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GUMBOS matrices of variable hydrophobicity for matrix-assisted laser desorption/ionization mass spectrometry

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RATIONALE: Detection of hydrophobic peptides remains a major obstacle for matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). This stems from the fact that most matrices for MALDI are hydrophilic and therefore have low affinities for hydrophobic peptides. Herein, 1-aminopyrene (AP) and AP-derived *g*roup of *u*niform *m*aterials *b*ased on *o*rganic *s*alts (GUMBOS) as novel matrices for MALDI-MS analyses of peptides were investigated for hydrophobic and hydrophilic peptides.

METHODS: A number of solid-phase AP-based GUMBOS are synthesized with variable hydrophobicity simply by changing the counterions. Structures were confirmed by use of ^{1}H NMR and electrospray ionization mass spectrometry (ESI-MS). 1-Octanol/water partition coefficients ($K_{o/w}$) were used to measure the hydrophobicity of the matrices. A dried-droplet method was used for sample preparation. All spectra were obtained using a MALDI-TOF mass spectrometer in positive ion reflectron mode.

RESULTS: A series of AP-based GUMBOS was synthesized including [AP][chloride] ([AP][Cl]), [AP][ascorbate] ([AP][Asc]) and [AP][bis(trifluoromethane)sulfonimide] ([AP][NTf_2]). The relative hydrophobicities of these compounds and α-cyano-4-hydroxycinnamic acid (CHCA, a common MALDI matrix) indicated that AP-based GUMBOS can be tuned to be much more hydrophobic than CHCA. A clear trend is observed between the signal intensities of hydrophobic peptides and hydrophobicity of the matrix.

CONCLUSIONS: MALDI matrices of GUMBOS with tunable hydrophobicities are easily obtained simply by varying the counterion. We have found that hydrophobic matrix materials are very effective for MALDI determination of hydrophobic peptides and, similarly, the more hydrophilic peptides displayed greater intensity in the more hydrophilic matrix. Copyright © 2014 John Wiley & Sons, Ltd.

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) is an important tool for the rapid and sensitive analyses of biomolecules.[1] In MALDI analysis, the matrix is an essential component, and selection of a suitable matrix is critically important because the optimal performance of many matrices has been found to depend on the chemical characteristics of the analyte.[2–4] Therefore, there have been intense efforts towards development and evaluation of novel and effective matrices for MALDI-MS analyses of different analytes.[5–17] In general, MALDI matrices must possess specific properties that include, but are not limited to, strong absorption of laser radiation, ability to effectively disperse analytes, good miscibility with the solvent and analytes, efficient energy transfer to analyte for ionization and desorption, ability to serve as a good protonating and deprotonating agent, and also vacuum stability.[9]

The majority of MALDI matrices studied so far possess hydrophilic characteristics, which are particularly well suited for detection of hydrophilic biomolecules.[17,18] However, detection of hydrophobic biomolecules remains an elusive limitation for MALDI analyses. This stems from the fact that hydrophilic matrices exhibit low affinities for hydrophobic biomolecules. It must be emphasized that a protein contains both hydrophilic and hydrophobic residues, and hence it is critical to also analyze hydrophobic peptides.[17,19]

An additional problem in the analyses of hydrophobic peptides is their limited solubility in commonly used solvents for MALDI-MS. To overcome this limitation, detergents have been employed to solubilize hydrophobic peptides in aqueous media.[17] These detergents, being amphiphilic, improve the solubility of hydrophobic proteins. However, use of surfactants at critical micelle concentrations can cause deterioration of the spectral quality of peptides, yielding poor signal-to-noise ratios, as well as poor mass resolution.[17,20]

Despite several attempts to develop new matrices for hydrophobic peptides and proteins,[16,19,21–26] limited success has been achieved in MALDI detection of hydrophobic biomolecules. For this reason, synthesis and evaluation of new matrices are still a priority for selective detection of hydrophobic biomolecules within complex biological samples. It has been observed that it is simple to detect hydrophobic biomolecules with hydrophobic matrices since

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hydrophobic matrices have good affinities for hydrophobic analytes.[17] Therefore, there have been several efforts to synthesize new hydrophobic matrices for analyses of hydrophobic biomolecules.[27–30] However, synthesis of such matrices often involves complex synthetic procedures, which are time consuming and at the same time often produce low product yields.

Ionic liquids (ILs) have been introduced as matrices for MALDI-MS due to several advantages when compared to conventional matrices.[8–11,16,31–34] ILs are organic salts with melting points below 100 °C and these molecules possess several favorable characteristics such as high thermal stability, negligible vapor pressure, and, most importantly, a simple approach to 'tuning' properties. Due to ease of synthesis, new matrices with varying hydrophobicity can be designed simply by modifying the cation or anion.[1] Based on results in the literature, a comparison of ionic liquid matrices (ILMs) with solid matrices in MALDI reveals that ILMs exhibit improved reproducibility, sensitivity, sample homogeneity, and detection limits.[8] However, no current studies can be cited which focus on tuning the hydrophobicity of ILMs through changes in the counterions.

In our laboratory, we are investigating a variety of applications for RTILs and related solid-phase organic salts with melting points up to 250 °C which we term a ***g***roup of ***u***niform ***m***aterials ***b***ased on ***o***rganic ***s***alts (GUMBOS). In other words, organic salts with melting points up to 25 °C are referred to as RTILs, while solids with melting points from 25 to 250 °C are referred to as GUMBOS. GUMBOS and RTILs have been used for a variety of applications, primarily due to the ease with which these materials can be engineered for specific needs.[35–38] The hydrophobicity of such compounds can be easily tuned simply by changing the counterion, which results in savings of time and energy. Thus, this approach is an economical method to obtain a desired product with excellent yield.

Herein, we report on an investigation of 1-aminopyrene (AP) and AP-based GUMBOS as MALDI matrices for detection of peptides. AP was selected for these studies because it exhibits strong absorption at the wavelength of laser radiation (337 or 335 nm), displays hydrophobic characteristics, and shows ability to protonate analytes. In this study, we synthesized a series of AP-based GUMBOS, i.e. [AP][Cl], [AP][Asc], and [AP][NTf_2], to achieve variable hydrophobicity simply by changing the anion. The relative hydrophobicities of these compounds and CHCA were obtained by measuring their 1-octanol/water partition coefficients ($K_{o/w}$), which indicated that these compounds are much more hydrophobic than CHCA. These compounds were then examined as MALDI matrices for analyses of hydrophobic (valinomycin and gramicidin) as well as hydrophilic (bradykinin, and angiotensin II) peptides. These new MALDI matrices showed better performance, and at the same time controllable affinity for different peptides, which is indicated by changes in the signal intensity for peptides of different hydrophobicities. As expected, we observed that hydrophobic matrices showed better signal intensity for hydrophobic peptides, and that hydrophilic matrices produced improved signal intensity for hydrophilic peptides. To the best of our knowledge, there are no literature reports on the use of AP and AP-based salts as matrices for MALDI-MS.

EXPERIMENTAL

Materials

The reagents α-cyano-4-hydroxycinnamic acid (CHCA), acetonitrile, acetone, methanol, ethanol, butanol, trifluoroacetic acid (TFA, 99%), bis(trifluoromethane)sulfonimide ($HNTf_2$, 99.95%), 1-aminopyrine (AP, 98%), bradykinin fragment 1-7, angiotensin II human, valinomycin, gramicidin from *Bacillus aneurinolyticus*, and ascorbic acid (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane (99%) (DCM) and hydrochloric acid (HCl) were purchased from Fisher Scientific. All chemicals were used as received without further purification.

Synthesis and characterization of GUMBOS

Equimolar amounts of AP and HCl were reacted to yield [AP][Cl]. Briefly, 1 g of AP (4.6 mmol) dissolved in 10 mL DCM was reacted with 5 mL of 0.460 M aqueous HCl and stirred for 3 h. The solid [AP][Cl] product was washed several times with water to remove excess acid. The DCM was removed using rotary evaporation and water was removed by lyophilization. [AP][Asc] was prepared by mixing equimolar amounts of AP and ascorbic acid in methanol and stirring for 24 h. Likewise, [AP][NTf_2] was prepared by reacting equimolar amounts of AP and $HNTf_2$ in methanol and stirring for 24 h. In both cases, methanol was removed by use of rotary evaporation. The melting points of these compounds were determined using a melting point apparatus (DigitMelt MPA 160, Stanford Research Systems).

Determination of relative hydrophobicity

The 1-octanol/water partition coefficient was used to gauge the relative hydrophobicities of CHCA, AP, and AP-based GUMBOS. The partition coefficients were determined using a procedure previously developed in our group with very slight modifications.[35] Briefly, 1-octanol and water were shaken for 24 h before use in order to correct for mutual solubility of the two solvents. A known amount of these compounds was then stirred in a mixture of saturated 1-octanol and water (volume ratio 1:1) for 24 h to allow partitioning. The concentration of the compound in the 1-octanol phase was then determined using UV-Visible spectroscopy and the concentration in water by use of mass balance. Finally, the partition coefficient was calculated by use of the following equation:

$$K_{o/w} = \frac{[X]_{o,e}}{[X]_{w,e}}$$

where $K_{o/w}$ is the octanol/water partition coefficient, $[X]_{o,e}$ is the concentration of solute in 1-octanol at equilibrium, and $[X]_{w,e}$ is the concentration of solute in water at equilibrium.

Sample preparation for MALDI

A solution of CHCA matrix at 10 mg/mL (ca 50 mM) was freshly prepared by dissolving an appropriate amount of the compound in a mixture of acetonitrile and water (2:1, v/v) containing 0.1% TFA.[31] Solutions of AP at a

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concentration of 60 mM and the GUMBOS at a concentration of 40 mM were freshly prepared in methanol. MALDI samples were prepared by using the dried-droplet method.[39,40]

MALDI-TOFMS data acquisition

All spectra were obtained using a MALDI-TOF mass spectrometer (UltrafleXtreme, Bruker, Bremen, Germany) in positive ion reflectron mode. A Nd:YAG laser at 355 nm wavelength with a pulse duration of 3 ns was used for ionization. The ion acceleration potential was 25 kV. One thousand laser shots were found to give an acceptable signal under nearly all conditions and were summed for each spectrum and 12 different positions were analyzed on each spot. Data were processed using FlexAnalysis 3.3 software (Bruker).

RESULTS AND DISCUSSION

Preparation and characterization of AP-based materials

One major requirement for a MALDI matrix is that it should possess a sufficient molar absorption coefficient at the wavelength of the laser used.[41] Another basic requirement is that the matrix should act as a proton source for a given analyte.[41] An important factor that affects the performance of MALDI matrices is the size of the matrix crystals. It has been reported that smaller crystals cause improved reproducibility, and resolution.[42–44] Smaller crystals also volatilize completely during laser irradiation.[42] Therefore, we have compared the sample morphology of our different matrices and all AP-based GUMBOS are found to be crystalline (See XRD graphs in Supplementary Figs. S1–S3, Supporting Information). The sizes of the matrix crystals are shown in the images depicted in Supplementary Fig. S4 (Supporting Information). The hydrophobic compound AP was chosen as a matrix because it absorbs UV radiation, has the potential to donate a proton, and satisfies other requirements for a MALDI matrix. In addition, it can be easily converted into a GUMBOS compound such that its hydrophobicity can be tuned by changing the counterion (the anion in this case).

In this study, three different GUMBOS were prepared using 1-aminopyrene: [AP][Cl], [AP][Asc], and [AP][NTf_2]. All three compounds are solids with melting points above 100 °C. These compounds were characterized by use of ^{1}H NMR (Supplementary Figs. S5–S8, Supporting Information). In addition, an ^{9}F NMR spectrum was acquired for [AP][NTf_2] and characterization-using ESI-MS was used to corroborate the NMR data (data not shown). The structures, molecular weights, and melting points of the compounds used in this study are shown in Table 1.

The absorption spectra of CHCA, AP, and AP-based GUMBOS in methanol are shown in Fig. 1. The absorbance band lies between 300 to 400 nm for CHCA, and between 300 to 420 nm for AP and AP-based GUMBOS. All of these compounds absorb over a similar wavelength range as compared to a CHCA matrix. The performance of our materials as MALDI matrices, reported in the subsequent sections, is compared with that of CHCA because CHCA is a commonly used MALDI matrix for low molecular weight biomolecule applications.[12,37] The molar absorptivities of all compounds at 355 nm are also listed in Table 1. It is observed that AP, [AP][Cl] and [AP][Asc] have lower molar absorptivities as compared to CHCA, and the molar absorptivity of [AP][NTf_2] is much lower than that of CHCA. Hence, the molar absorptivity is dependent on the counterion.

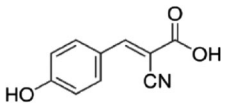
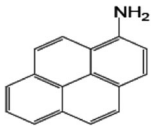
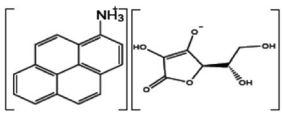
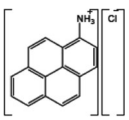
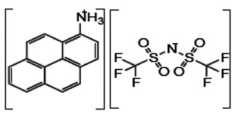
In order to estimate the relative hydrophobicity of all these compounds, 1-octanol/water partition coefficients ($K_{o/w}$) were determined. The logarithm of the partition coefficient (log $K_{o/w}$) for each compound is listed in Table 1. Hydrophobicity is directly related to the value of $K_{o/w}$. As seen from the values of log $K_{o/w}$, the relative hydrophobicity from lowest to highest increases as CHCA $<<$ [AP][Asc] $<$ [AP][Cl] $<$ AP $<$ [AP][NTf_2]. This observation demonstrates that a significant change in the hydrophobicity of GUMBOS can be attained by simply changing the counterion.

Evaluation of the performance of AP and AP-based GUMBOS as MALDI matrices for hydrophobic and hydrophilic peptide detection

We have evaluated the performance of this new class of materials using two hydrophobic (valinomycin and gramicidin) and two hydrophilic peptides (bradykinin and angiotensin II). Initially, the effectiveness of these AP-based matrices was evaluated by acquiring individual MALDI spectra for all four peptides, as shown in Supplementary Figs. S9–S28 (see Supporting Information). The dried-droplet method was used to prepare all MALDI samples. Briefly, 1 μL of sample solution was spotted on the MALDI target and 1 μL of matrix was added and mixed, followed by drying under ambient conditions. Valinomycin and gramicidin, in all matrices including CHCA, showed a clear signal at m/z 1133.62 and 1904.06, respectively, which corresponds to the $[M+Na]^+$ peak. Similarly, the $[M+H]^+$ peaks for bradykinin (1-7) and angiotensin II were observed at m/z 757.39 and m/z 1046.54, respectively, No matrix-derived peaks were observed in the spectral region of all peptides (Supplementary Figs. S10–S13, Supporting Information), indicating that these matrices do not interfere with peaks arising from the analytes.

The relative standard deviation (RSD), which is a measure of the spot-to-spot reproducibility, was evaluated by using the signal intensity measured at 12 different positions (Table 2). The laser settings used were the same for all matrices. In all measurements, only the spectra having a resolution greater than 10,000 were collected. Examination of Table 2 reveals that improved spot-to-spot reproducibility and significant enhancement (10–15 times) in signal intensity are obtained when using AP-based matrices as compared to the conventional CHCA matrix. The low signal intensity in the case of [AP][NTf_2] is attributed to the relatively low molar absorption coefficient of this salt as compared to AP and other AP-based GUMBOS. In addition, low signal intensity in [AP][NTf_2] could also be ascribed to formation of a hydrogen-bonding network between the highly electronegative atoms in the $[NTf_2]^-$ anion and the AP cation. The hydrogen bonding between cation and anion in [AP][NTf_2] may also inhibit proton transfer from matrix to the analytes, thereby accounting for poor performance.[45,46]

Table 1. Structures, molecular weights, log $K_{(o/w)}$ values, molar absorptivities, and melting points of matrices used in this study

Compound	Structure	MP (°C)	MW	Log $K_{(o/w)}$	Molar absorptivity (M^{-1} cm^{-1})
α-CHCA		240[a]	189.17	-1.72	23500
AP		117[a]	217.27	1.42	20000
[AP][Asc]		109[b]	394.13	0.81	18500
[AP][CI]		230[b]	253.73	1.02	15000
[AP][NTf_2]		190[b]	498.42	1.54	9000

[a]Melting points reported by Sigma-Aldrich and verified by us.
[b]Melting points determined in this study.

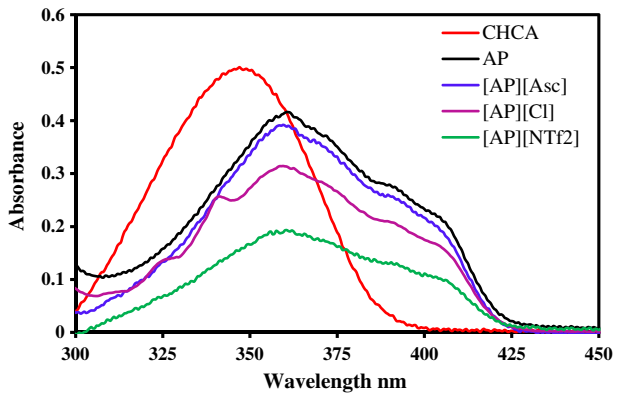


Figure 1. Absorbance spectra of CHCA, AP, and AP-based salts in methanol. The concentration of all these compounds is 50 μM.

The results obtained using AP and AP-based GUMBOS as matrices for hydrophilic (bradykinin, angiotensin II) and hydrophobic peptides, (valinomycin and gramicidin) are summarized in Fig. 2. The results are consistent with relatively high signal intensity for the hydrophobic peptides as compared to the hydrophilic peptides in the hydrophobic matrices (right-hand side of Fig. 2) and relatively high signal intensity for the hydrophilic peptides in the more hydrophilic matrices (left-hand side of Fig. 2). The propensity for hydrophobic/hydrophilic matrix favoring hydrophobic/hydrophilic analyte is not a strict rule. For example, angiotensin II and valinomycin have nearly the same intensity with [AP][Cl] and angiotensin and gramicidin have nearly the same intensity with [AP]. Nonetheless, the general trend of 'like favors like' holds when the results are taken in its entirety. Likewise, there is some variation in absolute signal intensity from matrix to matrix; for example, [AP][NTf_2] has a relatively low signal for all matrix compounds, most likely due to its low absorptivity. However, the hydrophobic [AP][NTf_2] matrix clearly favors the hydrophobic peptides in comparison to the hydrophilic peptides.

For all peptides, reproducibility is improved with GUMBOS matrices as compared to a conventional CHCA matrix. The improved reproducibility of the signal with

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Table 2. Signal intensities and relative standard deviations (RSDs) for $[bradykinin + H]^+$, $[angiotensin\ II + H]^+$, $[valinomycin + Na]^+$ and $[gramicidin + Na]^+$ ions in the presence of CHCA, AP, and AP-based GUMBOS as matrices

	Bradykinin		Angiotensin II		Valinomycin		Gramicidin	
Matrix	Average intensity	RSD	Average intensity	RSD	Average intensity	RSD	Average intensity	RSD
CHCA	2000	102	1200	63	1200	103	1100	90
[AP][Asc]	25000	16	9200	21	3300	29	2400	41
[AP][Cl]	25000	23	6724	26	7300	19	2900	6
AP	5000	41	5700	15	21000	36	7100	63
$[AP][NTf_2]$	2000	33	1800	16	5200	24	5500	13

Table 3. Intensities for mixtures of hydrophilic (angiotension II) and hydrophobic (valinomycin) peptides at equimolar concentration in different matrices of variable hydrophobicities

	(Angiotensin II)		(Valinomycin)	
	Average intensity	RSD	Average intensity	RSD
CHCA	3300	92	1000	100
[AP][Asc]	2700	72	600	63
[AP][Cl]	8300	32	2500	25
AP	1400	48	8400	30

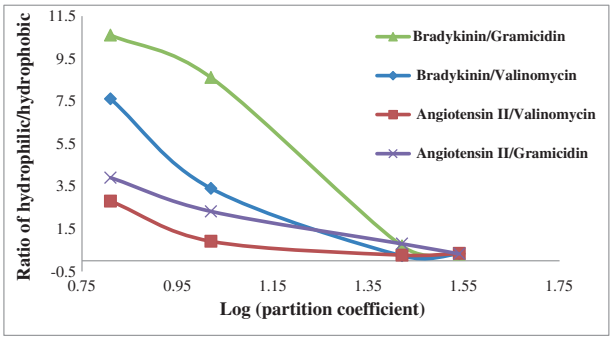

Figure 3. Intensity ratio for hydrophilic peptide/hydrophobic peptide as a function of log partition coefficient of AP and AP-based GUMBOS.

these matrix materials likely results from a more homogeneous distribution of sample and matrix as compared to CHCA.

We also analyzed the data by obtaining the ratios of signal intensity of each hydrophilic peptide to each hydrophobic peptide (bradykinin/gramicidin, bradykinin/valinomycin, angiotensin II/valinomycin, and angiotensin II/gramicidin) in order to normalize the effect. A plot of the ratio of signal intensity against the logarithm of partition coefficient of AP and AP-based GUMBOS shows a clear trend with hydrophobicity (Fig. 3). More specifically, the ratio of the signal intensity is found to decrease as the hydrophobicity of the matrix increases.

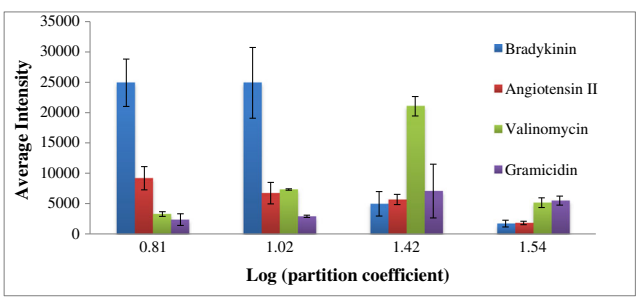

Figure 2. Average MALDI signal intensity as a function of the logarithm of the partition coefficient log $K_{(o/w)}$ for (left to right) [AP][Asc], [AP][Cl], [AP], and [AP][NTF2]; a larger partition coefficient indicates a more hydrophobic matrix.

Analysis of hydrophobic and hydrophilic peptide mixtures

Peptide mixtures were also analyzed using each matrix separately. An equimolar amount of hydrophobic (valinomycin) and hydrophilic (angiotensin II) peptide was mixed and the mixture was spotted on a MALDI target using the dried-droplet method. Each spot was analyzed at 12 different positions, and the average intensity as well as the RSD were calculated. The resulting signal intensities of the mixtures obtained with different AP-based matrices were compared with a conventional CHCA matrix (Table 3). In spite of the same concentration used for both peptides with the [AP][Asc] matrix, higher signal intensity was observed for angiotensin II than for valinomycin (Fig. 4). This suggests that [AP][Asc], a hydrophilic matrix, exhibits a higher affinity for the hydrophilic peptide as compared to the hydrophobic peptide. Similarly, both CHCA and [AP][Cl], hydrophilic matrices, showed higher intensity for angiotensin II and lower intensity for valinomycin (Supplementary Figs. S29 and S30, see Supporting Information). Thus, the hydrophilic matrices result in better signals for the hydrophilic peptides.

To investigate the hydrophobic peptides, a hydrophobic matrix was employed. Aminopyrene, a hydrophobic matrix, showed opposite results for a mixture of hydrophilic and hydrophobic peptides. In this case, higher signal intensity was observed for valinomycin as compared to the angiotensin II for the same molar concentration of peptides, as shown in Fig. 5. Thus, it is likely that the enhanced signal for the hydrophobic peptide is due to its higher affinity for the hydrophobic matrix.

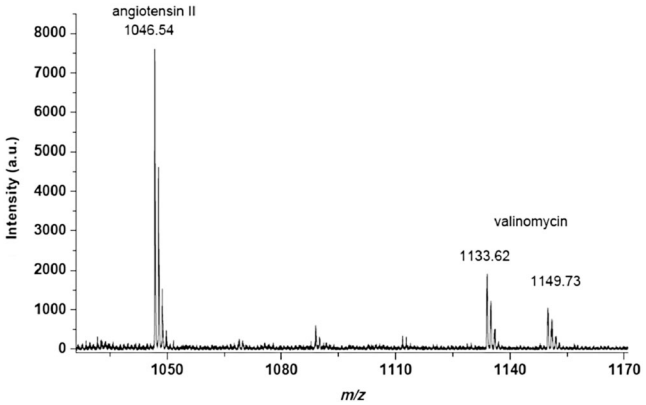

Figure 4. Positive ion mode MALDI MS spectrum of equimolar mixture of angiotensin II (hydrophilic) and valinomycin (hydrophobic) using [AP][Asc] matrix.

The average signal intensity obtained for the mixture was plotted against the logarithm of the partition coefficient (log $K_{o/w}$) of different AP-based matrices (Fig. 6). The RSD is highest in CHCA as compared to the AP and AP-GUMBOS matrices. In terms of reproducibility and signal intensity for both hydrophobic and hydrophilic peptides, AP-based matrices are better performing as compared with CHCA. In terms of relative intensity, the hydrophilic angiotensin II has approximately three times the signal intensity as the hydrophobic valinomycin for all but the most hydrophobic matrix [AP][NTf_2]. This supports the general trend of hydrophobic analytes requiring a hydrophobic matrix, but also suggests a nonlinear effect in which there is a threshold for matrix hydrophobicity above which ionization of the more hydrophobic components is favored. If this is the case, the ability to tune the matrix hydrophobicity within a narrow range will be critical for selective ionization of the hydrophobic components in a sample.

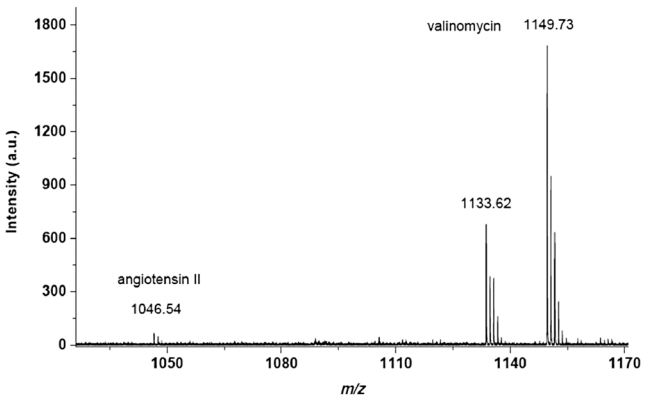

Figure 5. MALDI mass spectrum for an equimolar mixture of angiotensin II and valinomycin with AP as matrix.

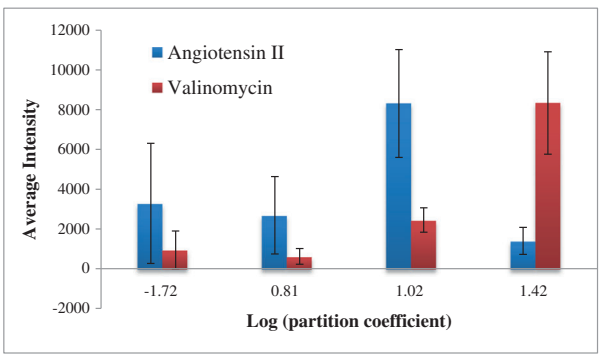

Figure 6. Signal intensities for a mixture of a hydrophilic (angiotension II) and a hydrophobic (valinomycin) peptide at equimolar concentration in matrices of different hydrophobicities. (CHCA: –1.72), ([AP][Asc]: 0.81), ([AP][Cl]: 1.02) and (AP: 1.42).

The result for the ratio of angiotensin II/valinomycin as a function of the hydrophobicity of the matrices is shown in Supplementary Fig. S31 (see Supporting Information). A plot of the ratio signal intensity against the logarithm of partition coefficient of matrices shows a clear trend: as the hydrophobicity of the matrices increases, the ratio of the signal intensity of the peptides decreases. This experiment was designed to normalize the effect of both hydrophobic and hydrophilic peptides at similar spots.

CONCLUSIONS

In this work, AP and AP-based GUMBOS of variable hydrophobicities were used as MALDI matrices. A series of AP-based GUMBOS were synthesized and characterized. A simple, facile and inexpensive approach was used to tune the hydrophobicity of AP-based matrices. The performance of these salts, along with AP, as MALDI matrices for detection of hydrophobic and hydrophilic peptides was evaluated. Tuning the hydrophobicity of the matrix by altering the counterions in AP-based GUMBOS changes its performance with peptides. Hydrophilic and hydrophobic peptides were determined using AP and AP-based GUMBOS and compared with conventional MALDI matrices that are often used with peptides. As expected, the performance of these matrices for different peptides depended on the matrix hydrophobicity. We have observed that increasing the hydrophobicity of a matrix increases the affinity to hydrophobic peptides after laser desorption. An increase in hydrophilicity of the matrix in the order of CHCA [AP][Asc] > [AP][Cl] > AP led to a higher affinity for hydrophilic peptides. Likewise, the signal intensity for hydrophobic peptides increased when the hydrophobicity of the matrix was increased. A mixture of hydrophilic and hydrophobic peptides demonstrated that these new matrices of variable hydrophobicities result in a similar trend in signal intensity, e.g. AP, a hydrophobic matrix, increased sensitivity of hydrophobic peptides, whereas the signal intensities for hydrophilic peptides were lower. Similarly, [AP][Cl] and [AP][Asc] and CHCA showed increased sensitivity for hydrophilic peptides and suppressed the detection of hydrophobic peptides. It was observed that CHCA, [AP][Asc] and [AP][Cl], showed good affinity for hydrophilic peptides. In ongoing work, these GUMBOS-based matrices and others are being used to enhance and selectively detect peptides in tissue samples for MALDI imaging.

Acknowledgements

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