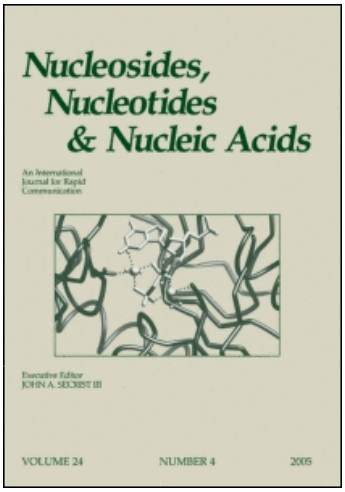

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Burgess, Kevin , Russell, David H. , Shitangkoon, Aroonsiri and Zhang, Alex J.(1996) 'MALDI AND FAB MASS SPECTROMETRY OF NUCLEOSIDE TRIPHOSPHATES: A COMPARATIVE STUDY', Nucleosides, Nucleotides and Nucleic Acids, 15: 11, 1719 — 1723
To link to this Article: DOI: 10.1080/07328319608002727
URL: http://dx.doi.org/10.1080/07328319608002727

NUCLEOSIDES & NUCLEOTIDES, 15(11&12), 1719-1723 (1996)

MALDI AND FAB MASS SPECTROMETRY OF NUCLEOSIDE TRIPHOSPHATES: A COMPARATIVE STUDY

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ABSTRACT: Comparison of MALDI and FAB mass spectra of dATP, dTTP, dCTP, and dGTP shows that the former technique gives clear molecular ions with minimal fragmentation whereas the latter gives more fragment ions and weaker parent peaks.

Nucleoside triphosphates are difficult to prepare in a high state of purity, and they are relatively unstable under ambient conditions. These problems are compounded by limitations on the methods available for their analyses. Proton and ^{31}P NMR spectra of nucleoside triphosphates, for instance, can be poorly resolved due to relatively slow exchange phenomena associated with the triphosphate counter ions. Mass spectrometric (MS) analyses of these important compound types therefore are of special significance. A method frequently employed for analyses of nucleoside triphosphates, and small oligonucleotides in general, is fast atom bombardment mass spectrometry (FAB-MS).[1-4] However, the published data indicate significant fragmentation occurs in FAB mass spectra of these compound types,[5] even if superior matrices are used,[6] and beneficial additives are included.[7] Recently, it has been shown that matrix assisted laser desorpsion ionization (MALDI) is superior to FAB with regard to

analyses of DNA and RNA.[8] Those observations led us to compare the performance of these two techniques for analyses of nucleoside triphosphates, and the results of that study are communicated here.

Commercially available (Sigma) samples of dATP, dTTP, dCTP, and dGTP were dissolved in 0.1 M triethylammonium acetate (pH 7) and purified by HPLC on a C18 column eluting with a 0.1 M triethylammonium acetate and acetonitrile gradient. After lyophilization, the purified triphosphates were redissolved to give solutions in 0.1 M triethylammonium acetate (20 mM for FAB, 200 μM for MALDI); this procedure gives volatile ammonium counter-ions in preference to sodium counter-ions.[9] The MALDI-TOF experiments were performed on a Voyager-Elite XL instrument (PerSeptive Biosystems, MS, USA) operated in the negative ion mode. In those experiments, 10 μL of freshly prepared 0.5 M ethanolic solution of 2,4,6-trihydroxyacetophenone (matrix) and 2 μL of the analyte solution were briefly mixed, then 0.5 μL of this solution was applied to the mass spectrometer autosampler plate and dried using a jet of cool air. The quantity of sample used in the MALDI experiments was about 10 pmol. In the FAB analyses, the analyte solution was applied to the probe using a diethanolamine/triethanolamine mixed matrix; the quantity of material used in these experiments was about 1 μmol.

Figure 1A and 1B, respectively, show the FAB and MALDI mass spectra for the samples of four nucleoside triphosphates. Two features complicated the FAB mass spectra. First, significant fragmentation was observed corresponding to loss of the heterocyclic base fragment $[M\text{-}BH]^-$. Other significant fragments in the spectra included those resulting from loss of $[H_2PO_3]^+$ and sometimes $[H_2PO_3 + H_2O]^+$ moieties from the triphosphate chain. Loss of base prevails over loss of phosphate, consistent with observations reported for FAB-MS studies of small oligonucleotides.[2,3] Second, all the molecular fragments were effectively "diluted" by corresponding ions associated with sodium cations. This effect was most noticeable in the spectrum of dGTP wherein only the mono-, di-, and tri-sodium adducts are observed, but not the pseudomolecular ($[M\text{-}H]^-$) ion peak. Sodium ions associated with psuedomolecular ions were not prevalent in the FAB spectrum of the matrix without analyte (not shown) so we suspect that occluded cations could be an intrinsic feature of the ionization technique when used in conjunction with these compound types.

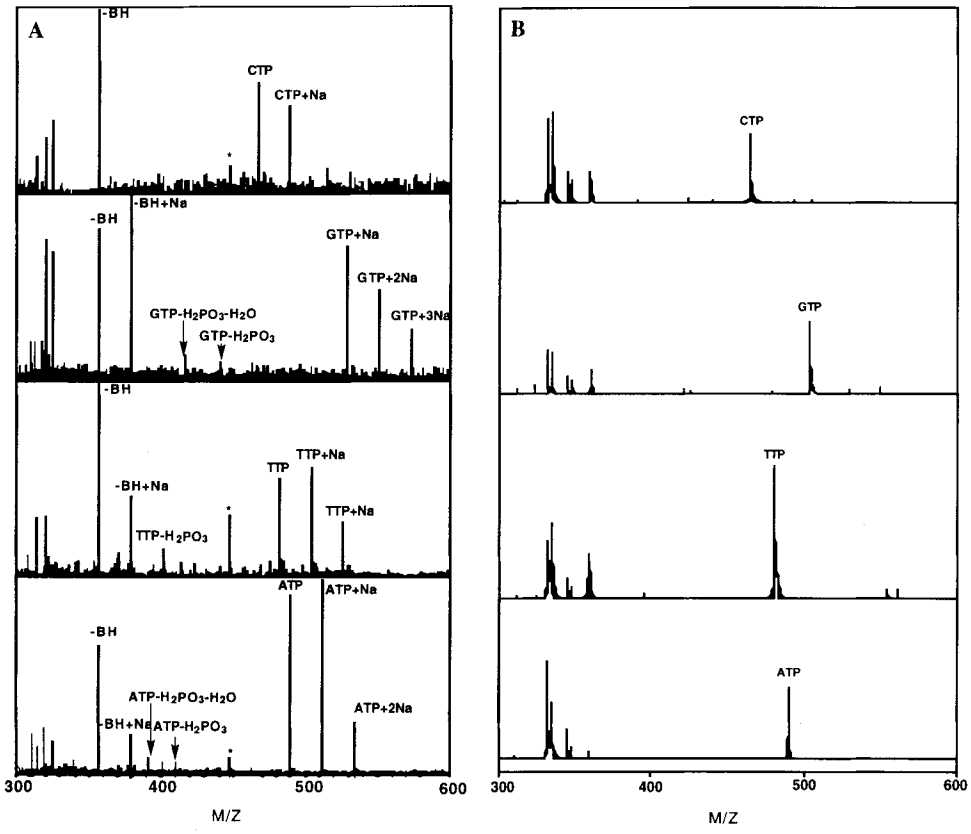

FIGURE 1. The denotation NTP used for the pseudomolecular ions is an abbreviation for $[dNTP-H]^-$, N = A, T, C, and G. The assignments of the form M+Na are an abbreviation for the more rigorous denotation, $[M-2H+Na]^-$. **A.** FAB MS spectra of triphosphates in negative ion mode. Peaks marked with an asterisk (*) are attributed to the matrix, and the peaks between 310 to 325 Da are tentatively assigned as matrix cluster peaks. **B.** MALDI MS spectra of triphosphates in negative ion mode. The peaks between 333 and 359 Da are matrix cluster ions.

In contrast to the FAB mass spectra, the MALDI mass spectra in Figure 1B show the pseudomolecular ion and matrix peaks to be the only significant signals. The mass spectra are relatively clean, being uncomplicated by fragmentation or sodium occlusion effects.

Essentially the same results were observed when FAB and MALDI mass spectra of ddATP, ddTTP, ddCTP, and ddGTP were compared. Once again, the data from the MALDI-MS analysis was remarkably free of ions other than those due to the complete molecule without sodium occlusion. Furthermore, throughout these studies, less sample was required to obtain the MALDI mass spectra due to the inherent greater sensitivity of this ionization technique in comparison with FAB. In general, we advocate the use of MALDI in mass spectrometric analyses of nucleoside triphosphates, unless spectra with fragmentation are preferred.

ACKNOWLEDGEMENTS

We would like to thank Dr Lloyd Sumner and Mr R. D. Edmondson for practical help and useful comments. Financial support for this research was provided by The Texas Advanced Technology Program, The U.S. Department of Energy, and The Robert A Welch Foundation. K.B. thanks the NIH for a Research Career Development Award and the Alfred P. Sloan Foundation for a fellowship. NMR facilities were provided by the NSF via the chemical instrumentation program. The MALDI-TOF instrument was purchased using funds provided by TAMU.

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Received June 10, 1996
Accepted July 31, 1996