Conformation Dependence of the $C^{\alpha}D^{\alpha}$ Stretch Mode in Peptides. 1. Isolated Alanine Peptide Structures

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Ab initio normal mode calculations have been performed on isolated alanine di- through octa-(i.e., blocked) peptides in uniform α_R, β, and polyproline II conformations to determine how the $C^{\alpha}D^{\alpha}$ stretch mode, which has been proposed as a possible determinant of the φ,ψ conformation at the C^{α} atom (Mirkin, N. G.; Krimm, S. *J. Phys. Chem. A* **2004**, *108*, 10923), depends on conformation and sequence length. This set of frequencies, including results on some kinked structures, demonstrates that such a discrimination is likely to be possible through experimental observations of peptides synthesized with successive deuteration at the H^{α} sites, on the basis of at least three properties: the values of the frequency at the first residue, the pattern of successive frequency differences, and the frequency differences between the first and last residues.

I. Introduction

Although many experimental techniques are available for the study of the structures of peptides and proteins in their native, and often crystalline, state, this is not generally the case for such molecules in unordered (or "unfolded") states. Such unordered states are of importance, and of increasing interest,[1] in understanding the broad range of functions of such molecules, yet experimental techniques for clearly defining their conformational states are limited, and fundamental questions still exist, such as whether local order does[2] or does not[3] persist along the unordered chain. It is clear that new experimental approaches to answering such questions are highly desirable.

It has long been established that vibrational spectroscopy is a powerful technique for studying the conformations of peptide chains.[4] Although many early studies have incorporated isotopic substitution in the spectral analyses (usually ND for NH), it has also been noted that "spectra of backbone isotopically substituted molecules in conjunction with normal-mode analysis provide a very powerful method for the determination of polypeptide chain conformation".[5] In a previous note,[6] we drew attention to the possibility that $C^{\alpha}D^{\alpha}$ stretch (s), ν(CD), which is essentially a relatively localized backbone mode in a clear region of the spectrum, could be a new tool for the determination of peptide conformation. In this paper, we report on the initial systematic normal mode study of the behavior of this frequency as a function of the local conformations at the C^{α} atoms in a peptide chain.

Although the characteristics of ν(CD) in aqueous solution are of most relevance to experimental studies of unordered peptide conformations (and will be reported in subsequent communications), it is useful to start such analyses with a study of the isolated peptide, since this permits investigating a number of common properties related to structure and providing a demonstration of the basic concepts. In this connection, we have performed ab initio normal mode calculations on a series of blocked peptides from the alanine dipeptide (ADP), CH_3-$CONHC^{\alpha}D^{\alpha}(CH_3)CONHCH_3$, to the alanine octapeptide (we label these as Ala-*n*, with $n = 1-7$ to indicate the number of C^{α} atoms). These have been studied in the α_R, β, and polyproline II (P) conformations, which reasonably span the range of ν-(CD) variability for such commonly occurring local structures in unordered peptides. In addition to the kinds of frequency differences between conformations noted in the earlier study of the ADP,[6] in which $n = 1$, we examine the dependence of ν(CD) on *n* and on the position of the C^{α} atom in the chain. These characteristics are analyzed for the ability of the $C^{\alpha}D^{\alpha}$ s mode to distinguish among such conformational states.

II. Computational Details

All calculations on the isolated peptides were performed at a hybrid density functional level of theory with the B3LYP functional and the (equilibrium planar-peptide predicting)[7] 6-31+G* basis set using Gaussian 03.[8] Geometries were fully optimized within the φ(CNC$^{\alpha}$C) and ψ(NC$^{\alpha}$CN) dihedral angle constraints of the three local conformations chosen for study: $\alpha_R(-60°, -40°)$, $\beta(-134°, 145°)$, and P$(-75°, 145°)$. Force constants in Cartesian coordinates were obtained by computing analytically the second derivative of the energy at each optimized geometry. These were then transformed into force constants in internal coordinates, and normal-mode frequencies and eigenvectors were calculated by the Wilson GF method.[9] We present the unscaled vibrational frequencies; if desired, scaling by 0.95 would bring these $C^{\alpha}D^{\alpha}$ s calculated frequencies roughly into the appropriate spectral region (for harmonic frequencies; anharmonicity may lower such frequencies by as much as 75 cm^{-1}).[10] We note that complete force field scaling[7] hardly affects the frequency differences.

III. Results and Discussion

A. Analysis Protocol. The proposed analysis of peptide conformation from ν(CD) is based on the following experimental protocol.[6] For a given Ala-*n* peptide, *n* samples would be prepared, the first with $C^{\alpha}D^{\alpha}$ substituted only on residue 1, $C^{\alpha}D^{\alpha}{}_1$; the second with $C^{\alpha}D^{\alpha}$ substituted only on residue 2,

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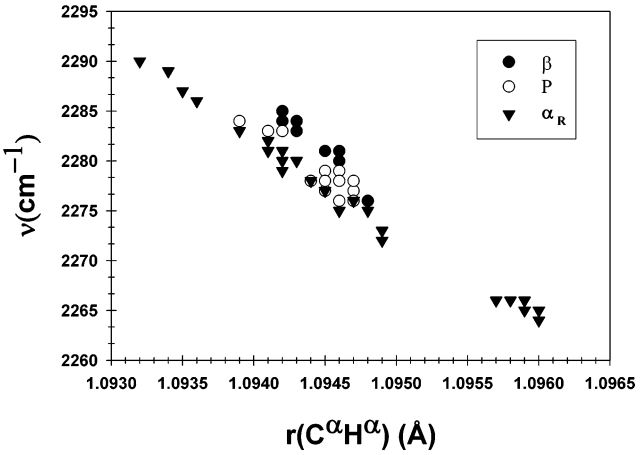

Figure 1. Dependence of $C^{\alpha}D^{\alpha}$ stretch frequency on $C^{\alpha}D^{\alpha}$ bond length in alanine peptides of varying length in uniform α_R, β, and polyproline II (P) conformations.

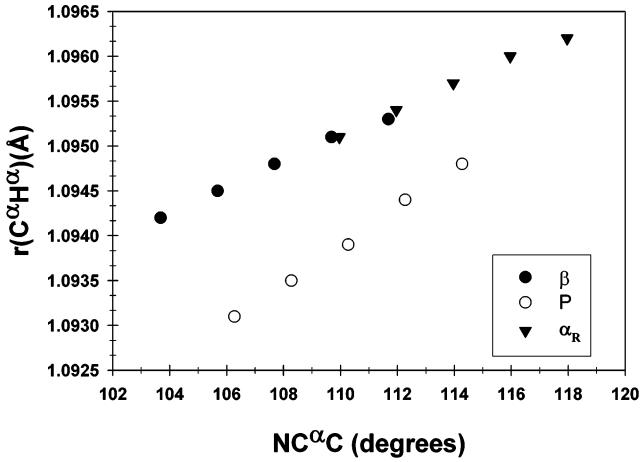

Figure 2. Dependence of $C^{\alpha}D^{\alpha}$ bond length on $NC^{\alpha}C$ angle in alanine peptides of varying length in uniform α_R, β, and polyproline II (P) conformations.

$C^{\alpha}D^{\alpha}_2$; and so on. Infrared (and possibly Raman) spectra would be obtained for these samples in the fundamental and (if possible) overtone regions (since the latter would yield greater frequency separations). The observed bands in conjunction with calculated normal-mode frequencies for the chosen system would constitute the basis for analysis of conformational states along the chain. We note that, although the frequency calculations are reported on the fully $C^{\alpha}D^{\alpha}$-substituted peptides, tests comparing, for example, tripeptides of $C^{\alpha}D^{\alpha}_1$,$C^{\alpha}D^{\alpha}_2$ composition with those of $C^{\alpha}D^{\alpha}_1$,$C^{\alpha}H^{\alpha}_2$ and $C^{\alpha}H^{\alpha}_1$,$C^{\alpha}D^{\alpha}_2$ structure showed identical residue ν(CD), thus demonstrating that there are no adjacent residue coupling interactions influencing the $C^{\alpha}D^{\alpha}$ s frequency at a given site.

B. Conformational Sensitivity of ν(CD). Before considering the specific correlations between ν(CD) and conformation, it is worth being reminded that, while such a stretch mode is usually considered to be totally localized in the $C^{\alpha}D^{\alpha}$ bond and therefore insensitive to environment, the major factor in determining the frequency is the bond length, which predominantly determines the diagonal force constant (off-diagonal force constants also contributing). This bond length should be sensitive to the local structure around the C^{α} atom (and therefore, the electronic environment), and this structure can thus be affected by the set of nonbonded interactions that are specific to the local φ,ψ conformation, including different local electric field influences on the bond length arising from the varying orientations of adjacent peptide groups.[11,12]

That in fact such an overall sensitivity exists is shown in Figure 1, which exhibits the range of $C^{\alpha}-H^{\alpha}$ bond lengths, r(CH), found in the various calculated structures and the range of associated stretch frequencies. The general known, essentially linear, trend of lower frequency with longer bond length is obvious (the gap between $r \approx 1.0950-1.0957$ Å is filled in by many (calculated) mixed conformation chains).[13] Within this trend, there are small variations: constant frequency for different bond lengths and different frequencies at constant bond length. These variations could be a result of conformation-dependent, though small, varying contributions of other coordinates to the eigenvector and, in particular, changes in the coupling between the $C^{\alpha}D^{\alpha}$ and C═O stretch modes.[10]

A specific demonstration of the sensitivity to local environment is illustrated by a calculation of the ADP r(CH) under a variety of conditions in which the $NC^{\alpha}C$ and $HC^{\alpha}C^{\beta}$ angles are varied. In calculations on the three conformations of the ADP, the $NC^{\alpha}C$ angle was constrained to $\pm 2°$ and $\pm 4°$ from its equilibrium value (113.96° for α_R, 107.68° for β, and 110.27° for P, order of magnitude differences already observed in experimental protein structures)[14,15] and the optimized $HC^{\alpha}C^{\beta}$ and r(CH) determined. The $HC^{\alpha}C^{\beta}$ angle changed by $-0.2°$ per $+1°$ change in $NC^{\alpha}C$ (an inverse relationship noted previously),[16] and the r(CH) changed by $\sim$0.0001 Å per 1° change in $NC^{\alpha}C$ for α and β and $\sim$0.0002 Å per 1° for P (see Figure 2). This corresponded to frequency changes of ~ -1.5 cm^{-1} for α and β, and ~ -2.4 cm^{-1} for P. When the $HC^{\alpha}C^{\beta}$ angle is similarly constrained from its equilibrium value (109.43° for α_R, 109.17° for β, and 109.90° for P), the $NC^{\alpha}C$ angle changes by $-0.1°$ per 1°change in $HC^{\alpha}C^{\beta}$, r(CH) *decreases* by ~ -0.0002 Å per 1° for α and ~ -0.0001 Å per 1° for β and P, and the respective frequency changes are $\sim$2 cm^{-1} for α and $\sim$1 cm^{-1} for β and P. The entire set of nonbonded interactions involved in a given local conformation clearly influences r(CH).

In the case of longer peptides, additional environmental influences are present. For example, in multiple structures of the tetrapeptide, we find that r(CH) in most cases differs from the equilibrium ADP value for all local conformations, and the ν(CD) varies from the ADP value in the expected manner (i.e., decreasing for increased r(CH) and increasing for decreased r(CH) in comparison to the ADP value). These results convincingly show that ν(CD) is a result of local interactions affecting the length of this bond, which are determined by the characteristic nonbonded interactions associated with the local φ,ψ conformation.

C. $C^{\alpha}D^{\alpha}$ Stretch Frequency Patterns for Ala-*n* Peptides. *1. Uniform-Conformation Peptides.* We first examine the characteristics of peptide structures containing repeated conformations at the C^{α} atoms, since it is possible that environmental conditions will tend to favor a predominant structure. In Table 1, we present the results of analyses of a series of Ala peptides, from the dipeptide through the octapeptide, in which all the C^{α} conformations are identical, that is, either α_R, β, or P. The ν(CD) of the first residue (i.e., at the $CH_3CONHC^{\alpha}D^{\alpha}$ end) is given plus the frequency differences, $\Delta\nu_{ij} = \nu_j - \nu_i$, between the subsequent residue pairs and the overall $\Delta\nu_{1n}$.

Regularities in these frequency sequences are apparent. First, the $\nu(CD_1)$ are the same (± 1 cm^{-1}) within all α_R, β ($n = 2-7$), and P peptides, the value of $\nu(CD_1)$ being $\sim$16 cm^{-1} lower for α_R than for the other two conformations. Second, the difference $\nu_n - \nu_1$ is characteristic of the conformation: for α_R, this is relatively large and ranges from 11 to 22 cm^{-1}, for β it is -1 cm^{-1}, and for P it is ~ -6 cm^{-1}. (Of course, the ability to

TABLE 1: Frequency Increments ($\Delta\nu_{ij}$)[a] in $C^{\alpha}D^{\alpha}$ Stretch of Isolated Uniform Ala-*n*[b] Peptides

		n						
	ij	1	2	3	4	5	6	7
α_R	ν_1[c]	2266	2265	2264	2265	2266	2266	2266
	12		17	12	12	14	14	14
	23			10	6	7	9	10
	34				−2	−12	−10	−9
	45					3	−6	−6
	56						4	−3
	67							5
	$\Delta\nu_{1n}$		17	22	16	12	11	11
β	ν_1[c]	2276	2281	2281	2281	2281	2281	2281
	12		−1	3	2	3	3	3
	23			−4	1	0	0	0
	34				−3	1	0	0
	45					−5	1	0
	56						−5	1
	67							−5
	$\Delta\nu_{1n}$		−1	−1	0	−1	−1	−1
P	ν_1[c]	2284	2283	2283	2283	2283	2283	2283
	12		−7	−6	−7	−5	−5	−5
	23			1	2	0	0	1
	34				0	0	1	0
	45					−1	0	−1
	56						−1	0
	67							0
	$\Delta\nu_{1n}$		−7	−5	−5	−6	−5	−6

[a] $\Delta\nu_{ij} = \nu_j - \nu_i$. [b] *n* refers to the number of C^{α} atoms in $CH_3CONH[C^{\alpha}D^{\alpha}(CH_3)]_nCONHCH_3$. [c] Frequency of the first residue at the $CH_3CONHC^{\alpha}D^{\alpha}$ end.

detect these as well as other small frequency changes will have to be determined experimentally.) Third, knowing *n*, it would be potentially possible in each case to further distinguish among the three structures. The $\Delta\nu_{ij}$ pattern for α_R is significantly different from those for β and P, and even for the latter two, the different signs and magnitudes of $\Delta\nu_{12}$, as well as the relative values of $\Delta\nu_{12}$ and $\Delta\nu_{n-1,n}$, should make it possible to distinguish between these two structures. Clearly, although the local interactions for α_R are significantly different from those for β and P (and particularly when intramolecular hydrogen bonding begins to appear at $n = 4$), the subtle differences in interactions between the latter two are sufficient to lead to potentially observable differences in their $C^{\alpha}D^{\alpha}$ s frequencies. Finally, it is tempting to infer that single isotopic substitutions at only residues 1 and 2 would distinguish between these regular structures, since $\Delta\nu_{12}$ is so significantly different: ≥ 12 cm^{-1} for α_R, ~ 3 cm^{-1} for β, and ~ -5 cm^{-1} for P. In the case of β and P, the regularity along the chain can be confirmed by the apparent persistence of $\Delta\nu_{ij} \approx 0$. In the case of α_R, the pattern of $\Delta\nu_{ij}$ is obviously more complex, but similarly, should be repetitive. Of course, these observations must be tempered by the necessity of determining the comparable results in the presence of the aqueous environment in which the peptides are being studied (hydrogen bonding of water to the peptide groups has a definite effect on ν(CD)).[13]

2. Kinked-Conformation Peptides. Although the characterization of peptides with repeated C^{α} conformations is an obvious starting point of our vibrational analysis, it is also important to know what the effect is of interruptions in the conformational sequence. This has become particularly relevant in light of the recent discussions concerning the nature of the early proposed predominance of P states in unordered peptides;[17] specifically, whether they occur in a dominant regular sequence[2] or in a mainly statistical arrangement.[3] Although the detailed answer to such a question is best based on the analysis of a specific system, it is useful to examine a simple case in order to develop an impression of possible spectral changes.

TABLE 2: Frequency Increments ($\Delta\nu_{ij}$)[a] in $C^{\alpha}D^{\alpha}$ Stretch of Isolated Kinked β and Polyproline II Heptapeptides

ij	β_6	$\beta_2P\beta_3$	$\beta_3P\beta_2$	P_6	$P_2\beta P_3$	$P_3\beta P_2$
ν_1[b]	2281	2280	2280	2283	2282	2282
12	3	2	4	−5	−1	−4
23	0	3	−1	0	1	3
34	0	0	2	1	0	2
45	1	−1	0	0	−2	−1
56	−5	−3	−5	−1	−2	−4

[a] $\Delta\nu_{ij} = \nu_j - \nu_i$. [b] Frequency of the first residue at the $CH_3CONHC^{\alpha}D^{\alpha}$ end.

In Table 2, we present the results of interrupting the C^{α} sequences of uniform β and P heptapeptides with a single replacement of the other conformer to yield two different arrangements of two and three identical conformations. Certain patterns are evident. First, the $C^{\alpha}D^{\alpha}{}_1$ s frequency is essentially unchanged in both cases. Second, the patterns of the $\Delta\nu_{ij}$ of the kinked structures are significantly different (again within the proviso of determined experimental possibility) from those of the uniform structures. Third, the sensitivity to the second-nearest-neighbor conformer seems to be different for β than for P, the latter being more sensitive at both ends of the peptide than the former. Finally, it is not clear, at least in this short peptide, that the $\Delta\nu_{ij}$ of the regular kink neighbors can be identified from the comparable sequences of the uniform peptides (such as given in Table 1). Further studies of this issue are needed, particularly in the aqueous environment.[13]

Conclusions

In order to elaborate on our proposal[6] that the $C^{\alpha}D^{\alpha}$ stretch frequency in peptides could be used to determine the φ,ψ conformation at the C^{α} atom, we have calculated the ab initio normal modes of isolated alanine peptides from the di- to the octa- in uniform α_R, β, and polyproline II (P) structures and β and P in some kinked configurations. This yields a set of frequencies that characterize these structures, in particular, the differences $\Delta\nu_{ij}$ between successive individual C^{α}-deuterated peptides that would be experimentally studied. These results provide a kind of "proof of principle" for the sensitivity of this mode to local conformation.

We find that the uniform-conformation peptides can be distinguished in several ways. First, the values of $\nu(CD_1)$ are significantly different: 2266 cm^{-1} for α_R, 2276 cm^{-1} for β, and 2284 cm^{-1} for P. (Of course, these values are particular to the studied blocking groups and will probably be different for other end groups.) Second, the patterns of the $\Delta\nu_{ij}$ are different in their details. Finally, the $\Delta\nu_{1n}$ are significantly different: 11−22 cm^{-1} for α_R, essentially zero for β, and ~ -6 cm^{-1} for P. This combination of characteristics makes it likely that such structures can be experimentally discriminated, and departures from regularity (as shown by our evidence from kinked configurations) can be identified.

As noted, our current work involves characterizing such peptides with water hydrogen bonded to the peptide groups, since this influences ν(CD).[13] Further studies will determine the influence of side chains other than alanine, as well as different terminal groups.

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