, affinity-complex dissociation becomes less pronounced when compared with other separation methods, such as size exclusion chromatography [19].

Subsequently, after passing the first RA column, the affinity complex is subjected to a "pH shock," which disrupts the affinity interaction between target protein and bioactive compound. After dissociation of the affinity complex, separation of the free bioactive compound and the target protein is easily accomplished by introducing a second RA column. Ligands, originating from the dissociated affinity complex, are trapped into the hydrophobic pores, whereas the target protein passes the RA column unretained and is directed toward waste. In this way, bioactive compounds are isolated from nonbioactive molecules as well as from the affinity proteins. After this loading phase, which typically takes 2 min, the second RA column is washed extensively with 1% acetic acid to remove the majority of the impurities, such as salts, originating from sources such as reagent solutions and samples. Subsequently the second RA column is switched into a 75% MeOH/2 mM ammonium acetate solution, which is constantly introduced into the ESI probe. Trapped bioactive compounds are eluted from the RA column in a well-defined matrix at a flow rate of 50 μL/min and are subsequently analyzed by MS, using data-dependent scanning. This way, characteristic MS and MS^n ($n = 2$ or 3) data of bioactive compounds are recorded during a single run. Molecular mass information is obtained from the MS data, whereas a mass fingerprint of the bioactive molecule is provided by MS^n spectra.

3.2. Optimization of bioassay parameters

3.2.1. Concentrations and reaction times

Besides the ionization efficiency of the bioactive compounds and the MS and MS/MS settings applied, the sensitivity of the MS-based bioassay largely depends on the ability to obtain those reaction conditions that lead to high reaction efficiencies. Ideally, the interaction between bioactive compound and target protein should result in a high percentage of affinity complex formed during the association reac-

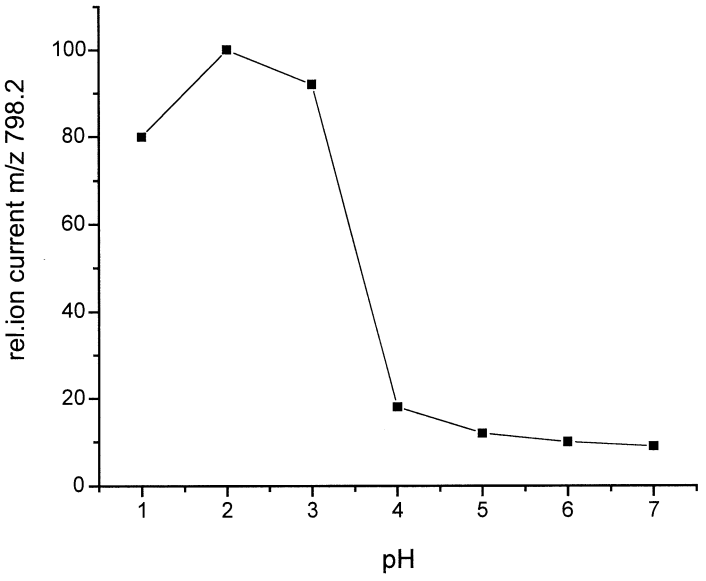

Fig. 2. Dissociation of the digoxin - anti digoxigenin FAb complex as a function of pH.

tion, whereas most of these affinity complexes should be disrupted again during the dissociation step. In general, bioreagent concentration and composition, as well as the reaction times employed, represent key parameters to optimize to achieve high reaction efficiencies.

The model affinity interaction between digoxin and antidigoxigenin FAb, which was used to demonstrate the suitability of this bioassay format to detect ligands for orphan receptors, is characterized by an association rate constant of $k = 10^7\ I\ \mathrm{mol}^1\ \mathrm{s}^1$ [20]. Using an equation that was described earlier [21], the extent of affinity-complex formation as a function of time can be calculated according to

$$[PL]_{t=1} = [P]_0[L]_o \frac{e^{-([L]_0-[P]_0)k_{+1}t} - 1}{[P]_0 e^{-([L]_0-[P]_0)k_{+1}t} - [L]_0}, \qquad (1)$$

in which k_{+1} represents the association rate constant, $[P]_0$ the concentration of target protein at t_o, $[L]_0$ is the concentration of ligand at t_o, and $[PL]_{t=1}$ is the concentration of affinity complex formed at $t = 1$.

Eq. 1 assumes that the influence of dissociation is limited during the reaction period applied and is, hence, neglected in the formula. When working with relatively high concentrations of digoxin and antidigoxigenin FAb (μM level) and a moderate association reaction time, this assumption is true. Using starting concentrations of 1 μM digoxin (15 times the detection limit when injected directly onto the second RA column) and 2 μM antidigoxigenin FAb, it can be calculated that most of the digoxin has bonded to the antidigoxigenin FAb within seconds. As the aim of the studies was to demonstrate the principle of the bioassay format, further optimization of the association reaction time was not conducted. During the studies, a sufficiently long association reaction time (30 s) was employed, which ensured a high association reaction efficiency.

In addition, Eq. 1 shows that in the case of orphan receptors, for which no association and dissociation rate constants are known, a high–protein target concentration as well as a long association reaction time should be employed to enhance reaction efficiencies as much as possible.

Dissociation was accomplished by mixing the affinity complexes with a solution of low pH value for 30 s. The optimum pH value for dissociation was obtained by determining the binding efficiency between digoxin and antidigoxigenin FAb as a function of pH value.

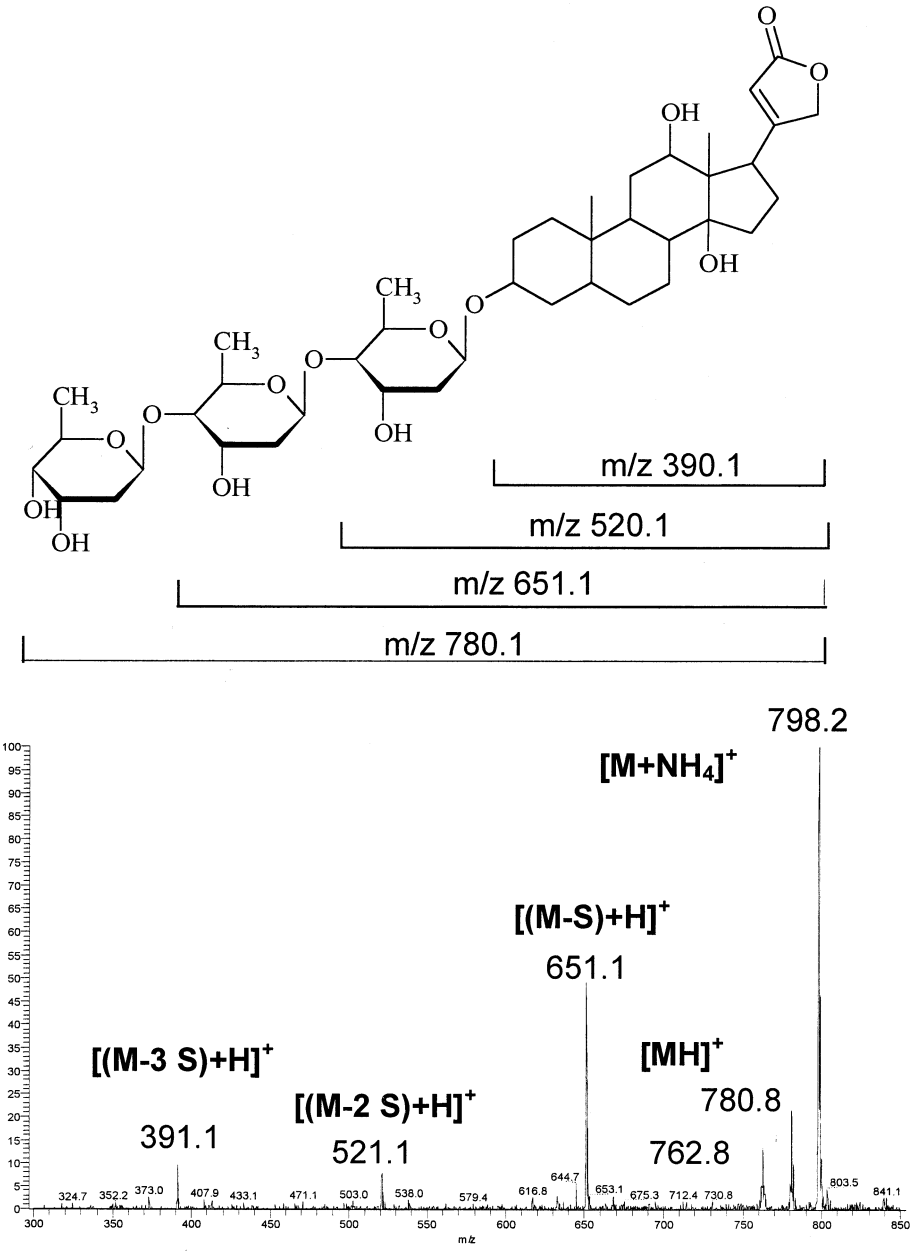

Fig. 3. Full scan MS analysis of digoxin. S represents a sugar moiety.

3.2.2. pH optimization affinity complex dissociation

Proper functioning of the bioassay strongly depends on the ability to dissociate affinity complexes during application of the pH shock. Incomplete dissociation results in loss of sensitivity as bioactive compounds are not efficiently trapped onto the second RA column. To ensure efficient dissociation of the digoxin–antidigoxigenin FAb complex, several batch incubations were performed to determine the most suitable pH value. The extent of affinity complex dissociation was evaluated by monitoring the intensity of the ammonium ion of digoxin at *m/z* 798.2. Fig. 2

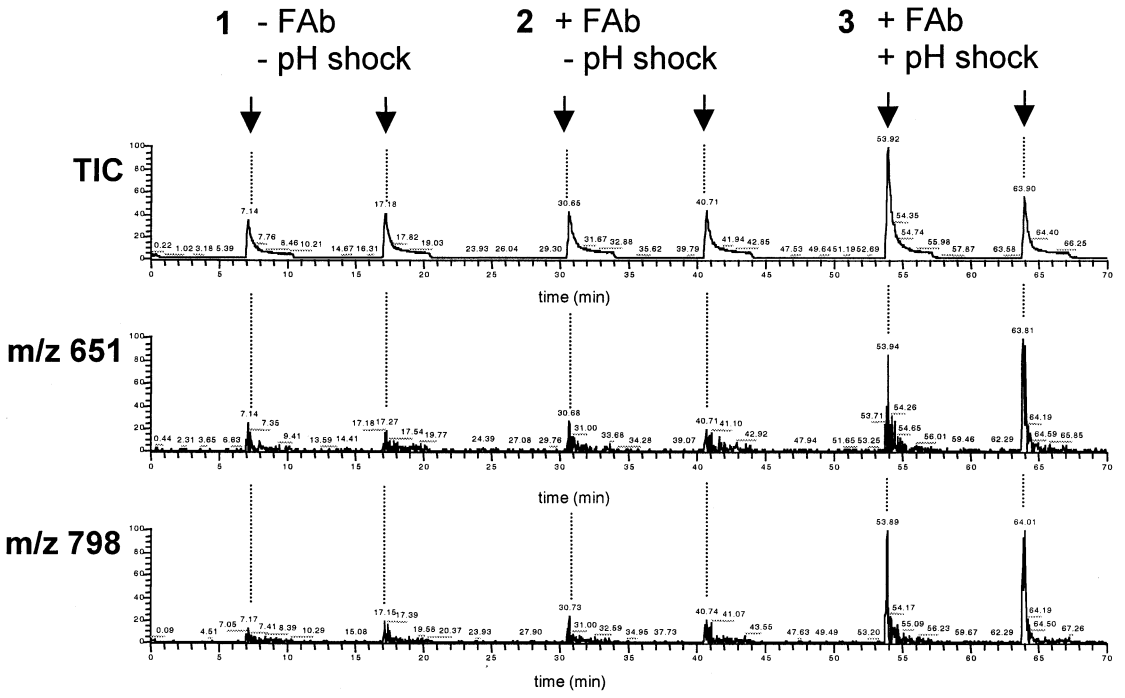

Fig. 4. Demonstration of MS-based bioassay functionality. Injections represent academic 1 μM digoxin samples. 1: Affinity protein and pH dissociation solution replaced by buffer solutions. 2: pH dissociation solution replaced by buffer solution. 3: All MS-based bioassay solutions installed.

represents the concentration of unbound digoxin as a function of pH value. As could be expected, the intensity of *m/z* 798.2 remained low at neutral and slightly acidic conditions, indicating that only a little dissociation of the affinity complex had occurred under these conditions. Using more acidic conditions, the ion intensity increased rapidly and reached a maximum at approximately pH 2. Accordingly, the pH value of the dissociation solution used during bioassay operation was adjusted in such a way as to obtain similar acidic conditions during the pH shock. During bioassay operation, the dissociation reaction was allowed to proceed for 30 s.

Compared to normal C18 column material, the implementation of a RA column, which rapidly separates large proteins from small ligands, presents an efficient way to reduce the introduction of target proteins into the MS detector and, consequently, decreases the background noise level. Working with proteins at low pH values, however, poses the problem of precipitation, which could affect bioassay performance. In case of antidigoxigenin FAb, however, 86% ($\pm$7.4%) passed the second RA column at pH 2, which was comparable to neutral conditions, thus indicating that only a small fraction of the antidigoxigenin FAb fragments was trapped as a result of nonspecific binding. The recovery of the antidigoxigenin FAb fragments when used at pH 2 was determined by injecting aliquots of FLUOS-labeled FAb fragments (100-nM injections).

In addition, 95% ($\pm$6.8%) of antidigoxigenin FAb fragments were recovered when passing the first RA column. This recovery percentage, compared to that of the second RA column, can be explained by the fact that nonspecific binding sites were blocked by BSA during the regeneration procedure. In the case of the second RA column, employing a similar blocking procedure would lead to an increase of background as BSA is eluted toward the MS detector together with the trapped bioactive molecules. To avoid nonspecific

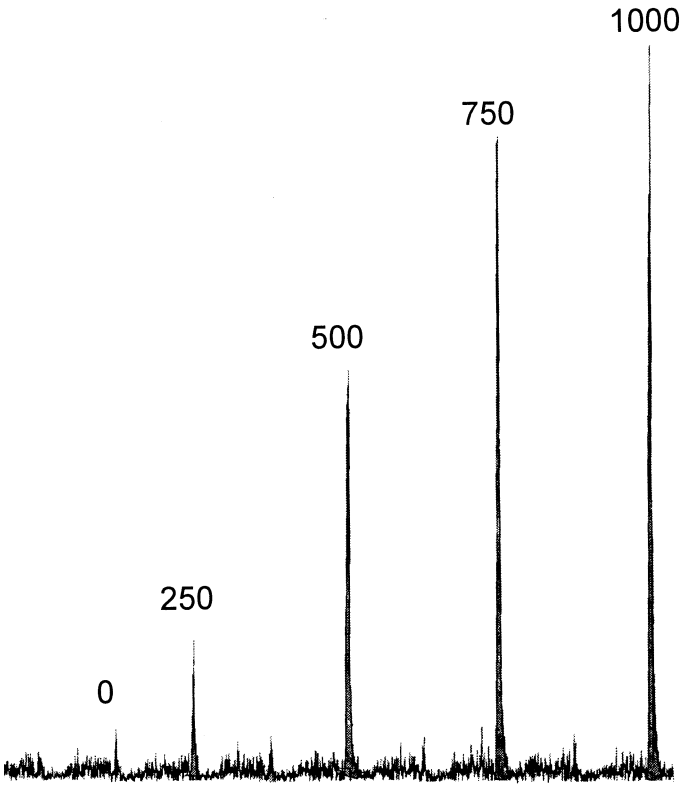

Fig. 5. Injection of various concentrations of digoxin (0, 250, 500, 750 and 1000 nM).

binding of target proteins as much as possible, the dimensions of the last RA column were minimized (2×5 mm).

3.3. Flow injection MS-based bioassay

To demonstrate proper functioning of the MS-based bioassay and illustrate the potential for orphan target screening, digoxin samples were injected under several bioassay conditions. The presence of digoxin onto the second RA column was evaluated by monitoring the reconstructed ion currents of two *m/z* values, 651.1 and 798.2, that were characteristic for digoxin under the conditions applied (Fig. 3). Fig. 4 shows the results of the digoxin injections (1 μM) using three different bioassay conditions.

First, digoxin was injected into a bioassay system in which the antidigoxigenin FAb as well as the dissociation solution were replaced by buffer (10 mM ammonium acetate, pH 6.7). The ion current traces for *m/z* 651 and 798 are comparable to those obtained for blank injections, thus indicating that digoxin is efficiently trapped onto the first RA column. The ion current peaks, which can be observed in the total, as well as reconstructed ion current profiles are caused by the introduction of residual ions and solvents (DMSO), which were not completely removed from the RA column during flushing.

During a second step, antidigoxigenin FAb was added to the bioassay, while the dissociation solution was still replaced by buffer. Again, the digoxin injections showed hardly any increase in ion current, indicating that the affinity complexes formed passed both the first and second RA columns.

Finally, digoxin was injected into a complete bioassay system containing both the affinity protein as well as the dissociation solution. The ion current traces of *m/z* 651 and 798 both clearly show a

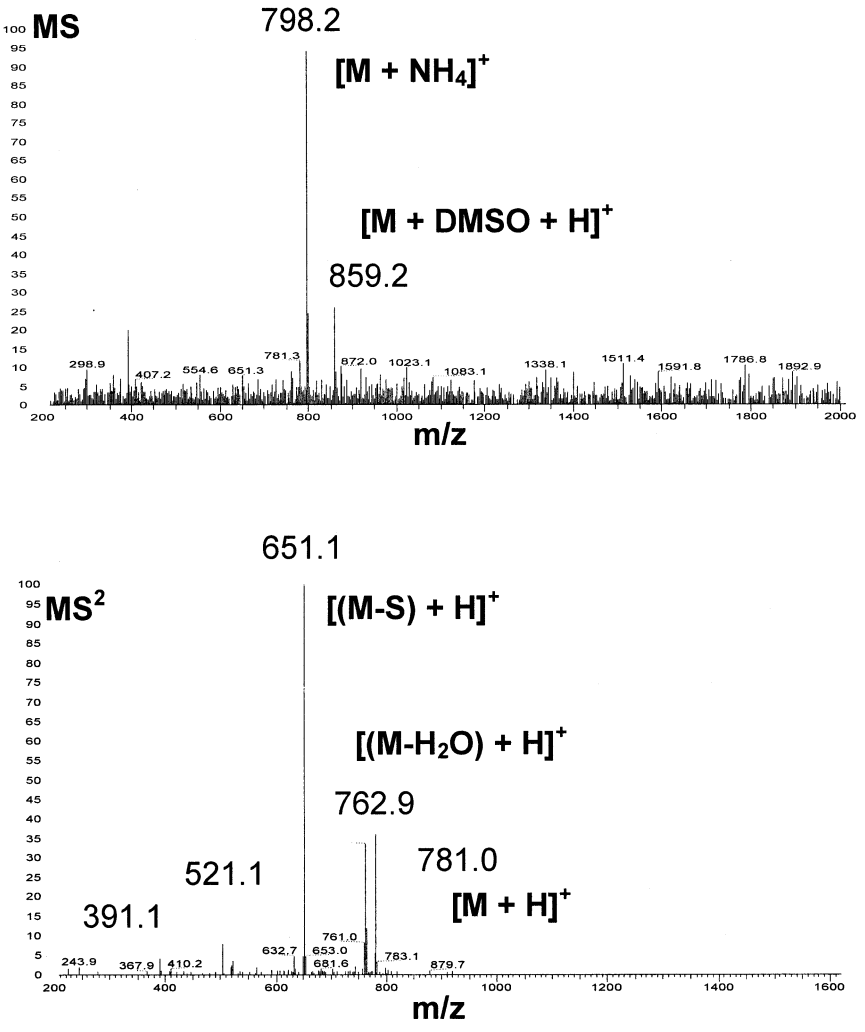

Fig. 6. Operation of MS-based bioassay in MS/MS data dependent scan mode. Injection of 1000 nM digoxin. S represents a sugar moiety.

significant increase in intensity compared with the previous digoxin injections. From these experiments it can be concluded that the affinity complexes between digoxin and antidigoxigenin FAb were indeed formed, passed the first RA column, and finally were dissociated by the pH shock applied. As a result, digoxin molecules were trapped onto the second RA column and were detected by full-scan MS analysis after column desorption. Under these semioptimized conditions, digoxin could be detected down to 250 nM (Fig. 5), whereas the precision of 1-μM digoxin injections equaled 13.0% ($n = 5$).

In addition, the Finnigan DECA mass spectrometer offers the opportunity to collect data in the data-dependent scanning mode. When applied in combination with the current bioassay format, compound characteristic data, such as molecular mass and mass fingerprints, are obtained during a single run. Fig. 6 presents an example of the MS-based bioassay using data-dependent scanning. Digoxin is most abundant as *m/z* 798.2 during MS scanning. In addition, depending on the distance of the spray tip from the heated capillary, digoxin adducts of residual DMSO and TRIS can be observed. Using the data-dependent

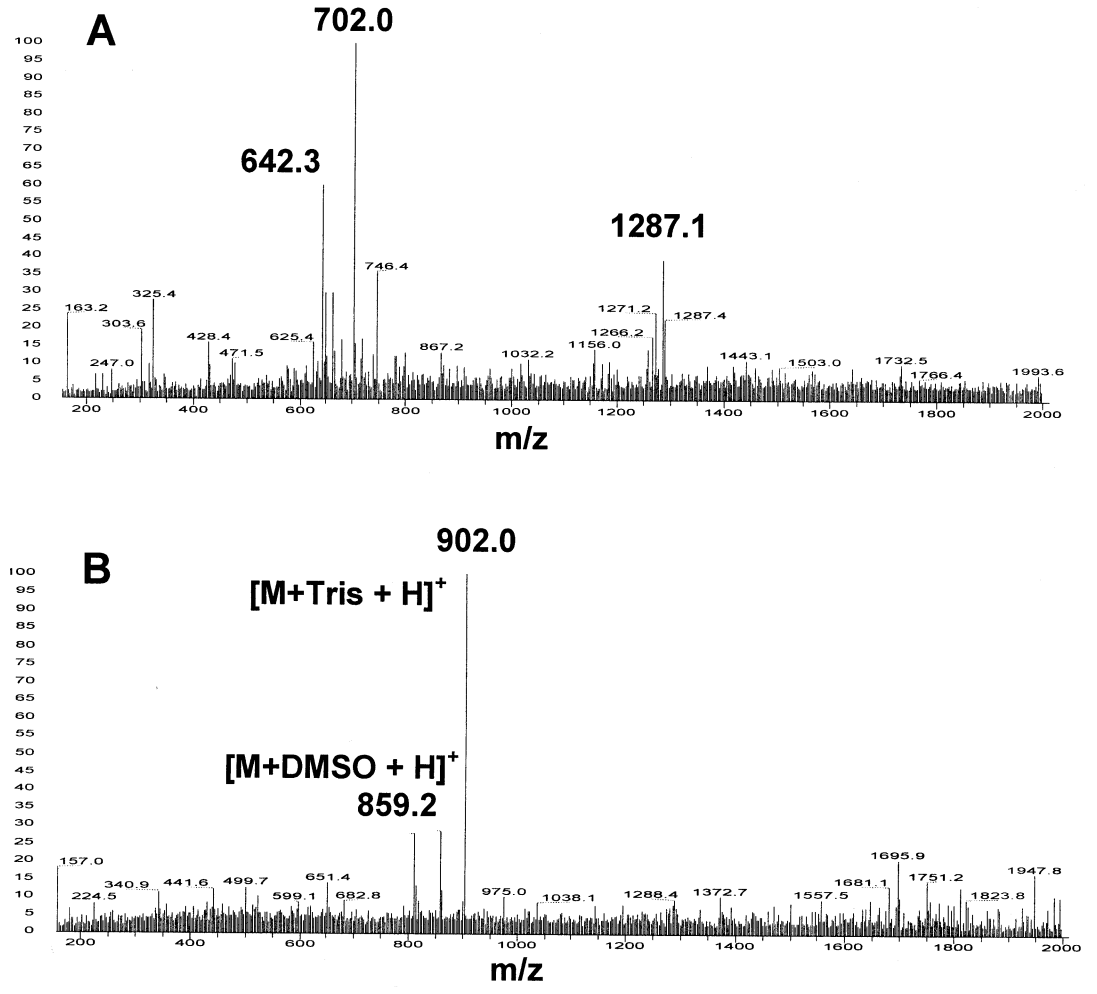

Fig. 7. Demonstration of MS-based bioassay functionality using a plant extract. A: MS analysis of pure extract. B: Analysis of digoxin spiked extract (250 nM).

scanning mode, the most intense ion obtained during MS scanning is designated as parent mass for MS/MS measurements. The MS/MS spectrum clearly shows the loss of ammonium (*m/z* 781.0), the loss of water (*m/z* 762.9), and the characteristic formation of *m/z* 651.1, which represents the loss of a sugar moiety. Similarly, MS and MS/MS data can be obtained from unknown bioactive compounds, which can provide the basis for compound identification using MS and MS/MS libraries. In addition, molecular masses and mass fingerprints that turn up frequently in the bioassay and that have been determined to be of no interest can quickly be recognized and ignored.

To demonstrate proper functioning of the MS-based bioassay using complex sample matrices, higher plant extracts were diluted 10 times with 10 mM TRIS buffer (pH 7.0), spiked with digoxin (250 nM), and subsequently analyzed. Fig. 7 shows an example of such a spiked extract. First, an unspiked aliquot of the extract was injected into the MS-based bioassay of which the first RA column was removed. Consequently, moderately polar to hydrophobic compounds present in the sample were trapped onto the second RA column and were eluted towards the MS detector. The principle of the MS-based bioassay was then demonstrated by reinserting the RA column

again, followed by the injection of a spiked aliquot of the same plant extract. As can be seen in Fig. 7, the first RA column efficiently traps the nonbioactive molecules, which were present in the plant extract. The bioactive compound digoxin, however, is successfully isolated from the plant extract and can clearly be observed in the MS spectrum (*m/z* 859.2 and 902.0). By injecting spiked and unspiked aliquots of natural extracts, the ability of the bioassay format to rapidly detect ligands for protein targets, such as soluble orphan receptors, is demonstrated.

4. Conclusions

The principle of the MS-based bioassay has been demonstrated using the model affinity interaction between digoxin and antidigoxigenin FAb and underlines the potential of the current method to detect ligands for orphan receptors. The use of RA columns as a tool to separate bound from unbound molecules results in short separation times, which reduce dissociation of the affinity complexes formed and, thus, enhance the detection of ligands. In addition, the range of sample sources suitable for screening is extended and is not limited to relatively clean and defined sample matrices. This way, elaborate fractionation efforts are circumvented, thus speeding up the screening process. Combined with data-dependent scanning, the bioassay format provides a rapid tool for detecting bioactive compounds for soluble orphan receptors while obtaining characteristic MS and MS^n ($n = 2$ or 3) data simultaneously. The MS and MS^n data obtained present a basis for identifying the detected bioactive molecules. In addition, compounds that are found frequently in the bioassay and have been determined not to be of any interest are rapidly recognized by their molecular weight and corresponding mass fingerprint and can be excluded for further studies.

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