Enhancement of Molecular Ion Intensity of Nucleic Acids in Negative Ion Fast-atom Bombardment Mass Spectroscopy

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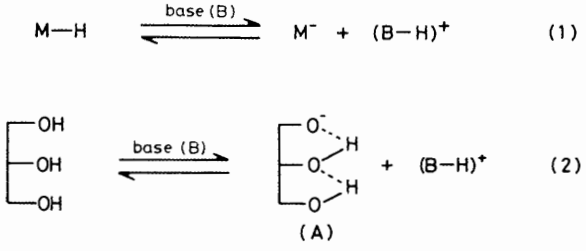
The addition of various organic bases to the glycerol matrix gives a considerable enhancement of the negative molecular ions of nucleic acids in fast-atom bombardment mass spectrometry.

Fast-atom bombardment mass spectrometry (f.a.b.-m.s.) is a useful technique for molecular weight and structure determination for large, nonvolatile compounds, particularly for molecules of biological interest.[1] The matrix is of great importance in f.a.b.-m.s. since it should preferably aid in ionizing the sample molecules and should also promote rapid diffusion of sample molecules to the near-surface region.[2—8] Glycerol has generally been used as a matrix, since it often facilitates the formation of molecular ions in both the positive and the negative ion detection modes, and also usually results in stable and long-lasting currents of molecular ions. It has been shown using glycine as a substrate by secondary ion mass spectrometry that the sample solution when acidified with nitric acid gives a maximum of $(M + H)^+$ emission while a maximum of $(M - H)^-$ emission could be observed for a sample solution made alkaline with sodium hydroxide.[9]



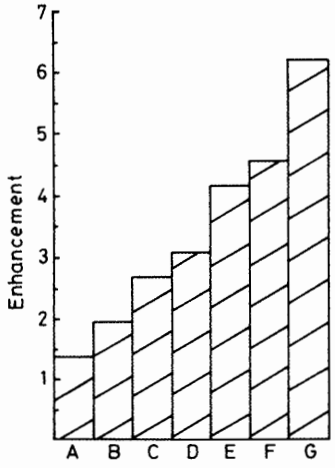


Figure 1. Enhancement of the sum of the intensities of the molecular/pseudomolecular ions at *m/z* 506, 528, and 550 caused by the addition of different bases to the glycerol matrix as compared with the glycerol-only matrix. In all experiments the ATP concentration was 20 mM, and the optimized amount of base was added: A, Pyridine added to give a 1.6 M solution. B, Di-isopropylethylamine added to give a 0.27 M solution. C, Triethylamine added to give a 0.34 M solution. D, Morpholine added to give a 0.55 M solution. E, Piperidine added to give a 0.25 M solution. F, DBN added to give a 0.20 M solution. G, DBU added to give a 0.16 M solution.

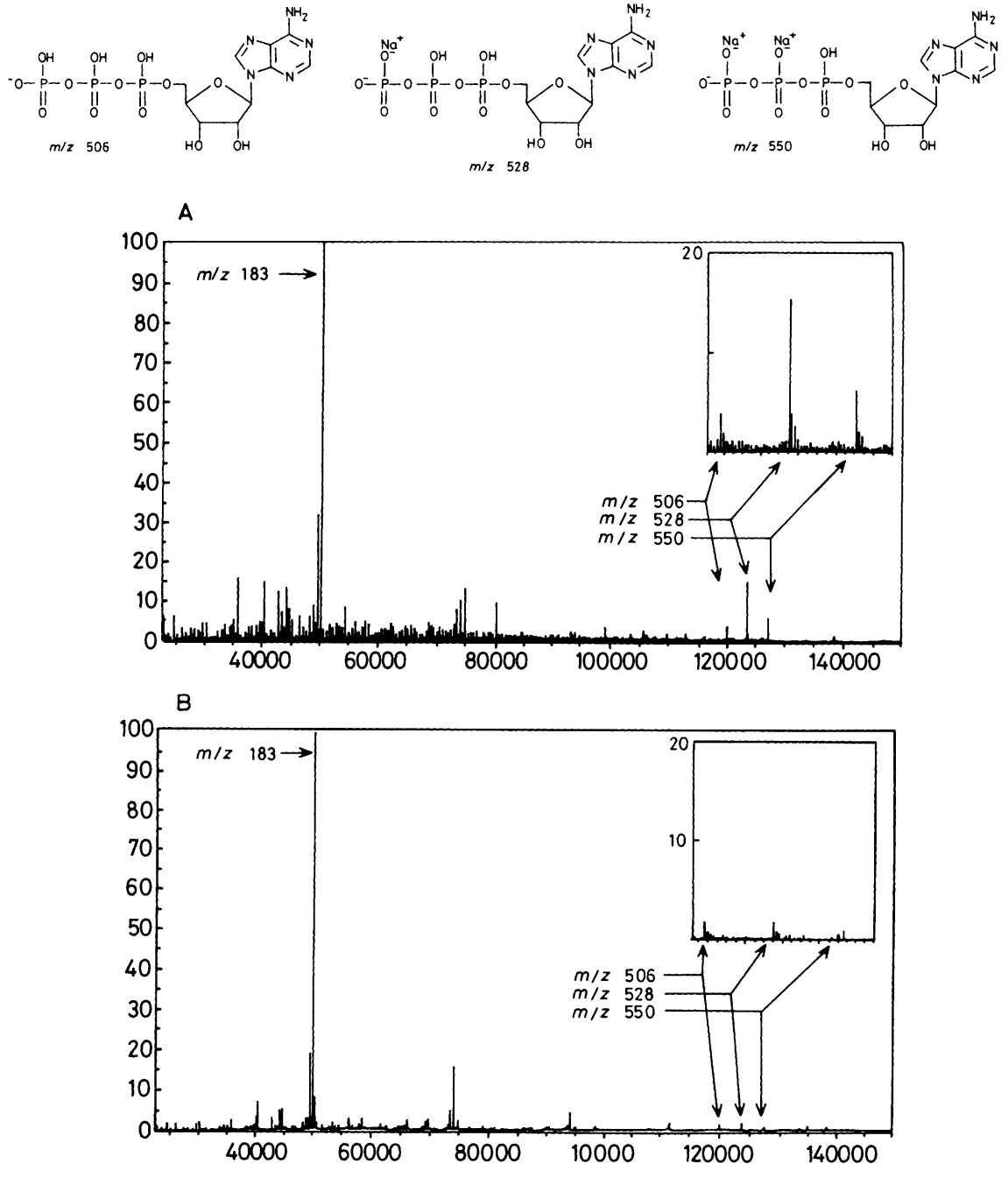


Figure 2. Negative f.a.b.-m.s. of ATP-disodium salt (20 mM); (A) with DBU (0.16 M) in glycerol; (B) in neat glycerol. The peak at *m/z* 183 orginates from glycerol.

However, to our knowledge, there have been no reports on the enhancement of negative molecular ions of nucleic acids upon addition of a base to a neutral matrix. Addition of alkali metal salts[2] or crown ethers can also enhance the intensity of molecular or quasimolecular ions.[10,11]

The addition of a particular organic base to the glycerol matrix containing a substrate with a dissociable proton will influence, depending upon its strength (pK_a), the formation of anionic species (M^-) owing to the shift of the equilibrium to the right as shown in equation (1). The equilibrium concentration of M^- in the matrix should be reflected in mass spectroscopic peak heights[3,11,12] in the negative mode. Clearly, the same base should also be able to ionize glycerol which is used as the matrix, as shown in equation (2). However, the relative abundance of M^- in equation (1) and species (**A**) in equation (2) should be dependent both upon the pK_a of the substrate (M–H, in equation 1) and the added base. This should also be true in a high-vacuum state in a mass spectrometer. The following factors are expected to influence the course of reactions in equations (1) and (2): (i) the

physicochemical properties (density, b.p., and viscosity) of the particular organic base; (ii) the nature of its solvation with a particular matrix; and (iii) its effect upon the substrate–matrix solvation. Furthermore, the stoicheiometry of the base will be quite difficult to control because of the loss of volatile organic base into the vacuum pumps. With these reservations, we have examined the effect of different organic bases and report that the intensity of negative molecular ion can be enhanced considerably for hydrophilic, polar, and nonvolatile nucleic acid molecules by adding an organic base to the glycerol matrix.

Figure 1 shows the effect of different organic bases variously enhancing the negative molecular ions of adenosine-5′-triphosphate disodium salt (ATP-Na_2) (the figure shown in the graph is the enhancement of the sum of molecular ions of ATP-Na_2 at *m/z* 506, 528, and 550) which shows that different organic bases influence the enhancement of the intensity of the negative ions quite differently. The concentration of a particular base added to the glycerol matrix was optimized in order to compensate for its loss in the pump of the mass spectrometer. Table 1 shows some of the physicochemical properties (density, b.p., and pK_a) of the different organic bases that were used in the glycerol matrix, containing ATP-Na_2. Despite the fact that DBU (1,8-diazabicyclo[5.4.0]-undec-7-ene) and DBN (1,5-diazabicyclo[4.3.0]non-5-ene) have comparable pK_a values (*ca.* 13) it is DBU that gives the best result for the enhancement of the negative molecular ions. This is probably due to the differences in their boiling points. A comparison of morpholine (b.p. 129 °C at 760 mmHg, pK_a 8.33), piperidine (b.p. 106 °C at 760 mmHg, pK_a 11.12), and triethylamine (b.p. 89 °C at 760 mmHg, pK_a 11.01) with respect to their effects on the enhancement of negative molecular ions clearly shows that piperidine is by far a better base than morpholine and triethylamine, showing that higher pK_a of the base is just one of the favourable factors for the enhancement of the negative molecular ions.

The optimum concentration of DBU is 0.16 M in glycerol in order to obtain the best result† for the enhancement of negative molecular ions of ATP-Na_2, as shown in Figure 2. An increase of concentration of DBU above 0.16 M in the glycerol matrix actually causes a reduction of the intensity of negative molecular ions.

Other experiments have shown that at least 4 molar equiv. of DBU to ATP-Na_2 salt were needed for the best result for the enhancement of negative molecular ions. Under these conditions, the molar ratio of DBU to glycerol is *ca.* 0.01 equivalent. However, it is difficult to explain the reason for the reduction of the intensity of negative molecular ions of ATP-Na_2 upon increase of the concentration of DBU.

Table 1.

Base	*d*	B.p./°C (*p*/mmHg)	pK_a
Pyridine	0.978	115 (760)	5.23
Di-isopropyl-ethylamine	0.742	127 (760)	*ca.* 10.6
Triethylamine	0.726	89 (760)	11.01
Morpholine	0.999	129 (760)	8.33
Piperidine	0.861	106 (760)	11.12
DBN	1.04	95—98 (7.5)	*ca.* 13
DBU	1.018	80—83 (0.6)	*ca.* 13

In order to establish the applicability of addition of DBU to the glycerol matrix for larger molecules such as synthetic oligonucleotides we prepared a solution of 0.16 M DBU in glycerol† and used this as a matrix and compared the spectra with those obtained with the neat glycerol matrix. We observed a seven-fold enhancement of the molecular ion intensity for the dimer d(ApT),[13] a 2.2-fold increase with the branched chain trinucleoside diphosphate[14] $A^{2'p5'G}_{3'p5'C}$, and a 5.2-fold increase with the pentaribonucleoside tetraphosphate, r(ApGpUpCpC).[15]

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† All experiments were performed with a JEOL DX-303 mass spectrometer connected to a JEOL DA-5000 data system. For all spectra the accelerating voltage was set to 3 kV except for the pentamer spectra where 2.5 kV accelerating voltage was used; resolution was set to 1500 and the target was bombarded wth xenon atoms at 6 keV. ATP-Na_2 stock solutions were prepared in glycerol with minimum amounts of water for solubility reasons. The concentrations of ATP in these stock solutions were 20 and 40 mM. Stock solutions of 20 μg sample per μl of water for d(ApT) and r[A(pC)pG] were prepared. For the pentamer a solution of 52 μg of sample per μl of water was prepared. 1 μl of sample solution was mixed with either 2 μl pure glycerol or with 2 μl of glycerol solution containing 0.16 M DBU. In the pentamer case 0.5 μl sample solution was mixed with 4 μl of matrix.

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