

Estimation of the Proton Affinity Values of Fifteen Matrix-Assisted Laser Desorption/Ionization Matrices under Electrospray Ionization Conditions Using the Kinetic Method

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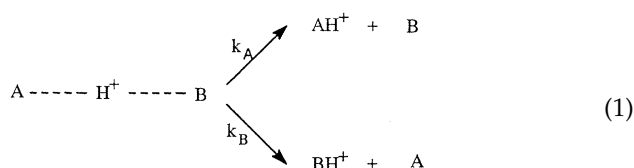
The kinetic method, which is known to be simple, fast and precise, is used for the measurement of proton affinity values of fifteen selected matrix-assisted laser desorption/ionization matrices under electrospray ionization conditions. The stabilization of $[M - H]^-/[M + \text{Matrix} - H]^-$ ions for β -cyclodextrin in negative ion mode has been rationalized based on the proton affinity values of the matrices. (J Am Soc Mass Spectrom 2004, 15, 431–435) © 2004 American Society for Mass Spectrometry

The most exciting innovations in the last decade for the analysis of non-volatile, high molecular weight compounds is the development of new mass spectral techniques like matrix-assisted laser desorption/ionization (MALDI) [1–3] and electrospray ionization (ESI) [4]. MALDI has been successfully applied for the analysis of peptides and proteins [5–11], oligonucleotides and nucleic acids [12, 13], carbohydrates [14–16], and synthetic polymers [17–21]. Despite its wide range of applications and many fundamental studies [22–30], there is a need for better understanding of the MALDI process, to improve ion yields, to gain access to new classes of compounds, and to provide rational guidelines for matrix selection, which play crucial roles in sample analysis. In the discussions of the ionization mechanism, proton-transfer reactions have a very prominent role. The protonated analyte is presumably produced by gas-phase reactions between matrix ions and analyte molecules. Thermochemical data like gas phase basicities (GB) and proton affinities (PA) of the matrices may contribute to better understanding of the MALDI processes.

To date, very few studies have been carried out for the measurement of GB and PA of MALDI matrices [31–34]. The PA values that have been reported for MALDI matrices are inconsistent with one another because of intrinsic errors in the different methods, due to sampling of ions of different internal energy, and because of the use of different ionization tech-

niques in these studies. So far, ESI has not been used for the estimation of PA values of MALDI matrices. In the present study, we estimated PA values of fifteen MALDI matrices under electrospray ionization conditions, which is easier and more broadly applicable than fast atom bombardment or chemical ionization techniques, using the kinetic method [35, 36].

Estimation of PA values by the kinetic method involves measurement of peak height ratios of the protonated monomers of the compound with unknown PA and that of the reference base with known PA values, generated from the collision induced dissociation of the corresponding proton-bound heterodimers. According to the kinetic method, the ratio of rate constants (k_A and k_B) for the competitive dissociation of a cluster ion comprised of the compound of interest and a reference compound, eq 1, can be expressed as eq 2 [37, 38].



$$\ln \frac{k_A}{k_B} = \ln \frac{[\text{AH}]^+}{[\text{BH}]^+} = \frac{PA_A - PA_B}{RT_{\text{eff}}} \quad (2)$$

where $[\text{AH}]^+$ and $[\text{BH}]^+$ are the abundances of the

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reference base Compound A and the Compound B with unknown proton affinity value, respectively. PA_A and PA_B are the proton affinity values of the respective Compounds A and B. R is the gas constant, and T_{eff} is the effective temperature of the activated dimer ion. This is a simple expression of the kinetic method where entropy effects are assumed to be negligible. The method was extended later by different groups to incorporate entropy effects by carrying out the experiments at various collision energies in cases where entropy contributions cannot be ignored [39–41]. It is also known that if the apparent free energy values [42, 43], which we obtain at different collision energies by the kinetic method do not change significantly with collision energy, there is no need to proceed with entropy corrections, and the average of the apparent free energies may be reported as the proton affinity [44].

Experimental

Materials

The MALDI matrices, 4-hydroxy- α -cyanocinnamic acid (4HCCA), 2,5-dihydroxy benzoic acid (gentisic acid, GA), 5-methoxysalicylic acid (MSA), *trans*-3, 5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA), 1,8,9-anthracenetriol (dithranol, DT), 2,4,6-trihydroxyacetophenone (THAP), *trans*-3-indoleacrylic acid (IAA), 3-hydroxypicolinic acid (HPA), 2-mercaptobenzothiazole (MBT), 6-mercaptapurine (MP), 2-(4-hydroxyphenylazo)benzoic acid (HABA), and nor-harmaline (NH) were obtained from Sigma-Aldrich Chemie GmbH, Sigma-Aldrich Chemicals, Steinheim, Germany, and Sigma (St. Louis, MO). 5-Ethyl-2-mercaptothiazole (EMT) was synthesized in our laboratory [45]. Two compounds under consideration as matrices in our laboratory (5-amino-2-mercapto-1,3,4-thiadiazole (AMT) from Sigma, and its acetylated analog, 5-Acetylamino-2-mercapto-1,3,4-thiadiazole (AAMT) from Nakoda Chemicals Ltd, Hyderabad, India, were also analyzed. All the compounds that are used as reference bases (Table 1) in these experiments, acetonitrile, water, trifluoroacetic acid (TFA), and formic acid (FA) were obtained from SD Fine Chemicals, Mumbai, India and used without further purification.

Mass Spectrometry

All the experiments for the determination of PA values by the kinetic method were performed on a Quattro LC mass spectrometer (Micromass, Manchester, UK) with Z-spray source using a Masslynx version 3.2 software. Sample solutions of 1 mM concentration were introduced into the ESI source using a direct infusion pump (Harvard Apparatus, Manchester, UK) at a rate of 5 $\mu\text{l}/\text{min}$. Capillary voltage was kept between 3.5–3.8 kV during the experiments and the cone voltage was kept at 7 V. Solutions of MALDI matrix and reference compound were made in 1:1 acetonitrile:0.01% FA-

Table 1. Proton affinity values of reference bases^a [46]

S. No	Reference base	Proton affinity (kcal/mol)
1	Benzaldehyde	199.3
2	γ -Butyrolactone	200.8
3	3-Cyanoaniline	201.3
4	Ammonia	204.0
5	Acetophenone	205.8
6	Acetamide	206.4
7	4-Nitroaniline	207.0
8	3-Chloroaniline	207.5
9	4-Fluoroaniline	208.3
10	Aniline	210.9
11	2-Toluidine	212.9
12	3-Toluidine	214.3
13	4-Toluidine	214.3
14	4-Anisidine	215.2
15	2-Bromopyridine	216.3
16	3-Bromopyridine	217.5
17	4-Bromopyridine	219.4
18	3-Hydroxypyridine	222.2
19	Pyridine	222.3
20	2-Methylpyridine	226.8
21	3-Aminopyridine	228.1
22	Di-n-butylamine	231.5
23	Di-i-propylamine	232.3
24	4-Aminopyridine	234.2
25	Triethylamine	234.7

^aThe uncertainty of 1.9 kcal/mol has been assigned by Lias et al [46].

water (vol/vol) and the proton-bound dimer ions were generated using nitrogen as nebulizing and desolvation gas. The precursor ion of interest was mass selected by the first quadrupole analyzer (MS1) and the product ion spectra were obtained by scanning the second quadrupole analyzer (MS2). The gas cell pressure was kept at 2 e-4 mBar to maintain single collision conditions. The experimental time window is of 10–100 ms for the detection of dissociation. Experiments were carried out at laboratory frame collision energy of 2, 5, 7, and 10 eV using argon as the collision gas. The results reported here were obtained from an average of 10 repeated experiments carried out successively and 20 scans were combined from each experiment. The experiments were repeated on two different days to check the reproducibility, and the measured PA values had ± 0.2 kcal/mol error.

For MALDI analysis, matrix solutions (4HCCA, GA, SA, MBT, EMT, and NH) of 10 mg/ml concentrations were prepared in acetonitrile:water (70:30 vol/vol). Dimethylsulfoxide (DMSO), 5% by volume was also added to make a clear solution of MBT and the final solvent medium is 67.5:27.5:5 (acetonitrile:water:DMSO vol/vol). Bovine insulin (5 pmol/ μl) was prepared in 0.1% TFA-water, while β -cyclodextrin of the same concentration was prepared in water without TFA. MALDI mass spectra were obtained using Kompact MALDI SEQ laser desorption time-of-flight mass spectrometer (Kratos Analytical, Manchester, UK) equipped with a pulsed nitrogen laser (λ_{max} : 337 nm, pulse width 3 ns).

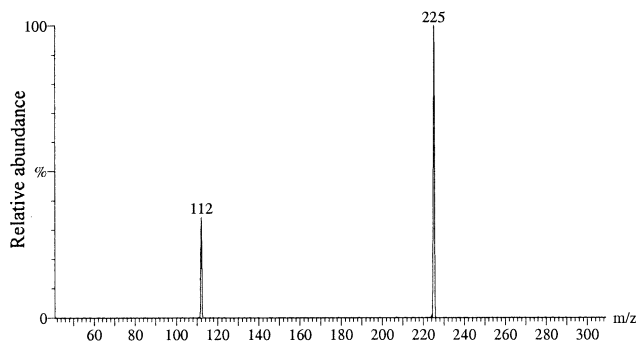


Figure 1. Daughter ion spectrum of the proton-bound dimers of sinapinic acid and 4-fluoroaniline at 7 eV of collision energy under ESI conditions.

Ions were accelerated into the analyzer at a voltage of 20 kV. To make an analyte-matrix deposit, typically 0.3 μl of the analyte solution was placed on a stainless steel sample slide (Kratos instrument part no. D 3665 TA) and the solvent was removed by forced air evaporation at room temperature. Subsequently, 0.3 μl of the matrix solution was placed above the sample spot covering the analyte film, and again the solvent was removed by forced air evaporation at room temperature. Both positive and negative ion spectra were recorded in linear mode setting a laser power of 100–110. All the mass spectra shown in this paper were accumulated over fifty laser shots across the sample spot. Data processing was carried out using Kompact version 1.2.2 software.

Results and Discussion

In the present study, mostly anilines and pyridines have been used as reference bases to determine the PA values of MALDI matrices. The reference bases used for each MALDI matrix and their PA values are given in Table 1 [46]. Proton-bound heterodimers of a pair of matrix compound and that of reference base were generated under ESI conditions. The daughter ion spectrum of the proton-bound heterodimer showed two prominent peaks due to the corresponding protonated monomers, other fragments being negligible (Figure 1). The peak heights of the protonated species were measured and processed for PA measurements by plotting the natural logarithm of the abundance ratio of protonated monomer ions ($\ln[\text{AH}]^+ / [\text{BH}]^+$) against the PA values of the reference bases (Figure 2). From the linear correlations of these plots, the effective temperature (T_{eff}) and apparent free energy values were determined from the slope and intercept, respectively. The apparent PA value of the unknown was obtained by extrapolating the $\ln[\text{AH}]^+ / [\text{BH}]^+$ value to zero (x-intercept).

The apparent free energy values at different collision energies and the average effective temperature obtained in this study are given in Table 2. It can be seen that the apparent free energy values do not change with the change in collision energy. Hence, we report the aver-

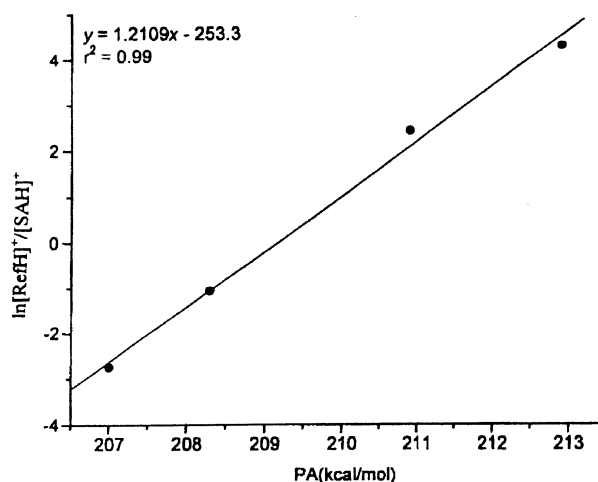


Figure 2. Plot of the logarithms of the ratios of product ions from dissociation of proton-bound dimers containing reference bases and MALDI matrix compound (SA) against the PA of reference base compounds at collision energy 7eV.

age of the values obtained at different collision energies as the PA values of the compounds studied [44]. The Table 2 also contains PA values reported in the literature for some of the matrices. It is observed that there are discrepancies in the PA values, even in the order of PA of MALDI matrices among the studies reported till date. In the present study, we used ESI technique which is a soft ionization technique like MALDI, and also, we followed the kinetic method which is simple, fast and even sensitive to small differences as little as 0.1 kcal/mol in the thermochemical quantity being determined for PA measurements [37]. It has been observed that the values for the common matrices (4HCCA, GA, and SA) are in good agreement with the values reported by Jorgensen et al. [31] and Nelson et al. [33]. The PA values for most of the other matrices are being reported for the first time.

Mechanistic Implications

Traditionally, for the analysis of carbohydrates, GA is used as a matrix of choice in positive ion mode [14, 16]. In due course, various mercaptobenzothiazoles have also been used for the analysis of carbohydrates [47]. Various β -carboline alkaloids were used as matrices for carbohydrate analysis in both positive and negative modes [48]. Among them, NH is reported to be more efficient. Recently, EMT has also been used both in positive and negative modes for the analysis of β -cyclodextrin in our laboratory [45].

To study the stabilization of carbohydrate ions in the negative mode, we carried out the analysis of β -cyclodextrin using four different matrices of varying proton affinities (Table 3). The matrices selected for this study are GA, MBT, EMT, and NH. With GA (204.4 kcal/mol), which is having the lowest PA among these, $[\text{M} - \text{H}]^-$ ion is not observed at all. Instead, it shows matrix

Table 2. PA values (kcal/mol) of MALDI matrices given in the present study and comparison of the same with literature values

Matrix compound	Reference bases used	Present study				Average Proton affinity ^a	Jorgensen et al. [31]	Burton et al. [32]	Steenvoorden et al. [34]	Nelson et al. [33]	
		Apparent free energy at collision energy (lab frame)									
		2 eV	5 eV	7 eV	10 eV						
4HCCA	1-4	201.5	201.0	200.9	200.6	201.0 ± 0.27 (±0.64)	817	201.0	183.0	223.0	203.0
GA	2-6	204.7	204.3	204.3	204.3	204.4 ± 0.17 (±0.36)	528	-	204.0	204.0	202.9
MSA	3-5,8	205.3	205.2	205.2	205.1	205.2 ± 0.52 (±1.22)	399	-	-	-	-
SA	7,9-11	209.3	209.2	209.2	209.2	209.2 ± 0.38 (±0.89)	427	212.0	204.0	214.0	210.0
DT	10,12-14	211.5	211.4	211.6	211.6	211.5 ± 0.77 (±1.81)	507	-	209.0	-	-
AMT	10-12,14	213.1	213.0	212.9	213.0	213.0 ± 0.31 (±0.73)	515	-	-	-	-
THAP	10-12,14	213.5	213.4	213.2	213.0	213.3 ± 0.65 (±1.53)	576	-	-	-	210.8
IAA	10-12,14	214.0	213.6	213.3	212.9	213.5 ± 0.26 (±0.61)	772	-	215.0	-	-
HPA	9-11,14	214.9	214.7	214.5	214.2	214.6 ± 0.33 (±0.78)	714	214.0	-	-	214.5
MBT	10-12,14	214.9	214.9	214.9	214.9	214.9 ± 0.23 (±0.54)	655	-	-	-	-
AAMT	10-12,14	215.5	215.7	215.8	215.8	215.7 ± 0.14 (±0.33)	619	-	-	-	-
EMT	15-19	218.1	218.0	217.9	217.9	218.0 ± 0.17 (±0.36)	432	-	-	-	-
MP	15-19	219.6	219.5	219.5	219.5	219.5 ± 0.19 (±0.41)	402	-	-	-	-
HABA	18-21	227.3	226.9	226.7	226.6	226.9 ± 0.26 (±0.61)	556	225.0	183.0	-	-
NH	22-25	233.1	233.1	233.0	232.8	233.0 ± 0.44 (±1.03)	395	-	-	-	-

^a±Average of standard deviation at different collision energies. Values in parentheses are uncertainties at 90% confidence limits [49].

adduct ion $[M + GA - H]^-$. Whereas, in case of analysis with NH (233.0 kcal/mol), which is having the highest proton affinity value among the MALDI matrices studied, $[M - H]^-$ ion is the only peak observed. With MBT (214.9 kcal/mol), peaks for both $[M - H]^-$ and $[M + MBT - H]^-$ ions were observed, $[M + MBT - H]^-$ ion being relatively more abundant than $[M - H]^-$ ion. When EMT (218.0 kcal/mol) is being used, $[M - H]^-$ ion is dominant. In addition, thiocyanate ion $[SCN]^-$, a fragment from EMT on laser irradiation adds to the substrate and gives rise to $[M + 58]^-$ ion [45]. From these observations, it can be explained that the PA of matrices play a vital role in stabilizing the $[M - H]^-$ ion of the analyte molecule and in the formation of the adducts with matrix or matrix fragments. A detailed study on effect of various MALDI matrices in stabilizing the anionic adducts under ESI and MALDI conditions for β -cyclodextrin and other linear and cyclic oligosaccharides is under progress in our laboratory.

Table 3. Percent relative abundance of various ionic species obtained for β -cyclodextrin in negative ion mode with four different matrices

Matrix compound ^a	% Relative abundance		
	$[M - H]^-$	$[M + \text{matrix} - H]^-$	$[M + (\text{matrix})_2 - H]^-$
GA (204.4)	-	100	4
MBT (214.9)	45	100	-
EMT (218.0)	100	-	-
NH (233.0)	100	-	-

^aValues in parentheses are the respective PA values in kcal/mol.

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