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Detection of ribose-methylated nucleotides in *Pyrodictium occultum* tRNA by liquid chromatography—frit-fast atom bombardment mass spectrometry

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Abstract

Ribose-methylated dinucleotides of the type NmpN′ derived from digestion of tRNA with RNase T_2 were separated and characterized by directly combined liquid chromatography—mass spectrometry (LC–MS) with a continuous-flow frit-fast atom bombardment (frit-FAB) interface. Prediction of NmpN′ peaks was readily made by comparison of the LC profile with that of comparative nuclease P_1 digest. The identity of the candidate peaks including NmpN′ was further recognized by the mass spectra, in which NmpN′ showed intense molecular-related ions, in addition to sequence-specific fragment ions, to verify the chemical structures in both positive- and negative-ion modes. The method was applied to screening NmpN′ (and NmpN′mpN″) in tRNA from the extremely thermophilic archaeon *Pyrodictium occultum*.

1. Introduction

Methylation of O-2′-ribose is one of the types of structural modifications in transfer RNA, and occurs at the polynucleotide level after the transcription of the tRNA genes, as in the case of the base modifications [1]. Ribose methylation alters the conformation in tertiary structure of tRNA, resulting in different impacts on tRNA activity, such as conformational rigidity and decoding efficiency, thus contributing to correct codon recognition [2–6]. Another significant role of ribose methylation has been shown to be associated with thermal stability of tRNA molecules [7]. In addition to the four common 2′-O-methyl ribonucleosides (Nm), many unusual nucleosides modified both in the base and by methylation in ribose, have been detected and characterized in tRNA from thermophilic archaea (formerly termed archaebacteria). The extent of ribose methylation tends to increase with increasing growth temperature of the thermophile [8].

The detection and characterization of modified nucleosides including Nm have usually been performed by two-dimensional thin-layer chromatography (TLC) of enzymatic digests of uniformly ^{32}P-labelled tRNA [9]. Although this procedure readily indicates the presence of new nucleotides, it is not useful for their direct identification. Liquid chromatography–thermospray mass spectrometry (LC–thermospray MS) has proved to be an efficient method for investigating post-transcriptional modifications in

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tRNA [10]. A number of new modified nucleosides, especially in archaebacterial tRNAs, have been detected and characterized by LC–thermospray MS [8]. In the method, identification of individual modified nucleosides in tRNA is made from their chromatographic retention times and from their mass spectra, which consist of the molecular and base ion species. Nm is formed as dinucleotides of the type NmpN′ (Nm-3′-phosphate linked with 3′ to 5′ with ribonucleosides) when using RNase T_2 for hydrolysis of tRNA, so that the LC–MS method has been extended to detect NmpN′ dinucleotides [11]. Mass spectra of NmpN′ acquired in nanogram amounts showed structurally useful fragment ions corresponding to both constituent nucleosides and their bases, but no molecular-related ions and sequence-specific fragment ions.

The introduction of continuous-flow frit-fast atom bombardment (frit-FAB) LC–MS interface affords new possibilities for determining non-volatile and thermally labile components in complex biological matrices [12]. The inherent mildness of FAB ionization significantly increases the range of applicability to highly polar and ionic analytes which are often unamenable to other LC–MS interfaces. Continuous-flow type LC–MS methods and their applications have been the subject of a recent review by Caprioli and Suter [13]. The feasibility of LC–frit-FAB-MS for the structural characterization of modified nucleosides in low microgram amounts of isoaccepting tRNAs has recently been evaluated using both positive- and negative-ion modes [14].

In this paper, we describe the detection and characterization of NmpN′ dinucleotides from enzymatically digested tRNA along with characterization of Nm nucleosides. *Saccharomyces cerevisiae* $tRNA^{Phe}$ is used as a model, which is composed of 76 nucleotides including 2′-O-methylcytidine-32 (Cm) and 2′-O-methylguanosine-34 (Gm) [15]. LC–frit-FAB-MS as applied to screening of Nm and NmpN′ in unfractionated tRNA from the archaeon *Pyrodictium occultum*, which is the most thermophilic organism presently known, with an optimum growth temperature at 105°C [16].

2. Experimental

2.1. Materials

The following materials were obtained from Sigma (St. Louis, MO, USA): transfer RNA^{Phe} from brewer's yeast (*Saccharomyces cerevisiae*); phosphodiesterase I (type VII, *Crotalus atrox* venom); ribonuclease (RNase) T_2 (grade V, *Aspergillus oryzae*); and alkaline phosphatase (AP, type VII-S, bovine intestinal mucosa). Nuclease P_1 was purchased from Yamasa (Tokyo, Japan). Unfractionated tRNA was a gift from P.F. Crain and J.A. McCloskey (University of Utah, Salt Lake City, UT, USA) and had been isolated from *Pyrodictium occultum* cells provided by K.O. Stetter, Regensburg University, by an earlier described procedure [17].

2.2. Enzymatic hydrolysis of tRNA

The tRNA was digested either by nuclease P_1 and alkaline phosphatase (AP) or by RNase T_2 and AP. The detailed protocol for enzymatic digestion of RNA has been published [8].

Nuclease P_1-AP digestion

Vacuum-dried $tRNA^{Phe}$ (100 μg) was dissolved in 100 μl of 10 m*M* acetate buffer (AcOH–AcONa) (pH 5.3) and 1 U of nuclease P_1 [1.0 U/μl in 10 m*M* AcOH–AcONa buffer (pH 5.3)] was added. The mixture was digested at 45°C for 16 h and then dried under reduced pressure. The residue was dissolved in 95 μl of 20 m*M* triethylammonium hydrogencarbonate (TEAB) buffer (pH 8.0), then 0.5 U of AP [0.1 U/μl in 20 m*M* TEAB buffer (pH 8.0)] was added and incubated at 37°C for 1 h.

RNase T_2-AP digestion

The same amount of $tRNA^{Phe}$ as above was digested by incubation with 2 U of RNase T_2 [0.05 U/μl in AcOH–AcONa buffer (pH 4.5)] in 100 μl of 20 m*M* AcOH–AcONa buffer (pH 4.5). The incubation was carried out at 37°C for 16.5 h. The incubation mixture was then evaporated by using a centrifugal freeze-dryer. The

residue was dissolved in 95 μl of 20 m*M* TEAB buffer (pH 8.0) and 0.5 U of AP was added, then the mixture was incubated at 37°C for 1 h.

P. occultum tRNA digestion

A 30-μg amount of unfractionated *P. occultum* tRNA was digested by incubation with 1.0 U of nuclease P_1 in 30 μl of AcOH–AcONa buffer (pH 4.5) at 45°C for 16.5 h. The mixture was further digested with 0.005 U of phosphodiesterase in 28 μl of TEAB buffer (pH 7.4) at 37°C for 2 h for complete hydrolysis [11,18]. The same amount of sample was also digested by using 0.6 U of RNase T_2 and 0.15 U of AP.

2.3. LC–MS

The LC and MS equipment and operating procedures used for these experiments have been reported previously [14] and are described briefly below. The gradient LC system consisted of two Shimadzu (Kyoto, Japan) LC-9A pumps, a Rheodyne (Cotati, CA, USA) Model 7125 loop injector and a Shimadzu SPD-6AV UV detector equipped with a laboratory-made micro flow cell (cell volume 0.1 μl). A flow-rate of 100 μl/min was split ca. 16:1 using a T-joint fitted to a microbore separation column (Develosil ODS-5, 150 × 0.5 mm I.D., 5 μm particles; Nomura Chemical, Seto, Japan) and a restriction column (Develosil ODS-5, 150 × 2.0 mm I.D.). This restriction column splitting provides a suitable flow-rate (4–6 μl/min) for the frit-FAB interface owing to the proper back-pressure differential of the restriction column, and allows the whole sample to enter the LC–MS interface [14,19]. Gradient elution was accomplished using a mixture of 0.1 *M* ammonium acetate buffer (pH 6.0) as solvent A and 40% methanol as solvent B. Both solvents contained 0.8% (v/v) of glycerol as FAB matrix. The gradient profile used is described in Ref. [14].

A Jeol (Tokyo, Japan) JMS-HX110 mass spectrometer equipped with an LC–frit-FAB-MS interface, with an intermediate modified SPD-6AV UV detector operated at 254 nm, was used to acquire the data. The mass spectrometer was operated at an 8-kV accelerating voltage in the positive-ion mode and 7 kV in the negative-ion mode. The FAB gun (Jeol) was operated at 6 kV with xenon. The ion source temperature was kept at 40–50°C. The mass spectra were obtained by linear scanning from 20 to 1000 u with a scan speed of 4 s per cycle and the background was subtracted with a Jeol JMA-DA5000 data system.

3. Results and discussion

3.1. Screening of Nm and NmpN′ in tRNA digest

Nuclease P_1 can usually cleave all phosphodiester bonds; an enzymatic digest of tRNA using nuclease P_1 in combination with AP should produce a mixture of mononucleosides including Nm. When nuclease P_1 is replaced by RNase T_2 in the digestion, Nm should be formed as dinucleotide NmpN′, because RNase T_2 cleaves RNA at all phosphodiester bonds except for those adjacent to Nm residues, as has shown to be the mode of action [20]. Therefore, screening of Nm and NmpN′ residues can be readily accomplished by comparing LC–MS profiles of the nuclease P_1-AP digest with the RNase T_2-AP digest of tRNA, if the tRNA has Nm residues. The analysis of isoaccepting tRNA has the additional advantage that sequence information in the form of the identity of the 3′-residue (N′) is also obtained.

Figs. 1 and 2 show examples of yeast $tRNA^{Phe}$ digests recorded under negative ionization conditions. The Nm residues included in the isoaccepting tRNA (Cm and Gm in this case) appear in the chromatogram of the nuclease P_1-AP digest (Fig. 1), and are tentatively assigned by combination of the relative retention times [21,22] and mass responses for the molecular ion species. Ion profiles of both the *m/z* 316 (Cm, $([M - H + AcOH]^-)$ and *m/z* 296 (Gm, $[M - H]^-$) responses are found at predicted retention times along with isobaric positional

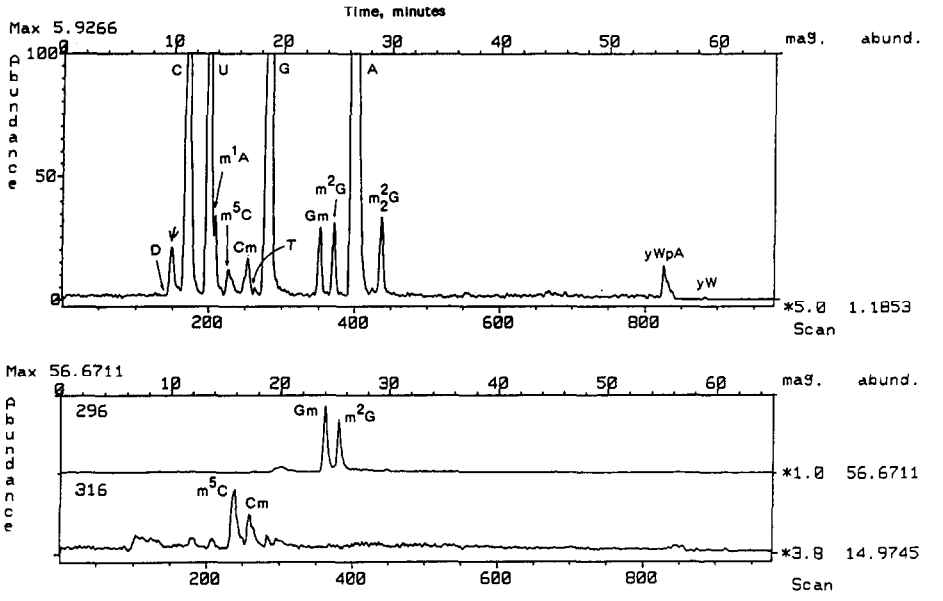

Fig. 1. Chromatographic separation of nucleosides from LC–MS analysis of enzymatic hydrolysate of 8 μg of *S. cerevisiae* $tRNA^{Phe}$ following digestion with nuclease P_1 and alkaline phosphatase. LC–UV trace (top) and mass chromatograms of *m/z* 296 ($[M-H]^-$ for monomethylguanosines) and *m/z* 316 ($[M-H+AcOH]^-$ for monomethylcytidines) (bottom). In the chromatograms, the mass channel time-scale lags behind the UV scale by approximately 32 s (eight scans), owing to the transit time between UV and mass detectors. Abbreviations: D = 5,6-dihydrouridine; ψ = pseudouridine; C = cytidine; U = uridine; m^1A = 1-methyladenosine; m^5C = 5-methylcytidine; Cm = 2′-O-methylcytidine; T = 5-methyluridine; G = guanosine; Gm = 2′-O-methylguanosine; m^2G = N^2-methylguanosine; A = adenosine; m_2^2G = N^2,N^2-dimethylguanosine; yWpA = wybutylyl-(3′,5′)-adenosine; yW = wybutosine.

isomers, m^5C and m^2G. Conversely, these Cm and Gm peaks disappear in the RNase T_2-AP digest chromatogram (Fig. 2), and additional peaks appear, some of which are expected to be NmpN′ (CmpU and GmpA in this sequence-known tRNA). The use of mass-specific detection greatly enhances structural specificity relative to UV absorbance alone particularly in such a case, because the relative LC retention times for various NmpN′ dinucleotides are not yet known. The dinucleotides, CmpU and GmpA, were detected by the mass responses of the $[M-H]^-$ ions at *m/z* 562 and 625, respectively, as shown in Fig. 2, where the chromatographic resolution for CmpU was not impaired by interference from the co-eluting component m^2G. Similar mass responses were obtained using positive-ion detection (data not shown).

3.2. Mass spectral characterization of chromatographic peaks

More rigorous identification of chromatographic peaks was achieved from the mass spectral data. The frit-FAB mass spectra of ribonucleosides including Nm have been examined and are characterized by the presence of definite molecular-related ions and the constituent base ion in both positive- and negative-ion modes [14]. The mass spectra of Cm and Gm obtained from LC–frit-FAB-MS analysis of the nuclease P_1-AP digest were consistent with those obtained from authentic standards in both positive and negative ionization modes. Structural confirmation of the two NmpN′ peaks assigned from the mass chromatograms can be made from frit-FAB mass spectra without comparison with those of

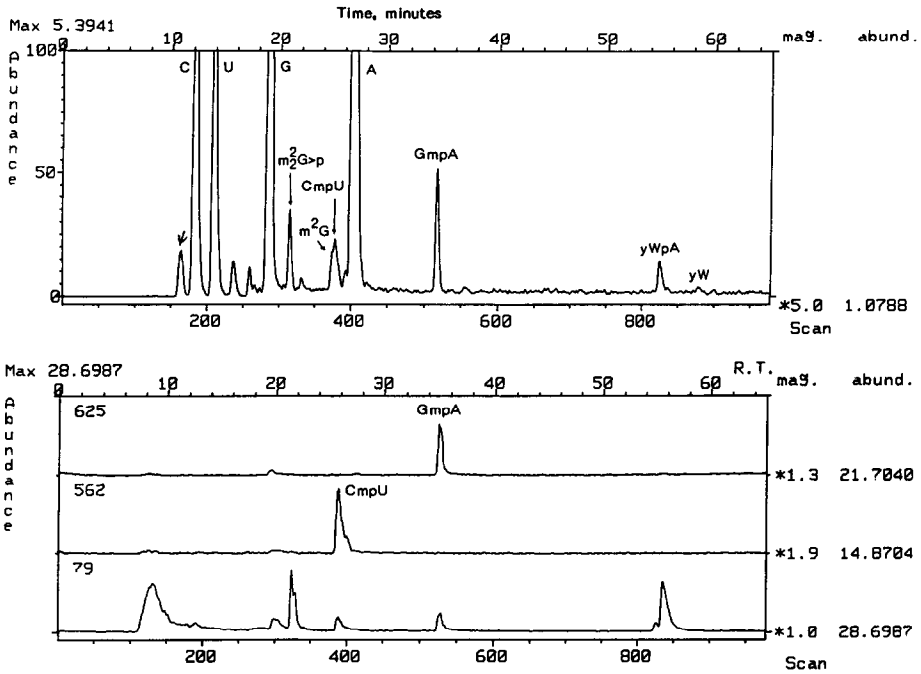

Fig. 2. Chromatographic separation of nucleosides and nucleotides from LC–MS analysis of an enzymatic hydrolysate of 8μg of *S. cerevisiae* $tRNA^{Phe}$ following digestion with RNase T_2 and alkaline phosphatase. LC–UV trace (top) and mass chromatograms of *m/z* 562, 625 and 79, representing the $[M-H]^-$ for CmpU, GmpA and $[PO_3]^-$ ions for nucleotides, respectively (bottom). Abbreviations: CmpU = 2′-O-methylcytidylyl-(3′,5′)-uridine; GmpA = 2′-O-methylguanylyl-(3′,5′)-adenosine; $m_2^2G>p = N^2,N^2$-dimethylguanosine 2′,3′-cyclic monophosphate; others as in Fig. 1.

authentic standards, because the mass spectra contain molecular-related ions, in addition to sequence-specific fragment ions to verify the structures. Negative ion frit-FAB mass spectra of CmpU and GmpA obtained from the LC–MS run in Fig. 2 are shown in Fig. 3, and their positive ion mass spectra are summarized in Table 1.

The principal fragmentations in negative ionization are essentially the same as those in positive ionization, but the observed ion species are all 2 u lower than in the positive ionization mode. Cleavage of the sugar–phosphate linkages gives nucleoside monophosphate anions representing 5′- and 3′-residues denoted $[Nmp]^-$ and $[pN']^-$, respectively. Comparative sequence-specific ions, $[NmpH_2]^+$ and $[pN'H_2]^+$, were observed in the positive ionization mode, but were less intense than in the negative ionization mode. Conversely, deprotonated base ion $[B]^-$ in the negative ionization mode was weak or often absent and the corresponding protonated base $[BH_2]^+$ in the positive ionization mode was usually abundant. Therefore, structural information obtained in both ionization modes complements each other. Additional fragmentations involving cleavage of the ribose ring of 5′- and 3′-residues were observed, giving another series of sequence ions corresponding to $[M-H-(B_1+73)]^-$ ($[MH-(B_1+73)]^+$) and $[M-H-(B_2+73)]^-$ ($[MH-(B_2+73)]^+$), respectively. The former cleavage involving the 5′-residue has been recognized in negative-ion FAB tandem mass spectrometry of dinucleotides [23,24]. These sets of fragment ions are not abundant in relative intensity and occasionally ambiguous, but their occurrence is of importance particularly when the unexpected peak formed in tRNA

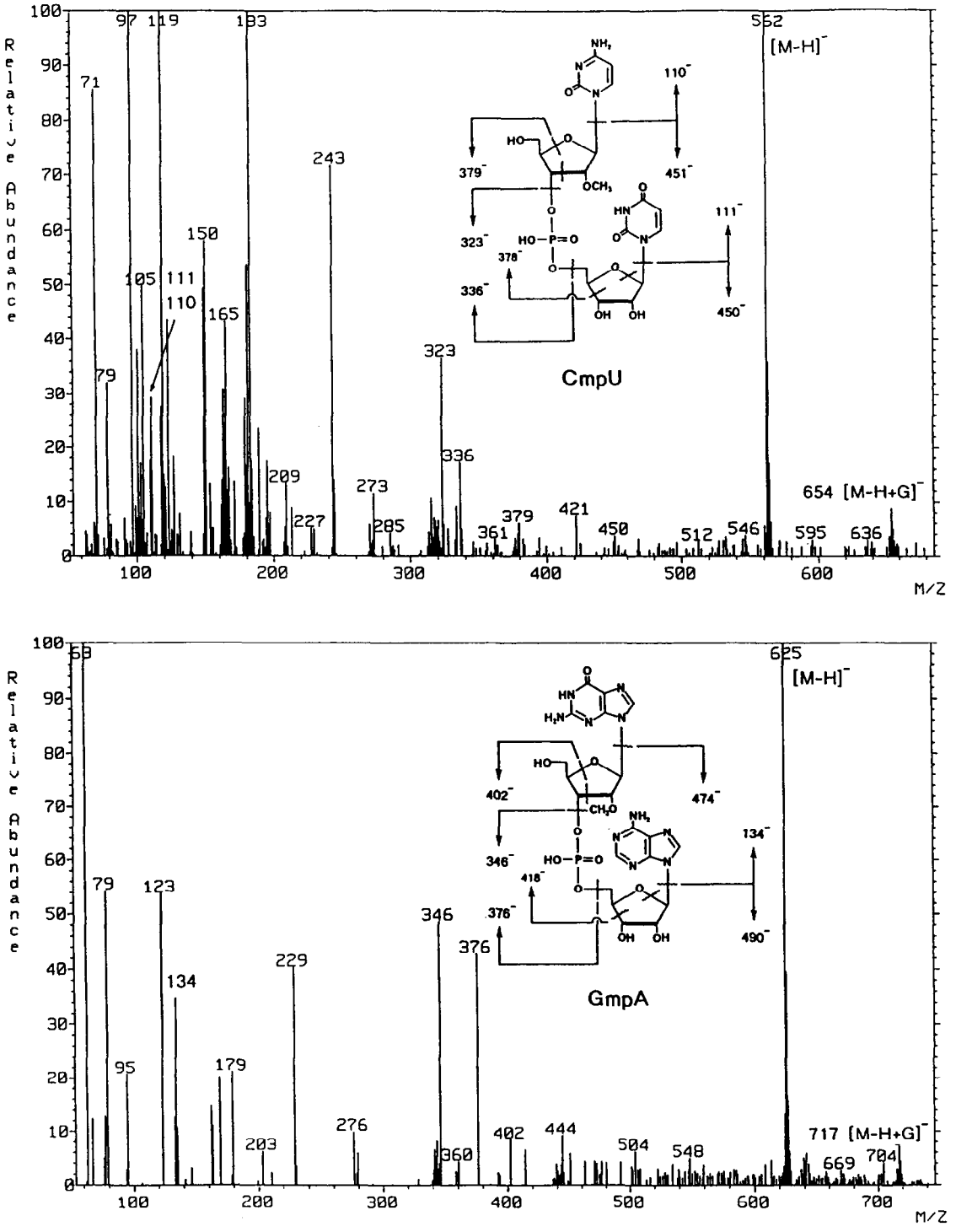

Fig. 3. Negative-ion frit-FAB mass spectra of CmpU (top) and GmpA (bottom) eluting at 25.0 and 34.5 min in Fig. 2, respectively, and possible cleavages involved in the fragmentation.

Table 1
Positive-ion frit-FAB mass spectra of dinucleotides (NmpN') obtained from hydrolysis of *S. cerevisiae* $tRNA^{Phe}$ with RNase T_2 and alkaline phosphatase

Dinucleotide	$[MH]^+$	$[MH - B_1H]^+$	$[MH - B_2H]^+$	$[NmpH_2]^+$	$[pN'H_2]^+$	$[B_1H_2]^+$	$[B_2H_2]^+$	$[MH - (B_1 + 73)]^+$	$[MH - (B_2 + 73)]^+$
GmpU	564(25)	453(0.1)	452(2)	338(3)	325(2)	112(100)	113(21)	381(1)	380(2)
GmpA	627(30)	476(5)	492(14)	378(9)	348(10)	152(100)	136(100)	404(−)	420(2)

Values are *m/z* with relative intensity (%) in parentheses.
B_1 and B_2 represent the constituent bases of Nm and N', respectively.

Table 2
Negative-ion frit-FAB mass spectra of dinucleotides (NmpN') detected in unfractionated *P. occultum* tRNA digested with RNase T_2 and alkaline phosphatase

Dinucleotide	$[M - H]^-$	$[M - H - B_1]^-$	$[M - H - B_2]^-$	$[Nmp]^-$	$[pN']^-$	$[B_1]$	$[B_2]$	$[M - H - (B_1 + 73)]$	$[M - H - (B_2 + 73)]^-$
CmpC	561(100)	450(2)	450(2)	336(−)	322(4)	110(−)	110(−)	378(2)	378(2)
CmpG	601(100)	490(−)	450(−)	336(−)	362(23)	110(4)	150(−)	418(−)	378(−)
CmpU	562(100)	451(4)	339(−)	336(20)	323(30)	110(49)	111(15)	379(2)	378(6)
CmpA	585(100)	474(−)	450(1)	336(−)	346(19)	110(−)	134(−)	402(1)	378(−)
UmpG	602(100)	490(13)	451(3)	337(20)	362(22)	111(19)	150(−)	418(−)	379(−)
UmpA	586(100)	474(−)	451(−)	337(−)	346(−)	111(−)	134(−)	402(27)	379(−)
GmpG	641(100)	490(−)	490(−)	376(37)	362(1)	150(−)	150(−)	418(4)	418(4)
GmpA	625(100)	474(−)	490(22)	376(30)	346(50)	150(−)	134(−)	402(−)	418(−)
m^2GmpU	616(100)	451(1)	504(3)	390(31)	323(−)	164(−)	111(−)	379(8)	432(−)
m^2GmpC	615(100)	503(12)	504(−)	390(16)	322(1)	164(−)	110(−)	378(−)	432(14)
m_2^2GmpG	669(100)	490(−)	518(−)	404(15)	362(8)	178(58)	150(4)	418(11)	446(−)
m_2^2GmpC	629(100)	450(1)	518(−)	404(27)	322(16)	178(1)	110(2)	378(2)	446(2)

Values are *m/z* with relative intensities (%) in parentheses.
B_1 and B_2 represent the constituent bases of Nm and N', respectively.

digestion is nuclease-resistant dinucleotides NpN′. As is distinct from the case of NmpN′, there are two possibilities according to the direction of the sequence, namely NpN′ and N′pN. A set of the two fragment ions gives specific *m/z* values, permitting the determination of the sequence direction [25]. The detailed results from systematic examination of dinucleotides NpN′ by LC–frit-FAB-MS will be reported elsewhere.

From the frit-FAB mass spectrum, the unexpected peak observed at a retention time of 21.3 min in the LC–UV trace of the RNase T_2-AP digest (Fig. 2) was found to be N^2,N^2-dimethylguanosine 2′,3′-cyclic phosphate ($m_2^2G>p$). The negative-ion mass spectrum is shown in Fig. 4, where $[M-H]^-$ and $[B]^-$ are observed at *m/z* 372 and 178, respectively. The result is consistent with the disappearance of m_2^2G in Fig. 2. It has been shown that some modified nucleotides are resistant to RNase T_2 digestion, leading to a 2′,3′-cyclic intermediate and prolonged treatment of m_2^2Gp with RNase T_2 may cause some kind of partial degradation [26].

It should be noted that negative-ion frit-FAB mass spectra of dinucleotides and cyclic nucleotide (Figs. 3 and 4) contain an *m/z* 79 ion, presumably assigned to $[PO_3]^-$ derived from phosphate group in the molecule. The mass chromatogram of the *m/z* 79 ion was then obtained for the RNase T_2-AP digest (Fig. 2) in order to see whether there is any mass response for nucleotides. The observed four mass responses are probably related to nucleotides, which demonstrates the utility of this approach for general screening of nucleotides in tRNA digest, and adds the advantage of performing negative-ion detection.

3.3. Detection of Nm and NmpN′ in *P. occultum* tRNA

By using the method described above, an attempt was made at the detection and characterization of Nm and NmpN′ in tRNA of the extremely thermophilic organism *P. occultum*. Chromatographic separations of nucleosides from LC–MS analysis of the unfractionated tRNA hydrolysates with nuclease P_1-AP and RNase T_2-AP are shown in Figs. 5 and 6, respectively. The modified nucleosides including Nm were characterized by their relative retention times and the frit-FAB mass spectra [14], and were indicated in the LC–UV profile (Fig. 5). Several of the ribose-methylated nucleosides are structurally unusual in terms of also being

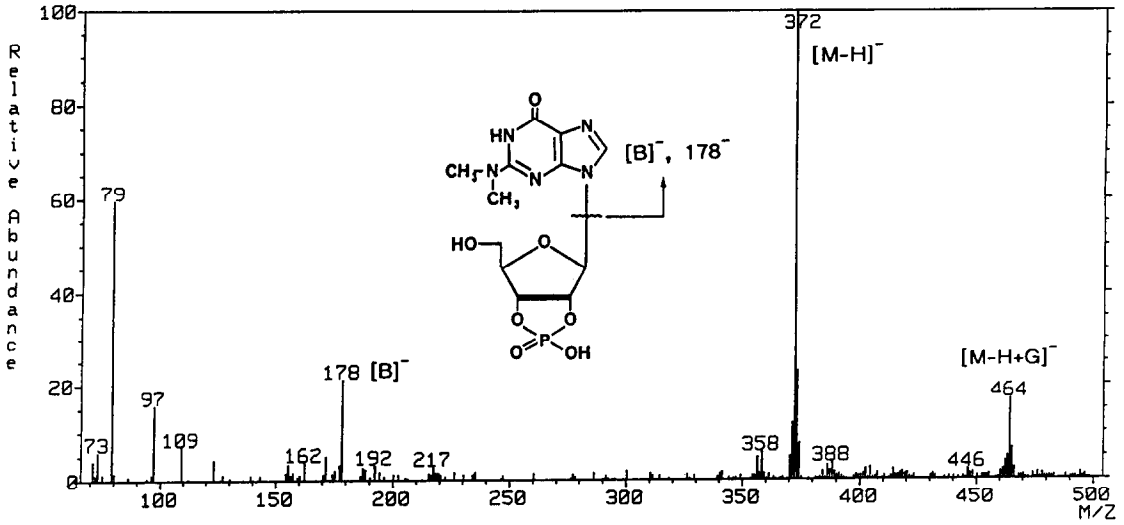

Fig. 4. Negative-ion frit-FAB mass spectrum of $m_2^2G>p$ eluting at 21.3 min in Fig. 2. The positive-ion frit-FAB mass spectrum also showed $[MH]^+$ and $[BH_2]^+$ ions with relative intensities of 100 and 93%, respectively.

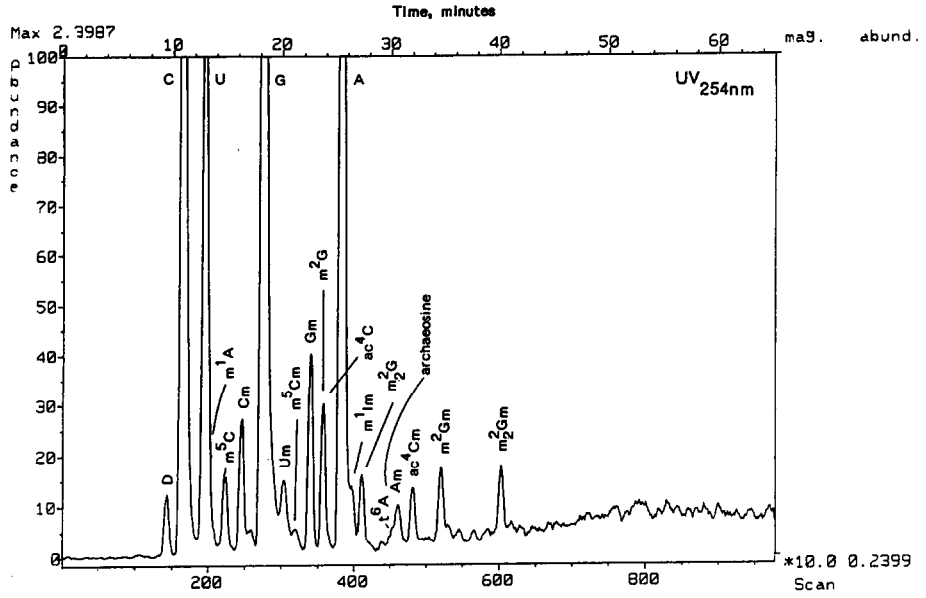

Fig. 5. Chromatographic separation of nucleosides from LC–MS analysis of enzymatic hydrolysate of 9 μg of unfractionated *P. occultum* tRNA following digestion with nuclease P_1, phosphodiesterase and alkaline phosphatase. Abbreviations: m^1A = 1-methyladenosine; Um = 2′-O-methyluridine; m^5Cm = 5,2′-O-dimethylcytidine; ac^4C = N^4-acetylcytidine; m^1Im = 1,2′-O-dimethylinosine; t^6A = N^6-threonylcarbamoyladenosine; Am = 2′-O-methyladenosine; ac^4Cm = N^4-acetyl-2′-O-methylcytidine; m^2Gm = N^2,2′-O-dimethylguanosine; m_2^2Gm = N^2,N^2,2′-O-trimethylguanosine; others as in Fig. 1.

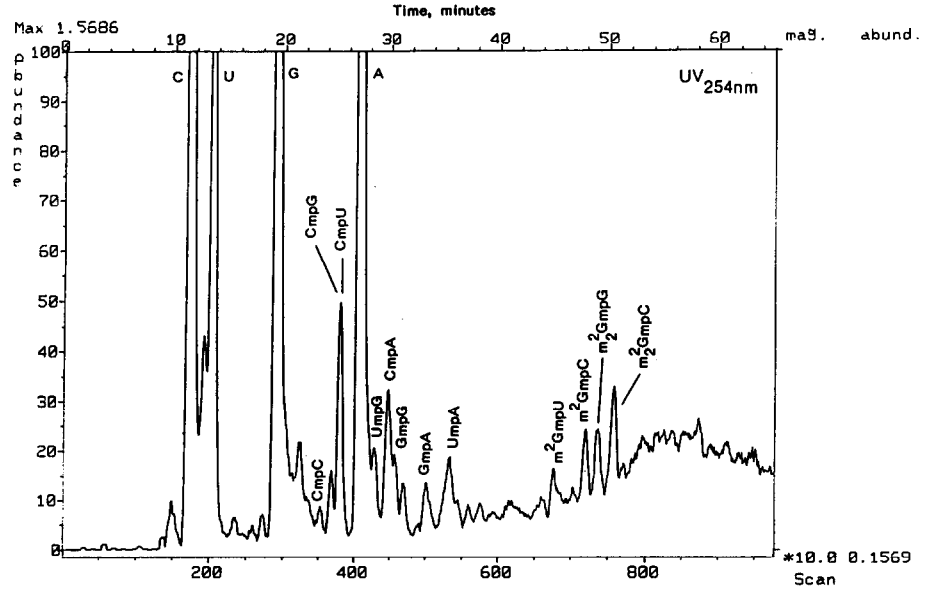

Fig. 6. Chromatographic separation of nucleotides from LC–MS analysis of enzymatic hydrolysate of 6 μg of unfractionated *P. occultum* tRNA following digestion with RNase T_2 and alkaline phosphatase.

modified in the base. These include m^5Cm, m^1Im, ac^4Cm, m^2Gm and m_2^2Gm, all identified by means of LC–thermospray MS [8], and are known to occur uniquely in archaeal tRNA. These Nm(s) should be formed in NmpN′ in the RNase T_2 digest. The assigned NmpN′ dinucleotides are indicated in Fig. 6 and the negative-ion frit-FAB mass spectral data are presented in Table 2. All the NmpN′ species, except for GmpA and UmpA, were detected in the positive ionization mode (data not shown).

In application to unfractionated tRNA such as in the present examination, the contents of individual modified nucleosides of interest are usually in the range 0.001–0.4% of the total nucleoside population of 40–50 different tRNAs [27]. Fig. 5 shows the entire nucleoside population in the total tRNA, and Fig. 6 shows the total distribution of 3′-nucleotide partners in a given population of NmpN′, so that less useful sequence information will generally result because of a significantly increased number of possible combinations of NmpN′ that resulted in the unfractionated tRNA [11]. However, certain post-transcriptional modifications are kingdom-specific and found at sequence-specific locations, so that we can survey specific components of potential interest to characterize this archaeon whose tRNA sequence is unknown. For example, Cm is present at positions 32, 34 and 56. The presence at position 56 is highly characteristic of archaeal tRNA, and there is a complete absence of modifications at this position in all currently known bacterial and eukaryal sequences [28]. m^1Im is likely to occur at position 57, which is deduced from the exclusive occurrence at the same position for the related nucleoside m^1I in the 27 published (every known) archaeal sequences [15]. Therefore, $Cm_{56}pm^1Im_{57}pN_{58}$ should be formed in the RNase T_2-AP digest. A comparative LC–MS analysis of *p. occultum* tRNA based on the thermospray interface strongly suggested the occurrence of $Cm_{56}pm^1Im_{57}pm^1A_{58}$ and $Cm_{56}pm^1Im_{57}pA_{58}$ [29]. A similar sequence $Cm_{56}pm^1I_{57}$ has frequently been found in archaea *Halobacterium volcanii* tRNA [30]. In the present examination, however, we failed to detect such a trinucleotide, whereas several dinucleotides CmpN (N = four major nucleosides) were observed instead. The non-detection of trinucleotides is due in part to the decreases sensitivity in FAB ionization with increased state of phosphorylation, and such phosphorylated components occur principally in minor tRNA isoaccepting species, which leads to difficulty in the detection of other di- or trinucleotide components including ac^4Cm and m^5Cm. Under the described conditions with 6 μg of the digest in the positive-ionization mode and 30 μg in the negative-ionization mode, they might be present at undetectable levels.

4. Conclusion

The results described above indicate that LC–frit-FAB-MS will be a valuable addition to the techniques available for studying post-transcriptional modification, particularly methylation at 2′-O-ribose in tRNA. The methylation can be recognized as Nm and NmpN′ in enzymatic digests with nuclease P_1 and RNase T_2, respectively, and their detection and characterization have been satisfactorily accomplished by a single LC–MS run with each digest even for unfractionated tRNA. Obviously, the potential value of the method with a frit-FAB interface lies in determining and sequencing oligonucleotides formed by appropriate nucleases. Further work is in progress on the direct measurement of oligonucleotide fragments by LC–frit-FAB-MS.

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