LETTER

MALDI-TOF Analysis of Binding between DNA and Peptides Containing Lysine and Tryptophan

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Abstract: Here, we demonstrate the use of MALDI-TOF as a fast and simple analytical approach to evaluate the DNA-binding capability of various peptides. Specifically, by varying the amino acid sequence of the peptides consisting of lysine (*K*) and tryptophan (*W*), we identified peptides with strong DNA-binding capabilities using MALDI-TOF. Mass spectrometric analysis reveals an interesting novel finding that lysine residues show sequence selective preference, which used to be considered as mediator of electrostatic interactions with DNA phosphate backbones. Moreover, tryptophan residues show higher affinity to DNA than lysine residues. Since there are numerous possible combinations to make peptide oligomers, it is valuable to introduce a simple and reliable analytical approach in order to quickly identify DNA-binding peptides.

Keywords: MALDI-TOF, DNA Binding Peptide, Lysine and Tryptophan-containing Peptide, Non-covalent DNA-Peptide Binding

Introduction

The genetic information in living organisms is regulated, replicated, and repaired by DNA-binding proteins that can recognize and bind specific DNA sequences.[1] In eukaryotes, DNA-binding proteins such as histones stabilize DNA by forming nucleosomes, which are essential in preventing unnecessary DNA damage and controlling gene expression. Thus, various DNA-binding proteins have been studied in an attempt to understand the biochemical control of gene expression.[2] More specifically, DNA-binding motif consisting of several peptides in a protein facilitates binding to double-stranded DNA, owing to interactions with DNA bases, without unwinding the double helix structure of the DNA. Therefore, the characterization of peptides from DNA binding motifs such as zinc fingers,[2] leucine zippers,[3] etc. is an active area of research. Moreover, DNA-binding peptides have enormous potential in a variety of biotechnological and biomedical applications for biosensors and peptide-based drugs, and others. Accordingly, the discovery of novel DNA-binding peptide motifs is of paramount importance for a variety of biochemical applications.

In the discovery of novel DNA-binding motifs, mass spectrometry (MS) has emerged as a powerful tool to investigate non-covalently bound complexes and presents advantages[4] over traditional techniques such as DNA foot-printing[5] and electrophoretic mobility shift assays.[6] Importantly, MS measurements are fairly simple to conduct and MS provides the molecular weight of intact non-covalent complexes, stoichiometry, and information on subunit interactions. Moreover, MS measurements require only a small amount of sample (femtomolar level). Two soft ionization methods, namely electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) have been utilized to provide MS peaks of intact, non-covalently bound complexes. ESI-MS is often preferred for non-covalent DNA-peptide complexes, which are formed within a buffer solution.[7] As an example, we previously reported the identification of non-covalent interactions between DNA and zinc finger motifs utilizing ESI-MS.[8] Although MALDI uses co-crystallized analytes within a matrix,[9] its use in the analysis of DNA-peptide complexes is attractive, because some of the difficulties encountered with ESI-MS can be avoided. First, MALDI spectra are much easier to interpret owing to the predominant singly charged species. Second, sample preparation for MALDI is much simpler than that for electrospray. Importantly, MALDI-TOF has been implemented to analyze numerous non-covalent complexes

since Juhasz and Biemann utilized MALDI-TOF to analyze non-covalent complexes between DNA and positively charged peptides for the first time.[10] Subsequently, a number of studies regarding DNA-peptide interactions were conducted using MALDI-TOF[11].

As a novel DNA binding peptide, we recently discovered that a neutravidin-coated surface could bind large DNA molecules without biotin[12]. Although it is well known that neutravidin is used to link biotin-labeled molecules, it was an unexpected result that neutravidin could bind DNA without biotin. From the crystal structure of avidin protein, we hypothesized that binding was facilitated by the protruding tryptophan (*W*) and lysine (*K*) residues (-DIGDD*WK*–) located outside of the neutravidin protein.[13] In addition, Firczuk *et al.* reported that aspartate (*D*) could work like lysine with the aid of divalent cation such as Mg^{2+}.[14] Early in 1970s, Helene and colleagues had pioneered in the investigation of DNA binding capabilities of KWK peptides in a series of studies.[15,16] From their experimental observation, they proposed a two-step DNA binding mechanism for such peptides: (1) the positively charged lysine residues show electrostatic interactions with the negatively charged phosphate backbone of the DNA molecules; and (2) the aromatic ring of the tryptophan residue intercalates with the adjacent bases on the DNA.[17] Although the peptides containing lysine and tryptophan have attracted quite an attention due to their biological meaning,[18] there hasn't been any study to conduct MALDI-TOF analysis for DNA binding with KWK or related peptides, to the best of our knowledge. Therefore, in this study, we employed MALDI-TOF to characterize the DNA binding affinity of peptides that include lysine (*K*), and tryptophan (*W*). Our analysis shows comparative binding affinity with varying peptides sequence and lengths, and selective preference for nucleotide sequence.

Experimental

Chemicals. All oligo-(deoxyribo)-nucleotides were purchased from Bioneer (Daejeon, Korea) and all peptides were purchased from GL Biochem (Shanghai, China). 2, 5-Dihydroxybenzoic acid was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Other chemicals were obtained from Sigma-Aldrich Korea (Yong-In, Korea).

Mass spectrometric analysis. For MALDI-TOF analysis, oligonucleotides were used as DNA substrates for peptide binding reaction. For these reactions, peptides were dissolved in water to a concentration of 100 μM. Peptide sequences used for the experiments are shown in Table 1. DNA sequences used for the experiments are shown in Table 2. Oligo-(deoxyribo)-nucleotides were dissolved in a TE buffer solution (10 mM Tris–HCl; 1 mM EDTA; pH 8.0) at room temperature to a concentration of 100 μM. DNA-peptide binding reactions were performed in 20 μL

Table 1 Peptides used in mass spectrometry

KWKWKK
KAKAKK
KKKKKK
AAAAAA
DIGDDWK
KWKWKWKK
KWKWKWKWKK
KWKWKWKWKWKK

Table 2 Oligonucleotides used in mass spectrometry

T_9	5'-TTT TTT TTT-3'
A_9	5'-AAA AAA AAA-3'
C_9	5'-CCC CCC CCC-3'

total volume: 10 μL of 100 μM oligo-(deoxyribo)-nucleotides and 10 μL of 100 μM peptides were mixed. The reaction was incubated at room temperature for 30 minutes. Samples were mixed with matrix solution onto a Bruker MTP 384 polished steel MALDI sample support target plate. The matrix solution was 130 mM 2, 5-Dihydroxybenzoic acid in methyl alcohol. MALDI-TOF analysis was performed using a Bruker Autoflex™ Speed (Bruker Daltonics Inc., Billerica, MA, USA) in the linear positive ion mode. Mass spectra were obtained as sums of 500 laser shots at 1000 Hz (at least 10,000 shots from different spots).

Results & Discussion

The mass spectra in Fig. 1 demonstrate the relative abundances of non-covalent complexes formed between DNA and positively charged peptides. Even though there are structural differences between single stranded DNA and double stranded DNA in the non-covalent binding to peptides, it is problematic to obtain mass spectra of double stranded DNA in MALDI-TOF, because of strand displacement; therefore, we conducted entire experiments using single stranded DNA instead of duplexes. Furthermore, in the series of studies, amino acid sequences are deeply related with their binding capability with single stranded oligonucleotides,[19-21] likewise with double stranded DNA. As a control, the mass spectrum of an alanine oligomer showed no complex formation (Fig. 1b). In contrast, Fig. 1c clearly shows the formation of a DNA-peptide complex. The positive charges on lysine facilitate the interaction between the DNA and peptide. In 1969, Shapiro *et al.* characterized polylysine-bound DNA via centrifugal precipitation and light scattering.[22] Subsequently, Helene discovered that DNA could quench the fluorescence of tryptophan,[23] and many experiments using tryptophan-containing peptides were conducted to quantitatively analyze DNA-peptide binding.[24] In this
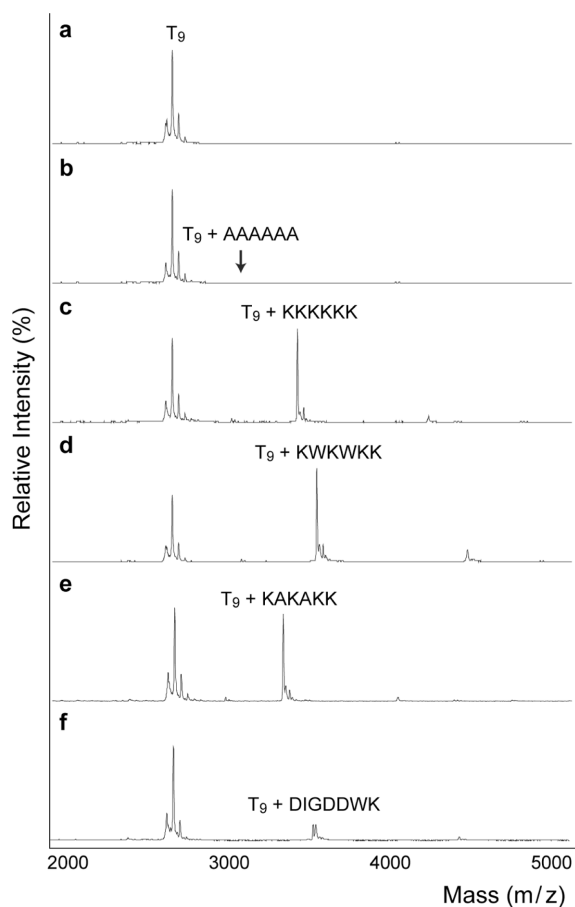
Figure 1. MALDI-TOF mass spectra demonstrating the formation of non-covalent complexes between DNA and peptides consisting of lysine (K) and tryptophan (W) with control peptides. T_9 is a thymine oligomer (nine-mer). A: alanine, K: lysine, W: tryptophan, D: aspartate, I: isoleucine, G: glycine. The peak intensity ratio of T_9-peptide complex over T_9 is (b) 0.0, (c) 1.1, (d) 1.4, (e) 0.9 and (f) 0.2.
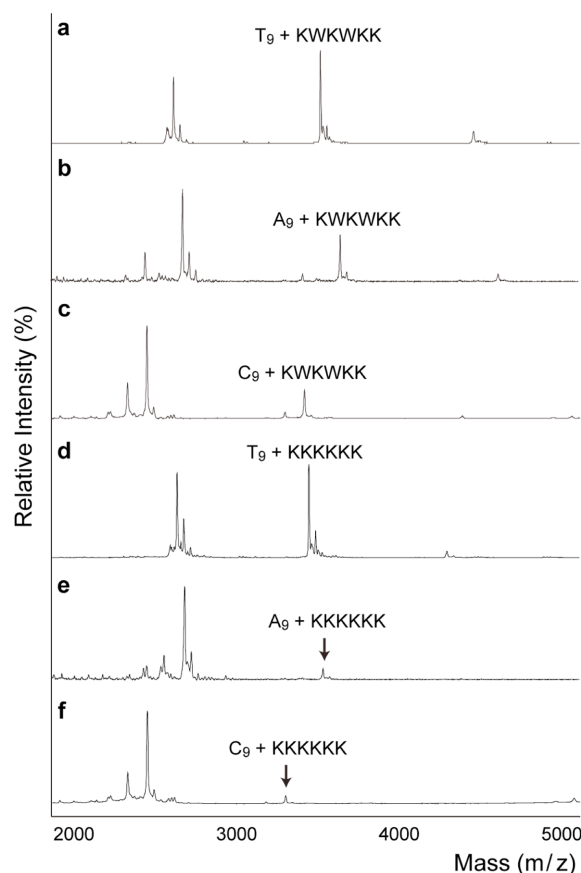

Figure 2. MALDI-TOF mass spectra demonstrating sequence selectivity of oligonucleotide with KWKWKK and KKKKKK. A_9 is an adenine oligomer (nine-mer), and C_9 is a cytosine oligomer (nine-mer). The peak intensity ratio of DNA-peptide complex over DNA oligomer is (a) 1.4 (b) 0.5, (c) 0.2, (d) 1.1, (e) 0.2 and (f) 0.1. The binding affinity decreased in the following order: $T_9 > A_9 > C_9$ in both peptides.

context, we measured MALDI-TOF analysis using tryptophan-containing peptide KWKWKK (Fig. 1d). Among the various MALDI matrixes, 2, 5-Dihydroxybenzoic acid (DHB) shows the highest intensity for DNA-peptide bindings, especially non-covalent bonds in lysine-tryptophan. We tried other matrixes to analyze DNA-peptide bindings such as α-Cyano-4-hydroxycinnamic acid (CHCA), and 6-Aza-2-thiothymine (ATT), however, these results showed very weak intensity peaks in mass spectra, even though tendencies of binding spectra seemed related with the data of DHB.

The ratios of the peak areas for the DNA-peptide complex over the unbound oligomer were 1.1 for KKKKKK (Fig. 1c) and 1.4 for KWKWKK (Fig. 1d). Previously, Mascoti and Lohman used a series of peptides (KWK$_n$; n=2~10) to characterize the effects of the number of positive charges.[25] Their results showed that there was a

linear relationship between peptide-DNA binding and the number of positive charges. Figure 1c and 1d show that replacing tryptophan with lysine increased DNA-peptide binding, despite the loss of positive charges. In order to evaluate the effect of tryptophan, we tested the peptide KAKAKK, which has the same number of positive charges as KWKWKK. The peak intensity ratio of the complex over T_9 was 0.9 (Fig. 1e), which was less than that of KKKKKK (1.1) and KWKWKK (1.4). The strong interaction between tryptophan and thymine is due to intercalation, as reported by Helene and colleagues.[15] Accordingly, our results are consistent with pervious results obtained using tryptophan fluorescence quenching.[16] In addition, we measured MALDI-TOF analysis using T_9 and DIGDDWK from neutravidin sequence to understand our previous observation of DNA binding on neutravidin without biotin.[12] Although the mass spectrum in Fig. 1f shows the formation of DNA-peptide
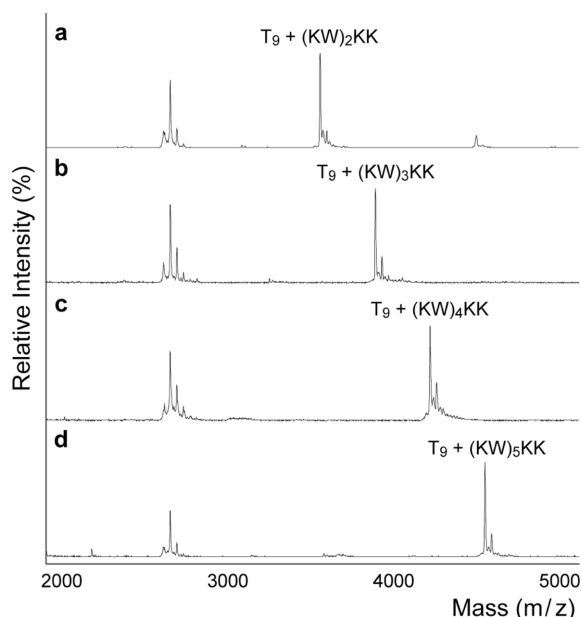
Figure 3. MALDI-TOF mass spectra demonstrating of DNA-peptide affinity enhancement by increasing the number of KW repeats. The peak intensity ratio of DNA-peptide complex over DNA oligomer is (a) 1.1 (b) 1.2, (c) 1.4, (d) 2.1

complex, the peak intensity ratio (0.2) is less than 15%, comparing with the strongest DNA binding peptide of KWKWKK (ratio=1.4).

The next question was whether the peptide has selective preference for DNA sequences. Figure 2a~2c shows the order of binding affinities of T_9>A_9>C_9, which was consistent with previous studies. Previously, using circular dichroism and fluorescence, Maurizot *et al.* explained that KWK exhibited a binding affinity to DNA with the order of AA>CC owing to the aromaticity of tryptophan.[26] This result was confirmed by Mascotti and Lohman who characterized the association constant of peptides containing lysine and tryptophan and poly A, poly C, and poly T.[27] They reported that the association constants of oligolysine containing tryptophan decreased in the following order: poly T > poly A > poly C. However, in contrast to previous hypothesis, Figure 2d~2f suggests that this sequence selectivity may not originate from the aromaticity of tryptophan. Instead, this selective preference for DNA sequences may come from lysine residues because KKKKKK shows similar sequence specificity like KWKWKK. Accordingly, the function of lysine cannot be simply explained by electrostatic interactions between positively charged lysine and the phosphate backbone of DNA. It is very interesting that there seems some interaction between nucleotide bases and lysine residues.

The purpose of this study was to discover novel strong DNA-binding peptides using MALDI-TOF. As mentioned earlier, increasing the number of positive charges enhanced the peptide-DNA binding affinity. Furthermore, the addition of tryptophan also increased this affinity by the base intercalation with indole rings. Therefore, we prepared a number of lysine and tryptophan (KW) repeats. Figure 3 demonstrates that the addition of KW repeats increased DNA binding capability. This trend is consistent with a previous study reported by Mascotti and Lohman.[27,28] In particular, (KW)$_5$KK formed a complex with DNA; the ratio of the peak intensity was 2.1 for the complex over free DNA, which was almost double that of (KW)$_2$KK (*ratio* =1.1).

Conclusions

In this paper, we demonstrated an approach to measure DNA binding affinity against the peptide consisting of lysine and tryptophan residues using MALDI-TOF. Although there are traditional ways to measure DNA-peptide binding such as footprinting, electrophoretic mobility shift assays, and tryptophan fluorescence quenching, MALDI-TOF is simple and fast compared with other methods. More importantly, since there are numerous combinations to make peptide oligomers, a simple and fast analytical approach, such as MALDI-TOF, is promising for quick identification of non-covalent DNA and peptides binding.

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