Observation of Conserved Solution-Phase Secondary Structure in Gas-Phase Tryptic Peptides

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Structure−function relationships are among the most difficult to probe in chemical biology;[1] however, H/D exchange[2] and ion mobility measurements[3−5] are becoming increasingly important as probes of secondary and tertiary structure of gas-phase peptide ions. Here we discuss results from ion mobility experiments on protein digest fragments where higher order structure of some gas-phase peptides is observed.

Spectra were obtained using a matrix-assisted laser desorption−ionization (MALDI) ion mobility (IM) time-of-flight (TOF) mass spectrometer (MS) as described elsewhere.[6,7] Briefly, ions formed at the operating pressure of the drift cell (10 Torr He) are separated by ion mobility and mass analyzed by TOF MS. Theoretical collision cross-section calculations incorporating molecular structures obtained from simulated annealing[8] were performed based on methods outlined by Jarrold.[9]

Figures 1 and 2 show data for two digested proteins: bovine hemoglobin and horse heart myoglobin (purchased from Sigma, St. Louis, MO). Note the near linear relationship between drift time and m/z illustrated in both figures. Signals in both IM-MS maps were mass identified and found to contain 16 digest fragments for bovine hemoglobin and 14 fragments of horse heart myoglobin.[10] In each data set, the mass−mobility relationship (established by linear regression analysis) for a single tryptic peptide deviates by greater than 10% from that of the other peptide ions in the plot. For example, the noncorrelated tryptic peptide (m/z of 1379.7) for myoglobin corresponds to residues 64−77 (HGTVVLTALGGILK), and in the case of hemoglobin, the noncorrelated peptide (m/z 1275.5) is identified as residues 104−115 (LLGNVLVVVLAR, β subunit). Peptide sequences were confirmed by a combination of MALDI-TOF accurate measurement[7] and ESI tandem mass spectrometry (MDS Sciex QSTAR Pulsar).

To obtain structural information for ions that deviate from the near-linear mass−mobility relationship to those that follow expected trends, mobility data were compared to structures generated by molecular dynamics. Proposed structures for the identified amino acid sequences are shown in Figures 1 and 2.[11] In the case of bovine hemoglobin, the peptides selected for further analysis, LLVVYP-WTQR (residues 30−39 of the β subunit) and the noncorrelated peptide LLGNVLVVVLAR, are close in mass, differing by only 9 Da, but have drift times in helium that differ by ca. 80 μs. Low-energy conformations for LLGNVLVVVLAR are helical, between 3 and 4 amino acids per turn as shown in Figure 1 (top right). On the other hand, the structures obtained for LLVVYPWTQR are

more random coil in nature (Figure 1, bottom right). Theoretical and experimental cross-sections were compared for the two molecules, both of which showed considerable differences in collision cross-section (6% difference by experiment, and 5% difference by theory in total collision cross-section).

Similar molecular dynamics calculations were performed on tryptic peptides from horse heart myoglobin. In this case the two peptides, HGTVVLTALGGILK and VEADIAGHGQEVLIR, appeared to have very similar corrected drift times (1.14 and 1.11 μs, respectively), but very different m/z ratios (1379.7 and 1607.8, respectively). The low-energy conformation obtained for HGTV-VLTALGGILK (helical structure between 3 and 4 amino acids per turn) and VEADIAGHGQEVLIR (random coil, with a high degree of folding) are shown in Figure 2. Theoretical and experimental results for the two myoglobin peptides agree within the error of the calculations (±2% total error), which is consistent for ions with similar collision cross-sections, even though the masses of the two peptides are very different ($\Delta m = 228.1$ Da). For further conformation of structural assignments, solution-phase H/D exchange experiments[12] were conducted. LLGNVLVVVLAR (from hemoglobin) exhibits a slower rate of H/D exchange relative to LLVVYPWTQR, indicating that a greater number of intramolecular hydrogen bonds are present in the noncorrelated peptide. Structural information obtained from Figures 1 and 2 is also consistent with data obtained by other investigators. For example, Clemmer[13] and Jarrold[4] have shown that extended structures (such as helices) have larger collision cross-sections than globular structures, and Clemmer has also suggested that N-terminal acetylation of peptide ions gives rise to extended (possibly helical) structures in the gas phase.[14]

A comparison between proposed gas-phase and solution-phase structures of myoglobin and hemoglobin[15] provides additional insight on the conformational stability of tryptic peptides. Of the peptides discussed, two correspond well with their solution-phase structure within the native protein: LLGNVLVVVLAR from hemoglobin and HGTVVLTALGGILK from myoglobin (both α-helical in the native protein). The other two peptides, however, appear to be less structurally confined in the gas phase. The peptide LLVVYPWTQR occupies an interfacial position between the α and β subunits of the intact protein. In solution, the peptide (within the native protein) is roughly one-half 3−10 helical and one-half α-helical. Apparently this complex structure is not conserved in the gas phase, which implies that LLVVYPWTQR exhibits low conformational stability. This is also the case for the mass−mobility correlated peptide in the tryptic digest of horse heart myoglobin, VEADIAGHGQEVLIR. The position of VEADIAGHGQEVLIR is within the B helix of the intact solution-phase protein (residues

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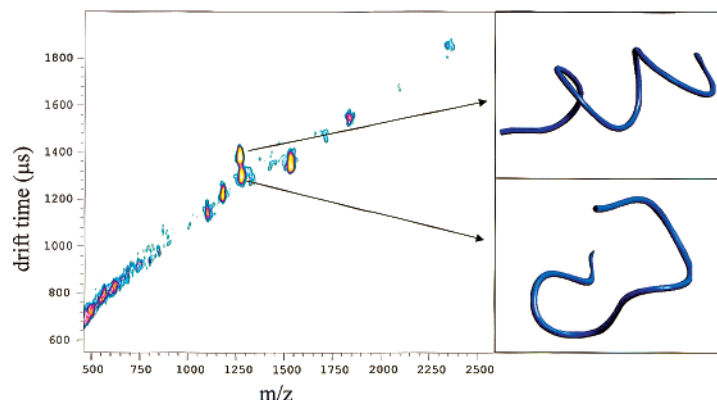‡ Laboratory for Molecular Simulation.

Figure 1. Ion mobility-mass spectrometry peptide map of bovine hemoglobin. Two low-energy MD calculated structures are assigned to peptide signals within the plot: (1) LLGNVLVVVLAR and (2) LLVVYPWTQR. The two peptide projections shown are 15 (top) and 10 Å (bottom).
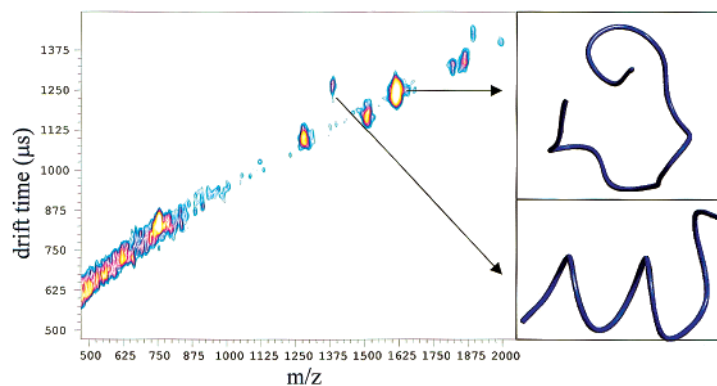

Figure 2. Ion mobility-mass spectrometry peptide map of horse heart myoglobin. Two low-energy MD calculated structures are assigned to peptide signals within the plot: (1) HGTVVLTALGGILK and (2) VEADIAGHGQEVLIR. The two peptide projections shown are 10 (top) and 15 Å (bottom).

17−31), and we find no evidence that the gas-phase ion prefers an α-helical conformation. These observations suggest that gas-phase peptide conformation, in some cases, is independent of the solution-phase conformation. Conversely, peptides such as LLGNVLVVV-LAR and HGTVVLTALGGILK appear to possess sufficient intrinsic stability to retain some memory of their solution-phase structure after thermal denaturation, digestion, and ionization processes.

Ion mobility presents an attractive method from which information on the intrinsic conformational stabilities of peptides outside of their native, protein environments can be determined, including structural effects of posttranslational modification.[16] The identification of such peptides could contribute to the current understanding of protein folding mechanisms. For example, protein folding theories put forth by Baldwin and others rely on the existence of peptides that have a high predisposition toward a given secondary structure, termed "autonomous folding subunits".[17] Data presented in Figures 1 and 2 indicate that IM-MS techniques can be used to obtain the position of these functional subunits within a protein, potentially adding protein function information to a high-sensitivity, high-throughput identification method.

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