Conformation Dependence of the $C^{\alpha}D^{\alpha}$ Stretch Mode in Peptides: Side-Chain Influence in Dipeptide Structures

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ABSTRACT:

We have shown by theoretical studies of alanine peptides that the $C^{\alpha}D^{\alpha}$ stretch frequency could be particularly useful for determining peptide structure because of its sensitivity to the φ,ψ torsion angles at the C^{α} atom. To demonstrate that this is a robust methodology worthy of experimental exploration, we have also shown that this mode is even more determinative of conformation in aqueous solution, mainly as a result of the development of differential $C^{\alpha}—D^{\alpha}\cdots O$(water) interactions. As further assurance, we now determine the influence of the side chain on this band, showing for aliphatic, a polar, and an aromatic side chains that the dependence is minor and explaining why this is also expected for other side chains. These results should stimulate new experimental methodologies in the field of peptide structure determination. © *2010 Wiley Periodicals, Inc. Biopolymers 93: 1065–1071, 2010.*

Keywords: infrared; Raman; spectra; structure; proteins; ab initio

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INTRODUCTION

Infrared (IR) and Raman studies of the structures of peptides, polypeptides, and proteins have historically focused on the peptide group frequencies as indicators of local chain conformation. This was the result of a natural evolution from the first IR experimental evidence of differences in the CO stretch (s) frequencies of (X-ray determined) "folded" (α) and "extended" (β) synthetic polypeptides[1] to the subsequent comprehensive theoretical studies aimed at understanding the origin of such conformational dependence of the vibrational spectra. The latter have included (a) early efforts to characterize, in *N*-methylacetamide, the so-called amide normal modes of the peptide group[2]; (b) development of a general perturbation treatment of the amide I (mainly CO s) and amide II (mainly CN s plus NH in-plane bend) modes[3] and its application to α-helical and β-chain polypeptides[4] and to proteins[5]; (c) elucidation of the physical basis of such perturbations in transition dipole couplings between amide modes in the polypeptide chain[6] and demonstrations of the quantitative validity of this interaction mechanism[7–9]; and (d) implementation of full normal mode analyses, based on a complete empirical spectroscopic force field, to provide systematic spectra-structure correlations.[10] These and other aspects of the theoretical understanding have been the subject of a number of reviews of the field.[10–14]

This long, intense, and fruitful concentration on the conformational sensitivities of the peptide-group frequencies has drawn attention away from investigation of the potentially useful structural dependence of other regions of the vibrational spectrum. We have explored this possibility and as a result have begun a theoretical investigation of the $C^{\alpha}D^{\alpha}$ s frequency, ν(CD), which is found in a clear region of the fundamental spectra of both peptides and water, at ~2300 cm^{-1}. In part this study was motivated by the fact that this bond lies at

the confluence of the two dihedral angles, φ(CNC$^{\alpha}$C) and ψ(NC$^{\alpha}$CN), which determine the local conformation of the chain, and in part by the understanding that this mode, though highly localized, cannot be completely noninteractive with its surroundings. In our first article we demonstrated, by ab initio calculations on the isolated so-called alanine dipeptide, $CH_3[CONH]_1C^{\alpha}D^{\alpha}(CH_3)[CONH]_2CH_3$, in α_R, β, and polyproline II (P) conformations, that ν(CD) exhibits a significant φ,ψ sensitivity and thus could be a new experimental spectroscopic tool for the determination of peptide structure.[15] We subsequently showed that this remains true in longer isolated[16] as well as hydrated[17] alanine peptides, the increased structural sensitivity in the hydrated case deriving from the formation of differential C^{α}—$D^{\alpha}\cdots O_w$ electrical and hydrogen-bonding interactions with water.[17,18]

The proposed experimental protocol for implementing this technique for individual $C^{\alpha}D^{\alpha}$ sites in peptides (whose synthesis via isotopically substituted amino acids should be feasible) was the following. A peptide that could be designated as $\Sigma_j (X_j - n_j)$, where X_j is the amino acid residue (glycine, alanine, etc.) and n_j is the number of such residues in a sequence, would be synthesized in Σ_j forms. The first would have H^{α} substituted by D^{α} only on residue 1, the second only on residue 2, and so on. IR (and/or Raman) spectra would thus provide the ν(CD) (or possibly its overtone to increase frequency separation) for each residue. A calculation of the various conformational-state frequencies for that residue would be the basis for the determination of the local φ,ψ structural distribution. Of course, if only an overall average conformational state is needed the spectrum of only a singly fully-D^{α} peptide would suffice, since we have shown that there is no coupling between the ν(CD) on adjacent C^{α} sites.[16]

Our studies[15–17] showed that for peptides of alanine the above uniform conformations could be distinguished spectroscopically on the basis of three criteria: the value of $\nu(CD_1)$, the values of successive $\Delta\nu_{ij}$, and the value of $\nu_N - \nu_1$ (N being the last residue). However, in assessing the practical aspects of experimental implementation of this methodology it is also necessary to know whether this conformational sensitivity might be compromised by a significant dependence of ν(CD) on side-chain composition. To evaluate this influence we present the dependence of ν(CD) on aliphatic (glycine through isoleucine), a polar (serine), and an aromatic (phenylalanine) side chains of the dipeptide, both for the isolated as well as for explicitly hydrated species.

CALCULATIONS

The ab initio calculations were done at the B3LYP/6-31+G* level, as justified in our previous study,[17] using Gaussian 03.[19] As before, the main-chain and water geometries were fully optimized within the φ,ψ constraints of the $\alpha_R(-60^\circ, -40^\circ)$, $\beta(-134^\circ, 145^\circ)$, and $P(-75^\circ, 145^\circ)$ conformations. Although the lowest energy side-chain structures are the ones mainly discussed in this article, we have also investigated those in which the side chain adopts stable higher-energy rotationally isomeric states; the effect on ν(CD) is minimal.

Most calculations were on $(H_2O)_4$-hydrated structures, that is, having at least one water molecule (w) hydrogen bonded to each peptide group CO and NH, which we have shown to capture the essential features of a realistic C^{α}—$D^{\alpha}\cdots O_w$ interaction.[17] As a further test, we calculated the ν(CD) of several structures with additional waters: a total of seven with valine, to provide some modeling of the effects of a second water layer, and five with serine, to accommodate additional interaction with its OH group. The water molecule hydrogen-bonded to CO_1 has two equilibrium positions, CO_1a and CO_1b, corresponding to O_w—$H\cdots O_1$ hydrogen bonds with the two orbitals on O_1.[18] Only in the CO_1b minimized position does O_w also interact with D^{α} to affect ν(CD). Therefore, in order to evaluate only the side-chain influence, all hydrated optimizations were started with the O_1 water near the CO_1b position and they fully equilibrated with this interaction. The reported ν(CD) are unscaled harmonic frequencies; expected experimental frequencies will have to incorporate effects equivalent to scaling and to anharmonic corrections.[20,21]

RESULTS AND DISCUSSION

To assess the influence of the side-chain residue on ν(CD), we have done ab initio calculations on dipeptides with a representative group of side chains. The aliphatic include glycine (GDP), alanine (ADP), valine (VDP), leucine (LDP), and isoleucine (IDP), chosen to test not only nonpolar character, but also the relative sizes and shapes of the side chain. For a polar side chain we have chosen serine (SDP), and with its capacity to form a hydrogen bond to water we have also added an additional water molecule to the four used for the aliphatic hydrated species. For an aromatic side chain we have chosen phenylalanine (FDP).

Glycine

Since its side chain (a hydrogen atom) is unique among the residues, resulting in a CD_2 group rather than an isolated $C^{\alpha}D^{\alpha}$ bond, we consider the glycine dipeptide (GDP) independently, noting however that its properties also share many features with those of the other side-chain dipeptides. Some properties of the isolated form have been investigated

Table I Properties[a] of $C^{\alpha}D_2$ Glycyl Dipeptides

	α_R			β			P		
	r	*v*	i	*r*	*v*	i	*r*	*v*	i
I	$l - 1.0945$	2324 a	5.1	1.0935	2309 a′	3.4	1.0930	2326 a′	4.8
	$d - 1.0945$	2239 s	10.8	1.1003	2207 s′	21.6	1.0956	2241 s′	13.8
	Δv	85			102			85	
	ΔE	3.5			0			0.8	
$(H_2O)_4$	$l - 1.0938$	2330 a	3.0	1.0887	2348 a′	1.1	1.0917	2336 a′	2.2
	$d - 1.0943$	2246 s	7.2	1.1010	2206 s′	17.2	1.0954	2248 s′	7.4
	$D_l \cdots O$			2.83, 2.74			2.64		
	Δv	84			142			88	
	ΔE	14.2			0			4.0	

[a] I: isolated molecule; $(H_2O)_4$: hydrated molecule; *r*: $C^{\alpha}—D^{\alpha}$ bond length in Å; *v* : frequency in cm^{-1}; i: infrared intensity in km/mol; *l*: L-amino acid deuterium; *d*: D-amino acid deuterium; a: antisymmetric stretch; s: symmetric stretch; a′, s′: mixed modes of analogous character, with larger displacement associated with *l* or *d* deuterium; $D_l \cdots O$: distance $< \sim 3.0$ Å between D_l and water oxygen, the first in pair interacting with the $[CO]_1$ water and the second with the $[NH]_2$ water[17]; ΔE: relative energy in kcal/mol.

by others.[21] Also, a structural analysis has been proposed of the IR spectra of $C^{\alpha}D_2$ backbone-deuterated glycines of a human adaptor protein domain, based on calculations on *N*-formyl glycine amide.[22] In Table I, we present relevant structural and vibrational properties of the isolated and the hydrated GDP in the α_R, β, and P conformations. These properties include the $C^{\alpha}—D^{\alpha}$ bond lengths (for both the D_l and D_d bonds, *l* being the L-amino acid and *d* being the D-amino acid positions); the associated CD_2 stretch frequencies and their character; their IR band intensities; the $D_l \cdots O_w$ distances $< \sim 3.0$ Å in the hydrated structures, at which, judging from the results for the water dimer,[23] the initial effects of wave function overlap in hydrogen bonding are likely to be evident (of course, the angular relation may also be important); and the relative energies of the conformers. The structures of the three hydrated conformers are shown in Figure 1.

For the isolated molecule $r(C^{\alpha}—D_l{}^{\alpha}) = r(C^{\alpha}—D_d{}^{\alpha})$ in the α_R structure, despite slightly different CHELPG charges of $q(D_l) = 0.098e$ and $q(D_d) = 0.082e$, and the modes are relatively pure antisymmetric (a) and symmetric (s) stretch. For the β and P structures these bond lengths are different (perhaps because of the significant loss of C_2 symmetry in the local electrical environment), as a consequence of which the modes are now mixed, although they retain much of the character of the antisymmetric (a′) and symmetric (s′) coordinates (a situation also observed in glycine[24]). Similar behavior is observed for the hydrated molecule, with the added effect in the β and P structures of significant $C^{\alpha}—D_l{}^{\alpha} \cdots O_w$ interactions and accompanying $C^{\alpha}—D_l{}^{\alpha}$ bond contraction[17] as a result of $D_l \cdots O$ distances $< \sim 3.0$ Å, leading to the larger charge difference of $q(D_l) = 0.064e$ and $q(D_d) = 0.037e$. It is important to note that with these distances in the ∼2.6–2.9 Å range, compared to the ∼2.2 Å distances associated with maximal $C^{\alpha}—D^{\alpha} \cdots O_w$ hydrogen bonding,[17,18] the small decrease in the $C^{\alpha}—D_l$ bond length is likely to be primarily due to the contracting force associated with the antiparallelism between the dipole derivative of the bond and the electric field it experiences from the local water molecules.[25,26] Thus, the peptide-group-to-water hydrogen-bonded structure, which is established by the φ,ψ constraints of the backbone, results in $C^{\alpha}—D^{\alpha} \cdots O_w$ interactions that can span the continuous range from an essentially electric-field-alone effect to that of the electric field plus the wave function overlap effect associated with $D^{\alpha} \cdots O_w$ hydrogen bonding.[18] The loss of C_2 symmetry in the local electrical environment of the CD_2 group is obvious for the β and P structures, but "enough symmetry" is obviously retained in the α_R structure (perhaps from the dipole moment environments of the adjacent peptide groups) to allow for almost pure modes. The relative intensities of a and s (as well as a′ and s′) modes in both isolated and hydrated species are similar for the α_R and P structures (in the range of 2–4), but is significantly different for the β structure (6–16). The actual intensities, as expected, are small compared to those of major peptide group modes (e.g., the combined intensities of the two amide I modes range from 259 to 613 km/mol for the three conformations), but this may be advantageous in that very thin aqueous solution samples will not be necessary for a definitive experimental analysis.

We see from Table I that in both the isolated and hydrated cases, the β conformer is easily identifiable by the larger frequency separation and the greater relative intensity of the two modes, but based on these properties the α_R and P conformers could probably not be distinguished from each other. However, it is interesting to note that (as expected by

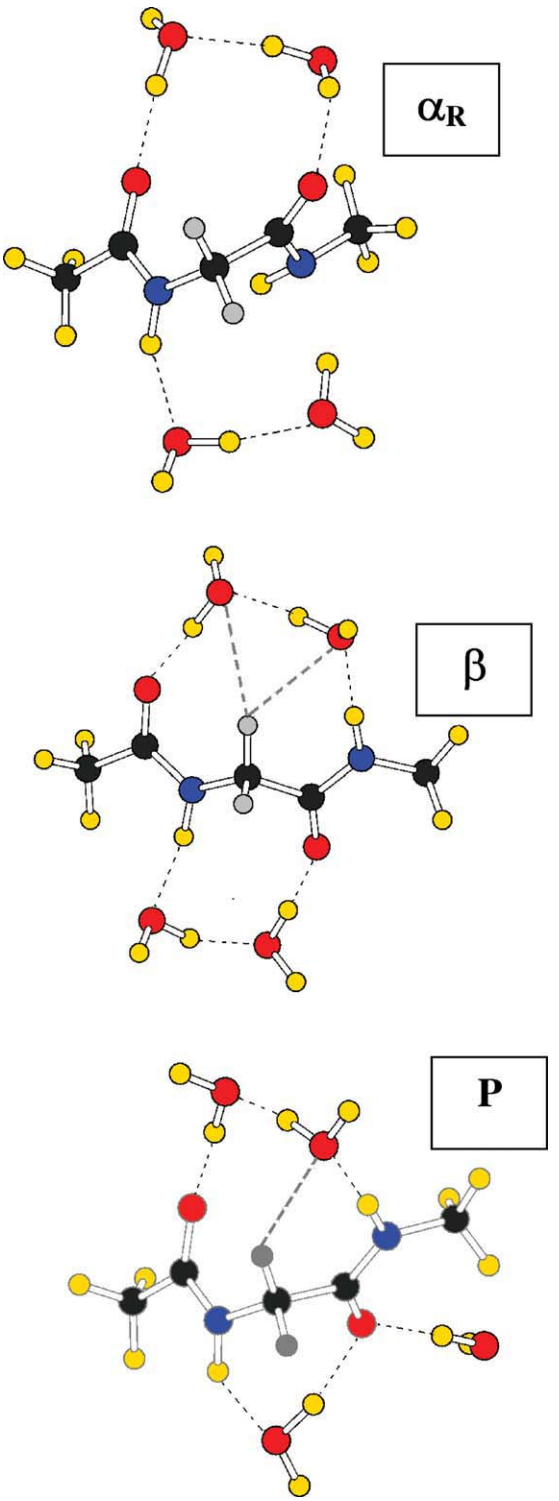

FIGURE 1 Structures of $(H_2O)_4$-hydrated $\alpha_R(-60^\circ, -40^\circ)$, $\beta(-134^\circ, 145^\circ)$, and polyproline II$(-75^\circ, 145^\circ)$ glycine-$C^\alpha D_2$ dipeptides. Light broken lines: standard peptide–water and water–water hydrogen bonds. Dark dashed lines: C^α—D · · · O (water) interactions < ~3.0 Å (see text).

analogy to the non-glycine cases), if the dipeptide could be synthesized with only one D atom in the L-amino acid position, these conformers would be readily distinguishable in the hydrated case, the ν(CD) then being α_R = 2288, β = 2335, and P = 2309 cm^{-1} (although not so in the isolated case, with α_R = 2280, β = 2292, and P = 2294 cm^{-1}).

Non-Glycine

In Table II, we present the properties of ν(CD) and the relative energies (in each row) of the isolated and $(H_2O)_4$-hydrated α_R, β, and P conformations of ADP, VDP, LDP, IDP, SDP, and FDP. (A calculation of the isolated ADP has also been reported.[21]) For all the isolated species, the ν(CD) for a given conformation are relatively unaffected by the side chain: the average frequencies for these dipeptides are 2260 ± 3 cm^{-1} for α_R, 2273 ± 6 cm^{-1} for β, and 2281 ± 7 cm^{-1} for P. Even the formation in the optimized α_R-SDP of a weak N_1—H_1 · · · O(ser) hydrogen bond in the near-planar $H_1N_1C^\alpha C^\beta O$ part of the structure (H_1 · · · O = 2.29 Å, $r(N_1—H_1)$ = 1.0144 Å compared to 1.0108 Å in the isolated ADP and 1.0277 Å in hydrated structures[18]) has not resulted in a significant change in ν(CD), viz., 2264 cm^{-1} vs. 2266 cm^{-1} for ADP, consistent with our observation that hydrogen bonding to the peptide groups has little effect on ν(CD).[17] As we noted before,[17] it might be difficult to distinguish between the conformations if all three were significantly present at the same time: the differences between the ν(CD) of β and P are within the variability limits.

For the hydrated species, the side-chain effect for a given conformation is also small: 2272 ± 4 cm^{-1} for α_R, 2326 ± 6 cm^{-1} for β (which includes results for $VDP(H_2O)_7$, with ν(CD) = 2322 cm^{-1}, and two $SDP(H_2O)_5$, with ν(CD) = 2327 and 2332 cm^{-1}, structures), and 2298 ± 7 cm^{-1} for P (which includes a $SDP(H_2O)_5$ structure). However, again as previously observed,[17] when water is present the differences in ν(CD) between conformations become larger, from a minimum (between the α_R and P structures of ADP) of 16 cm^{-1} to a maximum (between the α_R and β structures of SDP) of 74 cm^{-1}. It can thus be expected that even if all three conformations are present in solution their presence should be distinguishable. As we showed,[17,18] this large variation is due to significant though different C^α—D^α · · · O_w interactions between the conformations. The nature of these differences can be seen in Figure 2 for $VDP(H_2O)_4$, which is representative of the water interactions for the aliphatic and phenyl side chains. In the case of α_R such interactions are minimal ($r(D^\alpha$ · · · O) is > ~3.0 Å), and the small increase in ν(CD) on hydration is attributable to the small bond contraction arising from the antiparallelism between the dipole derivative

Table II Properties[a] of $C^{\alpha}D^{\alpha}$ Non-Glycyl Dipeptides

	α_R				β				P			
	r	*v*	i	ΔE	*r*	*v*	i	ΔE	*r*	*v*	i	ΔE
Alanine												
I	1.0957	2266	6.1	3.6	1.0948	2276	6.1	0.0	1.0939	2284	5.6	1.2
$(H_2O)_4$	1.0944	2280	4.3	15.1	1.0891	2330	1.7	0.0	1.0925	2296	3.3	0.3
$D\cdots O$						2.77, 2.69				3.03, 2.84		
Valine												
I	1.0967	2259	5.5	4.0	1.0960	2268	6.0	0.0	1.0952	2274	5.3	1.8
$(H_2O)_4$	1.0955	2270	4.0	17.9	1.0905	2320	2.1	0.0	1.0938	2288	3.8	3.0
$D\cdots O$						2.79, 2.82				3.05, 2.88		
Leucine												
I	1.0966	2253	6.2	3.9	1.0961	2261	6.4	0.0	1.0944	2274	4.6	1.9
$(H_2O)_4$	1.0951	2268	3.8	15.3	1.0903	2314	2.0	0.0	1.0925	2295	2.3	1.6
$D\cdots O$						2.74, 2.72				3.07, 2.69		
Isoleucine												
I	1.0967	2259	5.3	4.0	1.0958	2270	6.0	0.0	1.0951	2273	5.2	1.2
$(H_2O)_4$	1.0956	2270	4.3	17.5	1.0905	2319	2.2	0.0	1.0936	2289	3.2	2.9
$D\cdots O$						2.74, 2.79				2.71		
Serine												
I	1.0963	2264	6.1	3.6	1.0947	2281	4.8	0.0	1.0937	2292	3.5	2.9
$(H_2O)_4$	1.0945	2275	6.1	9.6	1.0889	2333	2.1	0.0	1.0921	2301	3.3	1.0
$D\cdots O$						2.68, 2.63				2.80, 2.73		
Phenylalanine												
I	1.0964	2260	6.1	3.9	1.0944	2279	5.6	0.0	1.0935	2288	4.8	2.5
$(H_2O)_4$	1.0949	2269	6.2	7.7	1.0884	2336	2.1	0.0	1.0918	2303	3.9	1.8
$D\cdots O$						2.58, 2.81				2.81, 2.91		

[a] I: isolated molecule; $(H_2O)_4$: hydrated molecule; *r*: $C^{\alpha}—D^{\alpha}$ bond length in Å; *v*: frequency in cm^{-1}; i: infrared intensity in km/mol; ΔE: relative energy (in row) in kcal/mol; $D\cdots O$: distance $< \sim$3.0 Å between D^{α} and water oxygen, the first in pair interacting with the $[CO]_1$ water and the second with the $[NH]_2$ water.[17]

of the $C^{\alpha}—D^{\alpha}$ bond and the external electric field.[25,26] In the cases of β and P, the differences derive from each backbone conformation enforcing it own distinctive water configurations that interact with D^{α}, thus resulting in different $D^{\alpha}\cdots O$ distances. The relatively small influence of the side chain for a given conformation can be attributed to the relative constancy in the hydrogen-bonding patterns of the two waters interacting with the $C^{\alpha}—D^{\alpha}$ bond in these minimum energy structures, seen by comparing Figures 1 and 2 and by noting the similarity between these water arrangements in β-$SDP(H_2O)_4$ and the two β-$SDP(H_2O)_5$ structures in Figure 3 (which even involve different hydrogen-bonding patterns to the serine OH). We have shown that the value of *v*(CD) for a $(H_2O)_4$-peptide may have to be modified slightly in a more fully hydrated peptide, since waters beyond the first layer can have a small influence on this layer and thus the pattern of the $C^{\alpha}—D^{\alpha}\cdots O_w$ interactions.[17] Nevertheless, in view of the results on $VDP(H_2O)_7$ and $SDP(H_2O)_5$, it is likely that when an extended water environment is involved the relative constancy of this pattern with different side chains will not change significantly since the side-chain environment will have only minimal influence on the interactions near the D^{α} atom. The detailed features associated with such many-water environments will probably be best determined, as we have noted,[17] by molecular dynamics studies.

The intensities of the *v*(CD) are also instructive. For the isolated species, the intensity of this mode is relatively independent of side chain and changes by a small amount with conformation. For the hydrated species, the situation is more variable. In all cases where interaction with the water molecules is evident, as indicated by significant frequency increases, the intensity decreases in comparison to the isolated value, as expected from the competition between the negative permanent dipole derivative of the donor and the positive dipole derivative induced in the donor by the acceptor.[25,26] There seems to be a general trend of larger intensity decreases being associated with larger frequency increases, but obviously this must be a limited correlation.

Since the side chain at a given C^{α} is known from the chemical structure of the peptide, we only need to compare the *v*(CD) associated with that side chain in Table II to evalu-

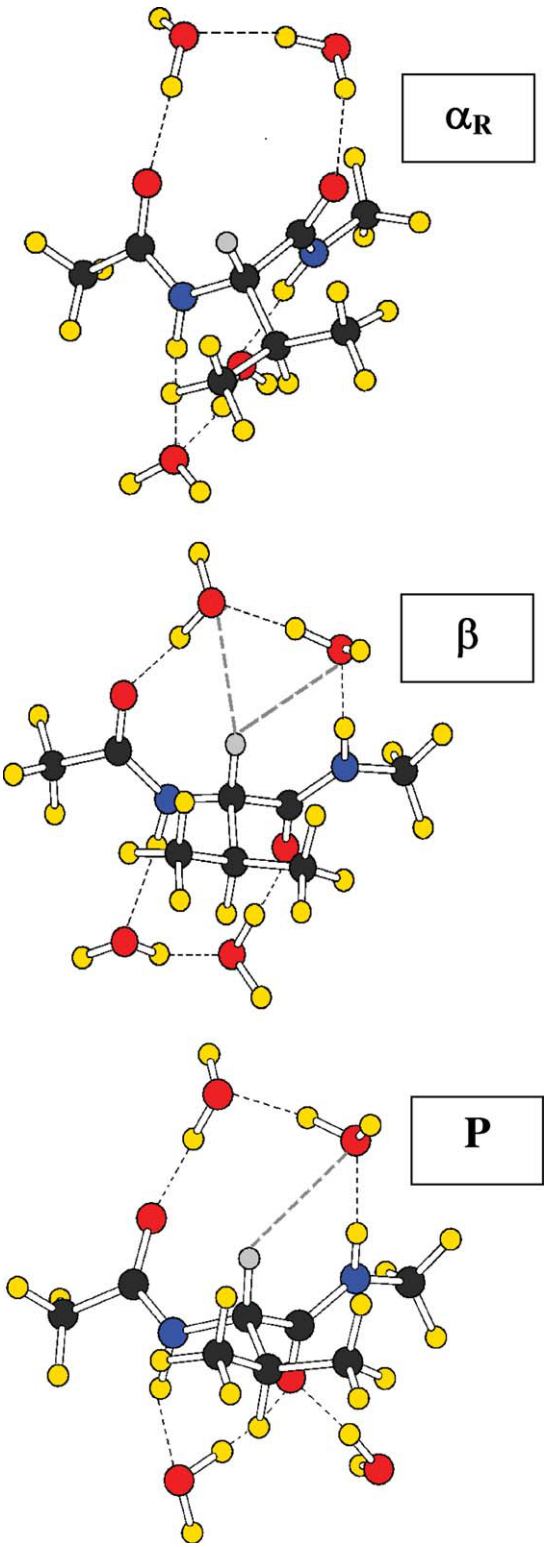

FIGURE 2 Structures of $(H_2O)_4$-hydrated $\alpha_R(-60°, -40°)$, $\beta(-134°, 145°)$, and polyproline II$(-75°, 145°)$ valine-$C^{\alpha}D^{\alpha}$ dipeptides. Light broken lines: standard peptide–water and water–water hydrogen bonds. Dark dashed lines: $C^{\alpha}—D^{\alpha}\cdots O$(water) interactions $< \sim 3.0$ Å (see text).

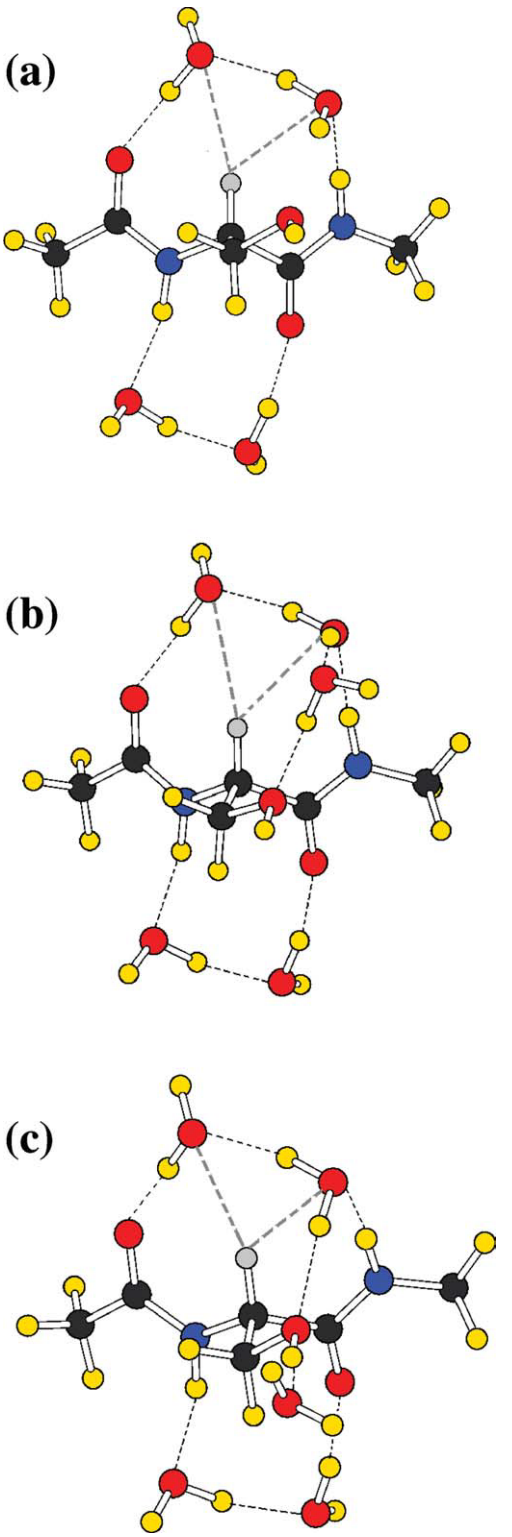

FIGURE 3 Structures of hydrated β-serine dipeptides: (a) $(H_2O)_4$ lowest energy structure; (b) $(H_2O)_5$ lowest energy structure; (c) $(H_2O)_5$ optimized structure 1.1 kcal/mol higher in energy than (b). Light broken lines: standard peptide–water and water–water hydrogen bonds. Dark dashed lines: $C^{\alpha}—D^{\alpha}\cdots O$(water) interactions $< \sim 3.0$ Å (see text).

ate the ability to distinguish between the three different conformations. Although as noted this may be problematic for the isolated peptides, the results show that discrimination should be expected for the hydrated species: the ν(CD) for the respective α_R, β, and P conformations are ADP = 2280, 2330, and 2296 cm^{-1}; VDP = 2270, 2320, and 2288 cm^{-1}; LDP = 2268, 2314, and 2295 cm^{-1}; IDP = 2270, 2319, and 2289 cm^{-1}; SDP = 2259, 2333, and 2301 cm^{-1}; and FDP = 2269, 2336, and 2303 cm^{-1}. Specific results for longer peptides, both of uniform as well as mixed conformation,[16] will of course have to be extended to peptides of mixed side-chain composition. Most likely, the practical parameters of this technique will be more specifically determined when experimental experience becomes available.

CONCLUSIONS

Our ab initio studies of the effects of different side chains on the conformation-sensitive $C^{\alpha}D^{\alpha}$ stretch frequency show that this influence is minimal, both for the isolated as well as the hydrated species of the dipeptide. This was found for all the aliphatic as well as for a polar (serine) and an aromatic (phenylalanine) side chains, a result that is consistent with the geometric isolation of the side chain from this bond in the isolated peptide and on the relatively common water structures in the vicinity of this bond in all of the (minimum energy) conformations examined. This commonality of water structure is likely to persist in water environments that are more extended than the $(H_2O)_4$-hydrated species examined here. The latter studies are needed, but are more likely to be best implemented via molecular dynamics calculations of peptides in realistic aqueous surroundings.[17] Nevertheless, present predictions suggest that this methodology is likely to be a robust technique for studying peptide conformation, and we hope that experimental studies will be pursued to fully evaluate its potential. This new tool, because it isolates the determination of structure to an individual C^{α}, could be instrumental in discriminating between current conflicting views of the role of the P conformation in the characterization of "unfolded" peptides.[27,28]

REFERENCES

1. Elliott, A.; Ambrose, E. J. Nature 1950, 165, 921–922.
2. Miyazawa, T.; Shimanouchi, T.; Mizushima, S.-I. J Chem Phys 1958, 29, 611–616.
3. Miyazawa, T. J Chem Phys 1960, 32, 1647–1652.
4. Miyazawa, T.; Blout, E. R. J Am Chem Soc 1961, 83, 712–719.
5. Krimm, S. J Mol Biol 1962, 4, 528–540.
6. Krimm, S.; Abe, Y. Proc Natl Acad Sci USA 1972, 69, 2788–2792.
7. Moore, W. H.; Krimm, S. Proc Natl Acad Sci USA 1975, 72, 4933–4935.
8. Cheam, T. C.; Krimm, S. Chem Phys Lett 1984, 107, 613–616.
9. Lee, S.-H.; Krimm, S. Chem Phys 1998, 230, 277–295.
10. Krimm, S.; Bandekar, J. Adv Protein Chem 1986, 38, 181–364.
11. Frushour, B. G.; Painter, P. C.; Koenig, J. L. J Macromol Sci Rev Macromol Chem 1976, C15, 29–115.
12. Barth, A.; Zscherp, C. Q Rev Biophys 2002, 35, 369–430.
13. Schweitzer-Stenner, R. Vib Spectrosc 2006, 42, 98–117.
14. Kim, Y. S.; Hochstrasser, R. M. J Phys Chem B 2009, 113, 8231–8251.
15. Mirkin, N. G.; Krimm, S. J Phys Chem A 2004, 108, 10923–10924.
16. Mirkin, N. G.; Krimm, S. J Phys Chem A 2007, 111, 5300–5303.
17. Mirkin, N. G.; Krimm, S. Biopolymers 2009, 91, 791–800.
18. Mirkin, N. G.; Krimm, S. J Phys Chem B 2008, 112, 15267–15268.
19. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennuccci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Lui, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chem, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, Revision C. 02; Gaussian, Inc.: Wallingford CT, 2004.
20. Wang, J. J Phys Chem B 2009, 113, 1813–1816.
21. Miller, C. S.; Ploetz, E. A.; Cremeens, M. E.; Corcelli, S. A. J Chem Phys 2009, 130, 125103.
22. Cremeens, M. E.; Zimmerman, J.; Yu, W.; Dawson, P. E.; Romesberg, F. E. J Am Chem Soc 2009, 131, 5726–5727.
23. Mannfors, B.; Palmo, K.; Krimm, S. J Phys Chem A 2008, 112, 12667–12678.
24. Bykov, S. V.; Myshakina, N. S.; Asher, S. A. J Phys Chem B 2008, 112, 5803–5812.
25. Qian, W.; Krimm, S. J Phys Chem A 2002, 106, 6628–6636.
26. Qian, W.; Krimm, S. J Phys Chem A 2002, 106, 11663–11671.
27. Makowska, J.; Rodziewicz-Motowidlo, S.; Baginska, K.; Makowski, M.; Vila, J. A.; Liwo, A.; Chmurzynski, L.; Scheraga, H. A. Biophys J 2007, 92, 2904–2917.
28. Chen, K.; Liu, Z.; Bracken, W. C.; Lu, M.; Kallenbach, N. R. In Unfolded Proteins: From Denatured to Intrinsically Disordered; Creamer, T. P., Ed.; Nova Science Publishers, Inc.: New York, 2008, pp 71–99.

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