Talanta 85 (2011) 1698–1702

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

DNA assay based on monolayer-barcoded nanoparticles for mass spectrometry in combination with magnetic microprobes

Fei Qiu[b,1], Kai Gu[c,1], Bing Yang[a], Yibing Ding[a], Dawei Jiang[c], Yan Wu[a], Lequn Lee Huang[a,*]

[a] Institute of Molecular Medicine, Nanjing University, 22 Hankou Road, Nanjing 210093, PR China
[b] Institute of Molecular Medicine, Huaqiao University, 269 Chenghuabei Road, Quanzhou 362021, PR China
[c] School of Chemistry and Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing 210093, PR China

ARTICLE INFO

Article history:
Received 21 February 2011
Received in revised form 14 June 2011
Accepted 15 June 2011
Available online 21 June 2011

Keywords:
DNA detection
Matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS)
Reporter mass molecules
Gold nanoparticle probe
Magnetic microparticle probe
Single nucleotide polymorphism (SNP) detection

ABSTRACT

Mass spectrometry (MS) based methodology offers simple, fast and sensitive diagnosis. While it has become the predominate approach in biomolecular analysis, it has not been suitable for analyzing nucleic acid due to its low ionization efficiency. We report herein on a DNA assay based on monolayer-barcoded nanoparticles that were encoded with reporter mass molecules, which act as surrogate molecules for the matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) identification of target DNA through mass spectrometry in combination with magnetic microprobes. This assay demonstrated high MS sensitivity, with the ability to detect target DNA at femtomolar (10^{-15} M) levels. This inaugural effort using combined techniques is significant because it showed an extraordinary analytical capability for differentiating the single nucleotide polymorphism (SNP), which comprises the most abundant source of genetic variation in the human genome. We also report herein the feasibility of MS detection of two target DNAs that have the same mass but different nucleotide base composition, which classic MS methodology is inherently unable to differentiate.

1. Introduction

Mass spectrometry (MS) has long been utilized to determine the molecular mass of small organic and inorganic molecules, and to characterize large biomolecules such as peptides and proteins [1–3]. In recent years, MS applications have been further extended to environmental, forensic and medical research [4–6]. Such widely accepted applications are due to the specificity, speed and sensitivity of MS [1,2]. However, compared to the detection of diverse sets of biomolecules, such as peptides, MS has not been the platform of choice for the direct detection of DNA molecules [7–9]. Of the many issues involved, two pose major hurdles [10–12]. First, MS sensitivity for DNA detection has not been achieved at low femtomolar (fM) range, as is typically achievable for biomolecules such as peptides and proteins. This is due to the low ionization efficiency of most DNA molecules. Second, unlike successful MS sequencing of peptide molecules against known protein databases, denovo DNA sequence specific information cannot be easily obtained by MS analysis due to the incomplete fragment ion readout nature of the DNA molecules by tandem MS.

Due to the lack of ionization efficiency of DNA molecules resulted in poor sensitivity, typically in a nanomolar (10^{-9} M) range [13], MS analysis of DNA molecules for molecular diagnosis is still a challenge. Many efforts to analyze DNA indirectly utilizing MS have been made by detecting small molecular tags or peptides [12,14–16] that encode DNA molecules. This encoding strategy is based on the indirect detection of a probe modified with a photo-cleavable linker coupled with a tag that is a small molecule of known mass easily detectable by means of mass spectrometry, and which is released just before the detection step. Tagged photo-cleavable linkers can be chemically attached to different classes of probes such as DNA, cDNA, single stranded cRNA, aptamer or antibody probes. Those methods are excellent for multiplexed detection, for rapid, simultaneous single nucleotide polymorphism (SNP) detection, and for proteomics. However, they introduced relative complexity in covalent bond formation of modifying tags to the DNA molecules.

Addition of non-covalent probes to enhance the specificity of the DNA detection has been reported in many studies in which nanoparticle probes (NPs) showed several advantages over others. NPs are frequently coupled with fluorescence [17] and other detection methods such as colorimetry [18] and Raman spectroscopy [19]. MS offers unparalleled detection specificity and resolution and

* Corresponding author. Tel.: +86 25 8368 6041; fax: +86 25 8340 2848.
E-mail addresses: huanglq@nju.edu.cn, lhuang@sinobiopharma.com (L.L. Huang).
[1] Both authors contributed equally to this work.

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.06.045

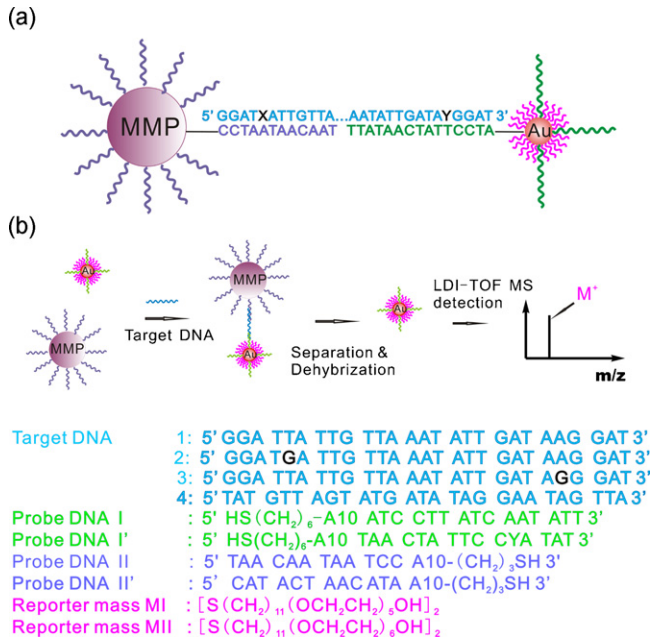

Fig. 1. (a) Schematic diagram of MMP–DNA–NPS. (b) MS based assay in combination of MMP–DNA–NPS. Reporter mass barcoded gold nanoparticles including 25 bases probing DNA I recognized the target DNA from 3′ terminal and function as a signal readout probe. The other 22 bases probing DNA II were covalently immobilized on the surface of MMP. MMP with DNA II was utilized as a capture probe for the target DNA from 5′ terminal and also functionalized as a concentration and separation tool for the target DNA in solution.

can serve as a better method for DNA detection when combined with NPs. Nanoparticles, such as Ag [20,21] and Au [22,23], which demonstrated as desorption/ionization matrices, have also been used for many years to facilitate analyses by mass spectrometry (MS).

In earlier proof-of-concept experiments, we demonstrated the use of monolayer nanoparticles for on-chip DNA hybridization assay [24]. It showed that MS analysis of DNAs can be realized at the 100 pM level. Unlike the previous study, in which the probe complex was bound on the silicon chip surface, we have recently developed an assay (illustrated in Fig. 1) in which the encoded mass nanoparticles and probing DNA strands are assembled on the magnetic microparticle (MMP) and served as a probe. Since MMP is a powerful tool for separation and enrichment of biomolecules [25,26], it was found that the MS detection limit of DNA using this assay is at a lower fM level, one that approaches that of familiar methods such as fluorescence [17], colorimetry [18], Raman spectroscopy [19] and PCR [27]. We have shown that our methodology can detect and differentiate the most frequently occurring single-base genetic mutations because dissociations of those three-component sandwich complexes of the MMP–DNA–NPs between perfectly complementary target strands and single nucleotide mismatches are differentiated [26,28] in the assay. We also report herein the feasibility of MS detection of two target DNAs that may have the same mass but different nucleotide base composition. Those possibilities are demonstrated because mass detection is not the direct detection of the target DNA, but rather the detection of the amplified "reporter mass" that acts as surrogate molecules.

2. Experimental

2.1. Materials and reagents

All DNA strands were synthesized and purified by Invitrogen Co. (Shanghai, China). $HAuCl_4{\cdot}4H_2O$ (99.9%) came from ShangJuly Chemical Co., Ltd. (Shanghai, China). Succinimidyl 4-[*p*-maleimidophenyl]butyrate (SMPB) was obtained from Pierce Biotechnology Co. (USA). Amino-functionalized MMP solution (Dynal Biotech Co., Dynabeads M-270 Amine, 2.8 μm) was commercially available from Invitrogen Co. Nanopure water (18.2 MΩ cm), purified by a Sartorius Arium 611 system, was used throughout the experiment. Mass-tagged molecules MI ($[S(CH_2)_{11}(OCH_2CH_2)_5OH]_2$) and MII ($[S(CH_2)_{11}(OCH_2CH_2)_6OH]_2$) were synthesized by previously reported methods [29,30].

A Bruker Ultraflex III mass spectrometer was used for MALDI-TOF MS detection. The mass spectrometer was operated in the positive ion, reflector mode under a nitrogen laser (337 nm) for desorption and ionization and an accelerating voltage of about 20 kV. For the limit detection, pure oligonucleotide-modified nanoparticles were used as the matrix and a Bruker MALDI target plate (AnchorChipR 400-384) was used as the target plate.

2.2. MMP–DNA–NPS-based DNA assay

Oligo-MMPs (MMP surface covalently immobilized HCV-DNA (5′-SH-$(CH_2)_6$-A AAA AAA AAA GCA CCC TAT CAG-3′) were prepared via reported protocol [31]. The double-functionalized gold NP with reporter mass-tagged molecules MI and HCV-specific oligonucleotides (5′-GCA GTA CCA CAA GGC AAA AAA AAA A-$(CH_2)_3$-SH-3′) was prepared as previously reported [24]. Oligo-MMPs (5 mg/mL, 50 μL) and the double-functionalized gold NPs (50 μL) were allowed to hybridize with 27 bp HCV target (5′-GCC TTG TGG TAC TGC CTG ATA GGG TGC-3′) in series dilution from 10^{-11} M to 10^{-15} M in 30 μL hybridization buffer (0.3 M NaCl, 10 mM phosphate buffer, pH 7.0), followed by the addition of 6 M NaCl solution (10 μL). The complex was first incubated at 45 °C for 30 min, and then rotated for 24 h at 25 °C. The sandwich complexes (MMP–target–NPs) were separated on a 12-well plate magnetic separator (Promega Co.) and washed seven times with 200 μL PBS buffer (0.6 M NaCl, 10 mM phosphate buffer, pH 7.0). The resulting complexes were dispersed in 10 μL of nanopure water, warmed to 75 °C and stayed for 5 min, and then immediately separated on a magnetic separator. Then the supernatants were analyzed by the mass spectrometer. Two μL of de-hybridization products (the released NPs) were dotted on the plate AnchorChipR 400-384). Then, the matrix was applied as a solution onto the plate and subsequently allowed to dry at ambient temperature. For MS detection, pure oligonucleotide (5′-GCA GTA CCA CAA GGC AAA AAA AAA A-$(CH_2)_3$-SH-3′)-modified gold NPs (prepared via reported protocol [32]) was used as the matrix. Each test was repeated three times.

For long chain of DNA detection, the 27 bp HCV-DNA was replaced with a 79 bp HCV DNA fragment of 5′-GTAGTGTTGGGTCGCGAAAG ***GCC TTG TGG TAC TGC CTG ATA GGG TGC*** TTGCGAGTGCCCCGGGAGGTCTCGTAGACCGT-3′. For detection of HBV genomic DNA in serum sample, isolation of the HBV DNA from the serum sample by the use of standard procedure of a restricted enzymatic digestion, extraction, and sample clean-up (see Supplementary Data). The procedure of the MMP–DNA–NPS-based DNA assay was the same as described for 27 bp HCV-DNA.

2.3. Detection of DNAs with the single nucleotide polymorphism

To evaluate the selectivity of the probes, four tests were run with different sequences of the target DNA. Oligo-MMPs (50 μL) (MMP surface covalently immobilized DNA II) (5′-TAA CAA TAA TCC AAA AAA AAA A-$(CH_2)_3$-SH-3′) were allowed to hybridize with target DNA strands 1 (5′-GGA TTA TTG TTA AAT ATT GAT AAG GAT-3′), or a single-base mismatched target DNA strands 2 (5′-GGA TAA TTG TTA AAT ATT GAT AAG GAT-3′), or 3 (5′-GGA TTA TTG TTA AAT ATT GAT AGG GAT-3′), respectively in 30 μL 0.3 M NaCl,

10 mM phosphate buffer (pH 7.0). The solutions were incubated at 45 °C for 30 min, and then rotated for 3 h at 25 °C. The MMP–target complexes were separated on a magnetic separator and washed three times with 200 μL 0.3 M NaCl, 10 mM phosphate buffer (pH 7.0). The complexes were re-dispersed in 50 μL buffer and 30 μL of the double-functionalized gold nanoparticle solution. The double-functionalized gold nanoparticle (NP) with reporter mass-tagged molecules MI and recognized DNA I (5′-SH-$(CH_2)_6$-A AAA AAA AAA ATC CTT ATC AAT ATT-3′) was prepared via reported protocol [24]. After hybridization for 2 h, the sandwich complexes were washed seven times at 38 °C with assay buffer to wash off single-mismatch bound and nonspecifically bound nanoparticles. The resulting complexes were dispersed in 10 μL of assay buffer, warmed to 75 °C and stayed for 5 min. They were then immediately separated on a magnetic separator. The procedure of MS detection was the same as that when HCV-DNA acted as the target.

2.4. Detection of DNAs with the same masses but different sequences

The capture DNA II and II′ (5′-CAT ACT AAC ATA AAA AAA AAA A-(CH2)3SH-3′) modified MMP were allowed to hybridize with target DNA strands 1 (10 nM) and 4 (5′-TAT GTT AGT ATG ATA TAG GAA TAG TTA-3′) (10 nM), or none of them, or one of them in 0.3 M NaCl, 10 mM phosphate buffer (pH 7.0), respectively. Following incubation and rotation, the MMP–target complexes were separated on a magnetic separator, washed with the buffer and re-dispersed in a hybridization buffer, and then 30 μL of the mass-tagged molecule M I and M II encoded with the recognized DNA I and I′ (5′-HS$(CH_2)_6$-A AAA AAA AAA TAA CTA TTC CTA TAT-3′) gold nanoparticle solution were added to the buffer. After hybridization for 2 h, the sandwich complexes were washed seven times at ambient temperature with assay buffer to remove nonspecifically bound nanoparticles. The procedure of MS detection was the same as that when HCV-DNA acted as the target.

3. Results and discussion

3.1. MMP–DNA–NPS-based DNA detection

As illustrated in Fig. 1, three-component sandwich complexes of the MMP–DNA–NPs were formed when the target DNA was detected from the homogeneous solution; MMP was functionalized with target-specific strands, while gold NP was functionalized with both target-specific oligonucleotides as well as reporter mass molecules. MMP can act as a convenient handle for easy separation and concentration of bio-molecules in many studies [25,26,31]. To access the limit of detection (LOD) of the method, the concentration of the mass-tagged gold NPs was held constantly at 1 nM, but the target *hepatitis C virus* (HCV)-DNA, was added in series dilution from 10^{-11} M to 10^{-15} M for hybridization, respectively. The MMP–DNA–NPs complexes were separated and non-specifically bound nanoparticles were washed off. The solutions were dispersed, warmed and stayed for dehybridization, and then immediately separated on a magnet separator. The resulting solutions were detected by the mass spectrometer.

Owing to the high sensitivity of MS detection, amplification of reporter masses and the pre-concentration nature of the magnetic particles in solution, it was demonstrated that the target HCV-DNA can be detected as low as 1 fM in homogeneous solution (Fig. 2). Since the MALDI TOF MS detection is not intended to ionize the DNA directly, but rather to detect the reporter masses 869, $[M+Na]^+$ (sometimes also 837, $[M+Na-S]^+$) assembled in MMP–DNA–NPs complex, the relative intensity of the reporter masses vs. the concentration of the HCV-DNA were shown in Fig. 2. It has been

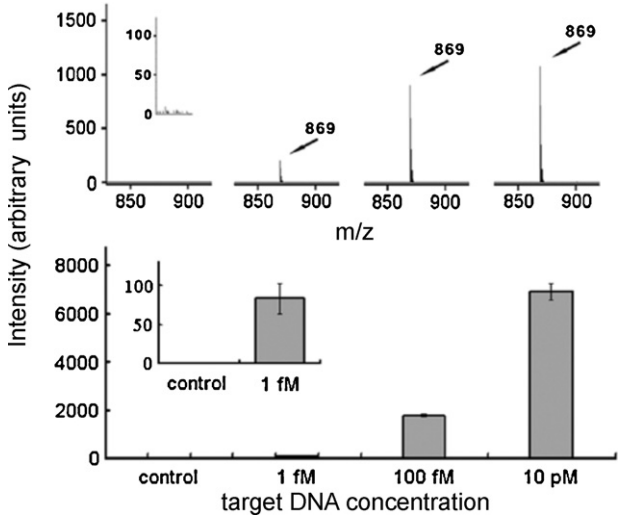

Fig. 2. Results of MMP–DNA–NPSs-based HCV-DNA detection. (a) Reporter mass was detected for DNA concentrations of 1 fM, 100 fM, and 10 pM. The 10 pM sample was diluted a factor of 10 prior to MS analysis avoiding the saturation of MALID-TOF MS so that the signal is actually higher than it appears to be by a factor of 10. (b) Signal profile of repeated analyses for DNA concentrations of 1 fM, 100 fM, and 10 pM. The signal intensity was calculated considering all dilution and concentration factors.

recently reported that gold nanoparticles have been used as a matrix for MALDI TOF MS to improve the MALDI ionization efficiency [9,22,23]. Therefore, we used gold NPs as a matrix for MS detection without the need of an organic matrix. Because it was found that citrate-stabilized gold nanoparticles could not be used directly in our MS detection, we used the nanoparticles that have just only capped with 3′- or 5′-(alkanethiol) oligonucleotides. It was found that the laser energy of ~30% is optimal for the analysis of this type of sandwich samples with a good reproducibility. Though the MS response is proportional to the concentration of the target DNAs as shown in Fig. 2, the variation in standard deviation is relatively large, which may be due not only to complexity in the probing process, but also to inherently poor quantitation performance of the MALDI TOF technique [33].

Nevertheless, the sensitivity of this new method reached to the lower fM range, thus approaching that of the polymerase chain reaction (PCR) [34–36], and those of the following methods: using gold nanoparticle probes in combination with fluorescence [17]; colorimetry [18]; and Raman spectroscopy [19]. Such high sensitivity in a low fM range should facilitate the analysis of nucleic acid in biotechnology research and may make this method feasible for the analysis of DNA without the need for target amplification schemes such as PCR [34–36]. Furthermore, in contrast to the previously reported DNA assay that showed nonspecific noise readout even when the target DNA is absent [17–19], there is no noise detected in the blank control in our method (Fig. 2) owing to the high specificity of MS detection. This feature is critically important in molecular diagnosis since the chance of false-positive results is very rare.

To further investigate the feasibility of the proposed MMP–DNA–NPS-based MS method for DNA detection in longer chain, and eventually genomic DNA fragments in real samples, we have initially attempted to test for probe capability for a much longer DNA chain, a 79 bp HCV DNA fragment of 5′-GTAGTGTTGGGTCGCGAAAG ***GCC TTG TGG TAC TGC CTG ATA GGG TGC*** TTGCGAGTGCCCCGGGAGGTCTCGTAGACCGT-3′. This DNA contains the key 27 bp HCV-DNA sequence at the middle with extra nucleic acids, 20 at the very 5′ end and 32 at the 3′ end of the DNA. The same probe for the short HCV DNA was utilized under identical experimental conditions for this longer

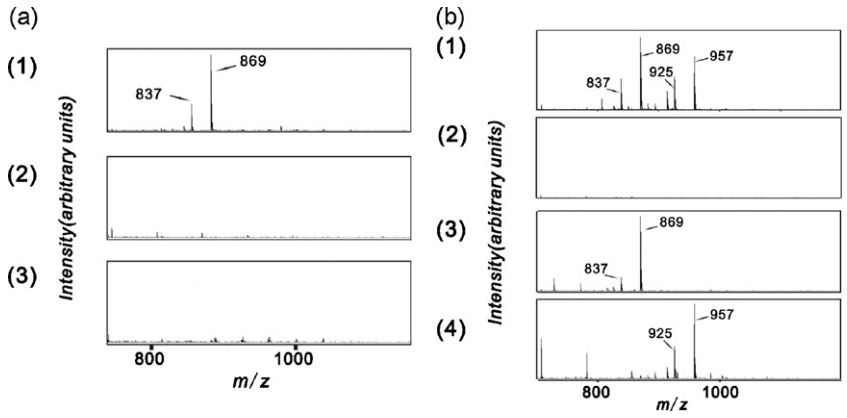

Fig. 3. (a) Results of single nucleotide polymorphism (SNP) detection. (1) The completely matched DNA 1. (2) A single mismatched DNA 2 mutated in 5′ terminal of X position from T to G. (3) A single mismatched DNA 3 mutated in 3′ terminal of Y position from A to G. (b) Analysis of DNAs with the same masses but different sequences. (1) Both target DNA 1 and 4 present. (2) The absence of any target DNA strands. (3) Only DNA 1 present. (4) Only DNA 4 present.

HCV DNA. The reporter mass was detected (see Supplementary Data). The result indicated that designed MMP–DNA–NPS can catch DNA with a longer chain as long as matched key DNA is present. This observation is consistent with the results using nano-probe detection previously published [37,38] without magnetic particles and encoded reporter mass. Blood samples containing HBV genomic DNA were then selected and analyzed utilizing the same MMP–DNA–NPS-based MS method. In the real bio-samples experiments, the samples went through standard extraction and pretreatment prior to MS detection. The reporter mass was detected, and the results were shown in Supplementary Data. However, this assay for real sample analysis and possible quantization will need to be further investigated.

3.2. Analysis DNAs with the single nucleotide polymorphism

SNPs represent the most abundant source of genetic variation in the human genome, and SNP is of importance in clinical diagnosis as many diseases are associated with such mutation [39,40]. Established SNP detection techniques are mainly PCR-based, which means that they involve complex, labor-intensive procedures, are easy contaminated, and can give false positive results [34–36]. We developed this simple and rapid MMP–DNA–NPS-based methodology for SNP discrimination. This methodology relies on the high sensitivity of two oligonucleotide probes, and the specificity afforded by the sequential hybridizations of two sequence-specific probes that selectively "sandwich" the completely matched target [26]. To demonstrate the capability of the assay, a single base was mutated (mismatched) in target DNA 2 with the mutation of T to G in position X, as compared with completely matched target DNA 1 (5′-GGA TXA TTG TTA AAT GAT AYG GAT-3′). Those experiments were carried out to hybridize with completely matched target DNA 1 and a single-base mismatched target DNA 2, respectively. At the hybridization step, single mismatched target DNA and nanoparticles were removed during the washing when the solution temperature was raised to 38 °C so that the "single mismatched target DNA sandwich" was eliminated. After being warmed and stayed subsequently for dehybridization, results showed a positive signal for the solution containing completely matched target DNA and a negative for the one with a mismatch. The solution color for completely matched DNA after dehybridization turned red owing to the formation of the sandwich as reported previously [24] (see Supplementary Data). The solution for mismatched DNA 2 was colorless (see Supplementary Data). Using the mass spectrometer, reporter masses peaks 869 ($[M+Na]^+$) and 837 ($[M+Na-S]^+$) for completely matched DNA were detected and shown in Fig. 3a(1). None of the reporter mass peaks for the single mismatched target DNA was detected in the identical experimental conditions (Fig. 3a(2)). The high-stringency washing steps were performed to remove unbound target DNA and nanoparticle probes, which reduce overall nonspecificity. Because we could not discern the color change at the lower concentration of the target than 1 nM, and we would like to assure that the non-detection is not due to our method, the experiment was performed at the 10 nM levels. MALDI TOF MS analysis revealed that our coding strategy could distinguish DNA sequences with a single-base mutation. To confirm the results, we analyzed another single-base variation DNA 3 (mutated from A to G in Y position). Again, the solution for mismatched DNA 3 was colorless (see Supplementary Data). None of the reporter masses peaks was detected for this mismatched DNA 3 through the same process (Fig. 3a(3)).

This approach permits direct SNP DNA typing without the need for primer mediated enzymatic amplification. Many typing methods based on various principles have been proposed, including allele-specific PCR [34], ARMS-PCR [35], RFLP-PCR [35], and ALM-ASA [36]. But all of those methods are based on DNA polymerase-mediated template amplification, which can yield several millions of copies of a target sequence within two hours. However, cross contamination caused by amplicons can lead to false positive results. As MS detection measures a very accurate molecular weight, resulting in a high specificity, typing accuracy was not affected by the impurities in a real genomic sample. Though this assay showed promise of capable of accurately identifying SNP genotypes from unamplified complex genomic DNA, detailed protocols need to be carefully and further investigated for real samples.

3.3. Analysis of DNAs with the same masses but different sequences

MS detects the m/z value for a molecule, but is inherently unable to differentiate two DNA molecules with the same masses but with a different sequence in a single assay. The key to this detection is the design of three-component sandwich complexes of the MMP–DNA–NPs for recognizing different target DNAs. To demonstrate that our strategy could be employed with such specificity of detection in the analysis, two strands of target DNA were chosen: DNA 1 (5′-GGA TTA TTG TTA AAT GAT AAG GAT ATT-3′) and DNA 4 (5′-TAT GTT AGT ATG ATA TAG GAA TAG TTA-3′). They both have the same mass but the nucleic acid sequences varied. To match the two different target DNA 1 and 4, two matched MMP–DNA–NPSs with probing DNAs and two sets of reporter masses (m/z 837 and 869 as well as m/z 925 and 957), respectively, were prepared.

Targets DNA 1 and 4, were added either together or individually to the solution containing both MMP–DNA–NPSs in separate experiments. The mass spectral readout results from the corresponding mass-tags correlating to the added target DNAs are shown in Fig. 3b. The color indication was also correlated with the formation of a sandwich in the experiments (see Supplementary Data). This result has demonstrated that DNA 1 and 4 can be simultaneously detected and differentiated in one sample, even though they have the same molecular weight but different sequences. When both of those target DNAs were not present in the solution (see Supplementary Data), none of the reporter masses peaks was detected. Again, the experiments were performed at the 10 nM level to observe the color change. It is interesting to note that the detection of DNA 1 and 4 did not interfere with each other even at this much higher concentration than that of the detection limit.

The results also demonstrated that this assay is very specific when multiple target DNAs are present in a sample. Since modern MS equipment has a very high mass resolving power, the detectable mass range of reporters should also be very large. The multiplexing capability could be achieved by utilizing distinct mass tags for each individual target. The high resolution of MS allows for almost unlimited choice of mass tags, which offers advantage over other multiplexing protocols. This assay includes two sequential hybridizations of the target DNA to the MMP probe and the AuNP probe, so that the design of these probes is critical to the specificity of the assay.

4. Conclusion

We have demonstrated that the MS method, in combination with the MMP–DNA–NPS, can detect SNP and multiple DNA with the same mass but different sequences with excellent sensitivity and selectivity. The method has the potential to revolutionize the MS detection of DNA in four distinct ways: (1) it lowers the detection limit to fM levels; (2) it is able to analyze SNP and multiplexed DNA molecules in a homogenous solution; (3) it is able to reveal specificity of the DNA sequencing information in complex mixtures; and (4) it confers the ability to perform the analysis using the small and amplifiable reporter mass readout that can be measured by a simple bench-top MS without the need of expensive tandem MS instruments.

The sensitivity may be further improved by optimizing the amplification factors of the assembled reporter mass molecules. The quantitation of DNA by this method may also be validated by standardizing the assay. Our on-going efforts focus on applying the assay for the detection of DNAs extracted from the biological samples. We are also undertaking the application of the assay for biomarkers and microbes.

Acknowledgements

This work has been supported by the project sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (0214168001), the National Natural Science Foundation of China (30900351), and the Natural Science Foundation of Fujian Province of China (2009J05029). We are especially thankful to Dr. Erol Gulcicek of Yale University for his thought-provoking discussions and manuscript revisions. We also acknowledge Prof. Guohua Zhou for consulting with us on the project and Fuqiang Wang for his technical assistance in some of the MALDI-TOF mass spectrometry experiments.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.06.045.

References

[1] P. Donnelly, Nature 456 (2008) 728–731.
[2] E. Lynge, M. Rebolj, Nat. Rev. Clin. Oncol. 6 (2009) 699–706.
[3] M.A.S. Khalid, A.A. Anees, A.A. Salman, J. Biomed. Biotechnol. 2010 (2010) 1–15.
[4] N. Blow, Nat. Methods 5 (2008) 741–747.
[5] R.D. Unwin, J.R. Griffiths, A.D. Whetton, Nat. Protoc. 4 (2009) 870–877.
[6] K. Chughtai, R.M.A. Heeren, Chem. Rev. 110 (2010) 3237–3277.
[7] E. Nordhoff, M. Schurenberg, G. Thiele, C. Lubbert, K.D. Kloeppel, D. Theiss, H. Lehrach, J. Gobom, Int. J. Mass Spectrom. 26 (2003) 163–180.
[8] Z.J. Zhu, V.M. Rotello, R.W. Vachet, Analyst 134 (2009) 2183–2188.
[9] D.R. Murdoch, L.C. Jennings, N. Bhat, T.P. Anderson, Infect. Dis. Clin. North Am. 24 (2010) 791–807.
[10] J.H. Banoub, R.P. Newton, E. Esmans, D.F. Ewing, G. Mackenzie, Chem. Rev. 105 (2005) 1869–1915.
[11] X.L. Kong, L.C.L. Huang, S.C.V. Liau, C.C. Han, H.C. Chang, Anal. Chem. 77 (2005) 4273–4277.
[12] X. Zhou, P. Cao, Y. Tian, J. Zhu, J. Am. Chem. Soc. 132 (2010) 4161–4168.
[13] T.A. Hall, K.A. Sannes-Lowery, L.D. McCurdy, C. Fisher, T. Anderson, A. Henthorne, L. Gioeni, B. Budowle, S.A. Hofstadler, Anal. Chem. 81 (2009) 7515–7526.
[14] M.L. McCloskey, R. Stoger, R.S. Hansen, C.D. Laird, Biochem. Genet. 45 (2007) 761–767.
[15] A. Mattes, O. Seitz, Angew. Chem. Int. Ed. 40 (2001) 3178–3181.
[16] R. Lemaire, J. Stauber, M. Wisztorski, C. Van Camp, A. Desmons, M. Deschamps, G. Proess, I. Rudlof, A.S. Woods, R. Day, M. Salzet, I. Fournier, J. Proteome Res. 6 (2007) 2057–2067.
[17] D.J. Maxwell, J.R. Taylor, S. Nie, J. Am. Chem. Soc. 124 (2002) 9606–9612.
[18] R. Elghanian, J.J. Storhoff, R.C. Mucic, R.L. Letsinger, C.A. Mirkin, Science 277 (1997) 1078–1081.
[19] Y.C. Cao, R. Jin, C.A. Mirkin, Science 297 (2002) 1536–1540.
[20] S.D. Sherrod, A.J. Diaz, W.K. Russell, P.S. Cremer, D.H. Russell, Anal. Chem. 80 (2008) 6796–6799.
[21] S.W. Cha, Z.H. Song, B.J. Nikolau, E.S. Yeung, Anal. Chem. 81 (2009) 2991–3000.
[22] Y.F. Huang, H.T. Chang, Anal. Chem. 78 (2006) 1485–1493.
[23] M.T. Spencer, H. Furutani, S.J. Oldenburg, T.K. Darlington, K.A. Prather, J. Phys. Chem. C 112 (2008) 4083–4090.
[24] F. Qiu, D. Jiang, Y. Ding, J. Zhu, L.L. Huang, Angew. Chem. Int. Ed. 47 (2008) 5009–5012.
[25] H.D. Hill, C.A. Mirkin, Nat. Protoc. 1 (2006) 324–336.
[26] J.M. Nam, S.I. Stoeva, C.A. Mirkin, J. Am. Chem. Soc. 126 (2004) 5932–5933.
[27] P. Song, B. Meibohm, A.O. Gaber, M.R. Honaker, M. Kotb, C.R. Yates, Clin. Pharmacol. Ther. 71 (2002) 103–111.
[28] R. Jin, G. Wu, Z. Li, C.A. Mirkin, G.C. Schatz, J. Am. Chem. Soc. 126 (2003) 1643–1654.
[29] C. Pale-Grosdemange, E.S. Simon, K.L. Prime, G.M. Whitesides, J. Am. Chem. Soc. 113 (1991) 12–20.
[30] B.T. Houseman, E.S. Gawalt, M. Mrksich, Langmuir 19 (2003) 1522–1531.
[31] L. Wu, L. Qiu, C. Shi, J. Zhu, Biomacromolecules 8 (2007) 2795–2800.
[32] J.J. Storhoff, R. Elghanian, R.C. Mucic, C.A. Mirkin, R.L. Letsinger, J. Am. Chem. Soc. 120 (1998) 1959–1964.
[33] K. Benkali, P. Marquet, J.P. Rrolle, BMC Genomics 9 (2008) 541–547.
[34] M.V. Myakishev, Y. Khripin, S. Hu, D.H. Hamer, Genome Res. 11 (2001) 163–169.
[35] D.C. Georgiana, S.G. Alexandra, C. Nicoleta, F. Stanciu, M. Dobre, B. Dorina, T. Rodica, P. Cristea, S. Veronica, Rom. Biotechnol. Lett. 14 (2009) 4845–4850.
[36] W.P. Wang, K.Y. Ni, G.H. Zhou, Chem. J. Chin. Univ. Chin. 27 (2006) 1856–1858.
[37] J.J. Storhoff, A.D. Lucas, V. Garimella, Y.P. Bao, U.R. Muller, Nat. Biotechnol. 22 (2004) 883–887.
[38] M. Huber, T. Wei, U.R. Müller, P.A. Lefebvre, S.S. Marla, Y.P. Bao, Nucleic Acids Res. 32 (2004) e137.
[39] Y.T. Chen, C.L. Hsu, S.Y. Hou, Anal. Biochem. 375 (2008) 299–305.
[40] Y.P. Bao, M. Huber, T.F. Wei, S.S. Marla, J.-J. Storhoff, U.-R. Müller, Nucleic Acids Res. 33 (2005) e15.