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Investigation of peptide microsolvation in the gas phase by radical directed dissociation mass spectrometry

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ABSTRACT

Peptide structure is often correlated with biological function, and recently interest in developing gas-phase based methods for examining peptide structure has grown. The relationship between solution and gas phase structures is unclear, partially due to removal of solvent during the transition. 18-Crown-6 (18C6) is a small molecule that can noncovalently attach to peptides in the gas phase via basic residues, perhaps replacing water and helping retain solution-like structures. Herein, we investigate structural differences between naked peptides and those solvated by 18C6 with radical directed dissociation (RDD), a structurally sensitive fragmentation method. Peptides with and without 18C6 attached often yield disparate RDD spectra, indicating significant structural differences between them. The effects of solvation by 18C6 were explored as a function of peptide size and sequence. Although general trends can be observed with regard to factors that influence solvation, the results suggest that solvation is unique for each peptide and should be examined on a case by case basis.

1. Introduction

Mass spectrometry (MS) is a powerful tool for protein characterization, capable of examining not only primary structure, but also higher order secondary, tertiary, and even quaternary structure. Importantly, analysis by mass spectrometry takes place in the gas phase following ionization of the analyte of interest. Electrospray ionization (ESI) is commonly employed [1], creating ions from highly charged droplets formed by high voltage at atmospheric pressure [2]. ESI is a soft ionization method, meaning that noncovalent bonds can be preserved, allowing for observation of noncovalent complexes in the gas phase [3]. However, the exact nature of the electrospray process is unknown, and it is difficult to predict how solution phase structures evolve during the transition to the gas phase [4,5]. Many previous investigations suggest that data from ESI–MS reflects protein solution phase structure, whereas other studies have found that proteins may undergo structural rearrangement while transferring from solution to gas phase [6–8].

One approach for preserving solution phase structure in the gas phase is to replace solvent interactions with noncovalent adducts [9–11]. 18-crown-6 (18C6) is a small molecule that binds cationic ions or functional groups. If a mixture of 18C6 and peptide/protein is electrosprayed, 18C6 will form noncovalent bonds with exposed basic sites such as the protonated side chains of Lys/Arg, or the N-terminus [12]. Recent work has demonstrated that 18C6 can replace the intramolecular interactions between positively charged side chains and backbone carbonyls that dramatically disturb the tertiary structure of a protein in the absence of solvent.[10] A simple example of this effect is illustrated in Fig. 1, which shows the structural rearrangement of a lysine containing peptide (KGGG) upon binding 18C6 in the gas phase. In solution, the protonated lysine side chain is solvated by water. As water is removed during ESI to yield the naked ion, the charged side chain seeks solvation by the peptide backbone, as illustrated by the structure on the left side of Fig. 1. However, solvation of the protonated lysine side chain can also be satisfied by 18C6 attachment, forming three hydrogen bonds between the protonated primary amine and 18C6 and yielding a different backbone structure for the peptide (Fig. 1, right side).

Examining the effects of microsolvation requires probing peptide structure in the gas phase. Several techniques for structural analysis are available including, spectroscopy [13,14], ion mobility [15], and energy-transfer based methods [16]. Additionally, radical chemistry can be used to investigate peptide/protein structure [17]. To use radical chemistry, a radical precursor such as a carbon-

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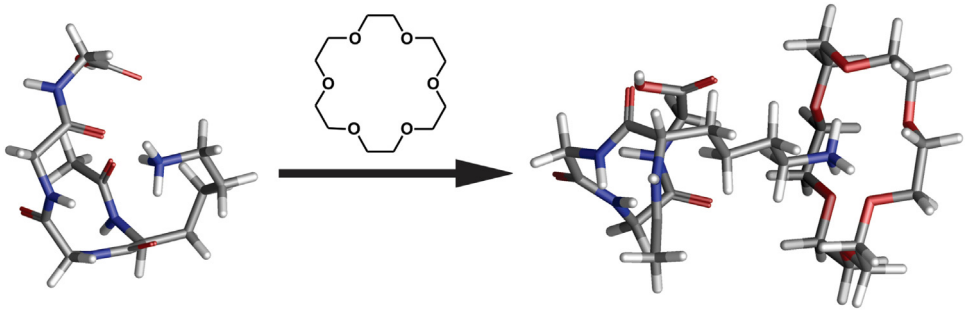

Fig. 1. Conformational change of a Lysine-containing peptide upon interaction with 18C6.

iodine bond is added site-selectively into the biomolecule and subsequently cleaved by photodissociation (PD). Photoactivation accesses a dissociative excited state, yielding homolytic cleavage of the C-I bond to generate a radical with atomic precision at the modified site [18]. Subsequent collisional activation of the radical leads to dissociation of the peptide/protein via radical directed dissociation (RDD). Fragmentation in RDD is dictated by the radical and yields characteristic fragments (a, c, z ions and side chain losses), primarily in spatial proximity to the nascent radical. Since the initial site where the radical is created cannot yield fragments directly, radical migration precedes observable RDD. Abundant RDD sites exist in peptides/proteins, therefore short migration distances are preferred, allowing RDD to identify close contact points in the three dimensional structure of the molecule [19]. In fact, the structural sensitivity of RDD is sufficient to detect very subtle structural variations such as changes in chirality [20].

In this manuscript, we utilize RDD to explore structural effects of solvation by 18C6 on peptides in the gas phase. For some peptides, dramatic differences in RDD are observed when the naked peptide is compared to the singly or doubly solvated peptides. In other cases, a single 18C6 adduct leads to significant change while the second 18C6 does not. We also observed peptides where the addition of 18C6 does not affect RDD patterns significantly, although this may result from lack of structure probing rather than lack of structural changes. The various factors affecting peptide solvation are discussed, in addition to the strengths and weaknesses of RDD for this type of structural probing.

2. Experimental methods

2.1. Materials and peptide synthesis

All organic solvents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Sodium iodide, chloramine-T, sodium metabisulfite, and all the other chemicals and solvents were purchased from Fisher Scientific (Fairlawn, NJ). All Fmoc protected amino acids and resins were purchased from Ana Spec (Fremont, CA). 18-Crown-6 was purchased from Alfa Aesar (Pelham, NH). Water was purified to 18.2 MΩ using a Millipore 147 (Billerca, MA) Direct-Q system.

2.2. Peptide synthesis and modification

All peptides except DRVYIHF (Angiotensin) were synthesized manually using standard fmoc procedures [21] with rink amide resin or Wang resin being employed as the solid support. Amino acids with protected side chains were used when needed. Angiotensin was purchased from American Peptide Company (Sunnyvale, CA).

Iodination of peptides was performed at room temperature using a previously published method [22]. Specifically, sodium iodide was used as the iodine reagent and chloramine-T was used for oxidation. After mixing sodium iodide, chloramine-T, and the peptide with 1:1:1 ratio for 1 min, excess sodium metabisulfite was added to the solution to quench the reaction. The reaction mixture was purified via peptide trap (Michrom Bioresource Inc.) to remove any excess reagent.

2.3. Mass spectrometry and dissociation of the peptides

Mass spectral data was acquired with an LTQ ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA) with a standard ESI source. 18C6 was added to peptide solution at 10 times excess and the mixture was directly infused into the mass spectrometer using 50/50 water/methanol as electrospray solvent. Isolation windows were varied (3–10 Da) to accommodate complex stability, wider isolation windows are needed to isolate weakly bound complexes. In order to perform RDD, the instrument was modified with a quartz window at the back of the ion trap, and an Nd:YAG laser (Continumm, Santa Clara, CA) was used to fire fourth harmonic pulses (266 nm) through the window. The laser fires at MS^2 level and yields the loss of iodine radical from the peptide. Further isolation and collision activation of the radical species induces side chain losses and backbone fragmentation of the peptide. 50 scans were acquired for each mass spectrum, and the standard deviation of fragmentation intensities were calculated using the data from each individual scan.

2.4. Δ Fragmentation

Δ Fragmentation was calculated to quantitatively compare the fragmentation patterns of two RDD spectra [23]. To calculate Δ fragmentation, the intensity of each fragment ion was first normalized to the total intensity of all the ions that were included in the calculation. Eq. (1) shows the normalization calculation:

$$NorInt_i = \frac{Int_i}{\sum_{i=0}^{n} Int_i} \quad (1)$$

Where $NorInt_i$ is the normalized intensity of fragment ion "i", and Int_i is the intensity of the fragment ion "i", and a total number of n fragment ions were included in the ΔFragmentation calculation.

The ΔFragmentation was calculated using Eq. (2):

$$\Delta Fragmentation = \sum_{i=0}^{n} |NorInt(Spectrum1)_i - NorInt(Spectrum2)_i| \quad (2)$$

Where the NorInt (Spectrum 1)$_i$ is the normalized intensity of fragment ion "i" in the first RDD spectrum for comparison, and NorInt(Spectrum 2)$_i$ is the normalized intensity of fragment ion "i" in the second RDD spectrum. A total number of n fragment ions were included in the calculation. Errors were included in the normalized intensity values in Eq. (2). For example, if ion i has relative