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Multiple Isomers and Protonation Sites of the Phenylalanine/Serine Dimer

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Supporting Information

ABSTRACT: Our investigation of the phenylalanine/serine (Phe/Ser) protonated dimer suggests that the intermolecular interaction between the two amino acids is more complex than could have been anticipated from previous studies of similar systems. Isomer-specific infrared (IR) spectra, recorded at an internal temperature of ∼10 K, demonstrate the presence of at least five isomers with nonzwitterionic structures. Moreover, isotopic substitution experiments provide evidence for different protonation sites among these isomers.

A complex of two amino acids in vacuum is possibly the simplest conceivable model system for the interaction of two proteins. While reduced to its very essentials, it still features the fundamental interactions that give rise to the complexity of larger systems. Protonated amino acid dimers in the gas phase have been investigated using IR spectroscopy.[1−6] Particular attention has been paid to the determination of the protonation sites, and it was found that most dimers are nonzwitterionic, with the exception of the proline dimer[3] and the mixed glycine/lysine dimer.[1] Different suggestions have been made for the structure of the glycine dimer. A geometry featuring a single intermolecular hydrogen bond between the ammonium group and the carbonyl oxygen of the neutral moiety now seems generally accepted.[1,3−5,7,8] Structures with the same hydrogen bonding pattern were also assigned to the alanine and valine homodimers and the alanine/glycine heterodimer, based on a strong resemblance of their IR spectra in the fingerprint region.[4] Likewise, the hydride stretch spectra of the Ser and threonine homodimers and the mixed Ser/threonine dimer were found to be similar to one another.[1,2] For the Ser dimer, several structures were discussed in which the ammonium group engages in several intermolecular hydrogen bonds.[2] The fingerprint spectrum of the protonated lysine dimer supports a structure that is also dominated by intra- and intermolecular solvation of the ammonium group, which is located on the side chain of one of the lysine moieties.[6]

These investigations paint a relatively simple picture in which the room-temperature IR spectra can be explained by the presence of primarily a single isomer. In contrast, proteins exist as a mixture of different conformers under physiological conditions.[9] In fact, the question has been raised of how much detailed information IR spectroscopic studies at room temperature can actually provide on protonated amino acid dimers.[10] In our investigation of the Phe/Ser protonated dimer, we obtain a more complex picture of the intermolecular interaction between the two amino acids. Isomer-specific IR spectra indicate the presence of at least five different species. Even more interestingly, ^{15}N labeling experiments provide evidence for different locations of the excess proton in different isomers.

As previously described, we obtain isomer-specific IR spectra by preparing ions at ∼10 K[11] and probing them with an ultraviolet (UV)/IR double resonance technique.[12] Briefly, the protonated 1:1 clusters of L-Phe and L-Ser (both Sigma Aldrich) are generated by nanoelectrospray from a 100 μM/1 mM methanol/water (1:1) solution. They are pretrapped in a storage hexapole, mass selected with a quadrupole mass analyzer, and injected into a cryogenic 22-pole ion trap, where they are cooled in collisions with helium. The ions are irradiated with a UV laser pulse, ejected from the trap and mass-analyzed with a second quadrupole filter. By detecting the occurrence of photofragments as a function of the laser wavenumber, a UV spectrum is obtained. To record an IR spectrum, the UV laser is fixed to the transition of a specific isomer, and an IR laser is fired ∼30 ns prior to the UV laser pulse. If the IR frequency is in resonance with a vibrational transition, it removes population from the ground state and thus depletes the fragment ion signal. An IR spectrum is obtained by recording this depletion as a function of the IR wavenumber.

Resonant UV irradiation of the protonated Phe/Ser dimer leads to the formation of photofragments, as shown in the mass spectrum of Figure 1. The peaks at m/z 106 and 166 result

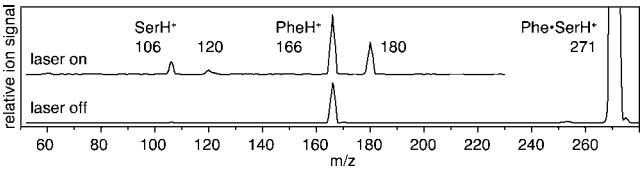

Figure 1. Fragment mass spectra obtained after irradiation of the dimer at 38432.8 cm^{-1} ("laser on") and without UV laser ("laser off").

from intermolecular dissociation of the dimer constituents, leading to protonated serine ($SerH^+$) and protonated phenylalanine ($PheH^+$), respectively. The feature at m/z 180 comes from loss of the phenylalanine side chain, and that at m/z 120 from the simultaneous loss of Ser, H_2O, and CO from the protonated dimer. Protonated phenylalanine is the only fragment that is also created by mild collisional activation,

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which occurs when the ions are injected into the 22-pole ion trap (bottom trace).

The UV spectrum of the dimer, recorded by monitoring the fragments at m/z 180 as a function of the laser wavenumber, is shown in Figure 2. The lowest energy transition occurs at

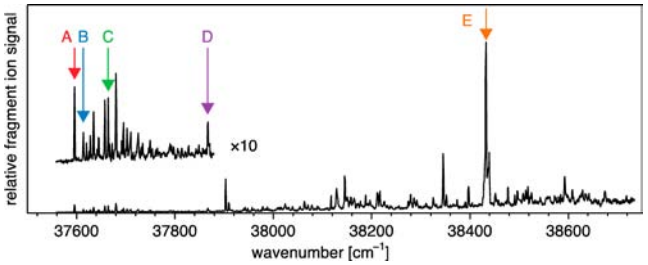

Figure 2. UV photofragment spectrum of the protonated dimer detecting the m/z 180 fragment ions.

37596.1 cm^{-1}, about 75 cm^{-1} blue-shifted compared to the band origin of PheH$^+$. As shown below by the IR spectra, several isomers contribute to the spectral features in this region.

Five distinct IR depletion spectra were obtained when several UV transitions were interrogated, indicating the presence of at least five different isomers under the present experimental conditions. The IR spectra A–E shown in Figure 3 correspond to the UV bands marked with arrows in Figure 2 (37596.1, 37613.7, 37664.4, 37866.6, and 38432.8 cm^{-1}, respectively).

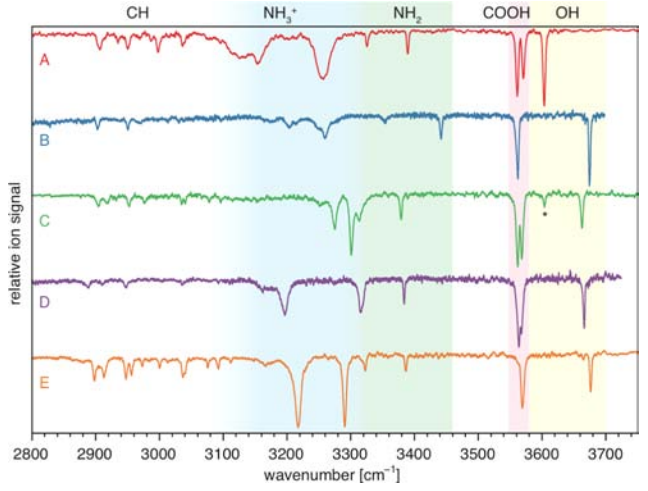

Figure 3. IR depletion spectra recorded with the UV laser tuned to the transitions marked in Figure 2. The shading indicates the spectral ranges of different types of hydride stretches.

The shading indicates the spectral ranges of the different hydride stretches. All isomers show a free serine OH stretch band between 3660 and 3680 cm^{-1} with the exception of isomer A, where a ~70 cm^{-1} red shift indicates a weak hydrogen bond, probably with the aromatic ring. The peak in spectrum C marked with an asterisk is attributed to a contribution to the spectrum from isomer A, which can occur if a weak UV transition of A overlaps with the UV band of isomer C that was interrogated. The free carboxylic acid OH stretches appear in a narrow range between 3550 and 3580 cm^{-1}. Isomers A, C, and D show two bands in this region, clearly indicating a nonzwitterionic structure, while for B and E, only one peak is observed.

The CH stretches, which occur below ~3100 cm^{-1}, are of little diagnostic value. The NH stretch range (~3100–3460 cm^{-1}), on the other hand, encodes most of the available information about the hydrogen-bonding network. For isomer A, the bands at 3326 and 3389 cm^{-1} are readily assigned to the symmetric and antisymmetric NH stretch of a neutral amino group, respectively, consistent with a nonzwitterionic structure. The spectral range, peak width, and intensity ratio of these peaks agree with the observations for neutral aromatic amino acids.[13−15] The peaks at 3128, 3155, and 3256 cm^{-1} exhibit the characteristic red-shift and broadening of hydrogen-bonded ammonium NH stretches.[12,16,17] Upon ^{15}N labeling of Phe, these three bands experience a ~7 cm^{-1} red shift,[18] which is not observed for the NH$_2$ bands, as shown in Figure 4, confirming

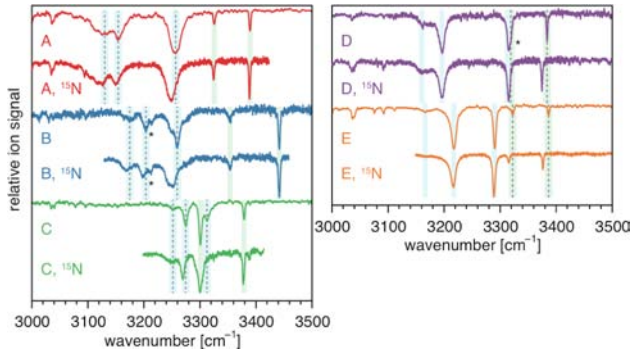

Figure 4. NH stretch spectra of the isomers A–D containing ^{15}N labeled Phe in comparison with their ^{14}N isotopologues.

their assignment. This observation also demonstrates that in isomer A, the excess proton is located on Phe, whose proton affinity is more than 8 kJ/mol higher than that of Ser.[19,20]

Similar to isomer A, ^{15}N labeling of Phe in isomers B and C reveals two different groups of vibrations. Three bands in the NH$_3^+$ range undergo a red-shift, while two bands in the NH$_2$ range remain essentially unchanged. In analogy to A, we assign them to the NH$_3^+$ and NH$_2$ vibrations, respectively. We conclude that B and C are both nonzwitterionic and protonated on the Phe amino group. For isomer B, an additional band appears at 3214 cm^{-1}, which does not shift upon ^{15}N substitution (marked with an asterisk in Figure 4). We tentatively assign it to the remaining carboxylic acid OH stretch vibration, which is red-shifted due to a strong hydrogen bond, as observed for some conformers of the neutral aromatic amino acids.[13−15] In isomer C, the NH$_3^+$ vibrations appear at higher wavenumber compared to isomers A and B, such that the ranges of the two groups of NH stretch bands overlap.

The IR spectra of isomers D and E show a different pattern of isotope shifts, which is most easily recognized for E. A red-shift is observed for the two NH vibrations with the highest wavenumbers, while the remaining transitions are unchanged. In D, the band at 3384 cm^{-1} clearly shifts to lower wavenumber. We identify a second transition at 3319 cm^{-1} (marked with an asterisk in Figure 4), which appears as a shoulder of the peak at 3316 cm^{-1} and which red-shifts upon isotopic substitution. The position of the peak at 3316 cm^{-1} does not change, while its shoulder disappears and its width decreases from 9 to 6 cm^{-1}, which is easily detectable at our IR resolution of ~1 cm^{-1}. Using similar arguments as above, we conclude that isomers D and E are both nonzwitterionic and

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protonated on the NH_2 group of Ser, the amino acid with the lower proton affinity.

It appears that for isomers D and E, intra- and intermolecular interactions override the inherent basicities of the two amino acids. The formation of an energetically more favorable hydrogen-bonding network obviously compensates for the energy cost of transferring the proton from Phe to Ser. For isomer E, a question remains about the assignment of the second carboxylic acid OH stretch. If involved in a strong hydrogen bond, it should appear in the NH_3^+ range, where it probably overlaps with one of the three broad and intense ammonium vibrations.

We also attempted to determine the detailed structures of isomers A–E by means of a commonly employed computational strategy (see the Supporting Information for details). Using the AMBER force field in MacroModel,[21] we performed a Monte Carlo conformational search for nonzwitterionic structures with the excess proton located on either amino group. The lowest-energy structures obtained were optimized on the M06/6-31G* level with the Gaussian package.[22] However, their calculated IR spectra show poor agreement with the experimental spectra, rendering a definitive structural assignment impossible. While the protonated dimer is sufficiently small to be tractable by DFT calculations, the conformational search of this highly flexible system proved a formidable task. Different structural families were obtained when different starting structures were employed, suggesting that the search algorithm does not sample the potential energy surface (PES) efficiently. If, moreover, the force field PES should be too coarse an approximation of the DFT surface, it is conceivable that the search might neglect relevant minima.

While the complexity of the conformational search clearly warrants more sophisticated tools, our calculations nevertheless provide some general insights. The most stable calculated structures that we obtained feature three or four strong hydrogen bonds involving heteroatoms (Figure S1). The solvation of the NH_3^+ group dominates the hydrogen bond network, while the OH and NH_2 protons are mostly bystanders, a finding that is consistent with the measured spectra.

In conclusion, we demonstrate that the intermolecular interaction in the Phe/Ser protonated dimer is more complex than could have been anticipated from previous studies. Cold, isomer-specific IR spectra show that at least five different isomers with nonzwitterionic structures are present under our experimental conditions. In two of them, the excess proton is located on Ser, the amino acid with the lower proton affinity, as isotopic substitution experiments indicate. These findings provide the first experimental evidence for the simultaneous existence of a large number of isomers in a protonated amino acid dimer. If other dimers should possess similar degrees of flexibility, it is obvious that isomer-unselective studies at room temperature could only yield an incomplete picture. Moreover, the Phe/Ser protonated dimer presents an interesting case in which structural rearrangement in a biomolecular cluster leads to intermolecular proton transfer. Even though at first sight an amino acid dimer may appear an oversimplified model for the interaction of two proteins, it captures the aspect of conformational heterogeneity and reflects the variety of interactions present in larger systems.

■ ASSOCIATED CONTENT

Supporting Information

Details on computations and complete ref 22. 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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