International Journal of Mass Spectrometry 300 (2011) 39–43

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Effect of esterification on MALDI–MS detection sensitivity for amino acids

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ARTICLE INFO

Article history:
Received 20 October 2010
Received in revised form 6 December 2010
Accepted 6 December 2010
Available online 13 December 2010

Keywords:
MALDI
Amino acid
Amino acid methyl ester
Protonation
CHCA

ABSTRACT

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI–MS) spectra for methyl esters H–X–OMe of 11 amino acid X were measured at various analyte/matrix mixing ratios using α-cyano-4-hydroxycinnamic acid (CHCA) matrix. For each amino acid, the effect of esterification on MALDI signals was examined by comparing the signal intensity ratio $(H–X–OMe)H^+/(CHCA)H^+$ for the ester H–X–OMe with the comparable ratio $XH^+/(CHCA)H^+$ for the corresponding amino acid X. For all 11 amino acids, the ratios for the esters are in line with a thermal equilibrium model [M. Tsuge, K. Hoshina, Investigation of protonation efficiency for amino acids in matrix-assisted laser desorption/ionization, Bull. Chem. Soc. Jpn. 83 (2010), 1188–1192.]. For 9 of the amino acids – Ala, Arg, Gly, Ile, Leu, Phe, Ser, Trp, and Val – the ratio for the amino acid agrees with the ratio for the ester, within the standard error. For the other 2 amino acids – His and Lys – the ratio for the amino acid is exceptionally smaller than the ratio for the ester, indicating that the effect of esterification is significant. This compensation by esterification suggests that for His/CHCA and Lys/CHCA systems, the coexistence of a carboxyl group and a basic side chain are responsible for suppression in the formation of $HisH^+$ and $LysH^+$, possibly by reducing the amount of desorbed analyte and/or decreasing the effective gas-phase basicity (GB) in the MALDI plume.

1. Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI–MS) allows highly sensitive detection of thermally labile samples such as biomolecules on a subfemtomole level. The soft-ionization technique involved has been applied to a variety of analytical methods such as peptide mass fingerprinting [1], proteomics [2], and polymer analysis [3]. However, although MALDI–MS is highly sensitive, it remains less than optimal for quantitative analysis owing to the complexity of protonated ion formation in the gas phase by laser desorption from the condensed phase.

In MALDI–MS spectra measured in positive mode, protonated ions XH^+ of analyte X are detected as the principal peak of the analyte and are commonly used for determining the molecular weight. The proton-transfer-reaction

$$MH^+ + X \leftrightarrows M + XH^+ \quad (1)$$

in the MALDI plume after laser desorption, where M denotes a matrix molecule and MH^+ its protonated ion generated in the desorption process, is thought to be a dominant process for XH^+ formation [4–7]. When this reaction reaches thermal equilibrium, the yield of XH^+ should correlate with the gas-phase basicity (GB) of the analyte X. Kinsel et al. demonstrated that the yield of XH^+ relative to $(MH^+ + M^+)$ in MALDI spectra increases with increasing amino acid GB for five amino acids – Ala, Gly, Ile, Phe, and Val – measured using α-cyano-4-hydroxycinnamic acid (CHCA) matrix [8].

Recently, we confirmed this model quantitatively by interpreting the XH^+ signal intensities in MALDI spectra for 17 natural amino acids measured using CHCA and 2,5-dihydroxybenzoic acid (DHB) matrices [9]. At the same time, we found that the three basic amino acids, Arg, His, and Lys, exhibit one- or two-order smaller XH^+ signal intensities, deviating from the model. Low protonation efficiency in MALDI–MS detection for a Lys/CHCA system has also been reported by Nishikaze and Takayama [10]. We considered that one possible cause of this deviation might be conformer-dependent effective GB. The GB of the basic amino acids is determined by the basicity of their side chains, and so could be affected by conformation changes such as by intramolecular hydrogen bonding.

In this study, we investigated the XH^+ formation efficiency of esterified amino acids to determine whether the presence and intramolecular interactions of carboxyl groups influence the properties of basic amino acids in MALDI processes. We measured the MALDI spectra for methyl esters H–X–OMe of 11 amino acids (hereafter abbreviated XOMe), where X = Ala, Arg, Gly, His, Ile, Leu, Lys, Phe, Ser, Trp, and Val, using CHCA matrix at various mixing ratios ranging from 1×10^{-4} to 1. We then compared the proto-

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doi:10.1016/j.ijms.2010.12.001

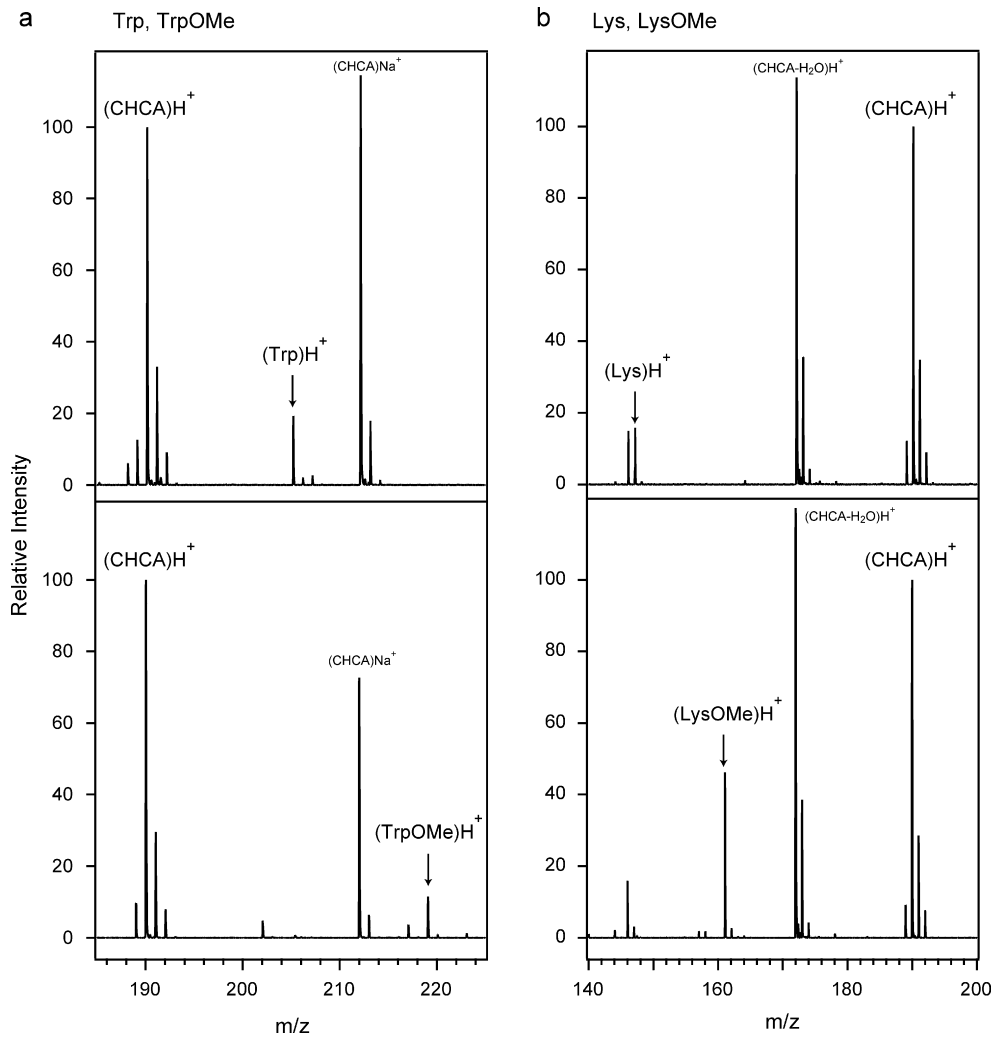

Fig. 1. MALDI–MS spectra of amino acid X and corresponding ester XOMe samples (analyte/matrix mixing ratio [X]/[CHCA] = [XOMe]/[CHCA] = 2×10^{-3}): (a) X = Trp; (b) X = Lys. Signal intensities for $(CHCA)H^+$ are scaled to 100.

nation efficiencies for the methyl esters, represented by the ratio $(XOMe)H^+/MH^+$, with those for the corresponding amino acids, represented by the ratio XH^+/MH^+.

2. Experimental

2.1. Materials and sample preparation

MALDI matrix (CHCA) and 11 amino acids (Ala, Arg, Gly, His, Ile, Leu, Lys, Phe, Ser, Trp, and Val) were purchased from Wako Pure Chemicals. Methyl ester hydrochlorides of the amino acids (AlaOMe·HCl, GlyOMe·HCl, HisOMe·2HCl, IleOMe·HCl, LeuOMe·HCl, LysOMe·2HCl, PheOMe·HCl, SerOMe·HCl, TrpOMe·HCl, and ValOMe·HCl) were purchased from Watanabe Chemical. Solvents (ultrapure water, acetonitrile, and trifluoroacetic acid) were also purchased from Wako Pure Chemicals. In place of ArgOMe, we used arginine d_3-methyl ester dihydrochloride ($ArgOCD_3 \cdot 2HCl$), which we synthesized employing a literature procedure [11], because for $ArgOCH_3 \cdot 2HCl$, a signal of $(ArgOCH_3)H^+$ overlaps with that of the radical cation $CHCA^+$ at $m/z = 189$.

MALDI samples were prepared by the conventional dried-droplet method [12]. In brief, 1 μL of analyte aqueous solution (5 nmol/mL–50 μmol/mL) and 1 μL of CHCA matrix solution (50 μmol/mL in 1:1, v/v water/acetonitrile with 0.1% trifluoroacetic acid) were deposited on a ground steel target plate. The mixtures were then evaporated at room temperature. More than three MALDI samples were prepared for each of the 13 analyte/matrix mixing ratios in the range 1×10^{-4} to 1.

2.2. MALDI–MS measurements

Positive ion mass spectra were measured using a MALDI time-of-flight (TOF) spectrometer (Autoflex III, Bruker Daltonics, Germany). Measurements were performed in reflectron mode at an acceleration voltage of 19 kV, and each spectrum was obtained by accumulating 2000 laser shots (355 nm, <100 μJ/pulse, 200 Hz). The laser-irradiation positions were displaced randomly on the MALDI samples every 10 shots to minimize fluctuations in signal intensity caused by non-uniform crystallization. Five spectra were measured for each sample for increased accuracy, resulting in >2000 items of data, which were securely analyzed to obtain signal intensities with standard errors.

3. Results and discussion

3.1. Observed MALDI–MS spectra

Fig. 1 shows typical MALDI–MS spectra for Lys, LysOMe, Trp, and TrpOMe samples, prepared at a mixing ratio of [X]/[CHCA] = [XOMe]/[CHCA] = 2×10^{-3}. The spectra are of two

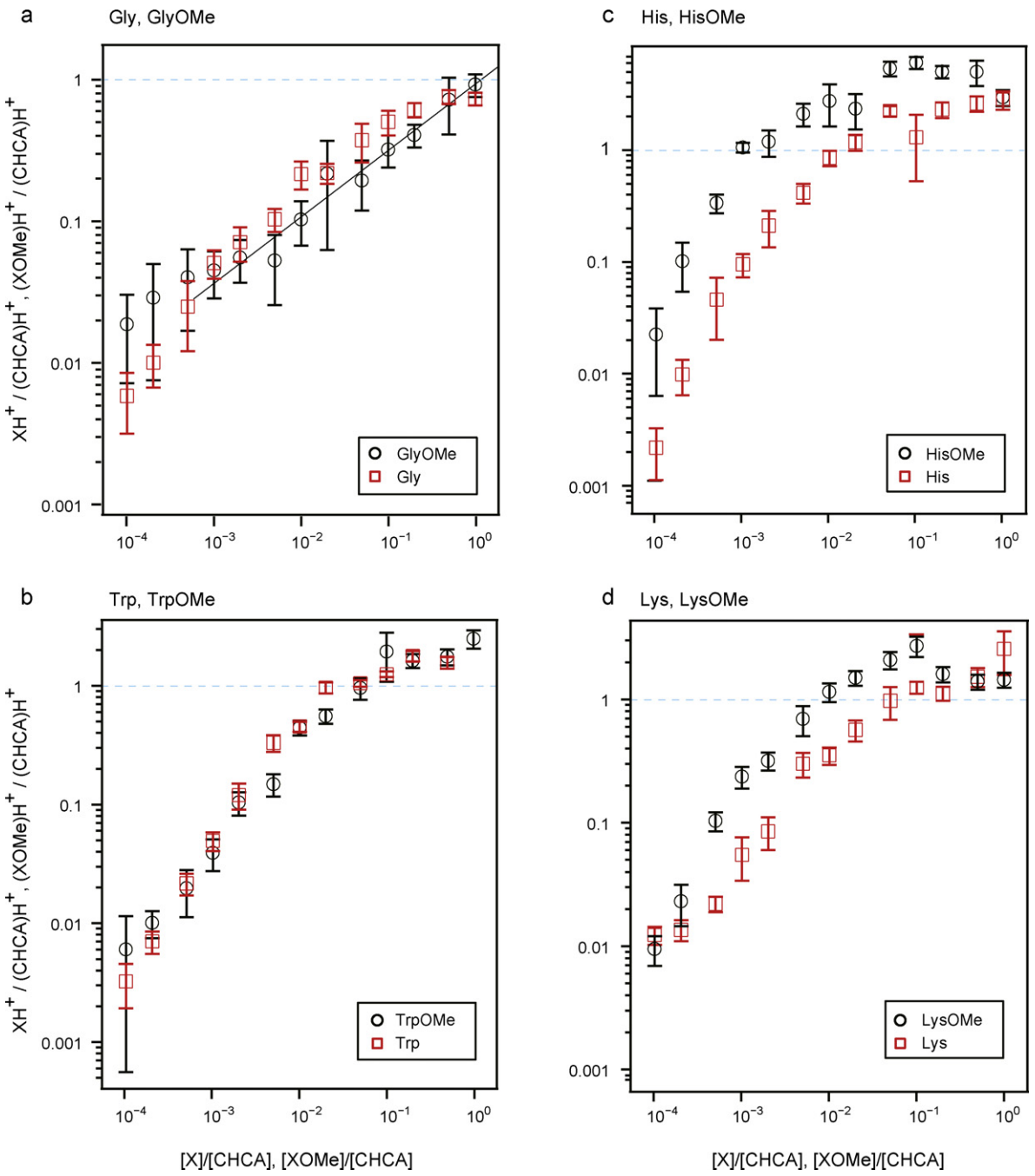

Fig. 2. Logarithmic plots of amino acid X MALDI signal intensity ratio $XH^+/(CHCA)H^+$ as a function of sample analyte/matrix mixing ratio [X]/[M] (open circles) and corresponding ester XOMe ratio $(XOMe)H^+/(CHCA)H^+$ as a function of [XOMe]/[M] (open squares): (a) X = Gly; (b) X = Trp; (c) X = His; (d) X = Lys.

types: the effect of esterification is either small (for Trp) or large (for Lys). In the mass region shown in the figure, the following are observed: protonated matrix $(CHCA)H^+$ at $m/z = 190$, and protonated samples $(Trp)H^+$ at $m/z = 205$, $(TrpOMe)H^+$ at $m/z = 219$, $(Lys)H^+$ at $m/z = 147$, and $(LysOMe)H^+$ at $m/z = 161$. The signals at $m/z = 212$ and 172 are for the cationized ion $(CHCA)Na^+$ and the dehydrated ion $(CHCA\text{-}H_2O)H^+$ of the matrix, respectively.

From the peak areas, the ion yields of XH^+, $(XOMe)H^+$, and $(CHCA)H^+$ were calculated for each spectrum. For Trp, the signal intensity ratios $TrpH^+/(CHCA)H^+ = 0.12(3)$ and $(TrpOMe)H^+/(CHCA)H^+ = 0.10(2)$ were obtained, indicating that the effect of esterification is negligible for the Trp/CHCA system. For Lys, the signal intensity ratios $LysH^+/(CHCA)H^+ = 0.089(26)$ and $(LysOMe)H^+/(CHCA)H^+ = 0.33(6)$ were obtained. These results imply that the role of the carboxyl group can be a key to MALDI–MS detection sensitivity, depending on the amino acid species.

3.2. Yields of XH^+ and $(XOMe)H^+$ as a function of sample mixing ratio

The protonated ion yield ratios $XH^+/(CHCA)H^+$ and $(XOMe)H^+/(CHCA)H^+$ were calculated from the MALDI–MS spectra. Samples were prepared at analyte/matrix mixing ratios [X]/[CHCA] and [XOMe]/[CHCA] ranging from 1×10^{-4} to 1×10^{-0} and a fixed CHCA concentration of 50 μmol/mL. Fig. 2 shows logarithmic plots of signal intensity ratio $XH^+/(CHCA)H^+$ as a function of sample mixing ratio [X]/[CHCA] for X = Gly, His, Lys, and Trp (red squares) and comparable plots for the corresponding

Table 1
Gas-phase basicity (GB) and obtained values of $x_{XOMe/CHCA}$ and $x_{X/CHCA}$ for 11 amino acids using CHCA matrix.

X	GB/kJ mol^{-1} [a]	$x_{XOMe/CHCA}$ [b]	$x_{X/CHCA}$ [b,c]	Ratio of change [d]
Gly	852.2	1.1×10^{-0} [e]	4.2×10^{-1} [e]	−0.62
Ala	867.7	2.1×10^{-1}	3.8×10^{-1}	0.81
Val	876.7	1.6×10^{-1}	2.2×10^{-1}	0.38
Leu	880.6	3.3×10^{-1}	3.2×10^{-1}	−0.03
Ser	880.7	5.0×10^{-2}	8.6×10^{-2}	0.72
Ile	883.5	3.6×10^{-2}	4.1×10^{-2}	0.14
Phe	888.9	7.8×10^{-2}	8.1×10^{-2}	0.04
Trp	915.0	5.2×10^{-2}	2.4×10^{-2}	−0.54
His	950.2	9.7×10^{-4}	1.4×10^{-2}	13
Lys	951.0	7.8×10^{-3}	4.9×10^{-2}	5.3
Arg	991.6	3.9×10^{-4}	2.1×10^{-4}	−0.46

[a] Evaluated values are taken from Ref. [13].
[b] Sample mixing ratio at which MALDI signals of XH^+ (or $(XOMe)H^+$) and $(CHCA)H^+$ have equal intensities (see text).
[c] Values are taken from Ref. [9].
[d] Calculated as $(x_{X/CHCA} - x_{XOMe/CHCA})/x_{XOMe/CHCA}$.
[e] Determined by extrapolation (see text).

methyl esters $(XOMe)H^+$ (black circles). These plots show monotonic increases, including a region in which $XH^+/(CHCA)H^+$ and $(XOMe)H^+/(CHCA)H^+$ increase linearly with a slope of nearly one. Similar variations were obtained for 20 natural amino acids/CHCA systems, as previously reported [9]. The region of linear increase was considered in light of the reversible proton-transfer-reaction (1) between desorbed amino acid and $(CHCA)H^+$, which satisfies the thermal equilibrium condition and the approximation that the ratio of neutral species X/CHCA in the MALDI plume equals [X]/[CHCA].

Comparison of the plots for Gly and GlyOMe (Fig. 2a) and for Trp and TrpOMe (Fig. 2b) show that the signal intensity ratios $(GlyOMe)H^+/(CHCA)H^+$ and $GlyH^+/(CHCA)H^+$ in the linear region agree with each other within the standard error. Similar results are observed for Ala, Arg, Ile, Leu, Phe, Ser, and Val. In contrast, plots for His and HisOMe (Fig. 2c) show clear differences in the linear region; the ratio for the latter is greater than the ratio for the former by nearly an order of magnitude. A similar difference is observed for Lys and LysOMe (Fig. 2d) acids. These results indicate that methyl esterification increases the formation efficiency of XH^+ for His/CHCA and Lys/CHCA systems but has little effect on Ala, Arg, Gly, Ile, Leu, Phe, Ser, Trp, and Val.

3.3. Effect of methyl esterification on MALDI–MS of amino acids

The analyte/matrix mixing ratio [X]/[CHCA] at which the MALDI spectra peak intensities of XH^+ and $(CHCA)H^+$ are equal, that is, at which the ratio $XH^+/(CHCA)H^+ = 1$, were determined from the log–log plots in Fig. 2. This ratio, designated $x_{X/CHCA}$, is given by the relationship

$$\ln x_{X/CHCA} = -\frac{GB(X)}{RT} + \frac{GB(CHCA)}{RT} \quad (2)$$

when the condition of the thermal equilibrium model is satisfied. As shown in the figure, $x_{GlyOMe/CHCA} = 1.1$, as determined by extrapolation of the linear increase obtained by least-squares fitting in the [GlyOMe]/[CHCA] region from 2×10^{-3} to 1. $x_{X/CHCA}$ values were taken for all the amino acids from our previous report [9]; $x_{XOMe/CHCA}$ values for esters determined in the present study are listed in Table 1.

Fig. 3 shows plots of $x_{X/CHCA}$ and $x_{XOMe/CHCA}$ as a function of amino acid GB [13]. GB values for methyl esterified amino acids (except for GlyOMe) were heretofore unknown. According to Locke and McIver [14], GB is about 16 kJ/mol larger for GlyOMe than for Gly. Lemoff et al. reported the proton affinity (PA) of Lys and LysOMe to be 996.5 and 1012 kJ/mol, respectively [15]. The rates of increase by methyl esterification are +1.9% for Gly GB and +1.5% for Lys PA. The effect of esterification on GB is expected to be comparable for other amino acids. Therefore, when comparing $x_{X/CHCA}$ values, it is acceptable to use plots of $x_{XOMe/CHCA}$ using the GB of the corresponding amino acids.

As seen in Fig. 3, $x_{XOMe/CHCA}$ values for X = Ala, Arg, Gly, Ile, Leu, Phe, Ser, Trp, and Val agree with the $x_{X/CHCA}$ values for the corresponding amino acids within the standard error. The red line is the regression line for the thermal equilibrium model, previously obtained by least-squares fits of Eq. (2) to $x_{X/CHCA}$ for 17 natural amino acids (excluding the basic amino acids Arg, His, and Lys) with optimized parameters $T = 1510$ K and $GB(CHCA) = 851$ kJ/mol [9]. Both $x_{X/CHCA}$ and $x_{XOMe/CHCA}$ for 9 amino acids follow the regression line, indicating that esterification of the carboxyl groups of these amino acids has little effect on protonation efficiency, in other words, the carboxyl groups on these amino acids do not play a crucial role in the desorption and protonation processes. This result also proves the validity of the thermal equilibrium model for amino acid esters XOMe as well as amino acids X.

Note that $x_{X/CHCA}$ values for the basic amino acids His and Lys, which deviate significantly from the thermal equilibrium model [9], are largely shifted downward, that is, toward $x_{XOMe/CHCA}$, by methyl esterification. These shifts reduce the deviations for His and Lys and cause $x_{XOMe/CHCA}$ to approach the regression line of the thermal equilibrium model; for His, the point of $x_{XOMe/CHCA}$ lies entirely on the line.

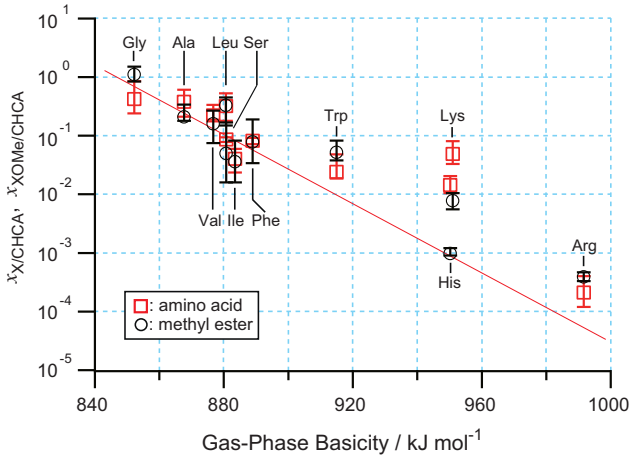

Fig. 3. Logarithmic plot of $x_{X/CHCA}$ (open circles) and $x_{XOMe/CHCA}$ (open squares) as a function of the gas-phase basicity (GB) of amino acid X. The regression line was obtained by least-squares fitting of Eq. (2) to $x_{X/CHCA}$ values of 17 amino acids taken from Ref. [9].

As a demonstration of how $x_{X/CHCA}$ is affected by amino acid species, the calculated ratios of change, defined as $(x_{X/CHCA} - x_{XOMe/CHCA})/x_{XOMe/CHCA}$, are listed in Table 1. The ratios of change for His and Lys are 13 and 5.3, respectively, which are remarkably higher than the ratios for other amino acids (the minimum is −0.62 for Gly; the maximum is 0.81 for Ala). This indicates that the presence of a carboxyl group on His and Lys significantly increases x, that is, significantly suppresses $LysH^+$ and $HisH^+$ formation for Lys/CHCA and His/CHCA systems. A possible explanation of why carboxyl groups suppress XH^+ formation is that they might decrease the amount of desorbed analyte and/or decrease the effective GB in the MALDI plume, although the mechanisms by which these phenomena would occur are not currently clear. Further investigation is needed to characterize matrix crystallization at the molecular level and to determine the conformation of the desorbed analyte in order to help us better understand the effect on MALDI signal intensity not only for His/CHCA and Lys/CHCA but also for other systems that behave similarly in MALDI analysis.

4. Summary

MALDI–MS spectra for methyl esters (XOMe) of 11 amino acid (X = Ala, Arg, Gly, His, Ile, Leu, Lys, Phe, Ser, Trp, and Val) were measured using CHCA matrix at various analyte/matrix mixing ratios between 1×10^{-4} and 1×10^{-0}. For most of the amino acids, the signal intensity ratio for the ester $(XOMe)H^+/(CHCA)H^+$ and for the corresponding amino acid $XH^+/(CHCA)H^+$ are well explained by the model protonation reaction $(CHCA)H^+ + X\,(\text{or XOMe}) \leftrightarrows CHCA + XH^+$ (or $(XOMe)H^+$), which reaches thermal equilibrium in the MALDI plume. The exceptions to this general observation are for the basic amino acids His and Lys for which the ratio for the ester agrees with the model but the ratio for the corresponding amino acid deviates significantly. This compensation by esterification suggests that some kind of interaction involving the carboxyl group, possibly with the basic side chain, suppresses $HisH^+$ and $LysH^+$.

Acknowledgements

We thank Dr. M. Tamiya and Prof. M. Ishiguro for the synthesis of arginine d3-methyl ester dihydrochloride. This research was supported by a Grant-in-Aid for Education and Research from Faculty of Pharmaceutical Sciences and by a Grant-in-Aid for Scientific Research (C) (grant no. 20550023) from the Japan Society for the Promotion of Science.

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