Higher-ordered structures based on the self-assembly of simpler molecules are of interest in a variety of fields including structural biology, nanotechnology and supramolecular chemistry.

Modified purine bases such as 3-methylxanthine (TMX) have been found to self-assemble in the presence of alkali metals and ammonium cations in the gas phase and in solution.

Miniaturised high-field asymmetric waveform ion mobility spectrometry (FAIMS) and travelling wave drift tube ion mobility spectrometry (IMS), both combined with mass spectrometry, have been used to investigate self-assembling, non-covalent complexes of TMX in the gas phase.

Travelling wave IMS (TWIMS) analysis has been used to determine collision cross sections (CCS) of selected TMX complexes.

3-Methylxanthine (TMX) is shown to self-assemble in the gas phase to form clusters around a stabilising cation (Fig. 2), which have been analysed using ESI-MS and ESI-FAIMS-MS. Supramolecular structures of TMX, where TMX forms tetrameric non-covalently bound structures around a Na\(^+\), K\(^+\) or Ca\(^{2+}\) cation have all been observed (Fig. 3). Clustering of TMX from single tetrameric complexes to higher-ordered quadruplex complexes of up to six TMX tetramers has been observed using FAIMS-MS in the presence of Na\(^+\) (Table 1). The focus of this preliminary study is on these monomeric and singly charged tetrameric complexes of TMX with sodium.

Table 1: TMX monomer and singly charged tetrameric complexes

<table>
<thead>
<tr>
<th>TMX Complex</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMX+Na(^+)</td>
<td>189.04</td>
</tr>
<tr>
<td>TMX+Ca(^2+)</td>
<td>203.75</td>
</tr>
<tr>
<td>TMX+K(^+)</td>
<td>1391.56</td>
</tr>
</tbody>
</table>

FAIMS-MS has been used for the analysis of the non-covalent complexes formed by TMX. The singly charged TMX\(^n-\) (n = 1-4) complexes show maximum FAIMS transmission at different CF values, with the optimum CF decreasing as the size of the cluster increases (Fig. 4).

The signal to noise ratio of low abundance multiply charged species (Fig. 5) can be improved using FAIMS-selection prior to MS analysis.

Separation of TMX complexes with different charge states can be achieved (Fig. 6) using FAIMS-selection of the appropriate charge state (Fig. 6 (d)).

IMS analysis combined with tandem MS analysis used to determine the CCS of the singly charged tetrameric complexes.

IMS analysis combined with tandem MS analysis used to determine the CCS of the singly charged tetrameric complexes.

The ion mobility spectra of the singly charged tetrameric complexes were determined using peptide standards of known CCS (Table 2).

Table 2: Experimentally measured CCS of [TMX+Na\(^+\)]\(^n\)- singly charged tetrameric complexes

<table>
<thead>
<tr>
<th>TMX complex</th>
<th>m/z</th>
<th>CCS (A(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMX+Na(^+)</td>
<td>189.04</td>
<td>181</td>
</tr>
<tr>
<td>TMX+Na(^+)+Na(^+)</td>
<td>267.2</td>
<td>181</td>
</tr>
<tr>
<td>TMX+Na(^+)+K(^+)</td>
<td>2037.6</td>
<td>338</td>
</tr>
</tbody>
</table>

IMS analysis combined with tandem MS analysis used to determine the CCS of the singly charged tetrameric complexes.

The ion mobility spectra of the singly charged tetrameric complexes were determined using peptide standards of known CCS (Table 2).

IMS analysis combined with tandem MS analysis used to determine the CCS of the singly charged tetrameric complexes.

The ion mobility spectra of the singly charged tetrameric complexes were determined using peptide standards of known CCS (Table 2).

Conclusions

The hyphenation of FAIMS-MS and IMS-MS has been used for the analysis of TMX complexes.

This preliminary study into the structural analysis of TMX complexes shows a complexity of non-covalently clustered structures.

FAIMS-selection has been used for the separation of overlapping charge states of TMX complexes.

Increased signal to noise ratio is observed for higher-order TMX complexes using FAIMS-MS.

TMX singly charged complexes formed in the presence of sodium show different CF values for maximum ion transmission.

Tandem MS combined with IMS has been used to obtain ion mobility spectra of TMX fragments.

Acknowledgements

We thank EMSS for providing a travel grant to attend this conference and Owlstone Ltd and Agilent Technologies for financial and technical support.