

Increased stability of nucleic acids containing 7-deaza-guanosine and 7-deaza-adenosine may enable rapid DNA sequencing by matrix-assisted laser desorption mass spectrometry

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ABSTRACT

The use of matrix-assisted laser desorption mass spectrometry (MALDI-MS) has been suggested as an ultrafast readout of Sanger DNA sequencing ladders in a manner analogous to that used with sequencing gels. Currently, a serious limitation of MALDI-MS for the analysis of DNA results from the tendency for oligonucleotides to undergo facile fragmentation in the gas phase. The present study was undertaken to gain an understanding of the influence of various chemical structural features of purine bases on the stability of oligodeoxynucleotide ions produced by MALDI. The study focused on the stability of model compounds of the type d(TTTTTTTTTTXXXXXXXXTTTT), where T designates deoxythymidine and X a purine-containing 2'-deoxynucleotide. A variety of different purine derivatives were chosen as the base in the nucleotide X. The mass spectra of the model compounds containing 7-deaza analogues of guanine and adenine reveal a significantly increased stability compared to the 7-aza analogues under the conditions of MALDI-MS. The previously reported incorporation of the 7-deaza-2'-deoxy-adenosine triphosphate and the 7-deaza-2'-deoxy-guanosine triphosphate into DNA by polymerases suggests their use in a Sanger dideoxy sequencing experiment. The dideoxy termination products with the 7-deaza-purines instead of the 7-aza-purines might be sufficiently stable to allow separation and detection of the sequencing ladder by MALDI-MS. Thus, an ultrafast (seconds) read-out of DNA sequence may become feasible.

INTRODUCTION

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has become an effective tool for the structural characterization of biopolymers (1). The utility of the method for the analysis of biopolymers such as peptides and proteins derives from its mass accuracy (0.1–0.01%), sensitivity (10^{-15} – 10^{-12}

mol), speed of analysis (10–100 s) and immunity to common impurities. In addition, the technique is capable of analyzing the components of complex mixtures. These properties of MALDI-MS are being used to great advantage in a host of biological applications (2,3). For example, the facility of MALDI-MS for measuring complex mixtures of peptides has been applied in a rapid new method for amino acid sequence analysis termed 'protein ladder sequencing' (4). The use of MALDI-MS has also been suggested for the rapid determination of DNA sequences (5–20). The proposed strategy for DNA sequence analysis has two steps. In the first step, conventional DNA ladders are produced by the method of Sanger (21). In the second step, the four sequencing reaction mixtures are separately analyzed by MALDI-MS. The DNA sequence is read off the four resulting mass spectra in a manner completely analogous to that used with sequencing gels. A potential advantage of the MALDI-MS readout scheme versus conventional gel-based readout schemes is the speed of the readout step—seconds rather than hours.

MALDI-MS has already been successfully applied to the structural characterization of small synthetic oligonucleotides (16–18). The determination of the molecular masses of intact oligonucleotides provides a convenient means for confirming the synthesis of natural or chemically modified oligonucleotides (16). Additional information concerning the nucleotide sequence and location of modifications can be obtained from an analysis of oligonucleotide ladders (17,18). Such ladders are frequently formed inadvertently during synthesis as a result of incomplete reactions or side-reactions (17). Alternatively, ladders can be generated by partial hydrolysis (17) or time-dependent exonuclease degradation (18). Recently, Smith and co-workers (6,7) reported a MALDI-MS measurement of four synthetic oligonucleotide ladder mixtures (ranging in length between 17 and 41 nt) to mimic the four ladders that are generated in standard Sanger dideoxy sequencing (21). The experiment confirmed that mass spectrometry can be used in a manner analogous with sequencing gels for ordering the sequence of bases. The experiment also revealed current shortcomings of the approach. These include the difficulty in obtaining high quality mass spectra from oligonucleotides larger than 40–50 nt in length (19,20) and the rapid falloff in sensitivity as a function of oligonucleotide mass. [A

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different experimental approach, employing ice as the matrix in place of UV wavelength absorbing matrices, was used to analyze two synthetic oligonucleotide ladder mixtures that ranged in length from 3 to 87 nt (terminated in C) and from 5 to 89 nt (terminated in G) and that mimicked the dideoxy C and G termination products of a Sanger sequencing experiment (14). Ablation of the frozen sample was induced through the absorption of the laser irradiation by an underlying metal substrate (15). Although this approach has produced, on occasion, high quality mass spectra, the investigators report that the reproducibility of the method is low (15).]

The present limitations in the analysis of oligonucleotides by MALDI-MS can be attributed to (i) the tendency of trace amounts of alkali ions to form adducts with the highly acidic sugar-phosphate backbone and (ii) the tendency for oligodeoxynucleotides to fragment upon MALDI. The tendency of trace amounts of alkali ions to form multiple adducts with the acidic phosphates can lead to severe peak broadening and diminution of the mass spectrometric response (8). This limitation has been overcome to a large extent by improvements in the methods used for preparing samples. In particular, removal of trace alkali ions has been effectively accomplished by the addition of cation-exchange resin beads (8) or an excess of ammonium citrate (18) or ammonium acetate (10) into the matrix-oligonucleotide solution prior to the drying step. The tendency of oligodeoxynucleotide ions to undergo extensive fragmentation upon MALDI can lead to weak or non-existent intact oligodeoxynucleotide ion peaks. This tendency for fragmentation is greatly influenced by the chemical structure of the oligodeoxynucleotides. For example, a comparison of homooligodeoxynucleotides revealed that polyTs gave strong mass spectrometric responses whereas polyGs, polyAs and polyCs gave very weak mass spectrometric responses (9,11). This behaviour has been attributed to the different propensities for fragmentation of the oligodeoxynucleotide backbone adjacent to the different nucleotides (11,13,22).

The present study was designed to gain an understanding of the influence of various chemical structural features of purine bases on the stability of oligodeoxynucleotide ions produced by MALDI. In particular, we were interested in identifying a modification that would inhibit fragmentation, but would still be compatible with the enzymatic environment of a Sanger DNA sequencing experiment. As model compounds we chose oligodeoxynucleotides with the sequence d(TTTTTTTTTT^XTTTTTTTTTTTTTTTT), where T designates deoxythymidine and X a purine-containing 2'-deoxynucleotide. The purine bases tested in X were guanine (G), adenine (A), hypoxanthine (I), 2-amino-purine (2Ap), 7-deaza-guanine, 7-deaza-adenine, purine and O6-methyl-guanine. This type of model compound was based on the design of the model compound d(TTTTGGGGTTTTTTTT) used in a previous study (22). Because no fragmentation is observed between Ts, this model system focuses all fragmentations adjacent to the test nucleotide X, so that the nature and extent of the fragmentation can be conveniently assayed.

MATERIALS AND METHODS

Synthesis and purification of oligodeoxynucleotides

All of the oligodeoxyribonucleotides used in this study were synthesized in the Protein Sequencing Facility at The Rockefeller University on a Applied Biosystem 380B synthesizer using

phosphoamidite chemistry (23). d(T₂₆) was subjected to purification by reverse-phase HPLC. The compounds d(T₁₀GT₁₄), d(T₁₀IT₁₄), d(T₁₀AT₁₄) and d(T₁₀2ApT₁₄) were synthesized with retention of the 5'-hydroxyl protecting group (dimethoxytrityl, DMT). d(T₁₀GT₁₄) was purified with a Oligodeoxynucleotide Purification Cartridge (OPC, P/N 40071, Applied Biosystems, CA). OPC-purified d(T₁₀GT₁₄) was further purified by polyacrylamide gel electrophoresis (15% acrylamide-8 M urea gel mixture), as were d(T₁₀IT₁₄), d(T₁₀AT₁₄) and d(T₁₀2ApT₁₄) d(T₁₀7-deazaGT₁₄), d(T₁₀7-deazaAT₁₄), N2 protected d(T₁₀O6-methylGT₁₄) and d(T₁₀NebularineT₁₄). The gel was covered with aluminum foil so that only the left and right extremities of the band were left exposed. The resolved band containing the intact 25mer was visualized by irradiating the exposed ends with UV light. The band was cut out after removal of the aluminum foil. Brief inspection of the whole gel under UV light confirmed that only the band of interest was excised. The oligonucleotides were extracted from the gel pieces with H₂O (three times) over a total of 2 days. After extraction from the gel, d(T₁₀GT₁₄), d(T₁₀IT₁₄), d(T₁₀AT₁₄), d(T₁₀2ApT₁₄), d(T₁₀7-deazaGT₁₄), d(T₁₀7-deazaAT₁₄), ibutyl-N2 protected d(T₁₀O6-methylGT₁₄) and d(T₁₀NebularineT₁₄) were further purified by anion-exchange chromatography. Desalting of the samples was performed by reverse-phase HPLC (Ultrafast Microprotein Analyzer, Michrom Bioresources, CA) on a 4.6 mm Vydac C4 column, buffer A: 10 mM ammonium phosphate pH 6.9, 2% acetonitrile, buffer B: 10 mM ammonium phosphate pH 6.9, 60% acetonitrile, gradient: 0-8 min 2% B, 8-10 min 2-30% B, 10-20 min 30-40% B, 20-23 min 40-90% B, 23-28 min 90-2% B, followed by lyophilization to dryness. d(T₁₀O6-methylGT₁₄) was deprotected following a protocol supplied by Glen Research, VA. Concentrated solutions (200 μM) were prepared by dissolution in high-purity water (Milli-Q UV plus, Millipore, MA) and were stored frozen at -20°C.

Mass spectrometry

Purified d(T₁₀GT₁₄), d(T₁₀IT₁₄), d(T₁₀AT₁₄), d(T₁₀2ApT₁₄), d(T₁₀7-deazaGT₁₄), d(T₁₀7-deazaAT₁₄), d(T₁₀O6-methylGT₁₄) and d(T₁₀NebularineT₁₄) were added to ferulic acid in 150 mM ammonium citrate (dibasic)/acetonitrile 1:1 (v/v) to yield a final oligodeoxynucleotide concentration of 20 μM. A volume of 1 μl of the resulting sample/matrix solution was loaded on to the sample probe tip and dried in the ambient atmosphere. The probe was then immediately inserted into the mass spectrometer and analysed. All spectra were obtained on a linear time-of-flight laser desorption mass spectrometer constructed at The Rockefeller University and described elsewhere (11,24,25). Laser pulses were produced in a Q-switched Lumonics HY 400 neodymium/yttrium aluminum garnet laser (355 nm wavelength, 10 ns duration). The spectra were obtained in the negative ion mode using a static electric potential of -30 kV. The mass spectra were acquired by adding the individual spectra obtained from 100 laser pulses. The spectra were calibrated with d(T₂₆). All mass spectra were obtained under identical experimental conditions, wherein the laser fluence was maintained close to the threshold for ion production. Under these conditions, repeated measurements of each compound yielded mass spectra with reproducible fragmentation patterns.

The solution of matrix and oligonucleotide sample in 150 mM ammonium citrate had a measured pH = 4.8. At this pH, no

Table 1. Comparison of calculated and measured molecular masses of the model compounds d(T₁₀XT₁₄) and their mass spectrometric fragments. Calculated fragment masses are shown in parentheses

X	[M-H] ⁻ calc.	[M-H] ⁻ meas.	[M-base] ⁻ (7418 u*)	[T ₁₀ P] ⁻ (3060 u)	[T ₁₀ P+s] ⁻ (3142 u)	[pT ₁₄] ⁻ (4277 u)
G	7568	7566 ^{sh}	7435	3059	3173	4276
I	7553	7555 ^{sh}	7428	3061	3143	4280
A	7552	7555	n.r.	n.o.	n.o.	4278
2Ap	7552	7553	7431 ^{sh}	3060 ^w	3170 ^w	4275
7-deaza-G	7567	7566	n.o.	3058 ^w	3156 ^w 3176 ^w	4275 ^w
7-deaza-A	7551	7551	n.o.	n.o.	n.o.	n.o.
O6-methyl-G	7583		n.o.	n.o.	n.o.	n.o.
Nebularine	7537	7534	n.o.	3059 ^w	3140 ^w 3180 ^w	4277 ^w

*u atomic mass unit

n.o., fragments not observed

sh, shoulder, peak not resolved

w, weak intensity

hydrolytic depurination is expected on the time scale of the experiment (a few min). Nevertheless, we checked for the occurrence of hydrolytic degradation by comparing spectra obtained directly after preparation of the oligonucleotide/matrix solution with spectra obtained from oligonucleotide/matrix solutions that were kept overnight at 4°C. No significant difference was observed in the resulting mass spectra, indicating that the observed fragment peaks are due to fragmentation in the mass spectrometer and do not arise from degradation in the matrix/DNA solution prior to the mass spectrometric measurement.

RESULTS AND DISCUSSION

The effect of the structure of purine bases on the amount of fragmentation induced in the MALDI process was investigated with a set of 25mer model compounds having the sequence d(T₁₀XT₁₄), where X designates a nucleotide containing a purine base. The long stretch of thymidines on the 5'- and 3'-ends of the purine nucleotide was chosen because rapid cleavage does not occur between thymidines under typical MALDI-MS conditions (8,10) and thus cleavage should be observed exclusively adjacent to X (22). The mass spectrum of d(T₁₀XT₁₄) thus provides a straightforward means to monitor the relative propensity for mass spectrometric fragmentation at the nucleotide X. The unambiguous identification of mass spectrometric fragments requires that the sample be absolutely free of impurities that arise from incomplete reactions that may occur during the synthesis. We therefore took great care in the purification of the model compounds and completely avoided pH conditions under which depurination and hydrolysis of the sugar-phosphate backbone can occur.

In our first set of experiments, we chose guanine, hypoxanthine, adenine and 2-amino-purine as the base in the nucleotide X. The mass spectra of the four compounds obtained using ferulic acid as matrix are shown in Figure 1. Two types of mass

spectrometric fragments were observed. The first was a fragment occurring through the loss of the purine base from the intact molecule, designated [M-base]⁻. The second were fragments that arise by cleavage of the sugar-phosphate backbone, designated [T₁₀P]⁻, [T₁₀P+s]⁻ and [pT₁₄]⁻. The masses of the fragments [T₁₀P]⁻ and [pT₁₄]⁻ indicate breakage adjacent to the purine-containing nucleotide at the 3'-CO and the 5'-CO bonds, respectively (Table 1). The fragment [T₁₀P+s]⁻ likely consists of the 5'-T₁₀ segment plus the sugar moiety of the X nucleotide. The large uncertainty in the mass determination (due to the broad and incompletely resolved peaks) precluded elucidation of the detailed structure of the [M-base]⁻ and [T₁₀P+s]⁻ fragments (Table 1).

The dominant species observed in the mass spectrum of the model compound d(T₁₀GT₁₄) was the [M-base]⁻ fragment (Fig. 1a). In addition, pronounced fragmentation of the sugar-phosphate backbone was observed to yield the 5'-fragments T₁₀P and T₁₀P+s and the 3'-fragment pT₁₄. Although the same fragmentation pattern was observed for the compounds d(T₁₀IT₁₄), d(T₁₀AT₁₄) and d(T₁₀2ApT₁₄) (Fig. 1b-d), the fragment peak intensities decrease from the G- to the I-containing compounds and further in the A- and 2Ap-containing compounds. Hence the tendency for fragmentation of the model compounds appears to decrease in the order guanine > hypoxanthine > adenine = 2-amino-purine.

In an attempt to correlate this order of stability of purine nucleotide-containing model system with the intrinsic properties of the various bases investigated, we considered the pK_a values and sites of protonation of the bases. In this regard, it has recently been suggested that the backbone fragmentation of oligodeoxynucleotides in MALDI-MS is correlated with base protonation (13). The pK_a values of adenosine-monophosphate and guanosine-monophosphate are 3.84 and 2.48, respectively (26) and the rates of hydrolysis of deoxy-adenosine and of deoxy-guanosine are 0.017/M/s and 0.0032/M/s, respectively (27). If the overall propensity for base protonation was the dominant factor in determining the degree of fragmentation of oligodeoxynucleotides, the values for the pK_as and rates of hydrolysis given above would indicate a higher propensity for fragmentation of the adenine-containing compound compared with the guanine-containing compound. This prediction is in conflict with the order of stability of the compounds determined in the present experiments. A more detailed consideration of base protonation reveals the N1 nitrogen as the site of the first proton attachment in adenine (28) and either N3 or N7 as the site of the second protonation (N3 in ref. 29). In contrast, the site of first protonation for guanine was found to be the N7 nitrogen (28). A comparison of the basicity for the N7 nitrogen in different purines reveals a pK_a of 2.48 for GMP and 1.30 for inosine-monophosphate (26). For adenosine the attachment of a single proton to the molecule at N7 cannot be directly measured, but was estimated to be -0.2 (30) [this value represents the microacidity constant of the H⁺ (N7) site of monoprotonated adenosine]. Hence, the order of instability of the model compounds in our MALDI experiments (guanine > hypoxanthine > adenine) appears to correlate with the decrease in basicity of the N7 nitrogen of guanosine, inosine and adenosine.

We therefore carried out a second set of experiments using our d(T₁₀XT₁₄) model compounds in which X contained bases with the nitrogen at position-7 replaced by a carbon, i.e., 7-deaza-guanine and 7-deaza-adenine. This substitution should preclude protonation at the 7-position. The resulting MALDI mass spectra

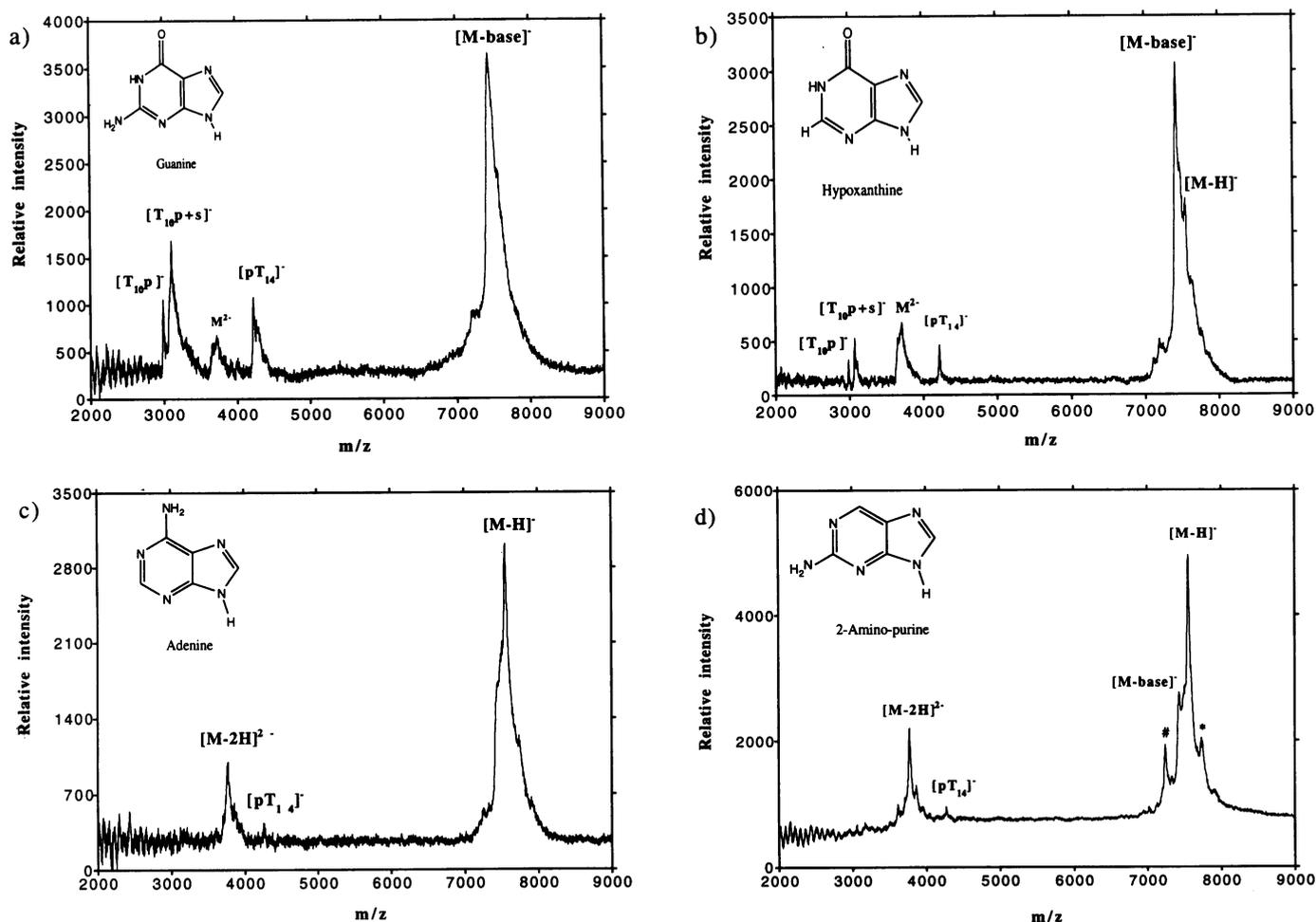


Figure 1. Negative ion MALDI mass spectra of the compounds (a) $d(T_{10}GT_{14})$, (b) $d(T_{10}IT_{14})$, (c) $d(T_{10}AT_{14})$ and (d) $d(T_{10}2ApT_{14})$ in ferulic acid. The peak marked with an * is due to photochemically-induced matrix adduct formation. The peak marked with a # is likely due to the synthetic side product $d(T_{24})$.

are shown in Figure 2. The dominant ion species in both cases correspond to the deprotonated intact molecule. No indication for the loss of the purine base was observed in either compound, although low intensity peaks corresponding to $[T_{10}P]^-$, $[T_{10}P+s]^-$ and $[pT_{14}]^-$ reveal a weak fragmentation along the sugar-phosphate backbone of the 7-deaza-G compound (Table 1). These results indicate a clearly increased stability of the 7-deaza-purine-containing compounds over their 7-aza-analogs. [The mass spectra of the $d(T_{10}O6\text{-methyl}GT_{14})$ and $d(T_{10}\text{Nebularine}T_{14})$ compounds also exhibited very little fragmentation (Table 1). Because the O6-methylG-triphosphate and the nebularine-triphosphate have limited use in Sanger dideoxy sequencing, results with these compounds are not discussed in detail here.]

The high signal intensity and the lack of fragments corresponding to the loss of base observed in Figure 2 suggest that substitution of 7-aza-purines by 7-deaza-purines in mixed-base oligodeoxynucleotides may significantly improve the quality of the MALDI mass spectrometric readout. Such improvements are especially noteworthy for the 7-deaza compounds because their ability for Watson-Crick base pairing is not hindered by the N7 modification and DNA chain extension can be readily performed with these modified nucleotides (31). Our results suggest the possible use of 7-deaza-ATP and 7-deaza-GTP in Sanger-dideoxy-sequencing with MALDI-MS as an ultrafast method for

the separation and readout of the 7-deaza-purine-containing termination products. Both triphosphate derivatives are commercially available and have already been successfully used in Sanger dideoxy sequencing experiments to resolve band compression (31,32).

With the 7-deaza-purine compounds, we have found a stabilizing modification for adenine and guanine bases. Because thymidines are stable under the conditions of MALDI, the only remaining nucleotide that may undergo extensive fragmentation (and therefore cause peak broadening and reduction of the mass spectrometric response of DNA) is cytidine. The fragmentation of 5-methyl-2'-deoxycytidine, 2'-deoxycytidine, and 5-bromo-2'-deoxycytidine have previously been studied in model compounds similar to those discussed in the present paper (33). An increased stability for the 5-methyl derivative over the normal cytidine compound was observed. Although fragmentation for the 5-methyl compound was still prominent, the use of 5-methylcytidine-triphosphate in a MALDI mass spectrometric Sanger dideoxy sequencing experiment may lead to improved results compared with cytidine. Other potentially interesting modifications of cytidines are the N4-methyl-2'-deoxy and the 5-fluoro-2'-deoxy derivatives. N4-methyl-2'-deoxy-cytidine 5'-triphosphate has been shown to prevent band compression in sequencing

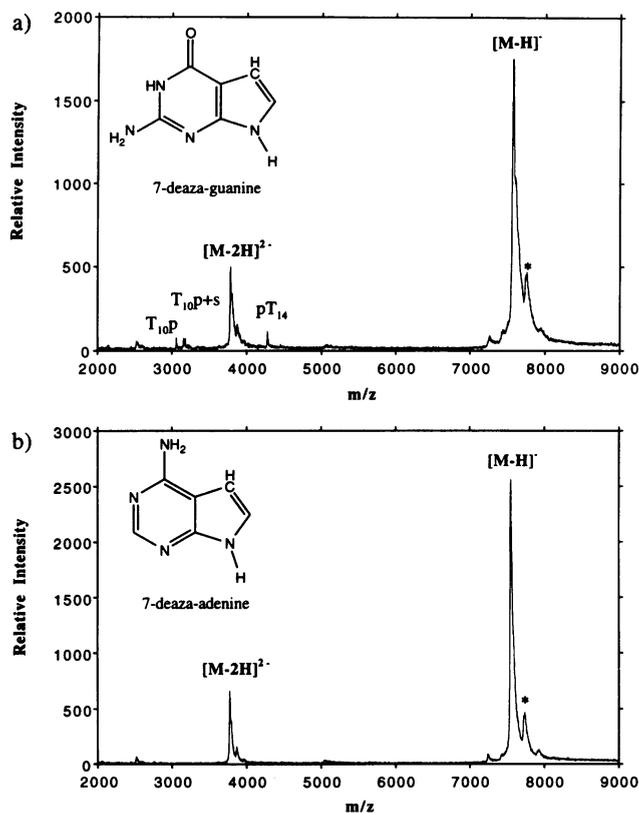


Figure 2. Negative ion MALDI mass spectra of the compounds (a) $d(T_{10}7\text{-deazaGT}_{14})$ and (b) $d(T_{10}7\text{-deazaAT}_{14})$ in ferulic acid. The peaks marked with an * are due to photochemically-induced matrix adduct formation.

gels (34) and both compounds have been incorporated into DNA by a number of polymerases (34,35).

It would appear that a straightforward way to further test the improved stability of nucleic acids containing 7-deaza-adenosine and 7-deaza-guanosine would involve the measurement of synthetic oligonucleotide ladders in which all 7-aza-purines are replaced by their 7-deaza-analogs. Unfortunately, a serious side reaction in the synthesis of oligonucleotides with more than 2–3 nt of 7-deaza-guanosine (36) renders this experiment impractical. An alternative means for testing the improved stability of nucleic acids containing 7-deaza nucleotides involves a scale up of the Sanger polymerase-catalyzed synthesis. Such a scale up is presently under way in our laboratory and will be the subject of a future manuscript.

For the mass spectrometric read-out of Sanger dideoxy sequencing employing 7-deaza-adenosine-triphosphate and 7-deaza-guanosine-triphosphate, the stability of the termination reagents has to be considered. Dideoxy-guanosine and dideoxy-adenosine are more amenable to hydrolysis than the deoxy-compounds (37) and may have a higher propensity for mass spectrometric fragmentation. To obtain the highest potential resolution in Sanger dideoxy sequencing with MALDI mass spectrometric read-out, we suggest the use of the 7-deaza-dideoxy-compounds as terminating reagents. 7-deaza-2',3'-dideoxyguanosine has been synthesized and exhibits a *N*-glycosidic bond that is very stable against hydrolysis (38).

CONCLUSIONS

We have studied the stability to MALDI mass spectrometry of a series of model compounds of the type $d(T_{10}XT_{14})$ in which a number of different purine derivatives were chosen as the base in the nucleotide X. In a first set of experiments, we found that the tendency for fragmentation decreased in the order guanine > hypoxanthine > adenine = 2-amino-purine. This order correlates with the decrease in basicity of the N7 nitrogen of the purine. We therefore carried out a second set of experiments in which the nucleotide X contained bases with the N7 nitrogen replaced by a carbon, i.e., 7-deaza-guanine and 7-deaza-adenine. The mass spectra of these compounds reveal a significantly increased stability compared to the 7-aza analogues under the conditions of MALDI-MS. The previously reported incorporation of the 7-deaza-2'-deoxy-adenosine triphosphate and the 7-deaza-2'-deoxy-guanosine triphosphate into DNA by polymerases suggests their use in a Sanger dideoxy sequencing experiment. The dideoxy termination products with the 7-deaza-purines instead of the 7-aza-purines might be sufficiently stable to allow the separation and detection of the sequencing ladder by MALDI-MS. Thus an ultrafast (seconds) read-out of DNA sequence may become feasible.

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REFERENCES

- Hillenkamp, F., Karas, M., Beavis, R. C., Chait, B. T. (1991) *Anal. Chem.* **63**, 1193A–1203A.
- Chait, B. T., Kent, S. B. H. (1992) *Science* **257**, 1885–1894.
- Aebersold, R. (1993) *Curr. Opin. Biotechnol.* **4**, 412–419; Wang, R., Chait, B. T. (1994) *ibid* **5**, 77–84.
- Chait, B. T., Wang, R., Beavis, R. C., Kent, S. B. H. (1993) *Science* **262**, 89–92.
- Beavis, R. C., Chait, B. T. (1992) United States Patent 5, 288, 644.
- Smith, L. M. (1993) *Science* **262**, 530–531.
- Fitzgerald, M. C., Zhu, L., Smith, L. M. (1993) *Rapid Commun. Mass Spectrom.* **7**, 895–897.
- Nordhoff, E., Ingendoh, A., Cramer, R., Overberg, A., Stahl, B., Karas, M., Hillenkamp, F., Crain, P. F. (1992) *Rapid Commun. Mass Spectrom.* **6**, 771–776.
- Parr, G. R., Fitzgerald, M. C., Smith, L. M. (1992) *Rapid Commun. Mass Spectrom.* **6**, 369–372.
- Currie, G. J., Yates, J. R. (1993) *J. Am. Soc. Mass Spectrom.* **4**, 955–963.
- Schneider, K., Chait, B. T. (1993) *Org. Mass Spectrom.* **28**, 1353–1361.
- Tang, K., Allman, S. L., Jones, R. B., Chen, C. H., Araghi, S. (1993) *Rapid. Commun. Mass Spectrom.* **7**, 63–66.
- Nordhoff, E., Cramer, R., Karas, M., Hillenkamp, F., Kirpekar, F., Kristiansen, K., Roepstorff, P. (1993) *Nucleic Acid Res.* **21**, 3347–3357.
- Schieltz, D. M., Williams, P. (1994) *Proc. 42nd ASMS Conf. on Mass Spectrometry and Allied Topics* Chicago, IL, 141.
- Schieltz, D. M., Chau-Wen, C., Cong-Wen, L., Thomas, R. M., Williams, P. (1992) *Rapid Commun. Mass Spectrom.* **6**, 631–636.
- Wang, B. H., Biemann, K. (1994) *Anal. Chem.* **66**, 1918–1924.
- Keough, T., Baker, T. R., Dobson, R. L. M., Lacey, M. P., Riley, T. A., Hasselfield, J. A., Hesselberth, P. E. (1993) *Rapid Commun. Mass Spectrom.* **7**, 195–200.
- Pieles, U., Zürcher, W., Schär, M., Moser, H. E. (1993) *Nucleic Acid Res.* **21**, 3191–3196.

- 19 Wu, K. J., Shaler, T. A., Becker, C. H. (1994) *Anal. Chem.* **66**, 1637–1645.
- 20 Tang, K., Allman, S. L., Chen, C. H. (1993) *Rapid Commun. Mass Spectrom.* **7**, 943–948.
- 21 Sanger, F., Nicklen, S., Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
- 22 Parr, G. R., Zhu, L., Fitzgerald, M. C., Smith, L. M. (1993) *Proc. 41st ASMS Conf. on Mass Spectrometry and Allied Topics* San Francisco, CA, 788a.
- 23 Caruthers, M. H. (1985) *Science* **230**, 281.
- 24 Beavis, R. C., Chait B. T. (1989) *Rapid Commun. Mass Spectrom.* **3**, 233–237.
- 25 Beavis, R. C., Chait B. T. (1989) *Rapid Commun. Mass Spectrom.* **3**, 432–435.
- 26 Sigel, H., Massoud, S. S., Corf, N. A. (1994) *J. Am. Chem. Soc.* **116**, 2958–2971.
- 27 Zoltewicz, J. A., Clark, D. F., Sharpless, T. W., Grahe, G. (1970) *J. Am. Chem. Soc.* **92**, 1741–1750.
- 28 Saenger, W. (1984) *Principles of Nucleic Acid Structure* Springer-Verlag, New York, p. 107.
- 29 Schindler, M. (1988) *J. Am. Chem. Soc.* **110**, 6623–6630.
- 30 Sigel, H., Corf, N. A., Ji, L.-n., Martin, R. B. (1992) *Comments Inorg. Chem.* **13**, 35–59.
- 31 Jensen, M. A., Zagursky, R. J., Trainor, G. L., Cocuzza, A. J., Lee, A., Ellson, Y. (1991) *DNA Sequence* **1**, 233–239.
- 32 Barr, P. J., Thayer, R. M., Laybourn, P., Najarian, R. C., Seela, F., Tolan, D. R. (1986) *BioTechniques* **4**, 428–432.
- 33 Nelson, C. M., Zhu, L., Fitzgerald, M. C., Smith, L. M. (1994) *Proc. 42nd ASMS Conf. on Mass Spectrometry and Allied Topics* Chicago, IL, 110.
- 34 Li, S., Haces, A. Stupar, L., Gebeyehu, G., Pless, R.C. (1993) *Nucleic Acids Res.* **21**, 2709–2714.
- 35 Tanaka, M., Yoshida, S., Saneyoshi, M., Yamaguchi, T. (1981) *Cancer Res.* **41**, 4132–4135.
- 36 Hugh Mackie, Glen Research, VA, personal communication.
- 37 J. L. York (1981) *J. Org. Chem.* **46**, 2171.
- 38 Seela, F., Muth, H.-P. (1988) *Liebigs Ann. Chem.*, 215–219.