International Journal of Mass Spectrometry 377 (2015) 646–654

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

An IMS–IMS threshold method for semi-quantitative determination of activation barriers: Interconversion of proline *cis* ↔ *trans* forms in triply protonated bradykinin

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ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form 27 June 2014
Accepted 1 July 2014
Available online 6 July 2014

Keywords:
Ion mobility spectrometry
Multi-dimensional
Peptide conformation
Activation energy

ABSTRACT

Collisional activation of selected conformations by multidimensional ion mobility spectrometry (IMS–IMS), combined with mass spectrometry (MS), is described as a method to determine semi-quantitative activation energies for interconversion of different structures of the nonapeptide bradykinin (BK, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg). This analysis is based on a calibration involving collision-induced dissociation measurements of ions with known dissociation energies (i.e., "thermometer" ions) such as leucine enkephalin, BK, and amino acid–metal cation systems. The energetic barriers between six conformations of $[BK+3H]^{3+}$ range from 0.23 ± 0.01 to 0.55 ± 0.03 eV. Prior results indicate that the major peaks in the IMS distributions correspond to specific combinations of *cis* and *trans* configurations of the three proline residues in the peptide sequence. The analysis allows us to directly assess pathways for specific transitions. The combination of structural assignments, experimentally determined barrier heights, onset of the quasi-equilibrium region, and dissociation threshold are used to derive a semi-quantitative potential energy surface for main features of $[BK+3H]^{3+}$.

1. Introduction

The biological activity of polypeptides is intrinsically tied to the structures and dynamics of the interacting species [1]. It is therefore important to consider not only the native structure, which is often assumed to be a single geometry of low energy, but also the ensemble of other structures that may exist transiently. A valuable means of understanding the multitude of possible structures and pathways associated with specific transitions is through examination of a biomolecule's energy landscape. In the past 20 years, conventional views of the "protein-folding problem" shifted from a strict pathway-dependent model involving a series of on- and off-route intermediates [2–4], to a more diffusion-driven funnel concept [5–8]. The latter idea involves a rough-surfaced landscape that consists of many local minima along a generally downhill path to the global minimum, i.e., the native structure [6]. These models help us to visualize the major structural transitions in proteins and guide computations; with a few exceptions [9,10], a detailed understanding of intermediate states (and transitions between them) is lacking – largely because of the inability of existing experimental methods to isolate and probe key intermediates, and assess barriers and pathways associated with specific transitions along the potential energy landscape.

In this paper, we analyze the nonapeptide bradykinin (BK), having the sequence Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg after it has been electrosprayed into the gas phase as $[M+3H]^{3+}$ ions. BK is a peptide hormone, which was successfully isolated by Andrade and Silva in 1956 [11] and in 1964 was used as the first biologically relevant application of Merrifield's solid-phase peptide synthesis approach [12]. Because of its relatively small size and the fact that it was available synthetically, BK has become an important model system for structural studies in solution as well as the gas phase. In the gas phase (the emphasis of this paper), Bowers and co-workers chose BK as the first peptide to be studied by ion mobility spectrometry (IMS) techniques [13]. The mobility of an ion through a buffer gas under the influence of a weak electric field depends upon the ion's shape and charge [14–18]. Their first measurements, combined with detailed molecular modeling [19] and collision cross section calculations [20–22], showed that singly charged BK adopts a tightly-folded globular structure. In other early studies of BK in the gas phase, Williams and co-workers investigated the reactivity of a series of chemically modified and amino acid deletion and substitution sequences, and showed that this ion exists as a "salt-bridged" structure [23]. Numerous other subsequent studies [24–29] appear to be consistent with these findings.

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http://dx.doi.org/10.1016/j.ijms.2014.07.012
1387-3806/

Recently, we extended the studies of BK in the gas phase to include structural insight into the triply charged ion. The IMS distribution for the $[BK+3H]^{3+}$ shows many peaks and thus is substantially more complicated than the single peak observed for the singly charged ion. In this paper, we use multidimensional ion mobility spectrometry–mass spectrometry (IMS–IMS–MS) measurements to obtain a semi-quantitative understanding of the potential energy surfaces associated with the multitude of stable $[BK+3H]^{3+}$ conformers. In this approach, electrosprayed $[BK+3H]^{3+}$ ions are separated in an initial drift tube, and enter a selection and activation region [30–33]. By studying the energetic thresholds necessary to convert one structure into another, we gain an understanding of the barriers and pathways between different $[BK+3H]^{3+}$ conformations.

We begin this study with a substantial amount of prior insight about the BK system. We reported evidence for at least ten independent conformer states that vary in abundance when solution compositions (aqueous and non-aqueous mixtures of varying proportion) are changed [34]. In additional studies we investigated a set of alanine-substituted BK-analogue peptides [35]. These results showed unambiguously that the major features in the $[BK+3H]^{3+}$ IMS distributions are associated with different combinations of *cis* (*C*) and *trans* (*T*) forms of the three proline residues, Pro^2, Pro^3, and Pro^7. The three most abundant $[BK+3H]^{3+}$ conformers (referred to as the A, B, and C states) were assigned as having the following *cis*/*trans* proline configurations: A as *CCC*; B as *CTT*; and, C as *TTC*.

Finally, we have previously investigated the transitions between different $[BK+3H]^{3+}$ conformations, and found that above a critical activation voltage all of the separated conformers that are produced by electrospraying BK will reach a gas-phase quasi-equilibrium (QE) distribution [36]. This is important as it allows us to distinguish between features that correspond to conformers coming from solution and those that are formed by activation in the solvent-free, gas-phase environment.

2. Experimental methods

2.1. Sample preparation

BK ($\geq$98% purity) and leucine enkephalin (YGGFL, $\geq$95%) were purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.), and used as received. Peptide ions were produced by positive-mode electrospray ionization (ESI) of 10^{-5} M solutions in 49:49:2 water: methanol:acetic acid. Amino acids (AAs) asparagine, aspartic acid, glutamic acid, glutamine, methionine, and proline (>99%, Fluka; Buchs, Switzerland) were dissolved in 50:50 (% volume) acetonitrile:water solutions at a concentration of $\sim 10^{-3}$ M. Sodium acetate (99.9%, Fisher Scientific; Fair Lawn, NJ, U.S.A.), potassium acetate (>99%, Aldrich; Milwaukee, WI, U.S.A.), and lithium acetate (99.99%, Sigma–Aldrich; St. Louis, MO, U.S.A.) were added to the AA solutions resulting in a concentration of $\sim 5 \times 10^{-3}$ M metal acetate.

2.2. Instrumentation

A schematic diagram of the two-meter IMS–IMS–time-of-flight MS instrument used in these studies is shown in Fig. 1. IMS theory [20,22,37,38] and applications [39–46] are described in detail previously. A brief description of the experiment is as follows: ions were produced by a chip-based ESI autosampler (Advion Biosciences, Inc., Ithaca, NY, U.S.A.) and trapped in an ion funnel [47,48] labeled F1 in Fig. 1. A 150 μs-wide pulse applied to G1 was used to gate a packet of ions into the drift tube. Driven by a $10\,V \times cm^{-1}$ electric field, the ions were separated on the basis of their low-field mobilities through 3.00 ± 0.01 Torr He. Upon exiting the drift tube, ions were focused through a differential pumping region and pulsed orthogonally into a two-stage reflectron-geometry time-of-flight mass spectrometer for nested drift time (m/z) measurement [49].

2.3. Multidimensional IMS

The drift tube illustrated in Fig. 1 is divided into two effective drift regions (D1 and D2) by ion funnel F2, which also contains 3.00 ± 0.01 Torr He buffer gas. One purpose of funnels F2 and F3 is to refocus radially diffuse ions toward the center axis to avoid ion loss through the instrument; this is accomplished through the use of an rf-focusing component, as well as operating at a slightly higher ($12\,V \times cm^{-1}$) linear electric field. F2 also contains non-rf ion selection (G2) and ion activation (IA2) regions utilized in these multidimensional IMS experiments. A delay pulse lowers a repulsive potential across G2 for 30–100 μs, thus allowing ions with a narrow range of mobilities to pass; all other ions reach an electrostatic-potential barrier and are neutralized. Timing of the delay pulse is adjusted with respect to the initial source pulse from G1 in order to isolate ions of a desired mobility from the total distribution. A new population of ions can be formed from the selected ions prior to entering D2 by application of a voltage drop across the 0.3-cm activation region (IA2); for these experiments, the ion activation voltage (V) was varied between 0 V and 260 V ($\sim$0–870 $V \times cm^{-1}$ above the drift field) to collisionally activate

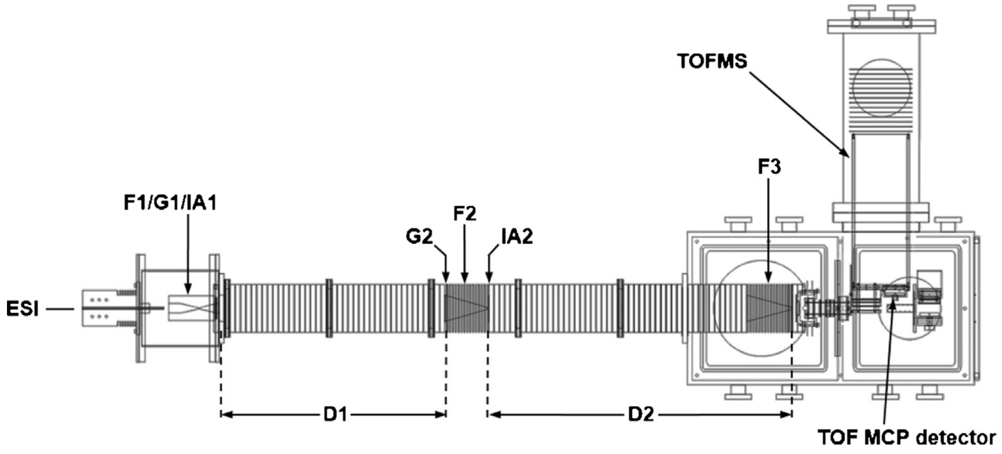

Fig. 1. Schematic diagram of the ~2-m IMS–IMS–MS instrument employed in these studies; the mid-drift tube ion funnel F2 includes mobility selection gate G2 and the 0.3-cm IA2 activation region where the electric field can be varied to tune the energy of ion–molecule collisions.