ECD/ETD-type Fragmentation Induced at (or near) Atmospheric Pressure

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INTRODUCTION

ECD/ETD-type fragmentation has been reported previously for peptides at atmospheric pressure in APPI source [1] and is hypothesized to be a consequence of ion/ion and/or ion/radical reactions. Herein, to investigate a mechanism leading to formation of c-type ion fragments at atmospheric pressure, we have designed and built a flow reactor which is constructed as a nozzle extension and can be mounted directly to the heated capillary of a mass spectrometer. The ultimate goal of this project is to deliver efficient method of fragmentation of the ESI-generated polypeptide ions at atmospheric pressure that would provide sequence information orthogonal to that obtained in CID and would be applicable in any mass spectrometer including low-cost bench-top instruments such as triple- and single- quadrupoles.

METHOD

Instrumentation

Peptide and protein ions produced by ESI and ions/radicals from corona discharge are mixed together within the flow reactor machined from stainless steel (similar to the design published by Frey et al [2]). Diameter of the flow reactor is 0.5 mm for the first 5 mm and 2.5 mm for the last 40 mm of its length. These dimensions should cause minimal pressure drop through the reactor because the majority of the pressure decrease will occur at the long and narrow capillary of MS instrument. The standard cartridge heaters are used to vary the temperature of the flow reactor in the range 20-500°C. The temperature of the gas flowing through the corona discharge can be adjusted separately using coiled tube wrapped with band heaters. The flow reactor front-end has a counter-current flow of "curtain" gas (nitrogen) to aid in dissolvation of droplets generated by ESI and to sweep away unwanted neutral species from the entrance aperture. All experiments were performed using Thermo LCQ "classic" instrument.

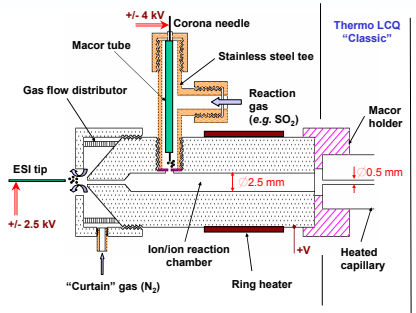

Figure 1. Schematic of flow reactor for ion/molecule reactions analysis.

Ion transmission efficiency

$$\frac{I}{I_0} = exp\left[-5.784\left(\frac{2l}{d}\right)^2 \frac{kT}{qU_{eff}}\right]$$

where l and d are the length and diameter respectively, and U_{eff} is the effective potential difference.

$$U_{eff} = \frac{vl}{K}$$

where v is the average gas flow speed and K is the ion mobility.

Using K=1cm²/Vs (typical ion mobility), equation gives **ion transmission efficiency** at room temperature through the proposed flow reactor of approximately **95.8%**. The increase in the temperature to **200°C** will lead to decrease in the ion transmission **down to 93%.**

Ion/molecule reaction time

The average linear speed of the gas flow in the reactor:

$$v = \frac{4G}{\pi d^2},$$

where G is the gas flow rate (17 cm³/s, experimental value), d is the diameter of heated capillary (0.5 mm).

It gives an average gas flow speed of 3.5 m/s through the central channel of Ø2.5 mm. Thus, a residence time (and therefore **ion/molecule reaction time**) can be estimated as approximately **10 ms**.

RESULTS

✓ The presence and intensity of the fragmentation depends on the temperature and gas flow through corona discharge.
✓ No fragmentation is observed for temperatures less than 380°C afterward the fragmentation efficiency increases roughly exponentially.
✓ The fragmentation occurs for a variety of pure gasses (Ar, N_2, O_2 etc) flowing through corona. No fragmentation was observed with helium.
✓ The addition of water (H_2O) or hydroxyl peroxide (H_2O_2) vapors to the corona discharge region significantly enhances the fragmentation efficiency.
✓ The occurrence of the fragmentation is independent on the corona polarity.
✓ c-type fragments are often observed along with the y/b fragments for a variety peptides and proteins.

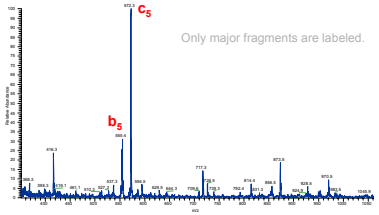

Figure 2. Fragmentation mass spectrum of bradykinin (positive corona, positive ESI, temperature 400°C, gas flow 250 cc/min).

Suggested chemistry

$$(M+H)^+ + OH^{\cdot} \xrightarrow{400°C} \text{fragments} \quad (1)$$

$$M^{\cdot}+ OH^{\cdot} \xrightarrow{400°C} \text{fragments} \quad (2)$$

Scheme 1. Suggested mechanism for the peptide fragmentation.

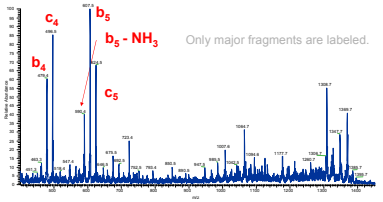

Figure 3. Fragmentation mass spectrum of Substance P (positive corona, positive ESI, temperature 420°C, gas flow 280 cc/min).

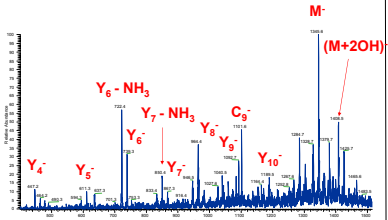

Figure 4. Fragmentation mass spectrum of Substance P (negative ESI, positive corona, temperature 420°C, gas flow 280 cc/min)

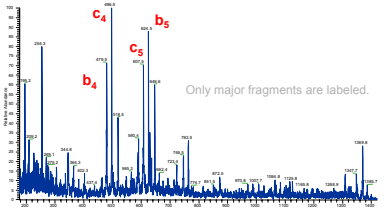

Figure 5. Fragmentation mass spectrum of Substance P (positive ESI, positive corona) with an addition of H_2O_2 vapor to corona discharge.

✓Hydroxyl radicals are common product of corona discharge. They can be generated from oxygen or water molecules always present in an ESI source.
✓ It is well-known that hydroxyl radicals can cause backbone cleavage of peptides and proteins although at room temperature reactions at side-chain sites are preferred and backbone cleavage reactions are observed only at high concentrations of hydroxyl radicals [3].
✓ An additional sign in favor of hydroxyl radical-induced fragmentation is the observed significant enhancement (few orders of magnitude) of fragmentation efficiency with an addition of hydroxyl peroxide vapors into corona discharge region.

CONCLUSIONS

✓ The novel method of peptide and protein fragmentation at atmospheric pressure is successfully demonstrated for a set of common peptides and proteins (bradykinin, substance P, fibrinopeptide A, γ-endorphin, insulin, myoglobin etc).
✓ Proposed mechanism involves interaction of ESI-generated peptide ions with OH· radicals originating from the corona discharge source.
✓ The frequent occurrence of c-type fragments along with y/b-type fragments in spectra brings an additional information orthogonal to conventional CID spectra that can potentially result in an improved sequence coverage.

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