Solvation State of the Tyr Side Chain in Peptides. An FT-IR and ^{17}O NMR Approach

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Received December 2, 1991

Abstract: Detailed studies of the ^{17}O NMR chemical shifts of the COH group of *p*-cresol and tyrosine derivatives as a function of pH and in a variety of solvents revealed an unusually large titration shift upon the deprotonation of the phenol group and a very small chemical shift variation as a function of solvent hydrogen-bonding ability. Similarly the ν_{CO} stretching frequencies exhibit a very small variation as a function of solvent hydrogen-bonding ability, and discrete hydrogen-bonded species could not be identified. In contrast, the in-plane COH bending frequencies of these compounds have been shown to be very sensitive to hydrogen-bond interactions, and discrete hydrogen-bonded species could be identified. The applicability of this novel methodology to peptide hormones is demonstrated in the case of Leu-enkephalin in aqueous solution.

Introduction

Several investigations have aimed at defining the molecular environment of the phenolic ring of the Tyr residue in peptides and proteins.[1-6] This is due to the fact that the orientation and dynamics of the side chains are known to be of key significance in determining the overall conformation and biological activity of peptides and proteins. More specifically, in the case of the peptide hormone Leu-enkephalin, Tyr-Gly-Gly-Phe-Leu, it has been suggested that the side chain of Tyr[1] might play the same role in the interaction with opioid receptors as the tyramine portion in morphine.[7]

Several spectroscopic methods, including CD and absorption spectra in H_2O,[8] laser Raman in H_2O,[9] and a ^1H NMR study in D_2O/DMSO mixtures,[10] have provided qualitative evidence that the Tyr[1] hydroxyl group of enkephalin in aqueous solution is fully exposed to the solvent environment and not engaged in any type of intramolecular hydrogen bond. On the contrary, ^{13}C NMR chemical shifts in H_2O were interpreted as indicative of the existence of an intramolecular hydrogen bond between the Tyr[1] COH group and the Gly[3] CO group.[11] The existence of this hydrogen bond, typical of the so-called hairpin-bend structure, was also suggested on the basis of the tyrosine fluorescence quantum yield.[12] This conclusion was furthermore substantiated from recent Monte Carlo studies based on statistical mechanics.[13]

It therefore appears that, although many attempts have been made to elucidate the nature of the H-bonded species involved in the tyrosine OH moiety using different physical techniques, the results have generally been ambiguous or at best qualitative in nature. It seems clear from the many problems encountered in characterizing the solvation state of the tyrosine OH moiety in peptides that a new spectroscopic methodology could prove useful for the investigation of specific hydrogen-bonded structures.

In view of the importance of oxygen atoms in the formation of intra- and intermolecular hydrogen bonds, ^{17}O NMR spectroscopy can be considered as an obvious candidate. Several investigations of solution interactions and molecular dynamics using ^{17}O NMR spectroscopy have been made in recent years,[14-21] but the utility of the ^{17}O probe to study the tyrosine OH side chain moiety is limited to a preliminary study of the ^{17}O NMR chemical shifts of Tyr as a function of pH in aqueous solution.[22]

The O–H stretching vibration of the tyrosine moiety can in principle provide information on specific hydrogen-bonding interactions, but due to strong H_2O absorption in this spectral region, it cannot be used in aqueous or aqueous-type solvents which present the biologically more relevant situation. Although the COH bending vibrations are in a transparent window for H_2O, it has been claimed that the presence of other absorption bands in the region complicates the spectra and makes interpretation of H-bonding effects difficult or impossible.[23,24] Furthermore, strong coupling of the deformation vibrations with other vibrations results in additional complications. These limitations have restricted the use of deformation modes in H-bonding studies.[23-25]

In this work, we have attempted to extend both ^{17}O NMR and IR COH bending vibrations in the study of the molecular environment of the tyrosine moiety in peptide hormones (the preliminary results of which have already been reported[26,27]). It is shown

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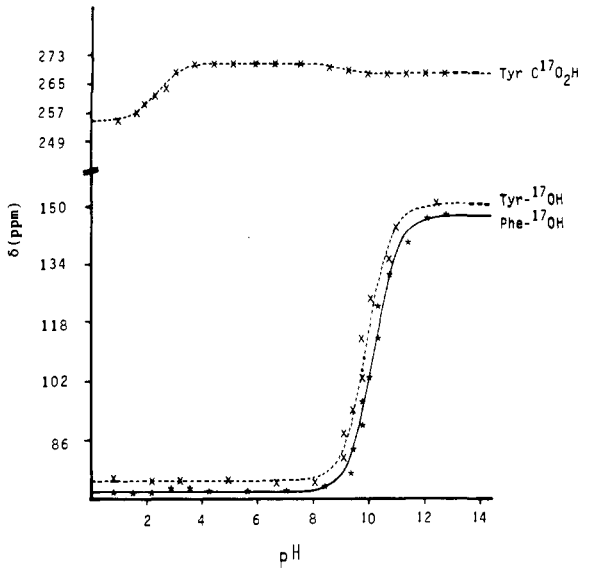

Figure 1. ^{17}O NMR chemical shift titration curves of 10^{-2} M aqueous solutions of [^{17}O]-*p*-cresol (—) and [^{17}O]Tyr (---) containing 5×10^{-4} M EDTA; temperature 30 °C. Lines were obtained from nonlinear least-squares fits of one-proton titration curves to the experimental data.

that ^{17}O NMR spectroscopy can provide valuable information on the ionization state but not on the solvation state of the tyrosine OH group. We have analyzed for the first time the IR COH bending vibrations of Leu-enkephalin, and this has led to the revision of literature assignments. An attempt was also made to quantify the number of water molecules bound to the tyrosine OH moiety. It is shown that the COH bending vibrations can provide unique information on the presence of discrete hydrogen-bonding interactions of the Tyr COH group in peptides.

Experimental Section

The ^{17}O and ^{18}O isotopically labeled, in the phenol group, *p*-cresol and tyrosine were obtained by hydrolysis of the corresponding diazonium salts in the presence of water enriched in ^{17}O (^{17}O content 20%) and ^{18}O (^{18}O content 98%). Tyrosine derivatives were synthesized by esterification and acetylation of the labeled tyrosine, according to classical procedures. *p*-Cresol-*O*-*d* was synthesized by simple exchange at room temperature with MeOD.[30]

^{17}O NMR spectra were recorded on a Bruker AM-400 spectrometer operating at 54.48 MHz. The spectrometer was equipped with a high-resolution probe accepting 10-mm sample tubes. The field was optimized using D_2O. No field lock was used during data acquisition. The chemical shifts were determined relative to external 1,4-dioxane. Data acquisition parameters: spectral width 50 kHz, 90° pulse, 32 μs, quadrature phase detection, $T_{acq} \approx 5T_2$, zero filling to 4 K before Fourier transform. Acoustic ringing effects were alleviated using the RIDE pulse sequence.[28] For the experiments in aqueous solutions, ^{17}O-depleted water (YEDA, ^{17}O content $\sim 10^{-3}$%) was used to circumvent the problem of dynamic range.

FT-IR spectra were scanned on a Bruker IFS-85 spectrometer with a 25-μm cell equipped with CaF_2 windows, using aqueous solutions of 20 mM concentration for Leu-enkephalin and near saturation for *p*-cresol. Each spectrum was recorded with 2 cm^{-1} resolution and was the result of the accumulation of 512 scans. Self-deconvoluted spectra were obtained using the Bruker self-deconvolution procedure.

Results and Discussion

^{17}O NMR Studies. In order to characterize the behavior of the ^{17}O chemical shifts of an isolated phenol-type OH group as a function of pH, we first turned our attention to the case of *p*-cresol. The ^{17}O NMR spectrum of *p*-cresol shows a single, proton-uncoupled resonance due to fast proton transfer with the solvent in protic solvents or through intermolecular proton transfer in nonprotic solvents. Figure 1 shows the pH dependence of the chemical shift of a 0.1 M aqueous solution of [^{17}O]-*p*-cresol. Upon deprotonation of the phenol OH group, we observe a shift to higher frequency with an inflection point at its pK_a and a least-squares titration shift of 75.6 ppm (Table I). ^{17}O NMR studies of [^{17}O]Tyr show that the chemical shift of the carboxyl group exhibits two inflection points at the pK_a values of the carboxyl and amino groups, while the chemical shift of the phenol OH group exhibits at its pK_a value one inflection point as a function of the pH. The titration shift of the carboxylic oxygens, $\Delta\delta \approx 16.5$ ppm, is in agreement with the trends observed for other amino acids.[15] The respective change of the phenolic site is 75.4 ppm (similar to that reported by Eckert and Fiat[22]) and in excellent agreement with the titration shift of *p*-cresol. This indicates that *p*-cresol is an appropriate model of the phenol group of Tyr. Furthermore, the large titration shifts reflect significant differences between the shielding parameters of the protonated and the unprotonated forms.

Within the range studied, the chemical shifts were found to be independent of concentration, while the line widths tended to increase with higher *p*-cresol or tyrosine concentrations, presumably due to an increase in the solution viscosity. Typical line widths (in 10 mM solutions) are 407 and 520 Hz for *p*-$CH_3C_6H_4OH$ and *p*-$CH_3C_6H_4O^-$, 650 and 850 Hz for the COO^- and COOH sites, and 1100 and 1300 Hz for the COH and CO^- sites of Tyr, respectively.

Contrary to the great sensitivity of ^{17}O chemical shifts to the ionization state, the effect of solvent-induced hydrogen-bonding interactions is surprisingly negligible and does not appear to correlate with the hydrogen-bonding strength of the solvent. Thus, upon transfer from CCl_4 to H_2O a chemical shift of only 1.9 ppm is observed (Table II). Since the COH group can act both as a proton donor and acceptor, one might hypothesize that the two modes of hydrogen bonding induce oxygen-17 chemical shifts of opposite directions, thus resulting in a significant reduction in the overall chemical shift. However, this hypothesis should be ruled

Table I. ^{17}O NMR Chemical Shifts[a,b] of Some Phenol-Type Compounds[a]

compd	functional group	δ_1[c]	δ_2[d]	$\Delta\delta$[e]	pK_a
[^{17}O]-*p*-cresol	OH	71.8	147.4	75.6	10.15
[^{17}O]tyrosine	OH	75.0	150.4	75.4	9.93

[a] Measured in 10^{-2} M solutions in H_2O which contained 5×10^{-4} M EDTA, temperature 30 °C. The chemical shifts were obtained from nonlinear least-squares fits of one-proton titration curves to the experimental data. [b] Chemical shifts were measured relative to 1,4-dioxane used as the external reference. The errors for chemical shifts were ±0.3 ppm. [c] δ_1 is the chemical shift for the protonated phenol oxygen atoms. [d] δ_2 is the chemical shift for the deprotonated phenol oxygen atoms. [e] Titration shifts.

Table II. ^{17}O NMR Chemical Shifts and Line Widths of [^{17}O]-*p*-Cresol and N^{α}-Ac-[^{17}O]Tyr-OMe in Various Solvents (Concentration 10^{-2} M, Temperature 30 °C)

compd	functional group	solvent	δ[b] (ppm)	$\Delta\delta$[c] (ppm)	$\Delta\nu_{1/2}$[d] (Hz)
[^{17}O]-*p*-cresol	OH	CCl_4	70.3	−1.9	167
		$CHCl_3$	68.9	−3.3	182
		acetone	71.4	−0.8	261
		MeOH	69.0	−3.2	334
		H_2O[a]	72.2	0.0	407
N^{α}-Ac-[^{17}O]Tyr-OMe	OH	$CHCl_3$	72.2	−2.3	977
		acetone	73.5	−1.0	641
		MeOH	70.9	−3.6	978
		H_2O[a]	74.5	0.0	1256
	ester C=O	$CHCl_3$	345.3	16.1	646
		acetone	349.7	20.5	342
		MeOH	342.8	13.6	518
		H_2O[a]	329.2	0.0	793

[a] Solutions contained 5×10^{-4} M EDTA.[14] [b] Chemical shifts were independent of concentration. [c] Chemical shifts relative to the values in H_2O solution. [d] Line widths of the resonances at half-height corrected for the line-broadening factors. Estimated error ≤10%.

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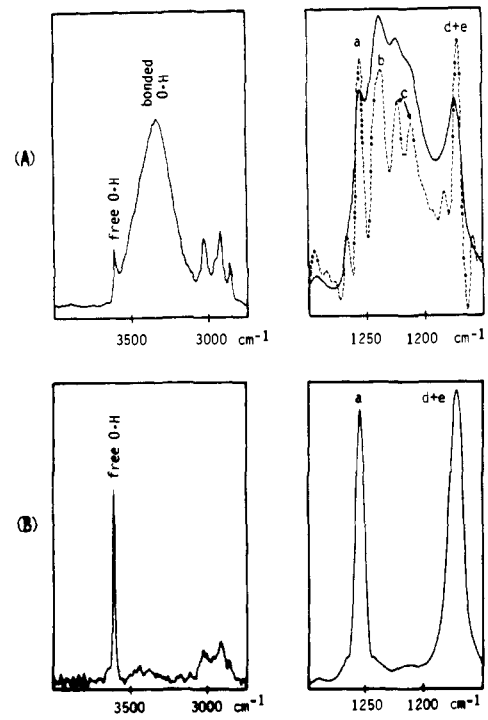


Figure 2. FT-IR spectra of *p*-cresol in $Cl_2C{=}CCl_2$: (A) concentration 2.1 M; (B) concentration 0.05 M. Self-deconvoluted spectra of *p*-cresol are presented with dotted lines: a, denotes ν_{CO} stretching vibration; b, COH bending vibration with the OH group acting both as donor and acceptor; c, COH bending vibration with the OH group acting as acceptor; d, COH bending vibration of the OH group free of hydrogen bonding; e, CH bending vibration.

out since the chemical shift of [^{17}O]-*p*-cresol in solvents that can act only as proton acceptors, e.g., acetone, is very similar to that in the absence of hydrogen bonding, for example, in dilute CCl_4. Similar results were obtained from ^{17}O NMR studies of N^{α}-Ac-[^{17}O]Tyr-OMe (Table II). Moreover, the behavior of *p*-cresol is at variance with that of alcohols.[29]

The above results suggest that ^{17}O NMR spectroscopy cannot provide accurate information about the solvation state of the COH group of the tyrosine moiety. This is in contrast to the substantial sensitivity to hydrogen-bonding interactions of the ^{17}O shielding parameters of several functional groups, especially those of amide and peptide oxygens.[16-20] This is presumably due to the fact that the hydrogen bond in which the phenol and tyrosine oxygens act as proton acceptor is rather weak and deviates significantly from linearity (see discussion below). However, the available data suggest that the changes in ^{17}O NMR chemical shifts for the COH tyrosine moiety produced by deprotonation may be used to measure changes in charge density on oxygen atoms. Therefore, ^{17}O NMR studies of this type may be useful in determining sites and degree of deprotonation of tyrosine-type oxygens in small peptide hormones as suggested by Eckert and Fiat,[22] but not in the search for hydrogen-bond interactions.

FT-IR Studies: Solvent and Isotope Effects on ν_{CO} Stretching and COH Bending Vibrations in *p*-Cresol. In Figure 2 the regions of the ν_{OH} stretching vibrations (3620–3300 cm^{-1}) and of the COH in-plane deformation (bending) vibrations (1265–1150 cm^{-1}) are presented. Although the study of the former region was not the primary purpose of the present work, interpretation of the spectra in this region greatly facilitates the assignment of the bending vibrations.

The COH group of *p*-cresol can act as both a proton donor and proton acceptor. Therefore, it can self-associate through H-bonds into a number of complex species (oligomers, multimers, cyclomers) which may coexist with one another in a series of complicated equilibria.[30,31] The progressive increase in the intensity

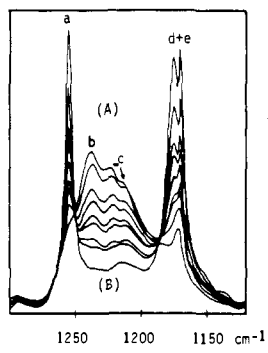
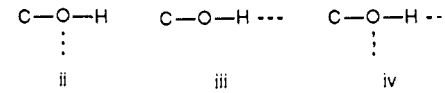


Figure 3. FT-IR spectra of *p*-cresol in $Cl_2C{=}CCl_2$ at concentrations from 4 (A) to 0.05 M (B). a–e have the same meanings as in Figure 2.

of the sharp monomer peak at ~3612 cm^{-1} (Figure 2B) with a decrease in the concentration of *p*-cresol in $Cl_2C{=}CCl_2$ and the concomitant decrease in the intensity of the very broad absorption due to the H-bonded OH group in the 3400–3250 cm^{-1} region are characteristic of self-associated compounds. Hall and Wood[31] have investigated in detail the self-association of phenol-type derivatives. They suggested that in solution four functionally different groups may result in distinct spectroscopic features: (i) monomer; (ii) acceptor end group, not acting as a donor; (iii) donor end group, not acting as an acceptor; and (iv) acting as both donor and acceptor.



The frequencies of groups ii and iii are likely to be independent of dimer or polymer attachment, and likewise the group iv frequencies are not expected to be sensitive to occurrence in a cyclic or open polymer. In the case of $Cl_2C{=}CCl_2$ solution, we failed to observe any resonance near the band at 3611 cm^{-1}, presumably due to the fact that few open acceptor end groups are present. Bands ii and iii are distinguishable only over a limited range of concentrations and usually appear as a composite, extremely broad resonance at ~3350 cm^{-1}.

Obviously as the concentration changes, the intensities of bands of the COH bending vibrations should follow that of their counterparts in the ν_{OH} region (Figure 2). Therefore, all of the in-plane COH fundamentals in the frequency range 1265–1150 cm^{-1} can be assigned to appropriate functional forms. The influence of the concentration and ^{2}D isotope effects on the absorption corresponding to the phenol COH bending vibration is presented in Figures 3 and 4. At low concentration, essentially three sharp absorption bands are present in $CCl_2{=}CCl_2$ (Figure 3). The absorption at ~1250 cm^{-1}, which is not shifted by O-deuteration, is assigned to the CO stretching vibration. The absorption at 1171 cm^{-1} disappearing by O-deuteration therefore can be attributed to the COH bending vibration. The sharper contribution at ~1165 cm^{-1}, which is distinguishable over a wide range of concentrations, is not sensitive to the concentration, and it is assigned to the β-CH vibration.[31]

When the concentration of *p*-cresol in $CCl_2{=}CCl_2$ is increased, some modifications due to self-aggregation appear with the occurrence of isosbestic points (Figure 3). The ν_{CO} stretching contribution broadens and decreases in intensity. However, it does not show splitting due to the different hydrogen-bonded species. Therefore the ν_{CO} stretching frequency, like the ^{17}O NMR chemical shift, is unlikely to be used as a probe for hydrogen-bonding interactions of the phenol OH moiety. The free COH bending contribution decreases, whereas the CH bending absorption does not shift. This intensity decrease is accompanied by the appearance of three absorption bands, which are shifted by O-deuteration and thus can be assigned to COH bending of the hydrogen-bonded PhOH groups in the aggregates. The band at 1245–1235 cm^{-1} increases, upon increasing the concentration,

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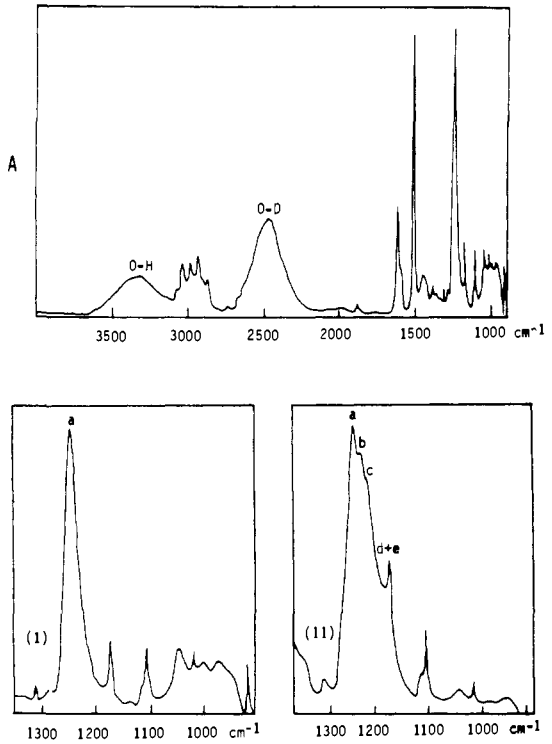

Figure 4. FT-IR spectrum of partially O-deuterated *p*-cresol in CCl_2=CCl_2, concentration 4 M (A); extended region of spectrum A (BI); and spectrum of *p*-cresol obtained under conditions identical to those of BI (BII). a–e have the same meanings as in Figure 2.

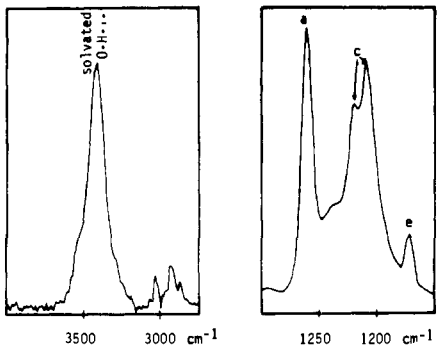

Figure 5. FT-IR spectra of *p*-cresol in CH_3CN, concentration 0.25 M. a, c, and e have the same meanings as in Figure 2.

more rapidly than the bands at 1225–1210 cm^{-1}, thus indicating that it is due to higher aggregates with the OH group acting both as donor and acceptor (iv). The bending mode of the hydrogen-bonded species with the OH group acting only as donor (iii) appears at ~1225–1210 cm^{-1}.

This interpretation is substantiated by the study of CH_3CN solution (Figure 5) in which the major species involved is the isolated solvated molecule with the OH group acting only as a donor with the solvent. As expected, the COH bending due to the free monomer is absent, making the CH bending vibration clearly visible at ~1172 cm^{-1}. The two bonded COH bending vibrations at 1220 and 1210 cm^{-1} are probably due to different solvation species by CH_3CN.

The IR spectrum of *p*-cresol in H_2O (Figure 6), near-saturated solution, exhibits after deconvolution a resonance at 1262 cm^{-1} which is not shifted by O-deuteration and thus can be attributed to a ν_{CO} stretching vibration, in agreement with our previous assignment in CCl_2=CCl_2. The strong band at ~1241 cm^{-1}, which is shifted by O-deuteration, can be attributed to the COH bending of the hydrogen-bonded species with the OH group acting both as donor and acceptor with the solvent (dihydrated species). It can be assumed that, due to the partial double-bond character of the C—OH bond, only one lone pair is available for hydrogen

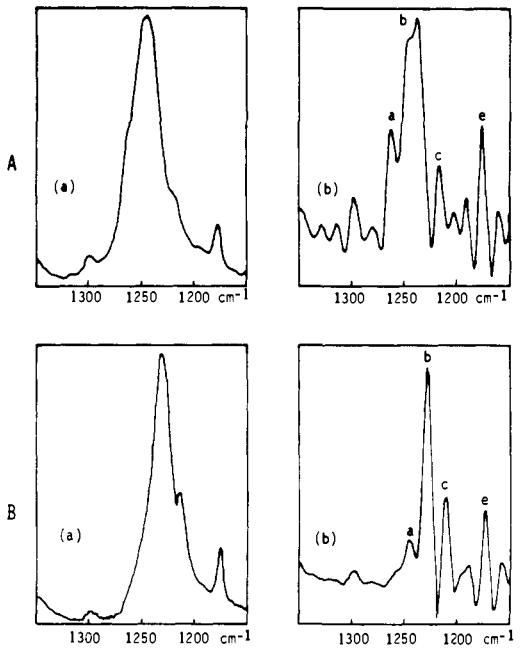

Figure 6. FT-IR spectra of *p*-cresol (A(a)) (A(b), self-deconvoluted spectrum) and of [^{18}O]-*p*-cresol (B(a)) (B(b), self-deconvoluted spectrum) in H_2O, near-saturated solution. a–c and e have the same meanings as in Figure 2.

bonding. A relatively weak absorption at 1218 cm^{-1} (~1216 cm^{-1} after band self-deconvolution), which is shifted in D_2O, can be attributed to the presence of a minor amount of monohydrated species. As expected, no absorption appears due to species free of hydrogen-bonding interactions. Under the same conditions [^{18}O]-*p*-cresol exhibits quite similar IR contributions (Figure 6). The band due to the ν_{CO} stretching vibration is shifted under the C^{18}OH bending vibrations. Strong deconvolution reveals that this band is probably at 1245 cm^{-1}, while the C^{18}OH bending vibrations of the dihydrated and monohydrated species are at 1229 and 1212 cm^{-1}, respectively.

From the above it is clear that the COH deformation modes are shifted to higher frequencies on increasing the strength of hydrogen bonding. The formation of hydrogen bonds constrains the deformation vibrations and therefore increases the force constants of these modes. Although these shifts are appreciably smaller than those found for the ν_{OH} stretching vibrations, the COH bending vibrations have three distinct advantages: (i) They do not exhibit any substantial band broadening when H-bonding occurs; therefore, discrete hydrogen-bonded species could be resolved and identified. On the contrary, the ν_{OH} stretching vibrations due to H-bonded species exhibit extremely broad absorption bands. (ii) The COH deformation absorption modes do not exhibit substantial changes in their intensity when H-bonding occurs.[32,33] (iii) Discrete hydrogen-bonding species can be identified even in aqueous solutions where the IR region of 3700–3100 cm^{-1} cannot be studied. *It is therefore clear that the region of bending vibrations has significant advantages over the classical region of the ν_{OH} stretching vibrations for phenol-type groupings.*

FT-IR Studies of Leu-Enkephalin in Water. The IR spectrum of Leu-enkephalin in water at neutral pH[34] exhibits a strong, composite absorption band centered at 1250 cm^{-1}, which is decomposed by self-deconvolution into two main contributions at 1265 and 1247 cm^{-1} (Figure 7). In D_2O the former band remains while the latter is eliminated. In this frequency domain, we expect the Tyr CO stretching and COH bending vibrations together with

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(34) Tyr at neutral pH resulted in spectra with an insufficient S/N ratio due to low solubility.

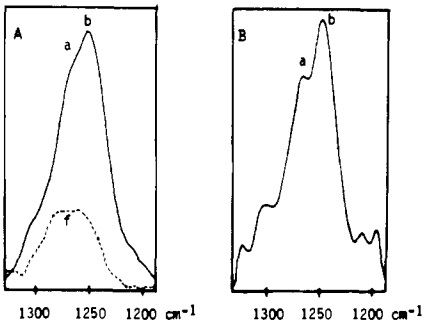

Figure 7. FT-IR spectra of Leu-enkephalin (—) and tetrapeptide Gly-Gly-Phe-Leu (---) in H_2O, concentration 0.02 M, pH 5.5 (A). Spectrum B represents the self-deconvoluted spectrum of Leu-enkephalin. a and b have the same meanings as in Figure 2; f denotes the amide III band of the tetrapeptide.

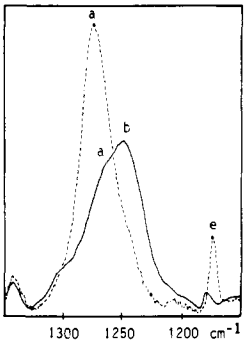

Figure 8. FT-IR spectra of Leu-enkephalin in H_2O, concentration 0.02 M at pH 5.5 (—) and pH 12.0 (---). a, b, and e have the same meanings as in Figure 2.

the amide III absorption. However, examination of the Gly-Gly-Phe-Leu tetrapeptide, lacking the Tyr residue, reveals that the amide III vibration is much weaker (by about one-third) than the observed absorption in Leu-enkephalin (Figure 7). It is therefore unlikely that the presence of a fourth amide group in Leu-enkephalin could explain the absorption difference. Moreover, the component at 1265 cm^{-1} shifts to ~1270 cm^{-1} upon deprotonation of the Tyr^1 OH group in Leu-enkephalin, whereas the component at 1247 cm^{-1} vanishes (Figure 8). These observations allow us to assign the bands at 1265 and 1247 cm^{-1} as contributions to the Tyr^1 ν_{CO} stretching and COH bending vibrations, respectively, with a small embedded contribution due to the amide III mode.

Han et al.[9] in extensive laser Raman spectroscopic studies of Leu^5-enkephalin in aqueous solution observed a variety of peaks in the amide III region. The band at 1266 cm^{-1}, which remains after N-deuteration and cannot be assigned to the amide III mode, was attributed to a CH_2 twisting mode and phenol ring vibrations of the tyrosine residue. The band at 1248 cm^{-1}, which disappeared completely after N-deuteration, was assigned to the amide III band. Renugopalakrishnan et al.[35] attributed the bands at 1263 and 1255 cm^{-1} (shoulder) in Leu-enkephalin in aqueous solution to an amide III band, in support of a β-turn structure. In view, however, of the conclusions of the present paper, some of the assignments and conclusions of the above papers should be reconsidered.

Table III. Hydrogen-Bond Dimensions of the Tyr^1 Hydroxyl Group of Leu-Enkephalin Trihydrate[37]

D–H···A	D···A (Å)	H···A (Å)	D–H···A (Å)
O–H···W1	2.706	1.68	174
O···W2	2.917	2.03	143

The similar IR data for Leu-enkephalin and *p*-cresol in water suggest that the phenol groups are exposed to the aqueous environment. Contrary to the proposition of Khaled et al.[11] the Tyr OH group does not seem to be involved in intramolecular contacts, but more probably is solvated by two molecules of water. This is confirmed by the fact that the *p*-cresol COH bending vibration is very sensitive to aggregation and the degree of hydration and absorbs near 1241 cm^{-1} when the hydroxyl group acts as both a donor and an acceptor group. It should be emphasized, however, that Leu-enkephalin does not exhibit a band at 1218 cm^{-1}, contrary to the case of *p*-cresol, presumably due to the fact that the amount of monohydrated species is small. Interestingly, recent X-ray structural studies[36,37] of Leu-enkephalin trihydrate revealed the existence of two molecules of H_2O hydrogen-bonded to the COH group of the Tyr moiety (Table III), in excellent agreement with our spectroscopic data in aqueous solution. The hydrogen-bonding dimensions are considerably different for the two molecules of water, W1 and W2. The one acting as proton donor, W1, forms a strong near-linear hydrogen bond, while the one acting as proton acceptor, W2, forms a weaker hydrogen bond which deviates considerably from linearity. The apparent weakness of this hydrogen bond can provide a reasonable interpretation of the weak variation of both $\delta(^{17}O)$ and the ν_{CO} stretching frequency on the formation of this hydrogen bond. Further, Thanki et al.[38] have recently reported a comprehensive study of the distributions of water molecules around the amino acid residues in peptides. Despite the problems in determining accurate sites of the water molecules from X-ray diffraction data and the complexity of the protein surface, distinct nonrandom distributions of water molecules were found. The stereoplot of water sites around the hydroxyl group of tyrosine residues shows two distinct clusters. Both clusters are in the plane of the ring because resonance appears to constrain the proton of the hydroxyl group and prevent free rotation.[39]

From the above discussion it appears that the Tyr COH bending vibration is an informative probe for studying, at a molecular level, the solvation state of the Tyr moiety in peptides in solution.

Acknowledgment. This work was supported by CEC (Grant ST2J-0184), the Research Committee of the University of Ioannina, the Greek General Secretary for Research and Technology, the Greek Foundation "Leonidas Zervas" (scholarship to N.B.), and EMBO (short term fellowship to I.P.G.).

Registry No. [^{17}O]-4-MeC_6H_4OH, 142867-36-5; H-[^{17}O]Tyr-OH, 104931-15-9; Ac-[^{17}O]Tyr-OMe, 142867-37-6; 4-MeC_6H_4OH, 106-44-5; 4-MeC_6H_4OD, 2876-04-2; H-Gly-Gly-Phe-Leu-OH, 60254-83-3; H-Tyr-OH, 60-18-4; ^{17}O, 13968-48-4; Leu-enkephalin, 58822-25-6.

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