Fitting of ETD Rate Constants for Doubly and Triply Charged Ions in a Linear Ion Trap

Dirk Nolting, Thermo Fisher Scientific, Bremen, Germany

Overview

Purpose: The rate constants characterize the ETD reaction. Knowledge of the rate constants gives information on fragment current yields and fragmentation pathways.

Methods: ETD reaction kinetics were measured and fitted to a consecutive reaction kinetics model.

Results: By fitting rate constants for different fragments it is possible to assign reaction pathways.

Introduction

Ion/ion reactions in the gas phase are a versatile tool for probing molecular properties. In mass spectrometry electron transfer dissociation has become a common fragmentation method, as it often results in complementary information in comparison to the widely used thermal dissociation methods by collisional heating.

The maximum product ion current, and thus the sensitivity are determined by consecutive reaction kinetics. As each reaction step involves charge reduction, it is important to stop the reaction at an early point where the number of remaining charges for product ions reaches is maximum. Both, the maximum product ion current and the optimal reaction time are determined by the rate constants. ETD reaction kinetics were measured and compared with the theoretical model.

Methods

Sample Preparation

All experiments were carried out with a 1 pmol/µl solution of Angiotensin I.

A mixture of 50:50 methanol/water (0.1% acetic acid) was used as solvent. All solvents were HPCL grade from Fisher Chemical. Angiotensin was purchased from Sigma-Aldrich (A9650) and used without further purification.

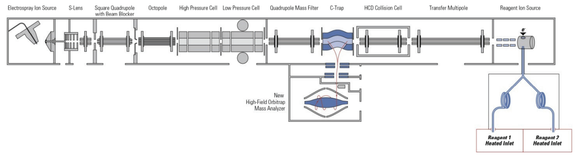
Mass Spectrometry

All measurements were done with an Thermo Scientific Orbitrap Elite hybrid mass spectrometer (Figure 1). The sample was directly infused using an IonMax ESI ion source. For the analyte ions a target value of $1x10^5$ was used and an anion target of $2x10^6$ if not noted otherwise. Orbitrap detection was used for all mass spectra to avoid saturation effects of the channeltron detectors. Transients were recorded using build-in software features.

Data Analysis

Transients were extracted from the recorded raw files using Thermo Scientific Xcalibur 2.1 Qual Browser. The extracted transients were imported into Origin 8 and fitted using its non-linear curve fitting routines.

FIGURE 1. Schematics of an Orbitrap Elite™ hybrid MS equipped with an ETD module. For ETD anions and analyte cations are trapped simultaneously in the linear trap, allowing an electron transfer from the fluoranthene anion to the analyte cation causing charge reduction and fragmentation.



Results

Modelling Consecutive Reaction Kinetics

ETD is a consecutive reaction where each reaction is a charge reduction:

$$[M+nH]^{n+} \xrightarrow{k_1} [M+nH]^{(n-1)+} \xrightarrow{k_2} [M+nH]^{(n-2)+} \xrightarrow{k_3} \dots$$

The reaction rates can be described by a system of differential equations:

$$\frac{d}{dt}\mathrm{a}(t) = -k_1\,\mathrm{a}(t) \quad (1)$$

$$\frac{d}{dt}\mathrm{b}(t) = k_1\,\mathrm{a}(t) - k_2\,\mathrm{b}(t) \quad (2)$$

$$\frac{d}{dt}\mathrm{c}(t) = k_2\,\mathrm{b}(t) - k_3\,\mathrm{c}(t) \quad (3)$$

Solving the differential equations gives the chronological sequence for each charge state:

$$\mathrm{a}(t) = a_0\,e^{-k_1 t} \quad (4)$$

$$\mathrm{b}(t) = \frac{a_0\,k_1\,e^{-k_1 t}}{k_2 - k_1} - \frac{a_0\,k_1\,e^{-k_2 t}}{k_2 - k_1} \quad (5)$$

$$\mathrm{c}(t) = \frac{a_0\,k_1\,k_2\,e^{-k_3 t}}{{k_3}^2 + (-k_2 - k_1)\,k_3 + k1\,k_2} - \frac{a_0\,k_1\,k_2\,e^{-k_2 t}}{(k_2 - k_1)\,k_3 - {k_2}^2 + k_1\,k_2} + \frac{a_0\,k_1\,k_2\,e^{-k_1 t}}{(k_2 - k_1)\,k_3 - k_1\,k_2 + {k_1}^2} \quad (6)$$

Fitting of the Precursor and the Charge Reduced Species

For the doubly charged Angiotensin I, rate constants can be fitted using the precursor and the total fragment current. In order to obtain the first rate constant k_1 the precursor intensity (Figure 2a) was fitted to eq. 4. The result shows a very good correlation. The second rate constant k_2 was obtained by fitting the total fragment current to eq. 5. To reduce the degrees of freedom, k_1 was taken from the previous fit and kept constant, thus, only A_0 and k_2 of eq. 5 were fitted.

Fragments of triply charged precursors can origin from different reaction pathways and may have different charge states obscuring the detector response. Thus, only the charge reduced species were used for fitting the rate constants (Figure 4b). This avoids ambiguities in the assignment of the fragments, i.e. to which reaction pathway the fragment current contributes. For fitting the $[M+3H]^{2+}$ species k_1 was taken from the previous fit and kept constant, i.e. only A_0 and k_2 of eq. 5 were fitted. Accordingly, $[M+3H]^{+\bullet}$ was fitted constant k_1 and k_2 from the previous fits and only fitting k_3 and A_0 to eq. 6. All fitted curves show good correlation to the experimental data.

Maximum Fragmentation Limit

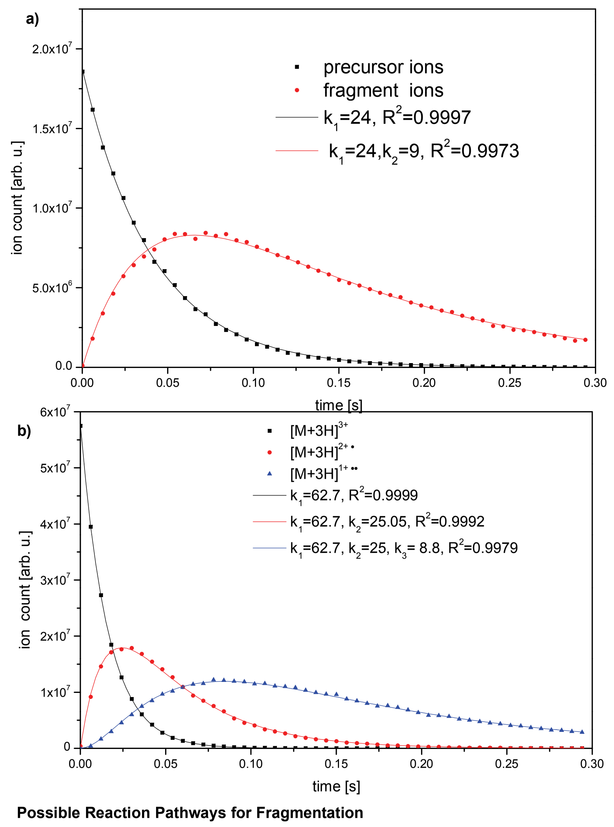
The ETD efficiency, i.e. the maximum number of fragments and accordingly the maximum product current (Table 1) is given by the rate constants. For the triply and higher charged precursor ions the time for the maximum number of product ions is different from time for the maximal product current.

Table 1. Maximal product ion current and product ion numbers for Angiotensin I calculated from the measured rate constants.

	Maximum number of product ions (reaction time)	Maximum product ion current (reaction time)
2+ precursor	56% (66 ms)	28% (66 ms)
3+ precursor	~86% (45 ms)	~45% (33ms)

For the triply charged precursor exist several pathways which makes it difficult to calculate the efficiencies as the branching ratio is not known. Most product ions (>95%) follow the main reaction pathway forming first a doubly and then a singly charged ion. Accordingly, only those ions were taken into account.

FIGURE 2. Reaction process of the precursor and the charge reduced species for a) doubly and b) triply charged Angiotensin I. Points are measured data and solid lines are results from fitting. Given are the fitted rate constants and the corresponding coefficient of determination.



Possible Reaction Pathways for Fragmentation

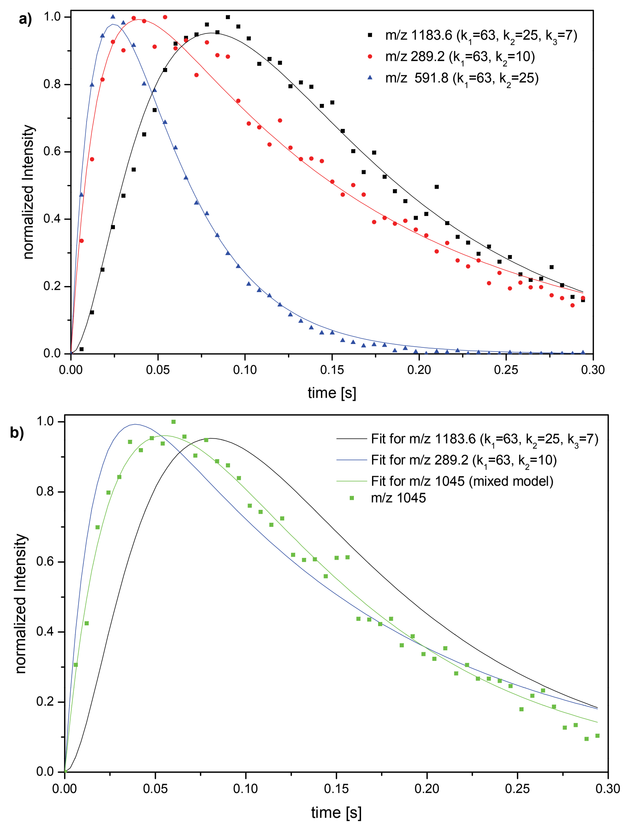
For a triply charged precursor exist several reaction pathways which can lead to fragmentation:

1. ETD forming one doubly charged fragment F_1
2. ETD forming two singly charged fragments F_2
3. Two charge reduction steps forming one singly charged fragment F_3

$$[M+3H]^{3+} \xrightarrow{k_1} [F_1]^{2+} \xrightarrow{k_2} [F_3]^{+} \xrightarrow{k_3} [\text{neutral}]$$
$$[M+3H]^{3+} \xrightarrow{k_1} 2[F_2]^{+\bullet} \xrightarrow{k_3} [\text{neutral}]$$

Each reaction pathway shows a characteristic reaction process, i.e. the corresponding species have maximum abundance at different points in time and different peak shapes (Figure 3).

FIGURE 3. Characteristic reaction processes for different reaction pathways. 3a) First generation ions show fast increase and a decay rate depending on their charge state (*m/z* 290.2, *m/z* 5912.8). Second generation ions start delayed and have a slow decay rate (*m/z* 1183.6). 3b) Some fragments as *m/z* 1045 can be formed after the first or second ETD step. The resulting kinetics can be modeled by the sum of first and second generation kinetics.

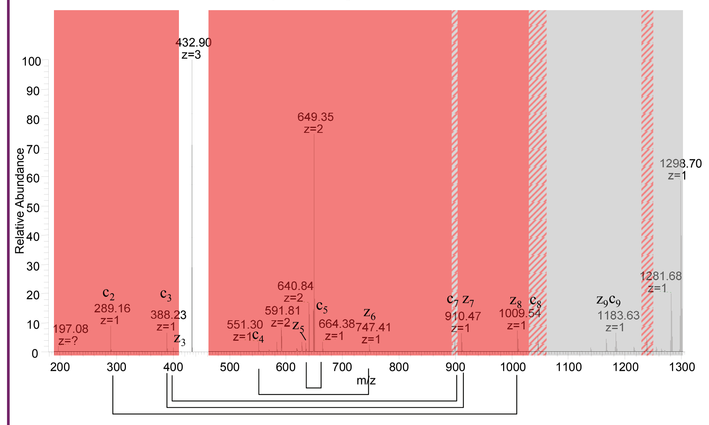


The mixed model (Figure 3b) is a combination of eq. 5 and eq. 6, i.e. $I_{m/z1045}(t)$=0.5*eq. 5 + 0.5*eq. 6 .

Mapping of First and Second Generation ions

The ETD spectrum of Angiotensin I can be divided into *m/z* ranges with first and second generation ions (Figure 4). A nearly complete series of c and z ions is formed by singly charged first generation ions. Most fragments are formed after the first ETD step. For most doubly charged fragments a charge reduced species is also present.

FIGURE 4. ETD spectrum of triply charged Angiotensin I. Different mass ranges show different kinetics. Red areas are first generation fragments, grey fragments are second generation fragments, hatched areas have mixed kinetics. Brackets denote complementary ions.



The ETD spectrum comprises a series of complementary c and z ions which contain most of the sequence information (Figure 4). Those ions are singly charged and are formed after the first ETD step. The optimal reaction for this reaction pathway is 35 ms which is close to time of the maximum product current.

Conclusion

- Measured ETD reactions kinetics can be described by rate equations for consecutive reactions assuming a first order reaction.
- Different reaction pathways can be assigned by the reaction kinetics.
- The maximum fragment yield for Angiotensin I can be calculated from the fitted rate constants (56% / 86% for the 2+/3+ precursor).
- Most sequence information containing ions are singly charged ions formed after the first ETD step.

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