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Short communication

Separation of amino acids by ion mobility spectrometry

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Abstract

The mobilities of the 20 common amino acids were determined by electrospray ionization ion mobility spectrometry. It was found that each amino acid had a different drift time and hence a different reduced mobility constant K_0. This difference in drift time was less than 0.1 ms in many cases. With the instrument used in this study it would not be possible to resolve mixtures of some of the amino acids. It would however be possible to determine any single amino acid. In addition, the detection limits were determined for the 20 amino acids. They ranged from 50 to 700 pg. This indicates that the detection limits were less than 3 pmol for all of the amino acids and that many amino acids had detection limits less than 1 pmol.

Keywords: Ion mobility spectrometry; Amino acids

1. Introduction

Amino acid analysis is important in understanding of both protein chemistry and in determination of protein sequences. There have been many strategies to analyze amino acids over the past 20 years. The most commonly used approach has been separation by ion-exchange chromatography followed by post-column derivatization using reagents such as ortho-phthaladehyde (OPA) [1] or ninhydrin [2–4]. Others have used high-performance liquid chromatography (HPLC) with post-column derivatization [5–11]. More recently, there have been numerous publications using derivatization with gas chromatography (GC) and GC–mass spectrometry (MS) methods [12–18]. All of these methods provide good separations of the 20 amino acids and show good detection limits. However, all of these methods require derivatization and generally require significant analysis times as well. This makes all of these methods timely and expensive.

Recently there have been a number of publications describing high-resolution ion mobility spectrometers fitted with electrospray ionization sources [19–21]. In this paper we investigate the possibility of using electrospray ionization high-resolution ion mobility spectrometry to analyze amino acids directly from liquid samples. We report in this work the drift times and the reduced mobility constants for all 20 common amino acids. In addition, the detection limits for each amino acid were determined. Interestingly it was found that all 20 amino acids had different drift times and that the sensitivity was in the low pmol to high fmol range depending on the amino acid. This

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demonstrates the possibility of using ion mobility spectrometry (IMS) to separate amino acids in less than a second.

2. Experimental

2.1. Instrumentation

The entire instrument was designed and built at Washington State University. The instrument contained three major regions, the electrospray ionization (ESI) source, the ion mobility spectrometer, and the mass spectrometer. The ESI source was a water-cooled device and has been described in detail previously [22]. In this source, a water-cooled jacket surrounds the spray needle and water-cooled nitrogen flows along the axis of the needle. The cooling was found to be necessary to prevent the heated spectrometer from causing the solvent to evaporate prior to reaching the end of the needle.

The ion mobility spectrometer contained two regions, the desolvation region and the drift region, separated by an ion gate. Both regions consisted of electrically conducting stainless steel and electrically insulating alumina rings stacked in an alternating and interlocking design. Each stainless steel ring was connected to the next by a series of 1 MΩ resistors. The desolvation region was 7.2 cm while the drift region was 13 cm in length. The specific details of this instrument have been described in considerable detail previously [20].

The ion mobility tube was interfaced to the mass spectrometer via a 40 μm pinhole leak. The mass spectrometer itself was a C50-Q (ABB Extrel, Pittsburgh, PA, USA) quadrupole mass filter fitted with an atmospheric pressure ionization (API) lens system. For the mobility studies in this paper the mass spectrometer was set in radiofrequency (RF) only mode. In this configuration all ions were allowed to pass and the mass spectrometer was essentially used as a detector. Mass spectra were obtained for each ion to confirm their identity.

2.2. General operating conditions

The electrospray needle was held at a voltage of +4000 V relative to the voltage of the mesh focus screen. The screen itself was held at a potential of 5500 V. This created an electric field of 280 V/cm in both the desolvation and the drift region of the IMS instrument. A counterflow of heated dry nitrogen gas was introduced at the end of the tube at a flow-rate of 800 ml/min. The drift tube was held at a temperature of 250°C at atmospheric pressure (~93 000 Pa). The amino acid solutions were introduced into the spray needle in a solvent of water–methanol–acetic acid (47.5:47.5:5). The flow-rate of the solvent was controlled to 5 μl/min. All spectra shown were the average of 256 individual spectra for a total analysis time of approximately 6.4 s. This indicates that each spectrum has a sample volume of approximately 500 nl.

The ion optics of the mass spectrometer were at the following voltages: pinhole leak +7.9, screen −4.9, first einzel element −20.1, second einzel element −7.4, third einzel element −99.9, and the ELFS plate −23.2. The quadrupole filter was biased at −5.2 V. The electron multiplier was operated at 1.75 kV and the collision dynode was at −4 kV.

2.3. Resolving power and resolution

In this paper we refer to both resolving power and resolution. Resolving power for ion mobility spectrometry is defined by a one-peak quotient:

$$R_p = \frac{t_d}{w_h}$$

where t_d is the ion drift time and w_h is the ion pulse duration at the detector measured at half of the maximal intensity. This measure of separation power is similar to that normally used in chromatography (theoretical plates) given by the relation:

$$N = 5.55 \cdot \left(\frac{t_d}{w_h}\right)^2$$

or

$$N = 16 \cdot \left(\frac{t_d}{w_b}\right)^2$$

When we refer to resolution in this paper we are using the standard two peak definition of resolution commonly used in chromatography:

$$R_s = \frac{t_{d2} - t_{d1}}{(w_{b1} + w_{b2})/2}$$

or

$$R_s = \frac{t_{d2} - t_{d1}}{w_{bavg}}$$

where t_d is the drift time of two consecutive peaks and w_b is the peak width at base.

2.4. Reagents

The solvents were all HPLC grade and were purchased from J.T. Baker (Phillipsburg, NJ, USA). The 20 amino acids were used without further purification and were purchased from Sigma (St. Louis, MO, USA).

3. Results and discussion

3.1. IMS spectra of amino acids

The 20 amino acids were sprayed into the IMS instrument at 1.0 μg/ml to determine their drift times. Fig. 1 shows the mobility spectra of all 20 amino acids. The drift time of each ion is shown in parentheses. As can be seen from the drift times, all of the amino acids had different drift times. The drift times were measured at least four times and in all cases the replicate measurements were the same. This would indicate that the standard deviation of the drift time measurement was less than the rate at which the measurements were taken. The data point were acquired every 0.02 ms indicating that the standard deviation of the measurement was 0.02 ms or less. It can also be seen from the spectra that many of the amino acids have similar drift times. In fact, from serine to methionine there are 15 amino acids that have drift times within 1.7 ms. With the current resolving power of the instrument (about 75 for singly charged ions) it would be impossible to see significant resolution of any two adjacent ions in this crowded region of the spectrum. The most difficult three ions to separate would be histidine, lysine, and methionine. The calculated resolving power required to achieve a separation of these ions at a resolution of 0.5 would be 240. Resolving powers this high have recently been reported for multiply charged ions using IMS [21].

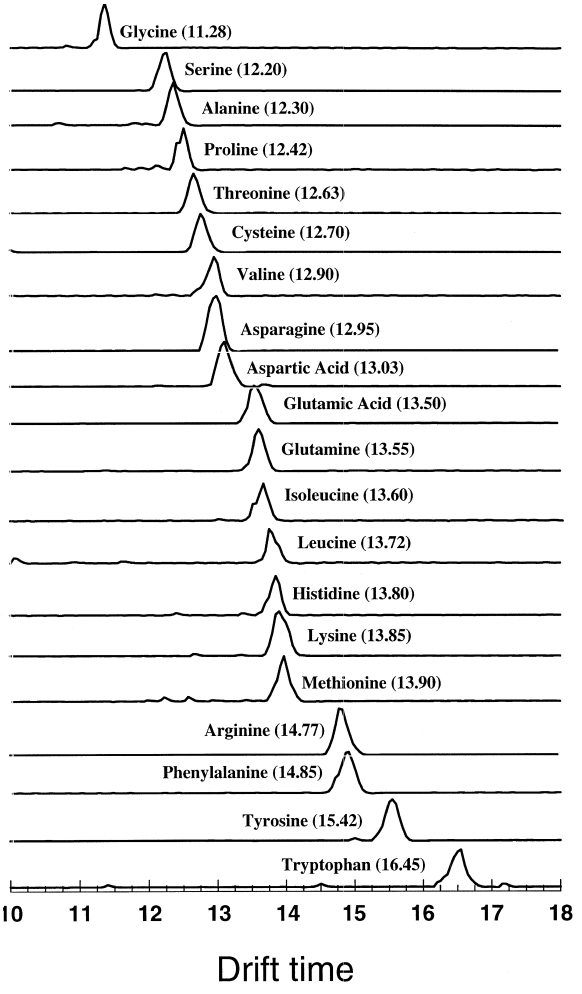

Fig. 1. Ion mobility spectra of the 20 common amino acids at a concentration of 1.0 μg/ml. Drift time scale in ms.

3.2. Ion formed, reduced mobility constant (K_0), and limits of detection

All of these analytical figures of merit are shown in Table 1. The identity of the ions formed was determined by mass spectrometry. Protonated molecules were formed for all 20 amino acids. In some cases at high amino acid concentrations sodium adduct ions were also seen in addition to the protonated molecules. The reduced mobility constant

Table 1
List of drift times, K_0 values, and limits of detection for the 20 common amino acids using electrospray ionization ion mobility spectrometry

Amino acid[a]	Molecular mass	Drift time (ms)	K_0	Absolute LOD[b] (pg)
Glycine	76	11.28	1.968	160±30
Serine	106	12.20	1.819	130±30
Alanine	90	12.30	1.805	130±10
Proline	116	12.42	1.787	170±20
Threonine	120	12.63	1.757	160±10
Cysteine	122	12.70	1.748	230±40
Valine	118	12.90	1.721	110±20
Asparagine	133	12.95	1.714	210±40
Aspartic acid	134	13.03	1.704	290±50
Glutamic acid	148	13.50	1.644	240±20
Glutamine	147	13.55	1.638	290±90
Isoleucine	132	13.60	1.632	120±10
Leucine	132	13.72	1.618	250±30
Histidine	156	13.80	1.608	120±10
Lysine	147	13.85	1.603	50±2
Methionine	150	13.90	1.597	320±80
Arginine	175	14.77	1.503	50±7
Phenylalanine	166	14.85	1.495	96±5
Tyrosine	182	15.42	1.440	160±20
Tryptophan	205	16.45	1.349	700±100

[a] All ions were protonated molecules $(M+H)^+$.
[b] Limit of detections (LODs) were the amounts required to produce signals three times those of the noise levels.

(K_0) values were all calculated using the usual method [23]. This paper presents the first published mobility constants for most of these ions. Finally the limits of detection for each compound were determined using the standard definition of the quantity required to give a signal-to-noise ratio of 3. For each compound a calibration curve was made using the following four concentrations: 5, 10, 20 and 50 ng/ml. The lowest value, 5 ng/ml was within one order of magnitude of the calculated detection limits for all of the 20 amino acids. The range of absolute limits of detection was from 50 to 700 pg. This showed a range in terms of moles of ~300 to 3 pmol. As might be expected the most sensitive amino acid was arginine, followed closely by lysine. These are the most basic amino acids and would be expected to be very sensitive using positive ESI. The acidic amino acids (aspartic and glutamic acid) were both near the least sensitive, but neither was as insensitive as tryptophan. At any rate the calculated values were all below 5 pmol.

4. Conclusions

Currently, IMS has the potential to distinguish any amino acid from another based solely on their calculate reduced mobility constant. With continued improvements of the resolving power of IMS instrumentation it should be possible to separate any mixture of amino acids. Based on this work, the required resolving power needed to get partial separation of all 20 amino acids is 240. This possibility coupled with the speed of IMS analyses makes it a very attractive tool for future amino acid analysis.

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