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Infrared Line Shape of an α-Carbon Deuterium-Labeled Amino Acid

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The utility of conventional vibrational spectroscopy as a tool for investigating the structure and dynamics of proteins is hindered by the inherent congestion of their infrared (IR) absorption spectra. Untangling detailed structural and dynamical information from the IR spectrum of a protein is particularly daunting because of the presence of coupled local vibrations with similar frequencies whose absorptions overlap to form broad and complex spectral bands. A strategy for circumventing these difficulties is the introduction of isotopically labeled bonds at specific sites, whereby the labeled vibrations are effectively decoupled from the unlabeled vibrations. In the case of isotopically labeled carbon−deuterium bonds (C−D), the resulting IR absorption frequencies (2000−2300 cm^{-1}) occur in an otherwise unoccupied region of the protein vibrational spectrum. This enormous simplification allows the nonperturbing C−D bonds to be used as reporters of *local* protein structure, dynamics, and energy flow.

In previous studies, C−D bonds were incorporated as vibrational probes at specific sites of amino acid side chains, and their IR spectra provided insights about static and dynamic processes in cytochrome *c*.[1] Another successful approach has been the use of isotopically labeled carbonyl bonds ($^{13}C{=}^{16}O$ or $^{13}C{=}^{18}O$) and IR spectroscopy to provide information on backbone structure and dynamics.[2] By analogy, direct deuterium labeling of the amino acid α-carbon (C_α−D) offers a convenient vibrational probe of the structure and conformational fluctuations of the protein backbone. In the present work, we demonstrate the potential viability of C_α−D labels through a systematic comparison of experiment, density functional theory (DFT), and combined quantum mechanical/molecular mechanical (QM/MM) simulations of the IR line shape of alanine deuterated at the α-carbon (Ala-d_1) in aqueous solution.

The curve in Figure 1a is the experimental IR spectrum of the C_α−D stretch of free Ala-d_1. The spectrum was recorded under ambient conditions and represents the difference in absorbance between a dilute solution (0.1 M) of Ala-d_1 in sodium phosphate buffer (50 mM, pH 7.0 ± 0.1) and an identical solution of nondeuterated alanine (Ala-d_0). See the Supporting Information (SI) for further experimental details. Of immediate note is the unexpected complexity of the experimental spectrum (Table 1 and Figure 1a): in addition to the strong absorption, $\omega_{C-D} = 2203$ cm^{-1}, there is a shoulder at $\omega_a = 2177$ cm^{-1} and absorptions at $\omega_b = 2102$ cm^{-1} and $\omega_c = 2262$ cm^{-1}. The spectrum does not depend on concentration, indicating that the multiple absorptions do not arise from alanine aggregation (see SI).

To aid in the assignment of the spectrum, a series of DFT calculations were performed in Gaussian 03. The DFT calculations used the B3LYP functional and the 6-311++G(d,p) basis set. A polarizable continuum model (PCM)[3] served as an implicit aqueous solvent ($\epsilon = 78.4$) for zwitterionic Ala-d_1 because it is not stable in the gas phase. A normal-mode analysis alone, which predicts a single absorption in the 2000−2300 cm^{-1} region ($\omega_{C-D} = 2210.2$ cm^{-1}), was not sufficient to assign the multiple absorbances observed in the Ala-d_1 spectrum.[4] Thus, anharmonic third-order couplings, *C*, between the C_α−D normal mode and all overtones and combinations that fall within ±100 cm^{-1} of ω_{C-D} were calculated.[5] Four combinations of lower frequency modes (Table 2) were identified as being strongly coupled (≥10 cm^{-1}) to the C_α−D stretch (i.e., forming Fermi resonances with the C_α−D stretch). Each of the low-frequency modes involved in the Fermi resonances is delocalized over the entire amino acid (Figure 2). Degenerate perturbation theory was then used to predict the locations of the five absorptions (2090.7, 2160.2, 2184.4, 2206.9, and 2276.8 cm^{-1}) depicted as triangles in Figure 1a. The DFT calculations agree qualitatively with the experimental results and suggest the spectral complexity results from Fermi resonances. In

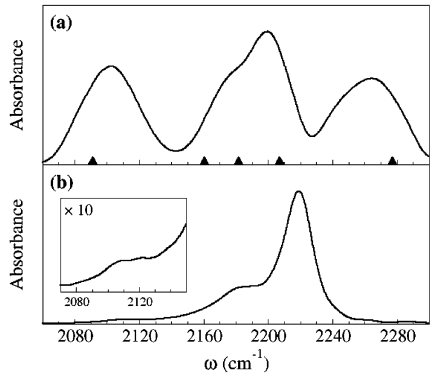

Figure 1. (a) IR difference spectrum of 0.1 M Ala-d_1 and Ala-d_0; (triangles) predicted peak positions from DFT for Ala-d_1 in implicit H_2O. (b) QM/MM simulation of the IR difference spectrum of Ala-d_1 in explicit H_2O.

Table 1. 0.1 M Ala-d_1 Spectral Assignments

assignment		position (cm^{-1})	width (cm^{-1})
ω_{C-D}	Exp.	2203	26
	QM/MM	2219.3	20.8
ω_a	Exp.	2177	35
	QM/MM	2186.5	45.5
ω_b	Exp.	2102	40
	QM/MM	2124.1	61.2
ω_c	Exp.	2262	47
	QM/MM		

Table 2. DFT Calculated Combination Bands for Ala-d_1 in Implicit Water

ω_i (cm^{-1})	ω_j (cm^{-1})	$\omega_i + \omega_j$ (cm^{-1})	|C| (cm^{-1})
978.7	1243.4	2222.1	38.7
942.0	1243.4	2185.4	22.7
978.7	1189.0	2167.7	31.0
942.0	1189.0	2131.0	60.2

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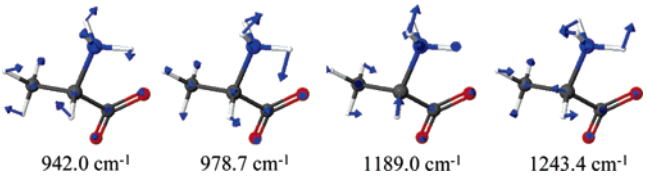

Figure 2. Ala-d_1 normal modes involved in Fermi resonances with the $C_\alpha-D$ stretch.

addition, they predict that the observed shoulder (ω_a) results from two overlapping absorptions not resolved in the experiment.

Despite the success of DFT in assigning the Ala-d_1 spectrum, computational cost prohibits its application to proteins. Furthermore, the DFT calculations are not sensitive to the dynamics of the local protein environment. Building on the recent success of Yang and Cho in using QM/MM methods for the calculation of the amide I vibrational line shape of *N*-methylacetamide in water,[6] we present in Figure 1b the first calculations of a C−D vibrational line shape in aqueous solution. Details of the QM/MM simulations are provided in the SI, but briefly (1) a 20 ns molecular dynamics (MD) trajectory with a 1 fs time-step of a system containing a single zwitterionic Ala-d_1 or Ala-d_0 molecule and 442 TIP3P water molecules is constructed in the NPT ensemble (300 K and 1 atm) using AMBER; (2) the configuration of the entire system is saved once every 4 fs; (3) for each saved configuration, the electric dipole moment of the alanine solute is computed by a Mulliken population analysis of a semiempirical (PM3) calculation of the alanine wave function in the presence of the fixed TIP3P water charges; (4) the classical IR absorption intensity is obtained as the power spectrum of the fluctuating electric dipole moment. This procedure allows the solute charge distribution (i.e., its dipole moment) to be polarized by the environment, which has been shown by Yang and Cho to be essential for obtaining reliable IR line shapes.

The lower panel of Figure 1 shows the IR difference spectrum for Ala-d_1 in explicit water simulated with the QM/MM strategy outlined above. The positions and widths of the spectral features are summarized in Table 1. Qualitatively, the simulated line shape mirrors the experiment. The two weak features on the red side of the main absorbance are classical analogues of quantum mechanical Fermi resonances and are present in the QM/MM simulations because of nonlinearities inherent in the classical force field (e.g., anharmonic terms describing intramolecular dihedral interactions and kinetic energy coupling).[7] Despite the fact that the MM force field was not specifically parametrized to reproduce such phenomena, it is remarkable that the QM/MM calculations give resonances in the correct relative positions: the splitting between ω_{C-D} and the two Fermi resonances, ω_a and ω_b, are 26 and 101 cm^{-1} in the experiment compared to 32.8 and 95.2 cm^{-1} in the QM/MM simulation.

To further support the interpretation that the Ala-d_1 spectral complexity results from Fermi resonances, a QM/MM simulation of the Ala-d_4 ($ND_3^+-CDCH_3-CO_2^-$) line shape in D_2O was also performed. Since the QM/MM simulations are based on classical molecular dynamics, the distribution of solute conformations and solvation environments is not affected by this isotopic substitution, but the low-frequency modes of alanine are altered. The simulated spectrum in D_2O is significantly different from that in H_2O (see SI), consistent with the assignment of the features in the Ala-d_1 spectrum to Fermi resonances and not to different solute conformations or solvation. Unfortunately, in D_2O, the experimental C−D line shape is obscured by the broad and intense O−D stretch.

It is encouraging that the QM/MM methodology provides an approximate description of Fermi resonances because the techniques are directly applicable to the study of C−D vibrations in proteins. Nevertheless, there are some notable differences between experiment and the QM/MM simulations. The relative intensities of ω_a and ω_b are not captured by the simulations, and ω_c is absent (Figure 1). Also, the QM/MM line widths do not quantitatively agree with experiment; ω_{C-D} is too narrow, while ω_a and ω_b are too broad (Table 1). For future studies, the QM/MM approach could be improved to give more accurate intensities and line widths by utilizing a more sophisticated population analysis technique to obtain partial charges for the solute dipole or by improving the parametrization of the solute's internal motions.

We have demonstrated that $C_\alpha-D$ labels offer a new direction for exploring backbone structure and dynamics with IR spectroscopy. The C−D absorption is split into several bands due to Fermi resonances, and the absorption frequency of each band remains within the transparent region of the protein's IR spectrum. These absorptions should be easily observable within a protein and should be sensitive to the protein's lower frequency collective vibrations that play a role in protein dynamics and function. Thus, the combination of experiment and theory holds great promise for the study of protein dynamics and its potential contribution to biological function.

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Supporting Information Available: Details regarding the experimental techniques and calculations are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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