Analysis of the Complex of Oligonucleotide Duplexes with Ligands by MALDI-TOF Mass Spectroscopy

Kenichi Yamashita, Shinobu Sato, Hiroki Takamiya, Makoto Takagi, and Shigeori Takenaka*
Department of Applied Chemistry, Faculty of Engineering, Kyushu University, Fukuoka 812-8581

(Received April 19, 2001; CL-010360)

The peaks for a complex of DNA-interacting ligands naphthalene monoimide (NMI), naphthalene diimide (NDI) and ethidium bromide and groove binding ligand Hoechst 33258 with a duplex of oligonucleotide 5'-CCCCGGGG-3' were observed successfully in MALDI-TOF mass spectroscopy. The appearance of the peak of the complex depended on both the binding mode (classical intercalation, threading intercalation, or groove binding) and the duplex structure (fully matched or mismatched).

By the completion of the human genome sequencing project, emphasis is now shifting to analysis of interactions of DNA binding proteins and organic ligands with DNA. Currently, such interactions are studied mainly by mass spectroscopy with the electrospray ionization (ESI) mode.[1–3] On the other hand, in the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopic method, a sample molecule is less prone to fragment or to generate multiply charged ions.[4,5] In addition, it can handle many samples at a time and in a short period of time.[5] These advantages make the MALDI-TOF-MS method an attractive alternative for analysis of biological material such as proteins and nucleic acids.[4,5] In the current MALDI-TOF-MS technology, the following devices were made to ease detection of double stranded DNA: use of a new matrix,[5,6] cooling the sample,[7] and mixing glycerol with the sample.[8] The samples analyzed to date were relatively long double stranded DNA fragments such as PCR products.[7,8] As for relatively short double stranded DNA fragments, their interaction with short peptides was reported as a model to study the interaction of a DNA binding protein with DNA.[9] It is also important to study various interactions of a small ligand with double stranded DNA. Moreover, the interaction of such a ligand with mismatched DNA duplex is also important. In this paper, we report for the first time detection of a complex of DNA with a DNA-binding ligand by MALDI-TOF mass spectroscopy. We succeeded in detecting the double stranded DNA complex with such DNA binding ligands as classical and threading intercalators and groove binders. The quantity of the bound ligand decreased significantly for mismatched DNA duplex, a finding making this method a promising tool for mismatch analysis.

Figure 1 illustrates the structures of DNA-binding ligands, ethidium bromide[10,11] and naphthalene monoimide (NMI) as a classical intercalator,[12,13] naphthalene diimide (NDI) as a threading intercalator,[13] and Hoechst 33258 as a groove binder[14] together with the oligonucleotides used in this study. A fully matched DNA duplex is formed from sequence **1**, whereas a duplex with a mismatch(es) is formed from sequence **2** or **3**.[15–17] All of the oligonucleotides were self-complementary with or without mismatches and formed a duplex by themselves in the experimental conditions described below. Mass spectrometric experiments were carried out in the following way with a Voyager DE spectrometer (Applied Biosystems Japan Ltd.) One μL of a solution containing 15.4 μM oligonucleotide, 7.7 g/L 3-hydroxypicolinic acid (3HPA) as a matrix, and 77 μM ligand was placed on the sample plate and dried slowly in the air to leave a crystal. It was ionized by a laser beam with the lowest power at 5×10^{-7} Torr. Mass spectra were obtained by the negative mode at 25 °C Torr (Intensity of laser, 1600; accelerating voltage, 20 kV).

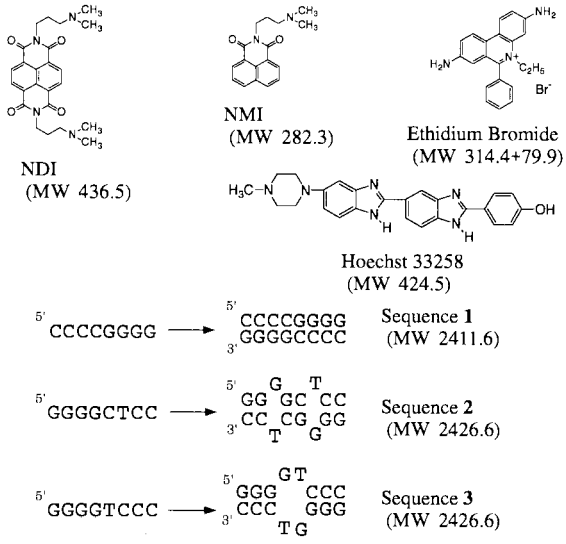

Figure 1. Structures of the ligands and oligonucleotides used in this article.

Two peaks appeared at around 2400 and 4800 Daltons in the mass spectrum of oligonucleotide alone (Figure 2). The former corresponds to the mass of single stranded DNA and the latter to double stranded DNA. In all the cases, the intensity of single stranded DNA was larger than that of double stranded DNA, but the ratio of single strand to double strand was almost constant for all of the DNA used. Since only a peak for single stranded DNA was observed for non-complementary oligonucleotide sequences (These data are shown in the supporting information), it is certain that the peak with the higher mass indeed represents a double stranded form of DNA.

In the next step, the complex of one of the ligands with either of the oligonucleotides was studied. Since these ligands are able to stabilize the DNA duplex, it is expected that such a complex may become detectable more easily in the MALDI-TOF mass spectroscopy. In fact, two peaks were observed at around 4800 and 5300 for the complex of NDI and sequence **1**. These mass numbers correspond to DNA duplex and its 1 : 1 complex with NDI, respectively. Although the duplex has seven intercalation sites for the ligand in principle, not all of them are usable for intercalation, as the intercalator binds to DNA only every other base pair according to the nearest neighbor exclusion model[18] and as the intercalator is hard to bind around the terminal base pairs.[19] By contrast, only a single peak was observed at around 4800 for NDI and sequence **2** or **3**, which represents the duplex form of DNA. In other words, no complex with NDI was detectable for the mismatched duplex. This result is also reasonable in the light of the fact that the intercalator cannot bind to the mismatched base pair regions.

NMI behaved nearly the same way as NDI: only a 1:1 complex

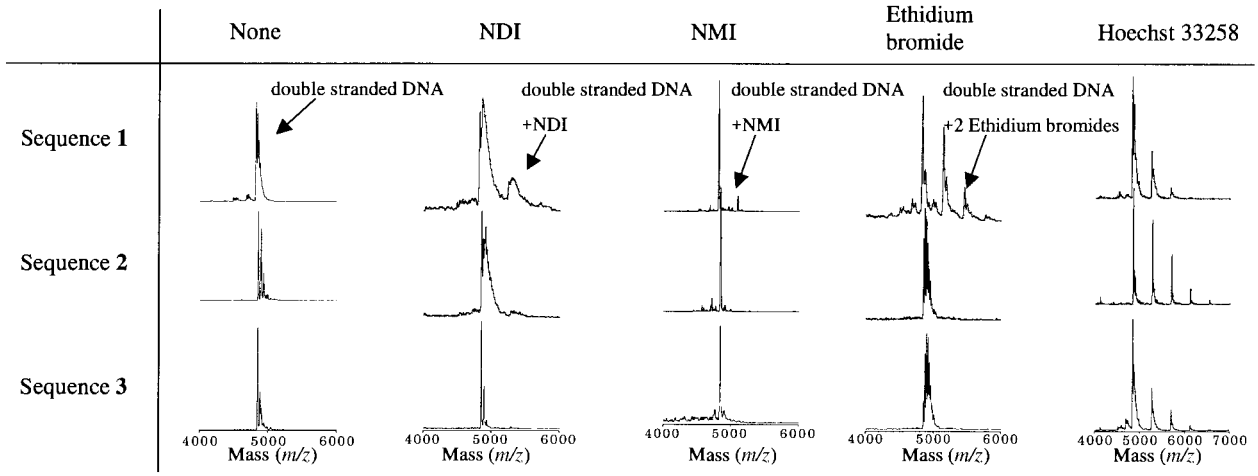

Figure 2. MALDI-TOF-MS spectrum of oligonucelotides **1–3** in the absence and presence of NDI, NMI, ethidium bromide, or Hoechst 33258.

with NMI and oligonucleotide duplex was observed for sequence **1** with no mismatch, though the intensity of the complex is smaller for NMI than that for NDI. This should reflect a difference in the binding constants for NDI and NMI: the binding constants for naphthalene diimide derivatives are 10–100 times larger than those for naphthalene monoimides.[13]

The behavior of ethidium bromide was slightly different from that of NDI and NMI: with sequence **1**, in addition to the duplex peak two complex peaks were observed whose masses correspond to the 1:1 and 2:1 complexes of ethidium to duplex. The reason for this difference in the behavior of ethidium and NDI or NMI is not clear now, but the difference in the binding mode between the former (classical intercalation) and the latter (threading intercalation) seems to be responsible. Where the binding affinity of a threading and classical intercalator is similar, the former appears to perturb the DNA structure to a larger extent. Incidentally, it is noted that the mass increments of 315 and 313 correspond to the ethidium part alone: the complex showed up with its counter anion off.

Finally, Hoechest 33258 was also tested with sequences **1**–**3**. Surprisingly, the peaks corresponding up to the 1:4 complex of the ligand were observed with either of the DNA, irrespective of fully matched or mismatched duplex. Hoechst 33258 is known as a groove binder and is also lying in the minor groove of DNA as a dimer.[20] However, there are seemingly contradictory reports to claim that Hoechst 33258 could bind to a duplex of 12-meric oligonucleotide as a tetramer under some conditions[21] and that it could intercalate into non-A T pairs of DNA duplex.[22] Additionally, Hoechst 33258 could bind to the unpaired bases such as a mismatched DNA duplex.[23–26] Taken together, Hoechst 33258 interacts with DNA in a complex way and that may be associated with the "extraordinary" behavior of this ligand in mass spectral measurements.

In summary, we were able to observe the peak based on the complex of a ligand with DNA duplex. Occurrence of such a complex peak depended heavily on the presence or absence of a mismatch(es) in the nucleotide sequence. This characteristic of the method will find use in the analysis of mismatches of DNA or single nucleotide polymorphisms (SNP) as far as oligonucleotides are concerned at least.[27]

The authors are grateful to Prof. Hiroki Kondo of Kyushu Institute of Technology for helpful discussions. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan. One of the authors (K. Y.) is also grateful to the financial support from the Japan Society for the Promotion of Science.

Supporting Information Available: The fluorimetric image of the spot containing ligand, DNA, and 3-HPA on the sample plate and the additional and reproducible MALDI-TOF Mass spectra data.

References and Note

1 R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga, and H. R. Udseth, *Anal. Chem.*, **62**, 882 (1990).
2 T. D. Veenstra, *Biophys. Chem.*, **79**, 63 (1999).
3 K. X. Wan, T. Shibue, and M. L. Gross, *J. Am. Chem. Soc.*, **122**, 300 (2000).
4 R. Bakhtiar and R. W. Nelson, *Biochem. Pharmacol.*, **59**, 891 (2000).
5 B. Gou, *Anal. Chem.*, **71**, 333R (1999).
6 P. Lecchi, H. M. T. Le, and L. K. Pannell, *Nucleic Acids Res.*, **23**, 1276 (1995).
7 D. P. Little, A. Jacob, T. Becker, A. Braun, B. Darnhofer-Demar, C. Jurinke, D. van den Boom, and H. Koster, *Int. J. Mass Spectrom. Ion Processes*, **169/170**, 323 (1997).
8 F. Kirpekar, S. Berkenkamp, and F. Hillenkamp, *Anal. Chem.*, **71**, 2334 (1999).
9 S. Lin, R. J. Cotter, and A. S. Woods, *Proteins, Suppl. 2*, 12 (1998).
10 J.-B. LePecq and C. Paoletti, *J. Mol. Biol.*, **27**, 87 (1967).
11 R. J. Douthart, J. P. Burnett, F. W. Beasley, and B. H. Frank, *Biochemistry*, **12**, 214 (1973).
12 K. A. Stevenson, S. F. Yen, N. C. Yang, D. W. Boykin, and W. D. Wilson, *J. Med. Chem.*, **27**, 1677 (1984).
13 S. F. Yen, E. J. Gabbay, and W. D. Wilson, *Biochemistry*, **21**, 2070 (1982).
14 G. A. Daxhelet, M. M. Coene, P. P. Hoet, and C. G. Cocito, *Anal. Biochem.*, **179**, 401 (1989).
15 T. E. Haran, Z. Shakked, A. H. J. Wang, and A. Rich, *J. Biomol. Struct. Dyn.*, **5**, 199 (1987).
16 T. Brown, O. Kennard, G. Kneale, and D. Rabinovich, *Nature*, **330**, 221 (1987).
17 G. Kneale, *J. Biomol. Struct. Dyn.*, **3**, 205 (1985).
18 W. Saenger, "Principles of Nucleic Acid Structure, " Springer-Verlag, New York (1983).
19 P. Samuelson, K. Jansen, and M. Kubista, *J. Mol. Recognit.*, **7**, 233 (1994).
20 F. G. Loontiens, P. Regenfuss, A. Zechel, L. Dumortier, and R. M. Clegg, *Biochemistry*, **29**, 9029 (1990).
21 A. Blasko and T. C. Bruice, *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 10018 (1993).
22 K. Utsuno, Y. Maeda, and M. Tsuboi, *Chem. Pharm. Bull.*, **47**, 1363 (1999).
23 L. Dassonneville, F. Hamy, P. Colson, C. Houssier, and C. Bailly, *Nucleic Acids Res.*, **25**, 4487 (1997).
24 I. V. Kutyavin, I. A. Afonina, A. Mills, V. V. Gorn, E. A. Lukhtanov, E. S. Belousov, M. J. Singer, D. K. Walburger, S. G. Lokhov, A. A. Gall, R. Dempcy, M. W. Reed, R. B. Meyer, and J. Hedgpeth, *Nucleic Acids Res.*, **28**, 655 (2000).
25 J. Cho and R. R. Rando, *Nucleic Acids Res.*, **28**, 2158 (2000).
26 X. L. Yang, R. B. Hubbard, IV, M. Lee, Z.-F. Tao, H. Sugiyama, and A. H.-J. Wang, *Nucleic Acids Res*, **27**, 4183 (1999).
27 K. Yamashita, M. Takagi, H. Kondo, and S. Takenaka, *Chem. Lett.*, **2000**, 1038.