

Gas-phase Infrared Spectroscopy of Monopeptides from 10 to 3 μm

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Infrared spectra of protected amino acids, *N*-benzoxycarbonyl-L-proline and *N*-benzoxycarbonyl-L-isoleucine were measured in the gas phase from 10 to 3 μm . 4 and 3 conformers were found, respectively. The spectra in the 3 μm region reveal the carboxylic OH and amide NH bonds do not participate in hydrogen bonding with neighboring amide groups despite the highest acidity in the molecule. The spectra in the 10–5 μm region imply the main chain structure is the same among the conformers.

Gas-phase spectroscopies intended to elucidate intrinsic structural behavior of peptide chains have been widely studied. The structural study of amino acids is of fundamental importance. Molecular structures of various amino acids have been studied by microwave and infrared (IR) spectroscopy.^{1–8} However, there have been several problems in previous studies of amino acids. Microwave spectroscopy gives very precise structure, but it is rather difficult to extend to larger peptides systematically. This point may be addressed by the use of vibrational spectroscopy such as IR, and several IR studies on middle size peptides have already been reported.⁹ The second problem is that of end-capping. Most spectroscopic studies on amino acids reported to date were performed on unmodified amino acids. If one wishes to obtain structural information of peptides from amino acids, the amino (or carboxy) group has to be bonded to a carboxy (amino) group. If the amino group is left as it is, it tends to act as a hydrogen bonding site and disturb the geometry. In fact, all previous studies of amino acids point to the formation of intramolecular-hydrogen-bonded structures, but it is highly plausible that the free amino group exaggerates the stability of the hydrogen-bonded structures. Thus the structure of amino acid with capped amino group is desirable. The third problem is spectral range and resolution. A free electron laser is a powerful light source covering from 20 μm to blue, but typical resolution is 20 cm^{-1} , preventing precise discussion of molecular vibrations. Conventional IR OPO or DFG lasers can generate an IR pulse with a resolution of <1 cm^{-1} , but can cover only regions shorter than 4 μm .

Recently, we have developed a table top coherent IR source that can cover a region from 10 to 2.5 μm .^{10,11} In this letter, we report IR spectra of end-capped mono-peptides, *N*-benzoxycarbonyl-L-proline (Z-Pro) and *N*-benzoxycarbonyl-L-isoleucine (Z-Ile) (Figure 1). Here, introduction of the aromatic end cap (*N*-benzoxycarbonyl group, Z-) enables us to apply UV laser spectroscopy with a minimum perturbation on the main chain structure.

The IR spectra were measured by applying IR–UV depletion in a spectrometer with a quadrupole mass filter, details of which have already been described elsewhere.^{10–12} Briefly, the samples were heated to 120 $^{\circ}\text{C}$ to achieve sufficient vapor pressure. The sample vapor was seeded in Ne gas (3 atm), and expanded into the vacuum chamber. The samples were purchased from Kanto Chemical Co., Inc., and used without further purification.

Figure 1 shows resonance-enhanced 2 photon ionization (R2PI) spectra of (a) Z-Pro and (b) Z-Ile. The strongest transition in the electronic origin region is located at 37588 cm^{-1} for Z-Pro and 37594 cm^{-1} for Z-Ile. These are shifted 61 and 67 cm^{-1} from that

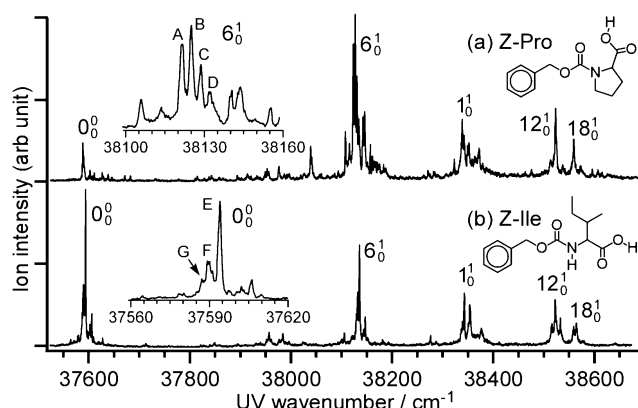


Figure 1. R2PI spectra of Z-Pro (a) and Z-Ile (b). Insets show the molecular structures and expanded views around 6_1^1 and 0_0^0 bands that were probed in the IR spectroscopy.

of benzyl alcohol (37527 cm^{-1}).¹³ This confirms the negligible interaction between the chromophore and the peptides. Several vibronic bands that are typical in monosubstituted aromatic molecules are observed and the assignments are also presented in Figure 1.¹⁴ One interesting point is that the origin-band intensities relative to the vibronic bands are largely different even though the same chromophore is in similar environment. Change in lifetime of the excited state is one possibility. Actually, lifetime shortening due to a minor conformational change around the chromophore has been reported for phenylalanine.⁴ In addition, all the vibronic bands split into 3 or 4 peaks, representatives of which are shown in the insets of Figure 1. These transitions are considered to stem from different structural isomers. This is verified by the IR spectra obtained by probing each band.

Figure 2 shows IR spectra recorded by monitoring the electronic transitions labeled (A)–(D) for Z-Pro and (E)–(G) for Z-Ile in the insets of Figure 1. Here, the 6_1^1 band of Z-Pro was probed because of the weak intensities of the origin band. Although the IR spectra in Figures 2A–2D (and Figures 2E–2G) are measured for the same molecule, each spectrum presents different a spectral feature. This means that the splitting arises from electronic transitions of rotational isomers (conformers) caused by rotations of single bond(s) in this case. The IR spectra imply at least 4 stable conformers for Z-Pro and 3 for Z-Ile in the gas phase. All spectra show sharp absorptions at around 3580 cm^{-1} . These bands are assigned to free OH stretching vibrations of the carboxylic acid.^{4,5,7,8} This indicates that the carboxylic OH bond does not participate in hydrogen bonding even though the acidity of the group is relatively high, and there exist neighboring electron-rich basic groups. It is largely different from previous reports on free proline studied by Fourier transform microwave spectroscopy with laser desorption source.^{1,3} Two major conformers are intramolecular-hydrogen-bonded structures and non-hydrogen-bonded conformer is only a weak component.^{1,3} It suggests the importance of

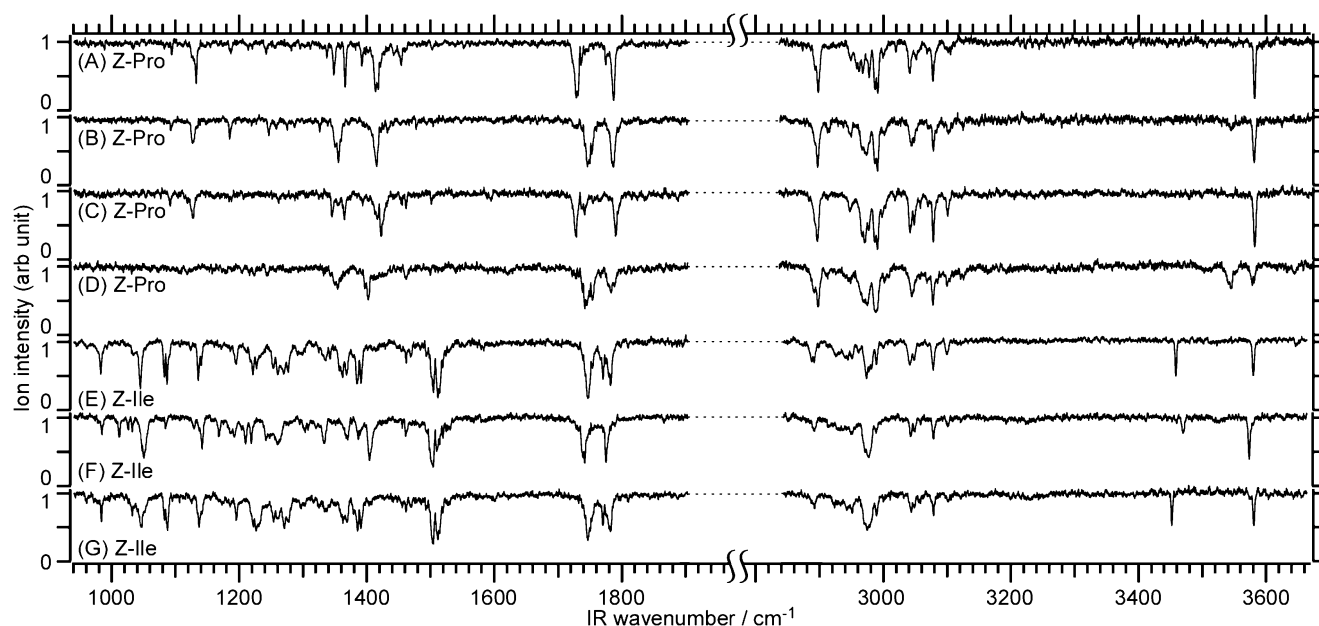


Figure 2. IR spectra of Z-Pro (A)–(D) and Z-Ile (E)–(G) recorded by monitoring electronic transitions labeled A–D for Z-Pro and E–G for Z-Ile in Figure 1, respectively.

the end cap upon the terminal amino group. The intramolecular-hydrogen-bonded structure is common for other free amino acids studied in gas phase, which always present stable conformers with hydrogen bond between a carboxylic OH and a lone pair on nitrogen.^{1–6,8} Thus the perturbation of the structure by a free amino group appears to be a common problem. Our preliminary calculation based on B3LYP/cc-pVDZ predicted the hydrogen-bonded conformers are stabilized more than non-hydrogen-bonded ones. To understand the specific stability of non-hydrogen-bonded conformers, advanced theoretical calculation is necessary. It is also necessary to assign the geometry of each conformer.

The trace (D) shows an additional OH stretching transition at 3545 cm^{-1} . We suggest that the higher component is caused by an overlap of the probed electronic transitions among the conformers and only this conformer forms a weak hydrogen bond on OH. However, it is clear from the shift of 35 m^{-1} that the interaction is not strong. In addition, the population of this conformer should be small because of the weak band intensity in the electronic spectrum, and it makes only a minor contribution to the population of the stable conformers. The sharp bands at around 3460 cm^{-1} are NH stretching vibration of the peptide bond. Z-Pro does not have the NH stretching due to its secondary amide structure. Similar to the OH stretching vibration, NH stretching vibrations are almost identical. From the position, the NH stretching can also be assigned as a free NH.⁹ The CH stretching vibrations at around 3000 cm^{-1} are complicated due to their numbers and anharmonic couplings, and it is difficult to give a firm assignment.

In the $10\text{--}5\text{ }\mu\text{m}$ region, strong doublets at 1780 cm^{-1} and bands at 1500 cm^{-1} of Z-Ile are assigned to the C=O stretching and NH bending vibrations, the amide I and II bands, respectively. Absorptions below 1400 cm^{-1} arise from a complicated mixture of CH bending and skeletal chain stretching motions. The butyl side chain of the Z-Ile may give many bands in this region. Again, the spectra closely resemble each other. This implies the main chain structure is common and difference in the conformers is ascribed to minor conformational changes in the side chains, like puckering of the proline ring and internal rotations inside the butyl group, which

are expected to have minor effects on IR spectral shifts. Quantum chemical calculations aiming to deduce sophisticated structure are now in progress.

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