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Strength of NH···S Hydrogen Bonds in Methionine Residues Revealed by Gas-Phase IR/UV Spectroscopy

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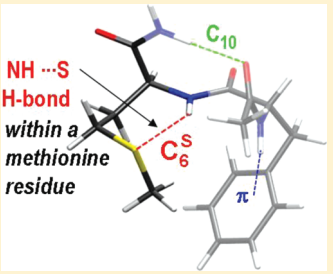
Supporting Information

ABSTRACT: Despite of being ubiquitous in proteins, $NH_{backbone}$···S hydrogen bonds linking the sulfur atom of methionine or cysteine to backbone NH groups remain poorly documented. Here, we report vibrationally resolved IR NH stretch spectra of two methionine-containing dipeptides (Ac-Phe-Met-NH_2 and Ac-Met-Phe-NH_2). The conformations observed for both molecules, assigned with the help of DFT-D quantum chemistry, provide spectroscopic evidence for the formation of $NH_{backbone}$···S H-bonds, surprisingly strong enough to challenge the classical intrabackbone NH···O═C H-bonds. The methionine side chain is found to fold locally, forming a H-bond with the neighboring amide groups (NH(i) or NH(i+1)). Comparison with protein data bank structural information shows that such a local folding is also common in proteins where it concerns 24% of the methionine residues that have a sulfur atom linked to a backbone NH group. This convergence between the strength of these NH···S H-bonds and protein structural data illustrates their contribution to the stability of protein chains.



SECTION: Kinetics, Spectroscopy

The role of hydrogen bonds[1] in governing the secondary and tertiary structures of proteins, protein−ligand interactions, and enzymatic activity is well-established.[2,3] Most of the studies about biologically relevant H-bonds have been devoted to the thorough characterization of X−H···Y H-bonds, where both donors (X) and acceptors (Y) are electronegative atoms, mainly nitrogen and oxygen. The sulfur atom, however, has not received as much attention due to its less electronegative character. Although the coded residues methionine (Met) and cysteine[4] are known for the capability of their sulfur atom to form H-bonds, these are commonly considered as weak interactions.[5] For instance, a recent statistical analysis of protein structures has shown that the side chain of methionine can form NH···S H-bonds with NH groups of backbone amides or of other side chains,[6] enabling H-bonding linkages between remote residues of the sequence or between distinct chains. Meanwhile, the same study mentions the poor H-bond acceptor properties of the sulfur atom of methionine. Besides these protein data, recent detailed investigations of small isolated intermolecular complexes, however, have suggested that H-bonds to sulfur can be as strong as their oxygen counterparts.[7,8] Although these systems are too small to model interactions occurring in a protein, the results are significant enough to question the presumably weak character of H-bonding to sulfur in proteins.

In order to address the intrinsic properties of such noncovalent interactions and their role in the stability of proteins, we have investigated Met-containing peptides prepared in a "solvent-free" environment, that is, isolated by using a supersonic expansion.[9] The technique employed allows the peptides to fold in a low electric permittivity environment, similar to what is encountered in hydrophobic biological media or protein cores.[10] The peptides are thus ideally frozen in one of their most stable conformers. Then, the IR spectrum of each of them is recorded separately in the NH stretch region by using IR/UV spectroscopy.[11,12] In addition to this conformer-selective technique, the cold conditions of the expansion ensure the resolution of each amide NH stretch, which will provide, at the end, a very precise characterization of each conformer. Because these vibrational modes are exquisitely sensitive to the environment of the NH groups, information about the H-bonding content of each conformer and, in particular, the H-bond strength can indeed be extracted from these IR spectra.[13−16] The full characterization of the conformations, including the peptide structure and the energetics, is completed by quantum chemistry calculations carried out on conformations selected for their low energy and their ability to reproduce the experimental spectra.

In order to document biologically relevant NH···S H-bonds, two Met-containing capped dipeptides, namely, *N*-acetyl-L-phenylalaninyl-L-methionine-amide (**FM**) and *N*-acetyl-L-methioninyl-L-phenylalanine-amide (**MF**), have been chosen as candidates of minimal size. In such peptides, several types of backbone H-bonded networks can already be anticipated (Figure S1, Supporting Information),[13] the relative stability of which is governed by extra interactions between backbone and side chains; in the present case, additional H-bonds are made

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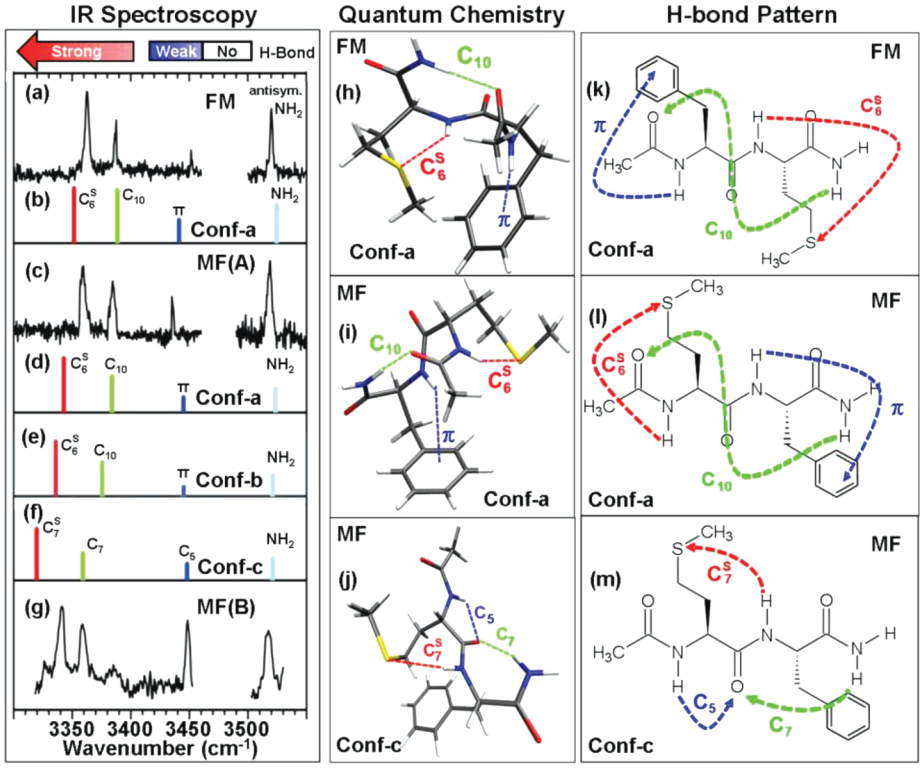

Figure 1. Experimental infrared spectra versus calculated vibrational frequencies, structures, and H-bond patterns of **FM** and **MF**. Experimental conformer-selective IR spectra of (a) the main conformer of **FM** and both conformers of **MF**, (c) conformer A and (g) conformer B, are compared with stick spectra (b,d–f) representing the computed NH stretch absorptions of conformations matching the IR spectra. For **FM**, Conf-a is the only one fitting the experiment; for **MF**, Conf-a best fits conformer A, Conf-b best fits conformer B, and Conf-c is the only one fitting conformer B with a C_5–C_7 H-bond sequence (Figures S7 and S8, Supporting Information). As a guide, the empirical H-bond strength range is also shown on the top. Three quantum chemistry structures (h–j) have been chosen to illustrate the H-bond patterns compatible with the experimental results (k–m). H-bonds involving the sulfur atom are colored in red and other H-bonds are in green for the stronger ones (C_7 or like C_{10}) and blue for the weaker ones (C_5 or NH···π).

possible by acceptors such as the π-electrons of phenylalanine or the sulfur atom of methionine.

The experimental techniques used in this work have been described elsewhere.[7] A pulsed laser desorption source (532 nm) is used to vaporize a solid sample made of peptide embedded in a graphite matrix.[17] The desorbed molecules are then cooled down by a pulsed molecular expansion of argon or a helium–neon mixture, which is skimmed prior to entering the ionization region of a reflectron time-of-flight mass spectrometer.[17] Mass-selective resonant two-photon ionization (R2PI) spectroscopy is used to record the ultraviolet (UV) spectra of the isolated peptides.[11,12,18,19] The low-temperature regime in the supersonic expansion allows us to distinguish the spectral contribution of each conformer, in the form of well-separated narrow vibronic bands. These bands are then used to probe the population of each frozen conformer and to perform conformation-specific infrared spectra using the IR/UV double resonance technique. The IR region of interest (i.e., the near-infrared NH stretch amide-A region) is scanned by an IR optical parametric oscillator, which causes a substantial heating of the peptides. IR absorption is then monitored through the depletion of the cold peptide signal generated by the UV laser, leading to a conformation-specific IR spectrum. The structural assignment of the peptides is based on both DFT-D[20] calculations and comparison with analogous model dipeptides already studied. Prior to DFT-D calculations, a systematic conformational exploration of the peptides has been carried out using different force fields to generate a large set of conformations (~600 for each). About 50 conformations were selected from each ensemble for their ability to match the number of strong and weak H-bonds (2 and 1, respectively) in the experimental IR spectra. The geometry optimization, electronic energy, frequency calculation, and zero-point vibrational energy of these selected conformations were carried out at the RI-B97-D/TZVPP level of theory. Finally, a set of 150 methionine-containing low-homology protein structures, selected from the protein structure database,[21] was used to search for the occurrence of intraresidue NH(i)···S and inter-residue NH(i+1)···S H-bonds for comparison with the observed gas-phase structures.

Gas-Phase Spectroscopy. The UV features observed (Figure S2, Supporting Information) combined with the IR signatures recorded (Figure 1) demonstrate that a limited number of stable conformers are observed in the expansion, only one for **FM** and two, of comparable abundances, for **MF** (labeled as **MF**(A) and **MF**(B)). Their IR spectra in the NH stretch region are depicted in Figure 1a, c, and g; four vibrational bands are observed for each conformer, as anticipated from the presence of two amide bonds and one C-terminus NH_2, this latter giving rise to both symmetric and antisymmetric combinations. These spectra resemble those obtained on the previously studied valine-containing peptide chains[22,23] (Figure S3, Supporting Information), suggesting similar secondary structures. However, each conformer observed for **FM** or **MF** is characterized by an extra band, which lies remarkably in the red part of the spectrum and clearly reveals an additional, strong H-bond.

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In order to assign these IR signatures, we take advantage of the excellent records of a recently developed quantum chemistry method, B97-D[20] which has demonstrated its ability in reproducing the structures and the energetic and the vibrational frequencies of isolated peptides faithfully enough to compare reliably with gas-phase experiments.[11,17] Among the hundred conformations calculated, only few structures have been found to be consistent with the experimental results based on both their energetics and predicted spectra (Figures S4−S8 and Tables S1 and S2, Supporting Information).

The resulting assignment of **FM** and **MF**(A) reveals that their backbone adopts a β-turn structure, stabilized by a C_{10} H-bond and, to a lesser extent, by a weak NH···π interaction (Figure 1a−d,h,i,k,l). The striking point is that the Met side chain folds back on itself, allowing the sulfur atom to link to the neighboring amide NH within the same residue (noted NH(i); Figure S1e, Supporting Information). The corresponding H-bond leads to the formation of an intraresidue six-membered ring (C_6^S), responsible for the most red-shifted band of the spectrum (~3360 cm^{-1}).

In **MF**(B), the assignment is less unambiguous because two types of structures are found to provide good agreement with the experimental results (Figure 1e−g). The assignment can then be made either to a β-turn backbone structure (Conf-d) stabilized by both a C_6^S ring and a NH···π interaction, like in **MF**(A), or to a C_5−C_7 backbone sequence, where an inter-residue NH···S H-bond between the sulfur atom and the NH of the next residue (noted NH(i+1)) leads to a C_7^S ring, giving rise to a different type of folding for the Met side chain (Figure 1j,m).

In all of these forms, the NH···S H-bond is characterized by the largest NH red shift, ranking this interaction among the strong H-bonds in peptides, together with the classical N−H···O=C H-bonds of the secondary structures.[13−16] As an illustration of the competition between these interactions, one will notice that the resulting backbone pattern of the isolated **FM** and **MF** is significantly different from their equivalent, in which Ala is substituted to Met, whose backbone adopts a C_5−C_7 and C_7−C_7 structure, respectively.[24]

Comparison with Protein Structures. In a recent statistical analysis of protein structures, it was found that in a large proportion of methionine residues (45%), the sulfur atom binds to an amide NH group of the protein backbone;[6] 24% of these $NH_{backbone}$···S H-bonds correspond to a local folding of the methionine side chain, forming either C_6^S intra-residue NH(i)···S H-bonds or C_7^S inter-residue NH(i+1)···S H-bonds. These two types of local folding correspond to the Met side-chain structures identified in the present peptides, C_6^S rings in **FM** or **MF**(A) and C_6^S or C_7^S in **MF**(B). C_6^S rings are characterized by a rather limited range of the Met φ Ramachandran dihedral angle ($\varphi \approx -70°$), whereas a large diversity occurs for the ψ dihedral (Figure 2). A similar observation can be made on proteins having a C_7^S, but in this case, the role of the dihedrals is reversed ($\psi \approx 150°$). With these limitations on the Ramachandran angles, the local folding of the methionine side chain can be seen as the softer and reversible version of the covalently bound side chain of proline, whose pyrrolidine ring brings a permanent local constraint ($\varphi \approx -60°$) on the backbone.[25] The striking result of the present study is that the gas-phase structural parameters that control the side-chain folding in the calculated structures (φ or ψ, depending on the type of rings formed, C_6^S or C_7^S) are quite close to the average data measured in proteins having the same

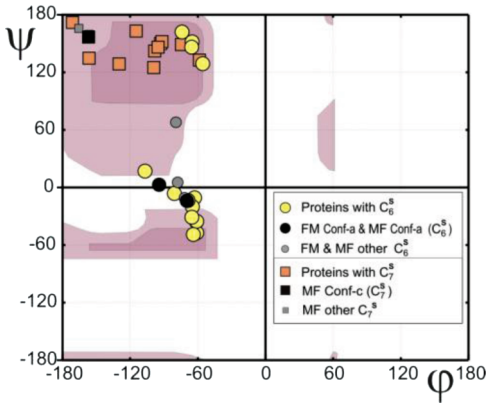

Figure 2. Ramachandran plot of the methionine φ and ψ dihedral angles (in degrees) when a local C_6^S (circles) or C_7^S (squares) side-chain folding occurs in proteins (colored symbols: protein data bank structures[21]) or in the investigated **FM** and **MF** peptides (black and gray: quantum chemistry calculations). Pink areas (adapted from ref 29) indicate the regions sterically allowed for any residue (apart from glycine and proline).

folding pattern (Figure 2 and Figure S9 and Tables S3−S6, Supporting Information). As an illustration of this convergence, an example of Met-Phe sequence conformations in proteins[21,26,27] is shown for comparison with the peptides studied (Figure 3). These results demonstrate that the peptides investigated turn out to be good models for locally folded methionine side chains, which qualifies them to estimate the properties of the $NH_{backbone}$···S_{Met} H-bonds occurring in proteins, in particular, their energetics.

Energetics. As the spectral shift of a NH stretch vibration probes the strength of the associated H-bond, it provides an ideal ranking tool. The observed NH···S H-bonds in peptides induce ~120 to ~140 cm^{-1} red shifts, taking 3480 cm^{-1} as a free amide NH vibration in peptides.[13] These red shifts are comparable to that observed for the H-bonded complex of indole with CH_3−S−CH_3 (~158 cm^{-1}),[7] where the H-bond strength was estimated from a quantum chemistry calculation of the binding energy (ΔH(0K)= 23 kJ mol^{-1}). In the present peptides, interestingly, the interamide NH···O=C H-bonds lead to ~95 and ~120 cm^{-1} red shifts for the C_{10} and C_7 bonds, respectively,[13] highlighting the fact that NH···S H-bonds have to be considered with at least the same importance as any other "classical" secondary structure H-bond in their contribution to the folding pattern of proteins.

Calculation of the energy difference between conformations with folded or extended Met side chains (Figure S10, Supporting Information) is also expected to evidence the stabilization of folded structures brought about by the NH···S H-bond, although additional, weaker interactions involving the methyl and methylene groups of the side chain are also at play. At 300 K, Met side-chain folding into C_6^S or C_7^S rings is found to provide a ~10 kJ mol^{-1} stabilization (Table S7, Supporting Information), illustrating the gas phase preference of Met residues to establish local NH···S H-bonds, although this process is not entropically favored.

The present study demonstrates that the highly selective gas-phase spectroscopic methods combined with quantum chemistry constitute a powerful approach to address basic biochemical issues at the molecular level. In the peptides investigated, the Met side chain is indeed found to fold locally by forming a strong H-bond with the neighboring NH(i) or

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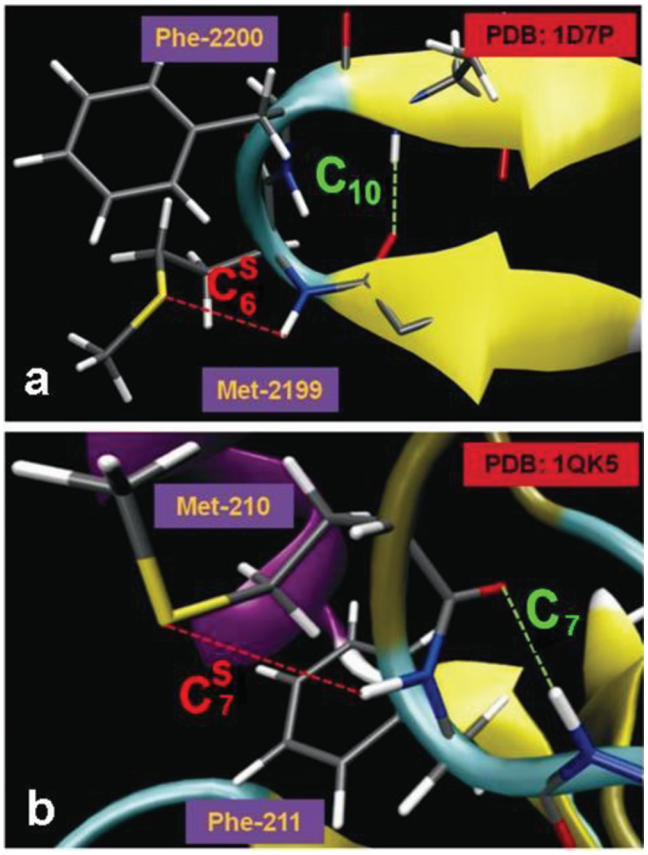

Figure 3. Examples of locally folded methionine residues in proteins as obtained from X-ray diffraction crystal structures. (a) Met-2199-Phe-2200 segment of the coagulation factor VIII Precursor protein (1D7P[26] in the Protein Data Bank[21]), exhibiting a C_6^S ring Met side-chain folding occurring on a β-turn of the Met-Phe sequence (C_{10}), resembling Conf-a of **MF** (Figure 1d,i,l), the form assigned to the observed gas-phase **MF**(A) conformer (Figure 1c); (b) Met-210-Phe-211 segment of the hypoxantine-guanine phosporosyltransferase (1QK5[27]), exhibiting the same C_7^S NH···S and C_7 H-bond pattern as that in **MF** Conf-c (Figure 1f,j,m), one of the structures that can be assigned to conformer **MF**(B) (Figure 1g).

NH(i+1) amide of the backbone, echoing the significant propensity for Met side chains to fold similarly in proteins. The existence of strong $NH_{backbone}\cdots S_{Met}$ bonds suggests that the role of the methionine residue as an intrinsically strong H-bonding acceptor in proteins has to be systematically considered by chemists and biochemists involved in protein simulation. This conclusion deserves to be emphasized in the context of the emergence of new classes of theoretical tools, like polarizable force fields,[28] which are currently targeting "biological accuracy" for the energetics of molecular recognition between ligands and receptors or between drugs and biological active sites of proteins.

■ ASSOCIATED CONTENT

Supporting Information

Appendix S1: Methodology details. Supplementary Figures S1–S9: H-bonding schemes, UV spectra of related molecules, most stable theoretical structures, comparison between experimental and simulated IR spectra. Supplementary Tables S1–S7: Spectroscopic data and comparison with theoretical predictions; comparison with protein structures. Corresponding references. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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