Spectroscopic investigation of hydrogen atom transfer in the McLafferty rearrangement

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Abstract. In this study, wavelength-selective infrared multiple-photon photodissociation (WS-IRMPD) was used to study the transfer of an H atom in a gas-phase unimolecular rearrangement reaction. The reaction studied was the McLafferty rearrangement, which involves transfer of a γ-position hydrogen atom to a carbonyl oxygen atom through a cyclic intermediate, followed by the elimination of an alkene. The IRMPD experiment allows for the collection of vibrational spectra of discrete gas-phase ions, which can then be compared to those predicted using density functional theory calculations to assign accurate composition and structure. For the experiment, the *tert*-butyl ester of a model peptide, nicotinic acid-glycine, was incubated in a mixture of deuterium oxide and deuterated methanol. This solution was the used to make ions by electrospray ionization. The McLafferty rearrangement was then induced by collision-induced dissociation. Following rearrangement, the product ion, consisting of the model peptide with 2 deuterium atoms and 1 H atom in exchangeable sites, was investigated by IRMPD. The infrared spectrum produced clearly shows that the H atom is transferred to the C-terminal acid group and no migration to amide positions occurs on the time scale of the experiment.

1. Introduction

Fragmentation pathways observed in the gas phase collision induced disassociation (CID) of protonated peptides under low energy conditions occur via intra-molecular rearrangement reactions that include cyclization, nucleophilic attack, and proton transfer [1-10]. The peptides of interest to this experiment are those that lack basic amino acids residues (e.g. histidine, lysine or arginine). In most low energy peptide disassociation reactions, amide bond cleavage requires the migration of the added proton, from the most basic site, to the point of nucleophilic attack and this general idea makes up the "mobile-proton" model of peptide disassociation [11-19].

Current protein identification in proteomics relies on CID and tandem mass spectrometry to characterize peptides derived from enzymatic digestion. For this reason it is important to clearly understand the cyclization, nucleophilic attack and proton transfer during peptide fragmentation. The specific aim was to use vibrational spectroscopy, via WS-IRMD, to determine whether the H atom remains at the acid group, or migrates to one of the exchangeable sites, thus probing directly the tendency for H/D scrambling. The experiments provide proof-of-principle results that demonstrate that the IRMPD approach can be used to study intra-molecular H atom transfer, and establish that the McLafferty-rearrangement of the peptide esters can be used to selectively label the C-terminus of a peptide for "isotope tracer" studies of intra-molecular proton migration.

2. Experiment, Results, Discussion, and Significance

Nicotinic acid, betaine hydrochloride, glycine *tert*-butyl ester hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO) and coupled to glycine *tert*-butyl ester to make NicGO*t*Bu and BetGO*t*Bu$^+$ using a resin-bound carbodiimide (PS-Carbodiimide, Argonaut, Foster City CA) suspended in dichloromethane. After a minimum of 8 hours the resin was filtered out of the solution, and then allowed to dry. The desired compound was dissolved and protonated with a 1:1 methanol/water mixture. Further evaluation of the compounds was conducted using a Fourier transform ion cyclotron resonance mass spectrometer and Infrared multiple photon dissociation (IRMPD) using the free electron laser at the FOM Institute for Plasma Physics. The combination of these techniques allowed the isolation of the compound of interest using its respective mass, production of infrared spectra via WS-IRMPD.

Theoretical spectra were generated using density functional theory calculations (B3LYP/6-311+g(,p) level of theory).

3. Conclusions

McLafferty rearrangement was used to generate the free-acid forms of the respective model peptides, which were then subjected to wavelength-selective IRMPD to produce vibrational spectra from 1200-1900 cm^{-1}. Comparison with DFT-generated spectra allowed definitive assignment of absorption bands corresponding to the free-acid carbonyl, amide I and amide II stretches, and enabled assignment of ion structure. For the nicotinyl-glycine system, the best match between IRMPD and theoretical spectra, both in terms of the respective absorption frequencies and relative intensities, is for a conformational isomer that is protonated at the pyridine ring N atom, and includes an intra-molecular hydrogen bonding interaction between the amide position H atom and the C-terminal carbonyl O atom. For the betaine-glycine system, the best agreement between theory and experiment was for a linear conformer with a stabilizing intra-molecular hydrogen bond similar to that for the analogous nicotinyl-glycine system.

The intra-molecular transfer of the H atom by McLafferty rearrangement, and in particular, the potential scrambling of the exchangeable H atoms, was proved using the model peptide *tert*-butyl esters incubated in a mixture of D_2O and CH_3OD to induce H/D exchange. For the deuterium exchanged peptide ester, CID and McLafferty rearrangement produces a heterogeneous isotopically labeled peptide ion containing two D atoms and one H atom at exchangeable sites. Comparison of the IRMPD results to theoretical spectra for different isotope labeled isomers clearly shows that the H atom is situated at the C-terminal acid group and migration to amide positions is minimal on the time scale of the experiment. Thus scrambling of H atoms situated on exchangeable sites is not occurring to any significant degree. In addition to demonstrating that the IRMPD approach can be used to study H atom transfer within discrete ions, the results suggest that use of the McLafferty rearrangement for peptide esters could be an effective approach for generation of H-atom isotope tracers, *in-situ*, for the investigation of intra-molecular proton migration during peptide fragmentation studies

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