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Heterogeneous cation induced clusters formed at micro-droplets surface

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Micro-droplets surface can be treated as a simplified model to study reactions on cell membrane. A novel group of heterogeneous cations induced clusters (HeteroCICs) that formed at micro-droplets surface is discovered by mass spectrometry (MS). This novel caregory of clusters are produced through two kinds of cations co-induced self assemblies of thymine molecules. Applying this phenomenon, an on-line derivatization and quantitation method of "ambient MS" style has been established, which can be used to real time monitor fish freshness changes during shelf-life.

Molecular recognition at cell membrane lays the foundation for intercellular communications.[1-3] The effort to shed light on the pathways of serious chemical reactions at cell surface would improve our understandings of basic living processes. Micro-droplets generated from electrospray or neutral spray, whose volume resemble those of single cells, can be regarded as a mimic model to study the chemistry both "on" and "in" cells.[4] Previously, the different pH values "on" and "in" micro-droplets have been stressed.[5] The unique acceleration effect within micro-droplets[6] and at the micro-droplets surface[7] has also been discussed. Specific reactions that only occurred at micro-droplets surface can be of significance. Although proton and electron transfers[8-10] on micro-droplet surface have been reported, the potential of non-covalent molecular recognitions at micro-droplet surface has not been considered. Non-covalent interactions play key roles in intercellular communications, so specific non-covalent reactions that only occurred at micro-droplets surface are supposed to be highly interesting and important.

The formation process of magic number clusters is a typical example of supramolecular recognition. Previously, the homogeneous cations induced thymine (and its analogs) clusters have been studied in our group.[11-14] Ammonium ion, alkali metal ions, and ions of organic primary amines can independently induce thymine (or its analogs) clusters. It has been shown such homogeneous cations induced clusters (homoCICs), in which only one kind of central cation is involved, are formed in liquid phase. In this work, we will show heterogeneous cations induced clusters (HeteroCICs), in which two kinds of central cations are involved, are formed only at micro-droplets surface. Moreover, a quantitation method applying this newly discovered phenomenon has also been established. This novel method is another example of "ambient mass spectrometry (MS)"[15-17] detection, which features in very few sample-preparation and quick procedures.

Experiments were conducted upon a home-built multichannel electrospray ionization (ESI) array[18] (MRESI, Scheme S1), upgraded with a neutral desorption (ND) system (Fig 1a). This design was similar to neutral desorption extractive electrospray ionization[19] (ND-EESI), except that multiple ESI emitters were used here. In the ND system, volatile compounds from liquid or solid samples can be desorbed and entrained in the neutral inert gas (nitrogen gas in this case) flow. The aerosol/molecules within the neutral gas flow can later be merged with charged micro-droplets generated from the ESI plumes, and after ionization process, the MS signals corresponding to the desorbed compounds can be obtained. The locations of the ND tube end (I.D. 2 mm), ESI emitters and their positions relative to the MS inlet are shown in Fig 1b.

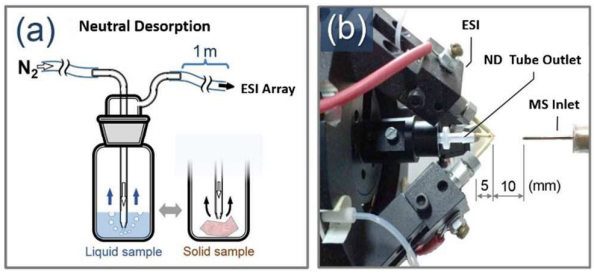

Fig 1 (a) The neutral desorption system coupled to the multichannel ESI array. Both liquid and solid samples can be desorbed by nitrogen gas flow. The neutral plume can be post-ionized by ESI spray plumes. (b) ND-MRESI configuration. The relative positions of the neutral desorption tube outlet, the three ESI sprayers and the MS inlet are demonstrated.

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Trimethylamine (TMA) is a typical example of tertiary amines, and according to our former experimental results, it cannot induce homoCICs in traditional ESI condition. Here, if TMA (10 mL, 0.6 mM) was desorbed by nitrogen gas flow and thymine (0.2 mM) solution was used in ESI post-ionization sprays, as shown in Fig 2a, no magic number clusters can be obtained. The ion m/z 610 was a background ion most probably originated from the plastic ND tube. However, when the TMA solution was spiked with ammonia (0.1 % v/v), and similar experimental procedure was applied, a TMA and ammonium co-induced thymine cluster, m/z 1110, can be obtained in the mass spectrum (Fig 2b). This is the first heteroCIC discovered in our lab. To our surprise, if TMA, ammonia and thymine were pre-mixed, and then the mixed solution was put to an ESI-MS analysis, only homoCICs of $[Na+T_4]^+$,$[NH_4+T_5]^+$ and $[2NH_4+T_{15}]^{2+}$ can be obtained in the mass spectrum, as shown in Fig 2c. These results indicated the heteroCIC m/z 1110 was unique compared to all the thymine clusters discovered previously. The structure, the way it formed and the applications of this special cluster are our major concerns.

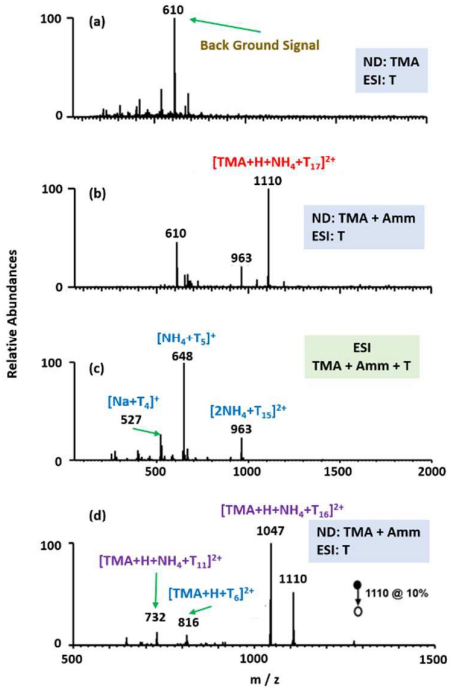

Fig 2 (a) ND-MRESI-MS spectrum obtained when the TMA solution was neutrally desorbed and the thymine solution was used as the ESI post-ionizing spray. (b) ND-MRESI-MS spectrum obtained when the TMA and ammonia mixed solution was neutrally desorbed and the thymine solution was used as the ESI post-ionizing spray. (c) ESI-MS obtained when mixed solution of TMA, ammonia and thymine was analyzed. (d) ND-MRESI-MS/MS spectrum of m/z 1110 in the experiment of (b).

Previously, we have evidenced that magic number thymine clusters are commonly constructed from building blocks of 5 or 6 membered thymine circles.[11-14] Central cations were usually located at the cavities of such circles. Fig 2d illustrated the fragments of the cluster m/z 1110 obtained under collision induced dissociation (CID) condition. Based on previous data and the MS/MS results, the plausible structure of the ion m/z 1110 is proposed in Fig 3. The model structure was optimized *via* molecular mechanical calculations as described previously[20]. The cluster is induced by one protonated TMA cation and one ammonium cation. The two cations are nearly aligned if we see them in the downward direction (the ammonium part defined as top, Fig 3b). 17 thymine molecules constitute, from top to bottom, "5+6+6" three consecutive circles, which surrounded the two central cations, as shown in Fig 3a. This structure model can well explain the obtained fragmentation pathways (Fig S1).

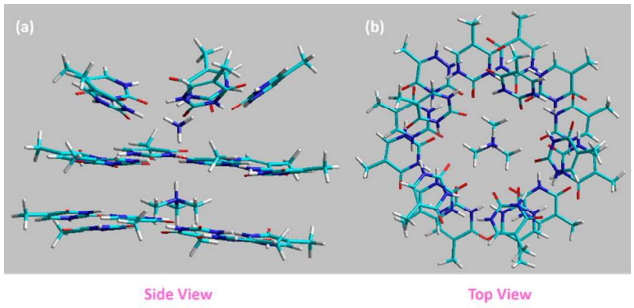

Fig 3 Proposed structure of the HeteroCIC m/z 1110. The structure has been optimized via molecular mechanical calculations. (a) Side view and (b) Top view of the HeteroCIC.

The next question is where this heteroCIC form. We propose three possibilities: 1) it is formed in liquid phase, 2) it is formed in gas phase, and 3) it is formed at the liquid/air interface, or described as the micro-droplets surface. EESI methods generate ions in milliseconds time scale. Recently, dual nano-ESI *via* theta capillary tips has been established as an *in situ* liquid-mixing method to generate micro-droplets with life-time of also milliseconds.[21-23] So we tried to implement dual nano-ESI *via* theta capillary tips to synthesize the cluster m/z 1110. As shown in Fig S2, under all possible conditions, no such cluster can be obtained. These experiments evidence that the cluster m/z 1110 is not formed in liquid phase. Next, the reagents in the neutral spray and the ESI sprays were systematically changed, and the influence on the signal intensity of m/z 1110 was investigated. The combinations of reagents and the experimental results are presented in Fig 4a. To ensure the concentrations of the reagents introduced into the ionization zone in all the conditions are the same, instead of the ND system, a neutral spray (NS) assisted by nebulizing gas is implemented (details in the Electronic Supplementary Information). When TMA and ammonia were co-sprayed in the NS plume and the thymine solution was applied as the ESI post-ionization plume (condition 1), the highest signal intensity of m/z 1110 was obtained. However, when TMA was sprayed in NS plume and ammonia/thymine mixed solution was applied as the ESI post-ionization plume, or when ammonia was sprayed in NS plume and

TMA/thymine mixed solution was applied as the ESI post-ionization plume (conditions 2 and 3, respectively), the signal intensities of the cluster m/z 1110 were an order of magnitude lower. When TMA/ammonia/thymine were all sprayed in the NS plume and pure solvent (1:1 M/W) was applied as the ESI post-ionization plume (condition 4), signal of m/z 1110 was absent. If the cluster m/z 1110 was formed in gas phase, under conditions 1-4, the obtained signal intensities of m/z 1110 would be similar. However, the experimental data denies such possibility, because under conditions 1-4, the signal intensities of m/z 1110 differed greatly. Considering the abovementioned evidences, we propose the heteroCIC m/z 1110 is formed at the liquid/air interface (Fig 4b).

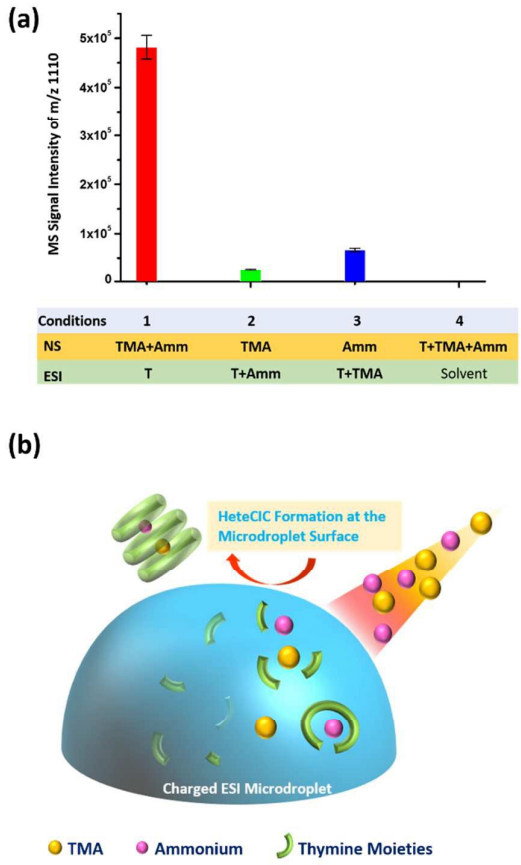

Fig 4 (a) Signal intensities of HeteroCIC in different conditions. (b) Schematic illustration of the HeteroCIC formation at micro-droplets surface.

The formation of the cluster m/z 1110 at micro-droplets surface can well explain the following two facts. First, in conditions 2 and 3, because some of the cations "buried" in the charged micro-droplets, the surface concentrations of the cations were lower than those in condition 1, which resulted in lower signal intensities of m/z 1110. Second, similar to condition 2, various ammonium salts were used instead of ammonia. With the carbon chain of the anion in the ammonium salts increasing, the signal intensities of both m/z 963, a cluster formed in liquid, and m/z 1110 decreased, but the later decreased much more intensely (Fig S3). As the carbon chain of the anion in the ammonium salts close to 10, they can be considered as surfactant, and the micro-droplets surface would be largely occupied by such anions. Since the heteroCIC is formed at the micro-droplets surface, the occupancy of surfactant at the surface would reasonably inhibit its formation. While for homoCIC (m/z 963, for instance), since it is formed "in" micro-droplets, the negative effect of the surfactant would be much less (Note that generally, surfactants would cause ion suppression in MS analyses).

TMA is an important biogenic amine and can be regarded as the indicator of fish freshness. According to the Chinese National Standard, when the concentration of TMA exceeds 10 ppm, the fish is not eligible for sell.[24] Fish meat of 3g was cut from a fish body, spiked with ammonia, and then was directly put into the ND bottle. The amount of ammonia spiked on the fish substrate and the concentration of thymine in the ESI spray solution were optimized (Fig S4). When the fish was fresh, as shown in Fig 5a, the signal of m/z 1110 was weak. When the fish was spoiled, as shown in Fig 5b, the signal of m/z 1110 was strong. As the EIC in Fig 5b shows, the signal intensity of m/z 1110 would decrease after a period of gas desorption, but if the position of the fish meat relative to the gas outlet in the ND bottle was change to another spot, the signal intensity of m/z 1110 would return to the similar level. For each sample, quantitative experiments were performed by repeating this procedure 5 times and the data were collected at the beginning 60 s at each spot. Calibration curve was established as shown in Fig 5c. The limit of detection (LOD) is 0.2 ppm, which is as well as or slightly better than those obtained from published methods.[25-27]

In real cases, the real time monitoring of fish freshness within the first few hours is challenging. The ideal method should be quick, sensitive and selective. By implementing our instrument, one fish sample can be analyzed within minutes and no labor-consuming sample preparations are needed. Since only TMA with ammonium cation can induce the cluster m/z 1110, high selectivity is guaranteed. Fig 5d shows the real time monitoring of the freshness changes of three kinds of fishes, under either room temperature or mild cold storage conditions. The detected concentrations were all below 10 ppm, demonstrating our method can be implemented to trace the spoiling process of fishes during their shelf-life. Dimethyl amine and imidazole can also induce heteroCICs with the assistance of ammonium, as shown in Fig S5-S6. Similar quantitation methods[28] can be established accordingly.

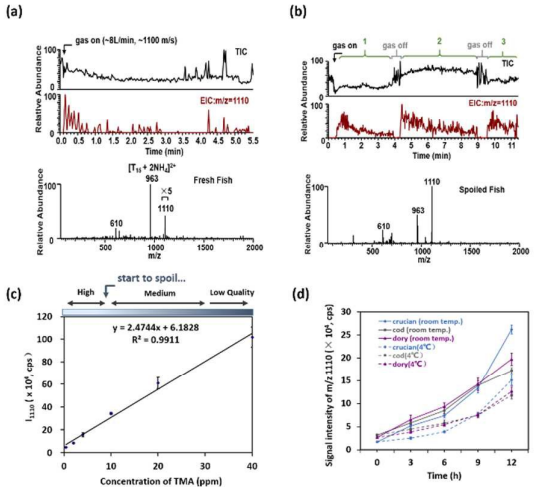

Fig 5 (a) ND-MRESI-MS when fresh fish pieces (Cod) were analyzed. The total ion chromatogram (TIC) and extracted ion chromatogram (EIC) are shown in the upper panels. (b) ND-MRESI-MS when spoiled fish pieces (Cod, 24 h at room temperature) were analyzed. The TIC and EIC are shown in the upper panels. (c) Calibration curves established using Crucian fish substrate. LOD: 0.2 ppm. (d) Real time monitoring of the fish freshness changes during their shelf-life.

Cell surface is a special platform for molecules/ions to react. Electrospray and neutral spray can generate micro-droplets that can be treated as simplified models to study reactions "in" and "on" cells. In this paper, the first example of non-covalent clusters formed only at micro-droplets surface is demonstrated. Moreover, an "ambient MS" quantitation method of TMA was established, and the real time monitoring of fish freshness has been conducted. The present work has offered a novel practical strategy to derivatize and quantify small molecules and will also intrigue forthcoming works to investigate supramolecular chemistry on the liquid/air interface.

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