

Far/Mid-Infrared Signatures of Solvent–Solute Interactions in a Microhydrated Model Peptide Chain

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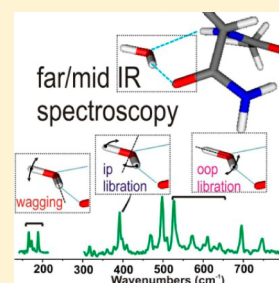
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Supporting Information

ABSTRACT: Far/mid-IR signatures of the first hydration step of a flexible biomolecule, the model peptide chain Ac-Phe-NH₂, have been investigated in the gas phase using the selective IR/UV double-resonance laser technique. The broad spectral region investigated with the free-electron laser FELIX (150–800 cm⁻¹/70–12 μm) provided a direct access to three intermolecular vibrational modes of monohydrates, in which the water molecule bridges neighboring NH and CO sites of the peptide backbone. The spectral features, analyzed with the help of quantum chemistry, are assigned to the IR activity of the libration and wagging motions of the water molecule together with a strongly mode- and conformer-dependent vibrational coupling between solute and solvent molecules. These resolved spectra obtained in a so far poorly documented spectral region provide benchmark data, which should enable theoreticians of molecular interactions to assess their methods, in terms of both intermolecular potentials and treatment of the vibrational anharmonicity.

SECTION: Spectroscopy, Photochemistry, and Excited States



The first hydration shell of a biomolecule, the so-called biological water, is thought to play a crucial role in the structural dynamics of polypeptides and proteins and in the end in their functionality.^{1–5} It has indeed been proposed that this critical layer, through its dense network of H-bonds, can efficiently mediate the dynamical coupling between the protein chain and its solvent environment.^{2,4} The basic foundations for such an idea can be elegantly tested on the molecular scale by investigating the influence of a few water molecules (and even a single one!) on a gas-phase isolated peptide. Sophisticated techniques, like IR/UV double-resonance spectroscopy, are indeed well-adapted to cope with the conformational diversity of flexible biomolecules, such as peptides and their hydrates.^{6–16} Used in conjunction with laser desorption and supersonic expansion to vaporize small biomolecules of interest, it has become a standard experimental approach to collect structural information in the gas phase, especially by interrogating the NH and CO stretch motions through their specific amide I and II or A spectral regions (1400–1800 and 3100–3500 cm⁻¹). Spectroscopists can thus isolate hydrates of biomolecules and characterize precisely their H-bonding network, which allows them to evaluate the role of the very first hydration molecules on the peptide folding. Several studies have recently reported how the conformational landscape of small isolated molecules is influenced upon the addition of the first solvent molecules.^{6,7,12,14–18} In the case of a short model protein chain, as, for example, the here-studied dipeptide analogue *N*-acetyl-phenylalanine-amide (AcPhe-NH₂, in short NAPA), a single water molecule has been proven to be efficient

enough to trigger the backbone folding by bridging two neighboring NH and CO sites along the chain.¹⁵

The same hydrate approach can, in principle, also allow experimentalists to access the intermolecular vibrational modes, which directly reflects the strength of the noncovalent forces responsible for cohesion of the hydrate. Here, targets of interest are the motions of the water molecule in the complex, especially water librations. These motions are expected to be IR-active and intense because of the large change in the water dipole moment orientation they induce. Their IR signatures, which are usually located in the far/mid frequency range (50–800 cm⁻¹), correspond to the so-called connectivity and libration regions of the absorption of bulk water.¹⁹ They have been investigated by IR or Raman spectroscopy in water complexes or hydrates of small molecules of biological interest like amides, either in an isolation matrix^{20–22} or in a supersonic expansion.^{23,24} However, these non-selective techniques do not allow disentangling easily the contributions of monomers and hydrates, especially in the case of a flexible molecule with multiple monomer conformations and hydration sites.²⁰ Similarly, information can be obtained from UV spectroscopy in a supersonic expansion, but these data pertain to the excited state.²⁵

Although, the IR region has been expanded previously toward the far-IR window,^{26,27} so far no explicit information

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about intermolecular modes in the electronic ground state of a hydrated protein/peptide chain could be systematically obtained. Therefore, taking advantage of the broad accessibility of a free-electron laser (FELIX at FOM Rijnhuizen, NL) together with its coupling to a state-of-the-art molecular beam machine,²⁸ we have recorded the far-mid-IR spectrum of a folded conformer of Ac-Phe-NH₂ and of its two main monohydrates using the IR/UV technique with a one-color resonant two-photon detection scheme.¹⁵ The major interest of the present approach stems from a comprehensive view of the spectral features induced by hydration, in particular the water libration motions and their coupling to the soft modes of the backbone. In addition, spectroscopic data from this poorly investigated spectral region also provide unique opportunity to theoreticians to assess the high-level biochemistry tools they develop for modeling proteins and their environment. These tools, namely, polarizable force fields, are usually adjusted against high-level quantum chemistry calculations on subsets of the system of interest. In turn, quantum chemical methods such as density functional theory (DFT) or DFT-D (explicitly including semiempirical dispersion terms) are struggling to properly describe long-range interactions. In such a context, any experimental relevant information about hydration structures and the low-frequency motions of hydrated systems is most welcome by theoreticians. In gas-phase experiments, two main conformations have been observed for the hydrate¹⁵ (labeled W and X), both based on the same folded Ac-Phe-NH₂ conformer (NAPA C, Figure 1a) that exhibits a so-called C₇ H-bond,

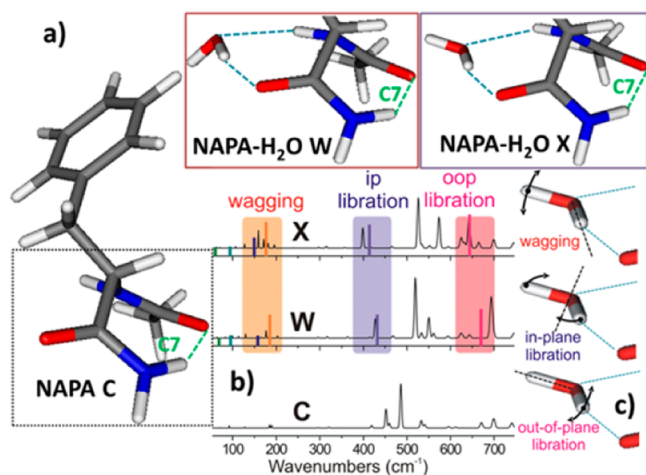


Figure 1. Calculated RI-B97D/TZVPP geometries (a) and far/mid infrared spectra (b) of hydrated W, X, and isolated C conformers. Intermolecular and intramolecular H bonds are indicated in blue and green, respectively. Sticks indicate the six intermolecular frequencies calculated assuming a frozen NAPA molecule. Three intense intermolecular modes are predicted in the ~ 160 (wagging), ~ 400 (in-plane libration, ip), and ~ 600 cm^{-1} (out-of-plane libration, oop) regions (colored areas). The three motions are schematized (c).

linking the two ends of the peptide backbone; all of these species having the same Phe side chain orientation. The two hydrates share the same H-bonding network, with the water molecule bridging the NH and CO sites of Phe, and mainly differ by the orientation of the water free OH moiety (Figure 1a). They have comparable stabilities, relative energies within less than 1 kJ. Quantum chemistry calculations (unscaled harmonic frequencies at the RI-B97D/TZVPP^{29–31} level of theory) predict for the complex three regions in which intense

absorption involving water motions are present. An overview of the calculated spectra for the hydrated complexes is shown in the top and middle panels of Figure 1b. The numerous features correspond not only to low-frequency backbone motions, which are already apparent in the spectrum of the C monomer (Figure 1b bottom), but also to displacements of the water molecule in the complex. An RI-B97D/TZVPP harmonic vibrational calculation test (sticks in Figure 1b) carried out assuming a frozen peptide enabled us to provide a rough picture of these latter intermolecular motions: (i) three intense modes: a wagging mode of the free OH moiety and two water librations, which correspond roughly to in-plane (ip) and out-of-plane (oop) water motions, taking the quasi planar H-bond bridge as a reference plane (see Figure 1c) as well as (ii) three modes of lower intensity and lower frequency: two bending (below 100 cm^{-1}) and one stretching mode (at ca. 160 cm^{-1}). The mid/far IR spectra of the two monohydrates W and X and of the C monomer have been recorded at FOM Rijnhuizen using the free electron laser FELIX.³² The spectra were recorded in regions where intense bands are predicted (Figure 1b), namely, $140\text{--}215\text{ cm}^{-1}$ (far-infrared) and $300\text{--}800\text{ cm}^{-1}$ (mid-infrared), with a spectral resolution of typically 1%. Narrow features corresponding to the IR absorption were obtained, confirming the feasibility of the IR/UV method in such a low-frequency region for molecules of this size.³³ In comparison with the monomer spectrum, the IR spectra of the two hydrates are richer and more intense. Besides a set of intense bands already present in the monomer (ca. 190 and 470 cm^{-1}), the hydrates exhibit new features, namely, additional bands around 160 cm^{-1} , an isolated strong band at ca. 390 cm^{-1} , and a set of medium bands in a broad region ($500\text{--}650\text{ cm}^{-1}$).

To provide a better insight into these intermolecular motions as well as to assess the robustness of the theoretical predictions, we have compared RI-B97D/TZVPP results with a series of DFT and DFT-D hybrid functionals (with the cc-pVTZ basis set³⁴), namely, the popular B3LYP³⁵ (although its known trend to underestimate the dispersion effects), ω B97XD³⁶ (which includes long-range correction (ω), exchange (X), and dispersion (D) terms), and M06-2X³⁷ (tested for noncovalent interactions). Although significant differences are found in the far-IR region, a reasonable agreement about frequency and intensity is observed. In the following, the experimental spectra will be discussed in light of the RI-B97D/TZVPP. Results from the other functionals are provided in the Supporting Information.

Monomer Spectroscopy. Figure 3 (left) presents the spectrum of the monomer C together with the RI-B97D synthetic spectrum. Calculations are very close to the experiment, and one can readily assign: (i) the far-IR band at 190 cm^{-1} and its shoulder at 187 cm^{-1} to collective torsional motions of the backbone; (ii) the two doublets ($538\text{--}549$ and $698\text{--}742\text{ cm}^{-1}$) to deformation and butterfly out-of-plane vibrations of the phenyl ring respectively; (iii) the four-feature massif in the 450 cm^{-1} region to coupled NH bending and NH₂ inversion motions, both of them roughly corresponding to motions out of the corresponding amide plane. The good agreement between experiment and RI-B97D harmonic calculations is quite promising with unscaled calculated frequencies off by $<8\text{ cm}^{-1}$ relative to the experiment for the phenyl deformation, butterfly, and backbone torsional motions (Supporting Information, Figure S1). One will, however, notice that the NH bends and NH₂ inversion frequencies are systematically

overestimated by the harmonic frequencies, whatever the method considered (Figure S1 in the Supporting Information), suggesting a large anharmonicity of these out-of-plane modes. In addition, their coupling pattern is not properly reproduced.

Hydrate Spectroscopy. Despite a moderate agreement between experiment and calculations on W and X (Figure 3, middle and right panels), a detailed analysis allowed us to assign the major part of the experimental spectra. For a better understanding of the effect of hydration, all calculated normal modes have been projected along virtual intermolecular motions involving the sole water molecule displacements (obtained assuming a frozen peptide), thus providing a qualitative contribution of the water displacements in each mode. This contribution is indicated by stars of various sizes in Figure 3, where larger asterisks imply larger water displacements.

Peptide Modes. Calculations indicate that NH bending and NH₂ inversion motions still lead, as in the monomer, to an intense IR absorption, which is significantly blue-shifted to the 500 cm⁻¹ region. This trend is indeed found experimentally for both hydrate conformers with, however, different manifestations: an isolated band at 479 cm⁻¹ in W, assigned to NH₂ inversion, and an intense doublet (at 498 and 527 cm⁻¹) in X, assigned to the NH₂ inversion and NH bending. One will notice again a significant overestimate of the theoretical frequencies compared with experiment, even more pronounced than in the NAPA C case, suggesting an increased anharmonicity in the hydrate. In the 700 cm⁻¹ region, the Phe butterfly doublet is still active and comparable to the monomer case, in agreement with the weak interaction expected between the phenyl ring and the water molecule.

In-Plane Water Libration. The isolated bands at 385 cm⁻¹ for W and 392 cm⁻¹ for X match well the features predicted for the in-plane libration, in terms of position, intensity, and weak coupling with peptide modes (blue stars in Figure 3). The agreement seems to be much better for X than for W (calculated frequencies are off by about 20 and 40–50 cm⁻¹, respectively), again suggesting a strong anharmonicity of this mode.

Out-of-Plane Water Libration. In contrast with the ip libration, calculations suggest that this oop motion has significant contributions (pink stars in Figure 3) spread within a broad region (500–750 cm⁻¹), where it is mixed with medium-frequency peptide backbone modes. This explains the experimental observation of numerous relatively intense bands in this region, not observed in the monomer but appearing in the hydrate due to a significant intensity borrowing from the oop libration (Figure 2). In other words, the IR oscillator strength of the oop libration is spread out all over the region by vibrational coupling. Such an extended coupling is reminiscent of the broad spectral region of the libration bands in water clusters, such as trimers,²¹ although in these cases identical molecules favor resonant couplings. In the present case, the coupling is favored by the density of the amide backbone modes in this region (oop bending and amide deformations), in contrast with the ip libration. Again, one can notice that whereas the coupling phenomenon is qualitatively accounted for by theory (pink stars in Figure 3) the pattern is far from being properly reproduced, partially because the coupling requires a correct estimate of the unperturbed backbone motions, which are probably not predicted with enough precision at this level of theory (whatever the method used, see Supporting Information Figures S2 and S3).

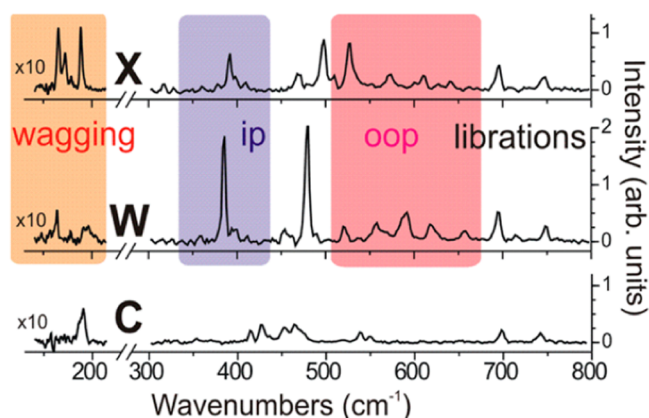


Figure 2. IR/UV double-resonance spectra of the W and X conformers of the NAPA hydrate (upper and middle panel) compared with that of the isolated NAPA C conformer (lower panel). New strong absorption bands together with enhancement of some peptide modes are observed in the wagging, ip, and oop intermolecular libration regions (colored areas) for hydrated W and X spectra. The UV wavenumbers used to monitor the ion signal in the IR/UV experiments are 37 533, 37 614, and 37 653 cm⁻¹ for monomer C and hydrated X and W, respectively.

Far-IR Region. The spectral signatures at ca. 180 cm⁻¹ are very different for the two hydrates, suggesting a high sensitivity of the corresponding modes to the water orientation. This observation is consistent with the fact that the wagging motion precisely concerns the orientation of the water molecule. For X, the band pattern found is comparable to the theoretical one, indicating a significant coupling of the water wagging to backbone torsional modes. For W, apart from a doublet already found in the isolated peptide (ca. 200 cm⁻¹), a single, isolated new band due to the presence of the water molecule is observed. However, in this case, owing to the large variability of the coupling pattern with the theoretical method, a further assignment of this band remains difficult: it may involve the water wagging motion (orange stars), the weaker stretching intermolecular mode of the complex (cyan stars in Figure 3), or any mixing with a peptide torsion mode. We cannot rule out the presence of stronger bands below 150 cm⁻¹ involving the wagging motion, especially taking into account that all tested functionals except RI-B97D seem to predict the intense wagging band at ca. 130 cm⁻¹ (Figures S2 and S3 of the Supporting Information). Comparison between unscaled harmonic calculated frequencies and experiment suggests to take into account anharmonicity effects. A first attempt in this direction has been performed using one of the less time-consuming approaches: the second-order perturbational approach (PT2).³⁸ The results fail in reproducing quantitatively their values (for example, in X, the ip libration harmonic frequency of 392 cm⁻¹ is overcorrected down to 181 cm⁻¹ (Supporting Information, Figure S4), showing that a more sophisticated approach is required to tackle the issue.

In conclusion, the present letter reports the first direct and extensive probing of the far/mid-IR signatures of the effects of hydration on a model peptide chain. The conformer-selective highly sensitive technique employed allowed us to propose a comprehensive overview of the absorption features of two Ac-Phe-NH₂⋯H₂O hydrates, in which the water molecule bridges the NH and CO sites of the Phe residue. Three out of the six intermolecular vibrations of these hydrates have been observed. These strongly IR-active modes are the in-plane and out-of-

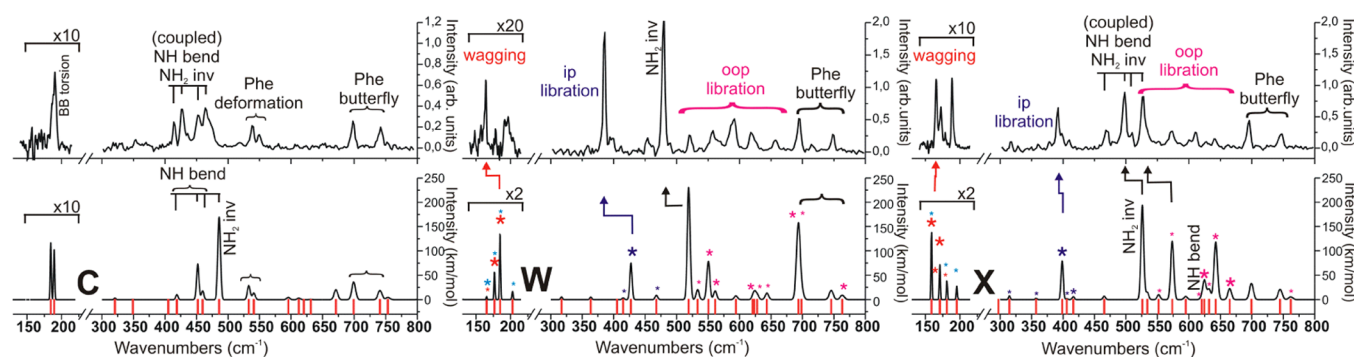


Figure 3. IR/UV double-resonance spectrum of isolated conformer C (left), hydrated W (middle), and X (right) compared with RI-B97D/TZVPP harmonic calculated spectra assign at 1% FWHM apparatus function (bottom). The red sticks indicate the calculated frequencies in this spectral region. Stars indicate quantitatively the contribution of the water modes (nature is color-coded), with the three sizes (by decreasing order) corresponding to >50%, between 30 and 50%, and between 10 and 30%, respectively.

plane librations of the water molecule as well as the wagging motion of the free OH bond of water. The IR signatures illustrate the interplay between peptide flexibility and molecular motions in the first solvation shell. They provide a very mode-dependent picture of the coupling between the water molecule and the solute motions, the out-of-plane libration being the most easily coupled to peptide backbone motions. They also show how these solute–solvent couplings are strongly dependent on the peptide conformation.

Finally, the comparison between experimental features and theoretical frequencies obviously shows that the far/mid-infrared region constitutes a real challenge to the theory, even for DFT with recent functionals. Identifying hydrated conformers from this spectral region alone (without benefiting from the NH stretch analysis of ref 15) would have been a challenging task. The present experimental results therefore provide benchmarks that should help quantum chemistry, especially DFT calculations, to improve the description of long-range interactions. They also demonstrate the necessity for anharmonic calculations to rely on advanced mode descriptions, with curvilinear motions,³⁹ for instance, or methods, like the variational one.⁴⁰

■ ASSOCIATED CONTENT

Supporting Information

Methodology details, comparison between exp. and simulated IR spectra at all tested levels (harmonic and anharmonic), spectroscopic data and comparison with theoretical predictions, 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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