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Ultrafast internal rotational dynamics of the azido group in (4*S*)-azidoproline: Chemical exchange 2DIR spectroscopic investigations

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ABSTRACT

The azido group in 4-azidoproline (Azp) derivative, SA (Ac-(4*S*)-Azp-NHMe), can form an intramolecular electrostatic interaction with the backbone peptide in the s-trans and C^{γ}-endo conformations of SA. As a result, the azido group exists as two forms, bound and free, which are defined by the presence and absence of such interaction, respectively. The bound and free azido forms are spectrally resolved in the azido IR spectrum of SA in $CHCl_3$. Using the two-dimensional infrared (2DIR) and polarization-controlled IR pump–probe methods, we investigated the internal rotational and orientational relaxation dynamics of the azido group and determined the internal rotational time constant of the azido group to be 5.1 ps. The internal rotational motion is found to be responsible for the early part of the orientational relaxation of the azido group in SA. Thus, the femtosecond 2DIR spectroscopy is shown to be an ideal tool for studying ultrafast conformational dynamics of SA.

1. Introduction

Proline residues confer unique conformational constraints on proteins, thereby influencing their structure, function, and dynamics [1–5]. In particular, the cis–trans isomerization of the aminoacyl-proline peptide bond plays distinct roles in the conformational control of proteins. The s-trans conformer is generally more stable than the s-cis conformer even though the s-trans/s-cis ratio can be modulated by backbone and side-chain modifications and solution conditions. For instance, the s-trans conformer is greatly stabilized in the seven-membered cyclic γ-turn (C_7) structure, which is favorably formed in polar aprotic solvents by an intramolecular hydrogen-bonding interaction between two peptide bonds [6–10]. In addition, the s-trans/s-cis ratio can be varied upon introduction of various substituents such as the azido group at the C^{γ} atom of proline via their stereoelectronic effects [11].

Recently, we studied 4-azidoproline (Azp) derivatives, RA (Ac-(4*R*)-Azp-NHMe) and SA (Ac-(4*S*)-Azp-NHMe), using NMR, FTIR, and IR pump–probe spectroscopic methods [12]. It was found that the s-trans/s-cis ratio is modulated by azido configurations. It turns out that the azido configurations affect the intramolecular interactions, which are responsible for ensuing conformational propensities. Interestingly, the azido group on the C^{γ} atom in the 4*S* configuration directly can form an intramolecular electrostatic interaction with the backbone peptide and thus stereoelectronically influences the stability of various SA conformers. As a result, the azido group in SA exists as two forms, bound and free, which are defined by the presence and absence of its intramolecular electrostatic interaction with the backbone peptide, respectively (Fig. 1). The internal rotation of the azido group around the $C^{\gamma}-N^{\delta}$ bond turns on and off such interaction (Fig. 2). However, such internal rotational dynamics have not been experimentally investigated in details.

Femtosecond two-dimensional infrared (2DIR) spectroscopic method has been used in studying a variety of chemical exchange processes occurring on picosecond timescales under thermal equilibrium conditions [13,14], such as solute–solvent complexation [15–18], hydrogen-bond exchange [19–21], carbon–carbon bond rotational dynamics [22], ion pairing dynamics [23,24], fast conformational switching dynamics of proteins [25,26], and other equilibrium dynamics [27–30]. In 2DIR spectroscopy, a specific target mode is vibrationally-labeled with an initial excitation frequency ω_τ. After a finite waiting time (T_w), the emission frequency ω_t of the initially vibrationally-labeled mode can be experimentally measured. The 2DIR spectrum, $S(\omega_t, \omega_\tau; T_w)$, is then displayed in a two-dimensional frequency space, i.e., ω_τ and ω_t. The spectral correlation between the initial excitation and final emission

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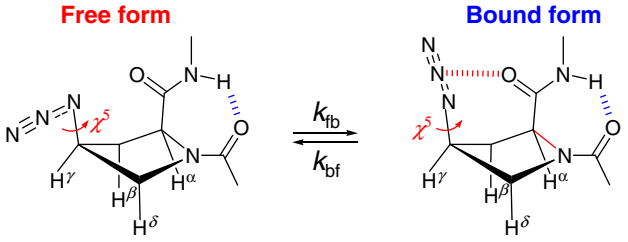

Fig. 1. Conformational dynamics between free and bound azido forms in SA. The energy minimum structure of SA depicted here adopts the s-trans and C^{γ}-endo conformations [12]. For the dihedral angles defining the geometry-optimized structures, see Table S1 of the Supplementary data. Free and bound azido forms are define by the absence and presence of the intramolecular electrostatic interaction between the azido central N^{ε} atom and methylamide carbonyl O atom. The intramolecular electrostatic interaction in the bound azido form is indicated by red hashed line, whereas the C_7-forming H-bonding interaction in the s-trans conformer is indicated by blue hashed line. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

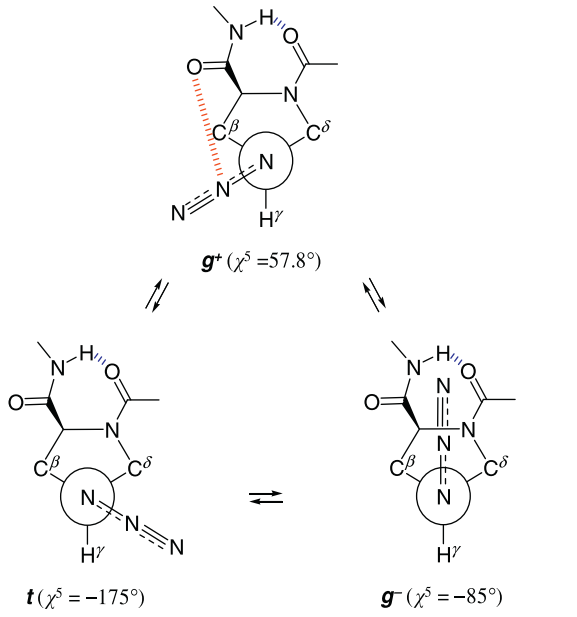

Fig. 2. Internal rotational dynamics of the azido group along the C^{γ}–N^{δ} bond axis in SA. The Newman projections of the three conformers (*trans*, *gauche*+, and *gauche*−) viewed down the N^{δ}–C^{γ} bond axis clearly illustrate the internal rotational relationship between the bound and free azido forms in the s-trans and C^{γ}-endo conformations of SA [12]. The g^+ conformer affords the bound azido form, whereas g^- and t conformers afford the free azido form. Note that the dihedral angles χ^5 (C^{β}–C^{γ}–N^{δ}–N^{ε}) for the geometry-optimized structures are indicated though the fully staggered conformations are depicted for simplicity. For the dihedral angles defining the geometry-optimized structures, see Table S1 of the Supplementary data. The intramolecular electrostatic interaction in the bound azido form is indicated by red hashed line, whereas the C_7-forming H-bonding interaction in the s-trans conformer is indicated by blue hashed line. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

frequencies is measured as a function of T_w. Therefore, the time-evolution of the 2DIR spectra as a function of T_w provides dynamical information about the molecular systems under study [31–34].

Here, we report the chemical exchange 2DIR studies on the internal rotational dynamics of the azido group along the C^{γ}–N^{δ} bond in SA. We found that the corresponding internal rotational time constant τ_{ir} is about 5.1 ps. The polarization-controlled IR pump–probe experiments were also performed to measure the orientational relaxation dynamics of SA by probing the azido stretch mode. It turns out that the initial orientational relaxation time constant is quantitatively similar to the internal rotation time constant. This result indicates that the internal rotational motion studied by the chemical exchange 2DIR spectroscopy is indeed responsible for the orientational relaxation of the azido group in SA.

2. Experimental

2.1. Two-dimensional infrared (2DIR) spectroscopy

The femtosecond laser systems employed in the experiments were described in great details before [23]. In addition, the principles and experimental details of 2DIR spectroscopy were presented elsewhere [35–38]. In brief, mid-IR pulses centered at ~2070 cm^{-1} with a bandwidth of ~250 cm^{-1} (in fwhm) were generated from our laser system and properly compensated to approximately produce a transform-limit pulse of ~60 fs at the sample position. Mid-IR beam with a pulse energy of ~800 nJ was split into three excitation beams and a local oscillator ($\mathbf{k}_1$, $\mathbf{k}_2$, $\mathbf{k}_3$, and $\mathbf{k}_{LO}$). Three mid-IR beams ($\mathbf{k}_1$, $\mathbf{k}_2$, and $\mathbf{k}_3$) were focused onto the sample in a boxcar geometry with a parabolic mirror (focal length = 10 cm) and after the sample the beams were collimated with another parabolic mirror (focal length = 10 cm). The spot size of the IR beams at the sample position was estimated to be less than 100 μm in diameter. The relative time delays between the three mid-IR pulses were varied with computer-controlled linear translational stages. The IR signal field emitted from the sample in a unique phase-matched direction was combined with a local oscillator pulse for subsequent heterodyne detection. The heterodyne-detected signal was spectrally-resolved in a monochromator onto a 64-element MCT (HgCdTe) array detector (InfraRed Associates Inc.) equipped with a high-speed data acquisition system (Infrared Systems Development Corp.). A small portion of the mid-IR beam was sampled and went around the sample and used as a reference beam to correct the fluctuation of the laser intensity during the experiments.

In 2DIR experiments, there are three experimentally controlled time variables. The time delay between the first and second pulses corresponds to the coherence evolution time (τ), the time delay between the second and third pulses is the waiting time (T_w), and the time delay after the third pulse is the detection time (t). The heterodyned 2DIR signal, $S(\omega_t, \tau; T_w)$, is collected by scanning τ at a fixed T_w and the resultant spectrum (ω_t) on a 64-element MCT array detector is measured. During the experiments, the monochromator essentially performs the Fourier transform along t, generating the ω_t spectrum. To obtain the 2DIR spectrum $S(\omega_t, \omega_\tau; T_w)$ at a given T_w, the Fourier transform of the signal $S(\omega_t, \tau; T_w)$ with respect to τ is numerically performed, which yields the ω_τ axis. Consequently, the 2DIR spectrum $S(\omega_t, \omega_\tau; T_w)$ is usually displayed with the initial excitation frequency ω_τ and final emission frequency ω_t at a given T_w. A purely absorptive 2DIR spectrum is obtained by adding the rephasing and nonrephasing 2DIR spectra measured with the dual scan method in which the corresponding signals are collected by using two different pulse sequences [39].

2.2. Polarization-controlled IR pump–probe spectroscopy

In IR pump–probe spectroscopy, a strong IR pump pulse, which is linearly polarized, is used to vibrationally excite the azido stretch mode to its first excited state ($v = 1$), and a subsequent time evolution of the excited state molecule is monitored with a time-delayed IR probe pulse whose polarization direction is experimentally controlled. This IR pump–probe signal originates from the ground-state bleach ($v = 0 \rightarrow 1$), stimulated emission ($v = 1 \rightarrow 0$),