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LETTERS

A New Vibrational Spectroscopic Tool for the Determination of Peptide Conformation: The Isotope-Edited $C^{\alpha}H^{\alpha}$ Stretch Mode

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Our studies of the alanine dipeptide (ADP) vibrational frequency map (*J. Phys. Chem. A* **2002**, *106*, 3391) reveal that the $C^{\alpha}H^{\alpha}$ stretch mode, and particularly its $C^{\alpha}D^{\alpha}$ isotope-edited form, is a potentially powerful tool for determining the φ, ψ conformation in peptide systems. In this communication, we present ab initio $C^{\alpha}D^{\alpha}$ and $C^{\alpha}H^{\alpha}$ stretch frequencies of the α_R, β, and polyproline II conformations of the ADP, showing that they give rise to well-separated frequencies, and discuss the kinds of experimental data needed to establish the desired structural information.

The effective use of infrared and Raman spectroscopies in determining chain conformation in peptides and proteins has been based on having a basic understanding of the normal modes of vibration of such structures, from the early studies of *N*-methylacetamide[1] through the development of detailed empirical polypeptide force fields[2] to the recent ab initio analyses of the alanine dipeptide (ADP) vibrational frequency map.[3,4] All of the associated theoretical results and the extensive associated experimental work to date have focused on the so-called amide modes of the peptide group,[1] primarily amide I (mainly CO stretch (s)), amide II (CN s plus NH in-plane bend (ib)), and amide III (whose main characteristic is the incorporation of NH ib, sometimes associated with CN s[2]). Because of the compound nature of these modes and their conformation-dependent interaction with other coordinates,[2] clear structure assignments are often ambiguous.

Our studies of the ADP map,[3,4] that is, vibrational frequency as a function of the φ(CNC$^{\alpha}$C) and ψ(NC$^{\alpha}$CN) dihedral angles, show that another normal mode is sensitive to these angles and may be additionally (and perhaps uniquely) capable of determining local conformation. This is the $C^{\alpha}H^{\alpha}$ s frequency, $\nu(C^{\alpha}H^{\alpha})$, and particularly its $C^{\alpha}D^{\alpha}$ s isotopic counterpart. In this communication, we introduce the theoretical basis for this correlation using three commonly studied conformations and the ADP to illustrate the assignments. Subsequent publications[5] will analyze in detail various aspects of this correspondence.

It may seem strange to expect that the $C^{\alpha}H^{\alpha}(D^{\alpha})$ s mode would be sensitive to the local φ, ψ conformation, even though this bond lies at the confluence of these two dihedral angles. Such an impression would be a result of a prevailing attitude that this is a completely localized mode, and in fact, the usual normal-mode analysis assigns to it a potential energy distribution (PED) of 100. Complete localization, however, is rarely the case, and this plus other factors suggest the possibility of a significant conformation dependence. First, our calculations[3−5] show that, even for the isolated ADP, the diagonal stretch force constant, $f(C^{\alpha}H^{\alpha})$, depends on φ, ψ. This undoubtedly reflects the differing local interactions as a function of φ, ψ, which result in different relative structures of the adjacent peptide groups and thus different electronic influences on the $C^{\alpha}H^{\alpha}$ bond (including different local electric field effects, which are known to influence the bond length and therefore its frequency[6,7]). Second, we find[3−5] that off-diagonal force constants, such as $f(C^{\alpha}H^{\alpha}, NC^{\alpha}C)$ and $f(C^{\alpha}H^{\alpha}, NC^{\alpha}H^{\alpha})$, are not insignificant and

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TABLE 1: Isotopically Isolated $C^{\alpha}D^{\alpha}$ and $C^{\alpha}H^{\alpha}$ Stretch Frequencies[a] of Conformers of the Alanine Dipeptide

conformation[b]	$C^{\alpha}D^{\alpha}$ [c]	$C^{\alpha}H^{\alpha}$ [d]
α_R	2153	2924
$\alpha_R(H_2O)_4$	2172	2951
β	2162	2936
$\beta(H_2O)_4$	2220	3018
PII	2170	2945
$PII(H_2O)_4$	2189	2973

[a] In inverted centimeters, scaled by 0.95. [b] (φ, ψ): α_R, ($-60°$, $-40°$); β, ($-134°$, $145°$); PII, ($-75°$, $145°$). [c] In $CH_3CONHC^{\alpha}D^{\alpha}(CH_3)CONHCH_3$. [d] In $CD_3CONHC^{\alpha}H^{\alpha}(CD_3)CONHCD_3$.

vary with φ, ψ. To the extent that these bond angles change with φ, ψ as a result of the different nonbonded interactions and that $NC^{\alpha}C$ or $NC^{\alpha}H^{\alpha}$ deformation, *d*, is part of the $C^{\alpha}H^{\alpha}$ eigenvector (which is the case for $NC^{\alpha}C$ d in $C^{\alpha}D^{\alpha}$ s to the extent of a 1−2 contribution in the PED), $\nu(C^{\alpha}H^{\alpha}(D^{\alpha}))$ will be affected. Third, since the nearest-neighbor hydrogen-bonded water structure is different for different φ, ψ,[5,8] differential bonding interactions to the two peptide groups would also be expected to affect the $C^{\alpha}H^{\alpha}$ bond and its stretching frequency.

Our computations for studying this relationship were done using density functional theory with the B3LYP functional and the (equilibrium planar-peptide predicting[9]) 6-31+G* basis set. The conformations examined were close to those now considered predominant in ordered and unordered peptides: α-helical, represented by α_R ($-60°$, $-40°$); extended chain, represented by β ($-134°$, $145°$); and "polyproline II", represented by PII ($-75°$, $145°$) (a local ∼3-fold left-handed helix structure proposed earlier on the basis of circular dichroism[10] and computational[11,12] studies and now recognized as a significant component of unordered peptides[13,14]). The molecules studied were the isolated ADP in the $CH_3CONHC^{\alpha}D^{\alpha}(CH_3)CONHCH_3$ and $CD_3CONHC^{\alpha}H^{\alpha}(CD_3)CONHCD_3$ forms (the latter to eliminate contributions from CH_3 modes to $C^{\alpha}H^{\alpha}$ s) as well as those molecules with four water molecules hydrogen bonded to the peptide groups (to account for important additional interactions occurring in aqueous solutions). In these calculations, only the φ, ψ were constrained, with all other parameters being optimized (a procedure we have shown to be meaningful for such stretching modes[4]). Roughly scaled frequencies are given in Table 1 (complete scaling[3,4] hardly affects the frequency differences).

We envision the experimental protocol to proceed as follows (using the $C^{\alpha}D^{\alpha}$ probe as an example, which is also favorable in that $\nu(C^{\alpha}D^{\alpha})$ occurs in an open region of the normal spectrum). For synthesized peptides, a series would be prepared with the $C^{\alpha}D^{\alpha}$ amino acid inserted sequentially at each position, with the φ, ψ at each site then being determined from the $C^{\alpha}D^{\alpha}$ s frequency of that sample (obviously, we need to know the dependence of $\nu(C^{\alpha}D^{\alpha})$ on the side chain,[5] but since it is hydrogenated, interaction with $C^{\alpha}D^{\alpha}$ s should be minimal). For fully ordered polypeptides (e.g., in the solid state), $C^{\alpha}D^{\alpha}$ amino acids would be inserted in an appropriately low concentration, with the regular structure making for a compelling determination of conformation. For proteins, to the extent that site-directed isotope labeling is possible, the above protocol can be implemented, which could be particularly useful in determining conformations in regions that are difficult to define securely by other methods.

For the proposed procedure to be effective, the $\nu(C^{\alpha}D^{\alpha})$ of probed conformations must be sufficiently distinctive. That this is so is indicated by the results shown in Table 1. We see that the $\nu(C^{\alpha}D^{\alpha})$ are well separated, even in the isolated ADP: 9 cm^{-1} between α_R and β and 8 cm^{-1} between β and PII. Hydrogen bonding to the peptide groups, as expected, shifts the frequencies, even reversing the order of β and PII. The frequencies are also more favorably separated: 17 cm^{-1} between α_R and PII and 31 cm^{-1} between PII and β. (These frequencies, not surprisingly, are somewhat sensitive to the details of the water structure.[5]) It should be mentioned that it might be the case that a given φ, ψ does not reside 100% of the time in one conformation: the overall band intensity profile would provide information on this distribution, which would be useful in comparisons with molecular dynamics simulations. In addition, our normal-mode analyses[5] show that discrimination between these structures is also evident in the $C^{\alpha}D^{\alpha}$ bend modes (in the 800−1000 cm^{-1} region), with the combined measurements making for an even more robust assignment of conformation.

Although most of the above observations also apply to $\nu(C^{\alpha}H^{\alpha})$, see Table 1, this version of isotope editing may be less favorable because all of the nonprobed amino acids need to be fully C-deuterated.

In addition to those mentioned, other relevant studies are in progress,[5] such as extension to other conformations (e.g., 3_{10}-helix), calculations of tri- and tetrapeptides (to characterize possible nearest-neighbor interactions), and comparison with experiment. It can be expected that these results will make the $C^{\alpha}D^{\alpha}$ s mode, because of its direct and sensitive coupling to φ, ψ geometry, an effective probe of peptide conformation.

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