

Room-Temperature Infrared Spectroscopy Combined with Mass Spectrometry Distinguishes Gas-Phase Protein Isomers**

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Gas-phase protein structures are the subject of extensive investigation by a number of techniques, e.g., tandem mass spectrometry (MS/MS), infrared multiphoton dissociation (IRMPD),^[1] and electron-capture dissociation (ECD).^[2] Typical IRMPD MS/MS experiments are carried out in instruments which allow ions to be trapped for a sufficient period of time to interact with IR irradiation, such as that in Fourier transform ion cyclotron resonance (FTICR) mass spectrometers. As the trapped ions absorb IR photons, their internal energy increases above the dissociation threshold, whereupon the ions undergo fragmentation. In proteins IRMPD usually leads to C–N bond dissociation, which gives N-terminal b-type and C-terminal y-type fragment ions.^[1] Lasers with an optical parametric oscillator (OPO) device have often been used for IRMPD-MS spectroscopy.^[3] With the introduction of bright, widely tunable IR sources such as free-electron lasers (FEL),^[4–6] the combination of IR spectroscopy and IRMPD-MS became especially powerful. In recent years, IR spectroscopy in combination with MS has been applied to probing structures of small molecules (e.g., metal–disaccharide complexes,^[7] metal–peptide complexes^[8]) and oligopeptides,^[9–11] and IRMPD spectra were obtained for cations of proteins as large as cytochrome *c*.^[3,12] Although absorption regions attributable to amide bands I and II have been found in the IRMPD spectra, these features were surprisingly similar for all proteins studied. Even different charge states of the same protein which have distinct gas-phase conformations produced similar IRMPD spectra.^[3,11,12] These results raised the issue of the suitability of IRMPD at room temperature for studying details of gas-phase protein structures. Unlike small molecules, large molecules easily isomerize in the gas phase because of their weakly bound secondary and higher order structures,^[3] absorb at most wavelengths, and require many photons for fragmentation. As a result, IRMPD of proteins requires much longer irradiation times, during which proteins may unfold and thus change their absorption spectrum.^[3,11]

Here we prove that IR spectroscopy at room temperature can provide unique signatures for gas-phase protein isomers.

In order to have a well-defined protein conformation in the gas phase, we chose as a model molecule Trp-cage, a minimal protein (20 amino acids, NLYIQWLKDGGPSSGRPPPS) which rapidly folds in solution^[13] and in the gas phase^[14] and which has been used as a model system in many detailed studies. When solution and gas-phase structures of several Trp-cage stereoisomers were studied both experimentally and theoretically,^[15] it was found that a single amino acid L/D substitution in Trp-cage induces structural changes. These changes become apparent when a mixture of two Trp-cage isomers is studied, one of which has a D substitution, and the other is a deuterated all-L isoform (substitution of stable H atoms by D does not change the protein structure).^[15] Molecular ions of these two species can be isolated together and subjected to IR irradiation, and the mass difference of 6 Da keeps the molecular ions and many of their fragments separated on the mass scale. Since the all-L isoform is common to all experiments with D substitutions, it serves as an internal standard for accurate quantitative measurements.^[15]

The highest absorption is observed at 1640–1660 cm^{−1} (Figure 1), which corresponds to the amide I band (mainly

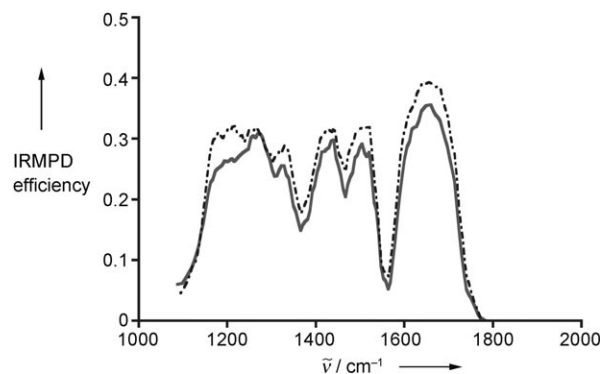


Figure 1. IRMPD spectra of Trp-cage 2+ ions measured via fragment abundances at conventional irradiation times. Solid line and dotted line represent two replicate runs.

the C=O stretching vibration with minor contributions from the out-of-phase CN stretching vibration, the CCN deformation, and the NH in-plane bend). The amide I band is less affected by the nature of side chains but sensitive to secondary structure of the backbone. Therefore, the amide I band is commonly considered to be useful in determining secondary structures of proteins in solution; its normal position is 1646–1655 cm^{−1} for α -helices and 1630–1640 cm^{−1} for β -sheets.^[16] An amide I band observed at 1650 cm^{−1} is

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possibly due to the α -helical structure of Trp-cage.^[13] Another broad band at around 1150–1350 cm^{-1} arises from the in-phase combination of NH bending and the CN stretching vibrations with minor contributions from the CO in-plane bending and the CC stretching vibration.^[16] There is also a pair of bands at about 1450 and about 1520 cm^{-1} ; at least one of them may correspond to the amide II mode.

The risk of unfolding was reduced by shortening the time of IR irradiation, but this weakened the fragment-ion signal (Figure 2). To compensate for this effect, we monitored

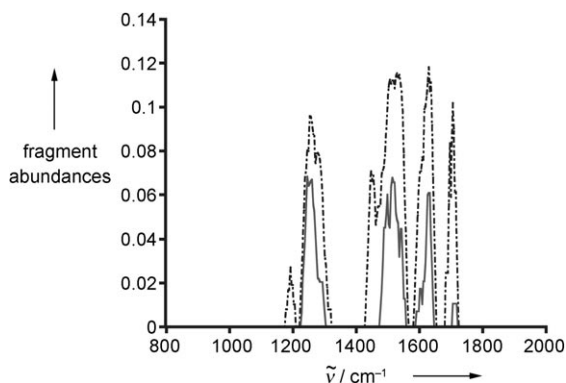


Figure 2. IRMPD spectra of two Trp-cage isoforms measured at low irradiation times via fragment abundances. Dotted line: All-L isoform, solid line: D-Trp⁶ isoform.

depletion of the precursor-ion signal, which is known^[1] to precede the onset of fragmentation. The structural differences between stereoisomers of Trp-cage were probed by measuring differential IRMPD mass spectra. A mixed solution of the deuterated all-L compound and a single-amino-acid D-substituted stereoisomer was electrosprayed, molecular dications were isolated together in the FTICR ion cell (the deuterated isomer was 6 Da heavier due to H→D replacement of two stable α -carbon H atoms in each of the three Gly residues), and irradiated with 1000–1900 cm^{-1} photons.

The abundance ratio of the molecular ions was taken as a measure of the relative IR absorption: a ratio greater than unity implies higher absorption of the all-L form compared to the D stereoisomer. This method of probing the structural difference between stereoisomers is less affected by instability of the electrospray current or variability of IR beam power.

Figure 3, which shows two independently acquired IRMPD spectra of the D-Trp⁶ stereoisomer, suggests that the experiment is reproducible. Figure 3 is much more informative than Figure 2, although the same data set was used in both cases. Both representations suggest higher absorption of the all-L molecule at most wavelengths. In ECD MS/MS experiments, the all-L form showed more structure than any other stereoisomer except D-Asn¹, which was not used in this study.^[15]

Figure 4 shows the differential spectrum of D-Tyr³, which has at least two regions with greater absorption than the all-L form. One of these “negative” regions is located between 1400 and 1600 cm^{-1} . The differential absorption spectrum of D-Leu⁷ is very different from those of the two first stereoisomers

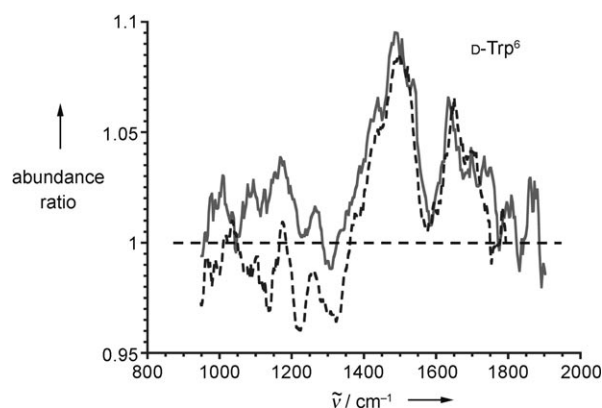


Figure 3. Differential IRMPD spectrum of 2+ ions of D-Trp⁶ and all-L stereoisomers of Trp-cage protein. Two independent runs are shown in overlap. The dashed line represents equal absorption.

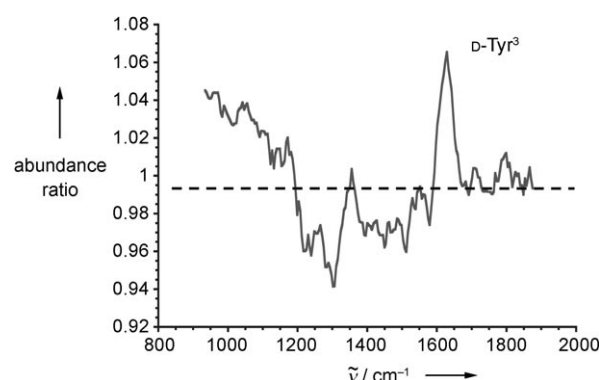


Figure 4. Differential IRMPD spectra of 2+ ions of D-Tyr³ and all-L stereoisomers of Trp-cage protein.

mers (Figure 5). It contains few negative bands, but shows instead strong, sharp, positive bands at 1400, 1500, and 1650 cm^{-1} . This result is consistent with the results of molecular dynamics simulations^[14] in which D-Leu⁷ 2+ ions differed more from all-L than any other stereoisomer.

In summary, we have performed differential absorption measurements based on precursor-ion signal depletion in FTICR MS. The vastly different differential spectra obtained for stereoisomers of the same small protein proved that

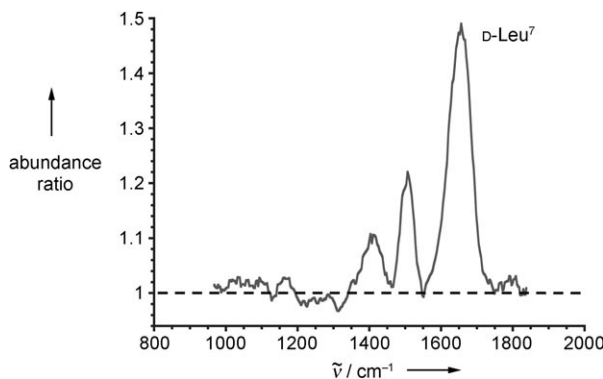


Figure 5. Differential IRMPD spectrum of 2+ ions of D-Leu⁷ and all-L stereoisomers of Trp-cage protein.

IRMPD results are sensitive to fine details of gas-phase protein structure. Therefore, IRMPD at room temperature can be successfully used for structural analysis of proteins, provided the differential spectra are rationalized, for example, by molecular dynamics simulations.

Experimental Section

A modified Bruker APEX-Qe FTICR mass spectrometer coupled to the CLIO (Centre Laser Infrarouge d'Orsay) IR-FEL was used for IRMPD experiments.^[17] The CLIO IR-FEL is based on a 10–50 MeV electron linear accelerator.^[18] At a given electron energy, the photon energy is tuned by adjusting the undulator gap which is placed in the optical cavity. The IR-FEL output consists of 8 μ s-long macropulses fired at a repetition rate of 25 Hz. Each macropulse is composed of 500 micropulses, each of which is a few picoseconds long and separated by 16 ns. The corresponding micropulse and macropulse energies were 40 μ J and 20 mJ, respectively, for a typical average IR power of 500 mW. The laser wavelength profile was monitored with a monochromator associated with a pyroelectric detector array (Spiricon) at each reading. The IR-FEL spectral width could be adjusted by tuning the length of the optical cavity, and the laser spectral width (fwhm) was less than 0.5 % of the central wavelength.

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- [1] D. P. Little, J. P. Speir, M. W. Senko, P. B. O'Connor, F. W. McLafferty, *Anal. Chem.* **1994**, *66*, 2809–2815.
- [2] R. A. Zubarev, N. L. Kelleher, F. W. McLafferty, *J. Am. Chem. Soc.* **1998**, *120*, 3265–3266.

- [3] H. Oh, K. Breuker, S. K. Sze, Y. Ge, B. K. Carpenter, F. W. McLafferty, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15863–15868.
- [4] J. Oomens, D. T. Moore, G. von Helden, G. Meijer, R. C. Dunbar, *J. Am. Chem. Soc.* **2004**, *126*, 724–725.
- [5] J. Lemaire, P. Boissel, M. Heninger, G. Mauclaire, G. Bellec, H. Mestdag, A. Simon, S. L. Caer, J. M. Ortega, F. Glotin, P. Maitre, *Phys. Rev. Lett.* **2002**, *89*, 273002.
- [6] J. Oomens, G. Meijer, G. von Helden, *J. Phys. Chem. A* **2001**, *105*, 8302–8309.
- [7] N. C. Polfer, J. J. Valle, D. T. Moore, J. Oomens, J. R. Eyler, B. Bendiak, *Anal. Chem.* **2006**, *78*, 670–679.
- [8] P. O. Balaj, C. Kapota, J. Lemaire, G. Ohanessian, *Int. J. Mass Spectrom.* **2008**, *269*, 196–209.
- [9] T. D. Vaden, T. de Boer, J. P. Simons, L. Snoek, S. Suhai, B. Paizs, *J. Phys. Chem. A* **2008**, *112*, 4608–4616.
- [10] T. D. Vaden, T. de Boer, J. P. Simons, L. C. Snoek, *Phys. Chem. Chem. Phys.* **2008**, *10*, 1443–1447.
- [11] H. Oh, C. Lin, H. Y. Hwang, H. Zhai, K. Breuker, V. Zabrouskov, B. K. Carpenter, F. W. McLafferty, *J. Am. Chem. Soc.* **2005**, *127*, 4076–4083.
- [12] J. Oomens, N. Polfer, D. T. Moore, L. van der Meer, A. G. Marshall, J. R. Eyler, G. Meijer, G. von Helden, *Phys. Chem. Chem. Phys.* **2005**, *7*, 1345–1348.
- [13] J. W. Neidigh, R. M. Fesinmeyer, N. H. Andersen, *Nat. Struct. Biol.* **2002**, *9*, 425–430.
- [14] A. Patriksson, C. Adams, F. Kjeldsen, J. Raber, D. van der Spoel, R. A. Zubarev, *Int. J. Mass Spectrom.* **2006**, *248*, 124–135.
- [15] C. A. Adams, F. Kjeldsen, A. Patriksson, D. van der Spoel, A. Gräslund, E. Papadopoulos, R. A. Zubarev, *Int. J. Mass Spectrom.* **2006**, *253*, 263–273.
- [16] A. Barth, C. Zscherp, *Q. Rev. Biophys.* **2002**, *35*, 369–430.
- [17] J. M. Bakker, T. Besson, J. Lemaire, D. Scuderi, P. Maitre, *J. Phys. Chem. A* **2007**, *111*, 13415–13424.
- [18] F. Glotin, J. M. Ortega, R. Prazeres, C. Rippon, *Nucl. Instrum. Methods Phys. Res. Sect. B* **1998**, *144*, 8–17.