Peptide Hydrogen Bonding. Conformation Dependence of the Carbonyl Carbon-13 Nuclear Magnetic Resonance Chemical Shifts in Ferrichrome. A Study by ^{13}C-{^{15}N} Fourier Double Resonance Spectroscopy[1a]

Miguel Llinás,*[1b] Donald M. Wilson,[1c] and Melvin P. Klein

Contribution from the Laboratory of Chemical Biodynamics (Lawrence Berkeley Laboratory) and the Department of Chemistry of the University of California, Berkeley, California 94720.
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Abstract: The carbonyl resonances in alumichrome and, by comparison, in the whole suite of homologues are unequivocally assigned by use of a novel ^{15}N–^{13}C double resonance technique which identifies the carbon resonances on the basis of a known, assigned ^1H NMR spectrum and sequential amide $^1H \leftrightarrow \{^{15}N\} \leftrightarrow {}^{13}C$ heteronuclear decoupling experiments. Comparison of spectra taken in nucleophilic $(CD_3)_2SO$ and in acidic protic CF_3CD_2OH solutions, and analysis of the resonance pattern on the basis of the known tertiary structure of the ferrichrome peptides, reveals the carbonyl chemical shift to be strongly dependent on the extent and type (donor or acceptor) of H bonding at the amide group. Thus, although a low field shift of the peptide carbonyl resonance results from carbonyl protonation (H bond acceptor role), *H bonding of the covalently linked NH group (H bond donor role) leads to an increased shielding of the carbonyl.* The latter effect is consistent with a facilitated electron charge density flux from the nitrogen lone pair toward the carbonyl and is dependent on the H bond stability at the NH as measured, e.g., by the temperature dependencies of the amide ^1H and ^{15}N chemical shifts. *Hence, NH and C=O H bonding oppose each other in their shielding effects on the peptide carbonyl resonance to such an extent that the H bond donor, high field shift can obscure deshielding resulting from a C=O H bond acceptor role.* As judged from the positions of the alumichromes' ornithyl resonances, the entire carbonyl chemical shift range accounted for by such effects can be as large as ~5 ppm. Such an interpretation of the carbonyl chemical shift is entirely consistent with literature data for other peptides of known conformation whose C=O resonances have been assigned. Water, which in an earlier study was shown to act as a good H bond acceptor solvent, stabilizes the carbonyl deshielding more than what would have been expected on the basis of its Brönsted acidity. From the overall ^1H and ^{13}C NMR effects, it is proposed that water favors maximal polarization of the peptide link, consistent with the high dielectric constant of the solvent.

Introduction

Allerhand and co-workers[2] have shown that nonprotonated aromatic carbons of proteins give resolved ^{13}C resonances which are sensitive to conformational and redox state effects. Since backbone carbonyl resonances are similarly narrow, they too should be of use as probes of solution structure, provided they may be assigned to individual amino acid sites and ^{13}C shift models are found which reproduce the observed spectral effects. Ferrichrome (Figure 1) offers an excellent prototype system for such an approach.

We have previously shown[3] that carbon-13 nuclear magnetic resonance (^{13}C NMR)[4] spectroscopy can be effectively used to detect the conformational change induced on the deferrichromes by Al^{3+} complexation. The event results in an overall spread of the resonances so that, e.g., the C=O region expands from 3.1 ppm in the metal-free peptide to 6.5 ppm in the coordination compound. Furthermore, by a comparative analysis of spectra from a number of alumichrome analogues, it was possible to assign most backbone resonances. Thus, in case of the carbonyl resonances, the identification was based on the appearance and disappearance of signals upon single residue substitutions as well as on the analysis of the concomitant nearest neighbor-induced (inductive?) shifts.[3b] The alumichromes provide a most adequate system to attempt such a carbonyl identification because of their isomorphism to the crystallographic model[5,6] (Figure 1). However, still remaining is the problem of understanding the pattern of carbonyl chemical shifts on the basis of the tertiary structure of the metallopeptide since current electric field and anisotropy models and peptide bond torsion effects fail to account for the spread in the carbonyl resonance positions.[3b,7]

In order to interpret the carbonyl spectrum on the basis of steric and H bonding effects, we have resorted to study solvent-induced chemical shifts by using typical H acceptor (Me_2SO) and H donor (TFE) solvents.[8,9] For this purpose it was felt necessary to substantiate the carbonyl resonance assignments in each solvent by a more unambiguous, direct procedure; hence, use was made of selective decoupling techniques.

The availability of ~99% ^{15}N-enriched alumichrome and the ^{15}N resonance assignment achieved on that peptide by a Fourier ^1H-{^{15}N} double resonance technique[9] suggest the plausibility of a similar experiment on the ^{13}C NMR spectrum, namely, identification of nitrogen-coupled carbon resonances by selective ^{13}C-{^{15}N} decoupling. In this communication we report the results obtained by use of this novel technique and propose a rationale to interpret the conformation and solvent dependence of the peptide carbonyl chemical shift on the basis of H bonding effects.[8,9]

Experimental Section

The ^{15}N-enriched alumichrome sample, obtained by growing the fungus *Ustilago sphaerogena* in a medium containing 99.2% [^{15}N]-ammonium acetate as the nitrogen source, belonged to a batch already described.[9] The carbon-13 NMR spectra were recorded with a Varian XL-100 spectrometer, modified for quadrature detection and double (^{15}N, ^1H) decoupling.[7,10] The sample (~100 mg of ^{15}N peptide) was dissolved in 1.6 mL of deuterated solvent and contained in 10-mL tubes. The deuterated solvents (^2H_2O, Me_2SO-d_6 and TFE-d_3) originated from Merck Sharp and Dohme of Canada.

Results and Discussion

The ^1H-noise decoupled carbonyl spectrum of the ^{15}N alumichrome is shown in Figure 2A. In contrast with the spectra of the ^{14}N peptides,[3b] the carbonyl resonances in the isotopically enriched alumichrome exhibit a definite doublet appearance due to direct spin–spin coupling to the $I = {}^1/_2$ ^{15}N nucleus ($^1J_{NC} \sim 12.8$ Hz). Figure 2B shows the appearance of the ^{15}N-coupled carbonyl spectrum as the ^{15}N frequency

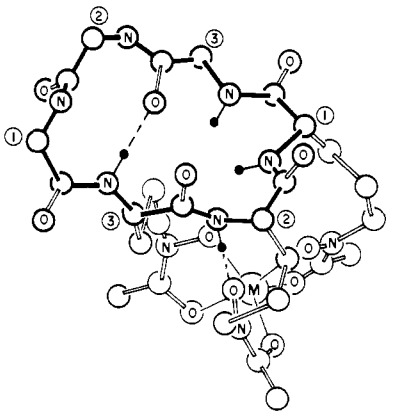


Figure 1. Structure of ferrichrome.[5,6] The numbers in circles label the glycyl residues occupying sites 1-3 and the sequential triornithyl peptide according to convention.[3,5,6] Only solvent-protected, "internal" amide hydrogen atoms are located on the model. Intramolecular H bonds of significance are indicated (- - -).

domain is CW scanned within the limits of the amide chemical shift range.[9] The carbonyl doublets experience from partial to total collapse as the f_2 wave approaches the resonance frequency of the proper, bonded ^{15}N nucleus. This general technique is more commonly used to connect ^{13}C and ^{1}H resonances directly, where there are no intervening heteroatoms.[11] The four (i.e. six, partially resolved) upfield resonances, which arise from the hydroxamate carbonyls of the ornithyl side chains,[3] are not affected by the applied ^{15}N irradiation because their coupled ^{15}N resonances are considerably shifted to lower fields from the amide region.[12] Figure 3 shows the assignments of the alumichrome carbonyls in Me_2SO and in TFE which were made using our technique. The carbonyl identification in Me_2SO fully agrees with the shifts and assignments resulting from single residue substitutions in isomorphic analogues.[3b] It should be recalled that selective enrichment has been used for carbonyl assignment in other peptides.[13] Our technique has the advantage of not requiring chemical synthesis of the natural peptide and of avoiding the complications of biosynthetic labeling at specific sites (metabolic branching, availability of aa$^-$ mutants, etc.). Furthermore, direct $^{13}C \leftrightarrow {}^{15}N$ decoupling reveals the link between residues at adjacent positions which has the potential of leading to primary structure elucidation.[14]

In 1963, Christ and Diehl[15] showed that the ^{17}O NMR shielding in acetone increases on going from a nonpolar to polar or protic acid solvents. Thus, on dissolving the ketone in phenol (pK_a = 9.89, 20 °C)[16] or formic acid (pK_a = 3.75, 20 °C)[16] the carbonyl ^{17}O resonance shifts by 28 and 48 ppm, respectively, to higher fields from its position in hexane solutions. The authors suggested that the more polar resonant structure (β) could be stabilized by solvent polarity or H bonding. Two years later, Maciel and Natterstad[17] reported the observation that in protic, H bond donor solvents, carbonyl groups in ketones are deshielded relative to the same group in esters. The authors found that on going from cyclohexane to trifluoroacetic acid, the acetone $^{13}C{=}O$ resonance shifts downfield by 16.5 ppm, while in ethyl acetate the corresponding peak moves only 8.2 ppm in the same direction. The suggestion was then made that the phenomenon could be a reflection of the relative importance of resonant structure γ in the case of esters. The imino (-NH-) group is a better electron donor for conjugation with the carbonyl in a peptide bond than the alcoholic (-O-R) group is for conjugation with the ester carbonyl. Hence, it appears very plausible that the carbonyl resonance shift induced by H bonding be further reduced on going from an ester to an amide and be even synergistically reversed (i.e., in direction to higher field) if the electron density deficiency at the

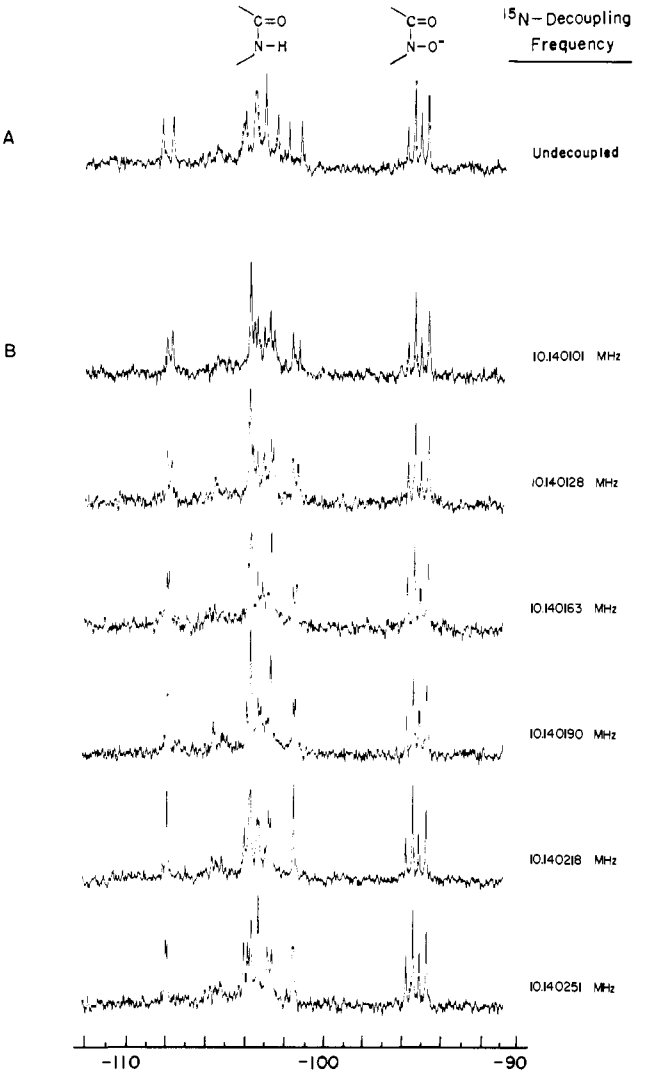
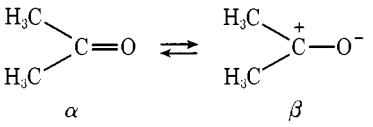
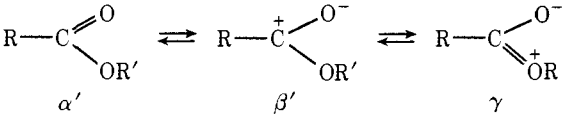


Figure 2. The carbonyl ^{13}C resonance region in ^{15}N-enriched alumichrome: (A) proton noise-decoupled spectrum; (B) proton noise- and ^{15}N continuous wave-double decoupled spectra. The collapse of all the amide ^{15}N-coupled carbonyl doublets can be observed at various ^{15}N frequencies. The solvent is Me_2SO-d_6 and the chemical shifts are referred to internal dioxane. The spectra were recorded at ~50 °C.

Scheme I



Scheme II



NH group is favored by, e.g., H bonding or dielectric effects.

We have shown[8,9] that the amide H bonding is sensitively detected by NMR spectra of the NH end of the peptide bond. Thus, the relative positions of both the ^{1}H and ^{15}N resonances shift to lower fields according to the extent of H bond donor role the amide plays in intramolecular or intermolecular (i.e., to the solvent) interactions. We have also demonstrated that

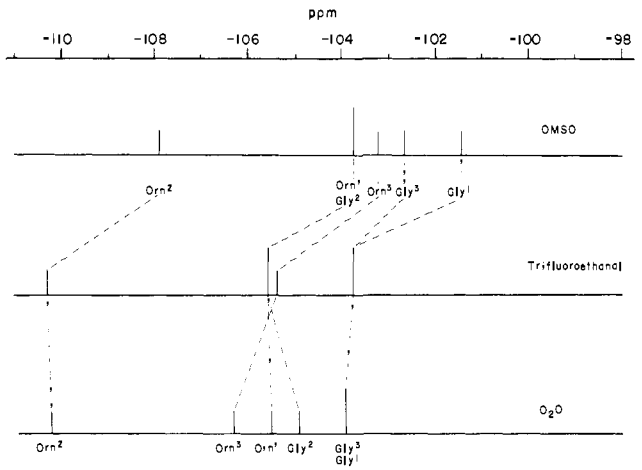


Figure 3. The solvent dependence of the alumichrome amide carbonyl ^{13}C NMR spectrum. In the case of the Me_2SO and TFE solutions, the carbonyl resonances have been directly assigned by ^{13}C-{^{15}N} decoupling (see Figure 2). In D_2O the assignments are only tentative, deduced as explained in the text. The experimental conditions are the same as for Figure 2.

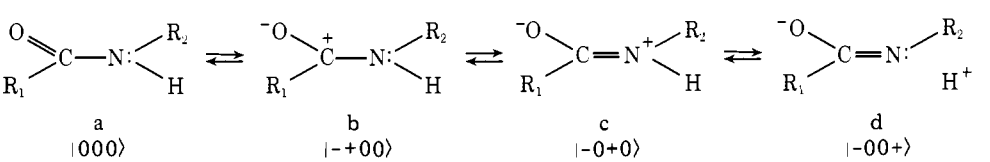
the ^{15}N-^{1}H deshielding is very sensitive to H bonding of the amide-linked C=O, the overall picture being consistent with a stabilization of the dipolar resonant structures c and d (canonical states $|-0+0\rangle$ and $|-00+\rangle$, respectively). The assignment of the ^{13}C=O resonances enables us to rationalize the carbonyl shifts in a consistent fashion keeping in mind that there are definite side chain inductive effects, peculiar of each residue type and independent of conformation, which dictate that ornithyl C=O resonances appear ~−3 ppm of the corresponding glycyl resonances. On the other hand, the lack of effect of a lysine-for-glycine substitution on the linked residue's ^{13}C=O resonance[18] strongly suggests that it ought to be of negligible spectroscopic consequence whether a resonance in alumichrome arises from an amide carbonyl peptide-bonded to a glycyl or an ornithyl residue.

We first note that in Me_2SO the Gly^2 C=O resonates more than 2 ppm downfield from the Gly^1 C=O (Figure 3). Both Gly^1 and Gly^2 have exposed carbonyls but, while the Gly^1 C=O is covalently linked to the intramolecularly H bonded Orn^3 NH (Figure 1), the Gly^2 C=O binds to the Gly^1 NH, an exposed amide involved in a weak, NH to solvent H bond [temperature coefficients, Gly^1 NH: -6.38×10^{-3} ppm/deg (^1H) and -18.05×10^{-3} ppm/deg (^{15}N); Orn^3 NH: -1.87×10^{-3} ppm/deg (^1H) and -6.60×10^{-3} ppm/deg (^{15}N)].[8,9] This indicates that C=O shielding is favored by stabilization of the NH H bond, which suggests the positive charge delocalization implied in resonant structures b ⇄ c (Scheme III) is that responsible for providing electron density to the carbonyl. The temperature coefficients of the Gly^2 NH chemical shifts [-5.12×10^{-3} ppm/deg (^1H) and -11.08×10^{-3} ppm/deg (^{15}N)][8,9] are smaller than those of the Gly^1 NH which implies that, relative to the Gly^2 C=O, the Gly^3 carbonyl should be more shielded since electron density flux from the (Gly^2) NH will be more favored. However, Gly^3 is unique among all the six residues in having an internal, intramolecularly H bonded C=O (Figure 1). Thus, although its linked (Gly^2) NH is exposed, as is the case with the Gly^2 C=O, the Gly^3 C=O also ought to experience some deshielding arising from a stable Orn^3 NH···O=CGly^3 H bonding. The result is that the Gly^3 $^{13}C'$ resonance appears centered between the corresponding Gly^1 and Gly^2 peaks (Figure 3).

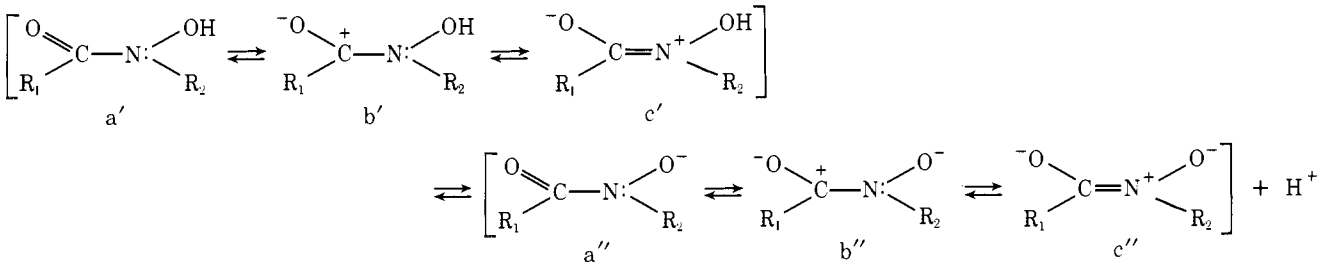
More explicit H bonding effects are exhibited by the ornithyl carbonyls (Figure 3). In Me_2SO, the Orn^2 C=O appears at ~4.7 ppm from the Orn^3 C=O. The exposed Orn^2 C=O is linked to the internal Orn^1 NH which is not H bonded,[5,6,8] while the also exposed Orn^3 C=O is linked to the strongly H bonded Orn^2 NH [crystallographic[5] N···O distance = 2.80 Å; NMR[8,9] temperature coefficients = -1.90×10^{-3} ppm/deg (^1H) and 0.00×10^{-3} ppm/deg (^{15}N)]. This suggests that a + charge excess at the carbonyl carbon (state $|-+00\rangle$, Scheme III) is only favored if an electron density deficiency at the NH group (states $|-0+0\rangle$ and $|-00+\rangle$) is *not* stabilized, e.g., because of lack of a suitable H acceptor group.[8] This interpretation is reinforced by the location of the Orn^1 C=O resonance, intermediate between the corresponding Orn^2 and Orn^3 peaks, whose linked Gly^3 NH is only slightly deshielded by weak H bond or electrostatic interaction with the Orn^3 C=O.[8] In this regard it is interesting to note that the Orn^3 C=O resonance moves to higher fields as the number of serine-for-glycine substitutions (alumichrome → alumicrocin → alumichrysin) increases.[3b] This effect correlates with a strengthened stability of the overall peptide conformation (decreased rates of amide H exchange[6]) which results in low field shift of the linked Orn^2 NH ^1H NMR, thus providing further evidence that the electron density at the peptidyl C=O increases the more the NH deshields.

The direct effects of C=O protonation can become very important in the presence of a good H bond donor solvent such as TFE, an acidic alcohol ($pK_a \simeq 12.4$ at 25 °C).[8] As seen in Figure 3 the transition from Me_2SO to TFE causes a downfield shift of ca. 2.12 ppm on all the amides except on the Gly^3 C=O which shifts only 1.09 ppm. The lesser shift of the Gly^3 carbonyl is consistent with its orientation in the molecule, pointing inward, away from the solvent and H bonding the Orn^3 NH (Figure 1). It should be noticed that the Orn^2 and Gly^2 ^{13}C=O resonances, which are unresolved in Me_2SO, shift exactly the same upon going to TFE exhibiting in this solvent also identical chemical shifts (Figure 3). This is of interest because while both the Orn^2 and Gly^2 carbonyls are exposed, their linked NH's are buried and protected in case of Orn^2 (Orn^1 NH) but external and solvated in case of Gly^2 (Gly^1 NH) which indicates that the weak peptide NH-to-solvent H bond plays a negligible role in determining the ^{13}C=O resonance position which is not the case for the strongly, as judged by $\Delta\delta/\Delta T$, intramolecularly H bonded amides. This observation is of relevance in interpreting solvent induced ^{13}C=O shifts in particular in those cases where peptide bonds containing prolyl residues are investigated. Urry and collaborators[19] have observed that when the set of peptides NAc-[1-^{13}C]Gly-Pro-OMe, NAc-[1-^{13}C]Gly-Ala-OMe, NAc-[1-^{13}C]Gly-Gly-OMe, NAc-[1-^{13}C]Val-Pro-OMe, NAc-[1-^{13}C]Val-Ala-OMe, and NAc-[1-^{13}C]Val-Gly-OMe are transferred from Me_2SO to TFE, the average shift of the residue 1 carbonyl is essentially the same for peptides containing or lacking prolyl residues, indicating there exists negligible difference between the behavior of the -CO-NR- and the -CO-NH- moieties in this regard. Thus, in general, it would appear that when there is no NH to interact with the solvent, the solvent-induced carbonyl shift is exactly the same. This contrasts the noticeable

Scheme III



Scheme IV

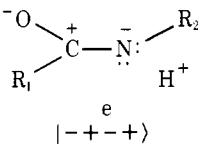


dependence of both the ^{15}N and ^{1}H NH chemical shifts on the state of solvation of the carbonyl.[8,9]

The reported[13b] carbonyl chemical shifts of gramicidin S in Me_2SO agree very well with the pattern discussed above. In gramicidin S, the two carbonyl resonances positioned at highest fields arise from the ornithyl and prolyl residues which happen to be covalently linked to the two residues (valine and leucine, respectively) whose NH's are involved in intramolecular H bonds. In contrast, the valyl and leucyl carbonyls are relatively more deshielded, consistent with their being peptide bonded to exposed NH's (involved in weak residue-to-solvent H bonds) and with their own H bond acceptor role within a β-pleated structure. Significantly, on going from Me_2SO to TFE, all the carbonyls become more deshielded, being the internal leucyl and valyl resonances those that move the least.[20] Such solvent perturbation effects have been found to be most useful to empirically delineate exposure of amide carbonyls in elastin oligopeptides.[21] In the protected elastin peptide Boc-Val-Pro-Gly-Gly-OMe, the prolyl carbonyl, itself not H bonded and linked to an exposed NH, resonates at −172.5 ppm from TMS, i.e., about 2.5 ppm to lower field from the same residue's C=O resonance in gramicidin S (−170.0 ppm from TMS) where it is covalently linked to an internally H bonded NH. Another example where the carbonyl resonances have been assigned is provided by *cyclo*-(Gly-Gly-Pro)$_2$.[13a,22] In this cyclohexapeptide the prolyl C=O, external and linked to an exposed (glycyl) NH, resonates at −172.3 ppm from TMS, in close agreement with the prolyl C=O resonance in the elastin peptide, which is in a similar configuration and shows up at −172.5 ppm from TMS. An interesting feature of the ^{13}C spectrum of the cyclohexapeptide is the position of the intramolecularly H bonded Gly^1 carbonyl (peptide linked to proline), ~2.5 ppm to higher fields relative to the exposed Gly^2 C=O (peptide linked to Gly^1). This shift may result from a more efficient carbonyl conjugation to the prolyl imino group (pK_a = 10.6) which should be a better electron donor (Lewis base) than the glycyl amino group (pK_a = 9.6), as would be suggested by a simple inductive effect considerations, *in spite of the fact that the* Gly^1 *NH is internally H bonded.* A related phenomenon is shown by the hydroxamic acid carbonyls which, as described elsewhere,[3] yield a typical peptide resonance appearing at positions intermediate between the glycyl and ornithyl amide C=O signals in the metal-free ferrichromes. Upon Al^{3+} binding the hydroxamic resonances shift from −103.7 to ~ −95.2 ppm[3b] (see Figure 2). It is clear that whatever electron-withdrawing substituent effect the amine hydroxyl exhibits will become less pronounced upon formation of the Al^{3+} complex. In other words, the stability of resonant structure b″ relative to a″ (Scheme IV) is less than that of b′ relative to a′ due to the one electron charge excess the hydroxamate hydroxyl oxygen gains upon ionization. In contrast, upon K^+ complexation by valinomycin, the liganding ester carbonyl resonances shift ~4 ppm to lower fields,[23] consistent with the lesser electronic lability of the -CO-O- bond discussed above. Thus, both the "anomalous" behavior of the prolyl-linked C=O resonance and the high field shift of the hydroxamate signal lend further support to our interpretation that *the carbonyl chemical shift is significantly controlled by the efficiency of negative charge density transfer from the linked nitrogen atom.*

The manner by which water interacts with amides is still an unsolved problem. On the basis of effects on the NH ^{1}H NMR chemical shift we have concluded that water behaves very much like a nucleophile.[8] Thus, in water the amide proton spectrum of alumichrome C is intermediate, in its overall appearance, to the spectra for the peptides dissolved in chloroform (a very weak acid, $pK_a \sim 24$) and Me_2SO (a good H bond acceptor, $pK_a \sim 0$).[8] In contrast to these results, the carbonyl ^{13}C NMR spectrum (Figure 3) reveals that water is about as good as TFE in stabilizing the carbonyl deshielding. Considering the spectral analogy between the aqueous and TFE solutions we tentatively assign the resonance at −110.2 ppm in D_2O to the Orn^2 C=O (−110.3 ppm in TFE) and the two overlapping resonances at −103.8 ppm to Gly^3 and Gly^1 (−103.7 ppm in TFE). However, the group of three resonances at −105.6 ppm (2×) and −105.4 ppm (1×) in TFE become spread and resolved in D_2O where they occur at −106.3, −105.5, and −104.9 ppm.

Taken separately, the proton and carbon-13 NMR results indicate what would have been expected a priori, namely, that amphoteric water behaves both as an H bond acceptor ($pK_{a_1} \sim -1.8$) and as an H bond donor (pK_{a_2} = 15.3).[8] The fact that water stabilizes the positive charges at C^+-O^- and -$(NH)^+$- independently suggests that canonical forms $|-0+0\rangle$ and $|-+00\rangle$ (Scheme III) and even the very polar canonical structure $|-+-+\rangle$ assume some importance in this solvent.



e

$|-+-+\rangle$

This could occur, e.g., by water molecules aligning against the peptide backbone so as to achieve maximal charge and dipole pairing. There exists in the literature circumstantial evidence which supports such an interpretation: (1) It is known[15] that relative to hexane solutions, water shifts the acetone carbonyl ^{17}O resonance by 45 ppm, i.e., about the same effect as that caused by formic acid, a much stronger proton donor (pK_a = 3.75). A consistent deshielding of −9.1 ppm is detected on the ^{13}C=O resonance in the same solvent transition.[17] This can be understood by assuming that water stabilizes the ^+C-O^- dipole in a stronger way than would have been expected on the basis of H bonding only [dielectric constant of water, ϵ = 80 (20 °C)]. (2) Spectroscopic studies indicate that in dilute aqueous solutions the amide nitrogens H bond the solvent.[25] (3) There exists substantial evidence which suggests that both the carbonyl oxygen and the amino nitrogen can act as H acceptors, depending on the solvent.[26] Resonant structures b, c and e suggest that the exposed Orn^3-CO-HN-Orn^2 amide dipole could be further stabilized in water vis-à-vis TFE, resulting in a deshielding consistent with assigning to Orn^3 the carbonyl resonance at −106.3 ppm. Similar considerations lead

to tentatively assign the −105.5 and −104.9 ppm signals to the Orn[1] and Gly[2] carbonyls (Figure 3).

Water ^1H, ^2H, and ^{17}O NMR relaxation dispersion studies of aqueous protein solutions have revealed a significant interaction between the solvent and solute.[27] The proposal was formulated that the interaction could impose a slow component of rotational correlation velocity on that fraction of water molecules which presumably is in contact with the randomly tumbling protein.[27] It might well be that the "friction" mechanism transmitting angular momentum from the polypeptide to solvent water is the kind of amide-water pairing effect suggested by the present study.

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(4) Abbreviations: ^{13}C NMR, ^{13}C nuclear magnetic resonance; ^1H NMR, ^1H nuclear magnetic resonance; NMR, nuclear magnetic resonance; Me_2SO, dimethyl sulfoxide or dimethyl-d_6 sulfoxide; TFE, trifluoroethanol or trifluoroethanol-d_2; ppm, parts per million; CW, continuous wave.
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Dielectric Studies on Retinal and Ionone

P.-J. Bauer* and P. Carl

Contribution from the Institut für Neurobiologie, KFA, D-5170 Jülich, West Germany.
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Abstract: The dipole moments of *all-trans*-retinal and 11-*cis*-retinal as well as of the analogs α-ionone and β-ionone were determined in the nonpolar solvent methylcyclohexane. No tendency of the molecules to aggregate in this solvent was observed. The calculations of the dipole moments were based on Onsager's theory of dielectric constants of a two-component system. The dipole moment of *all-trans*-retinal was determined to be 4.02 ± 0.05 D. The dipole moment of the sterically hindered 11-*cis*-retinal was found to be 3.89 ± 0.05 D, i.e., only 0.13 D lower than that of *all-trans*-retinal. This is much higher than one would expect if the preferred conformation of 11-*cis*-retinal in solution would be the same as in the crystalline state, viz., a distorted 12-s-cis conformation. This conclusion is based on the finding that dipole moments of conjugated polyene aldehydes strongly depend on the electric polarizability of the π-electrons. In nonpolar solvents, therefore, a polar conformation of 11-*cis*-retinal prevails, probably a distorted 12-s-trans conformation. Assuming the presence of two conformers in thermodynamic equilibrium, an upper limit for the relative amount of the less polar species is estimated. Mixtures containing various relative amounts of 9-*cis*-, 13-*cis*-, 9,13-*dicis*-, and *all-trans*-retinal were produced by photoisomerization of *all-trans*-retinal in nonpolar solution. No formation of 11-*cis*-retinal was detected by high-pressure chromatography. The dielectric constants of all these mixtures were identical with that of pure *all-trans*-retinal. This suggests that the 9-cis, 13-cis, and 9,13-dicis isomers, which are expected to possess planar polyene chains like *all-trans*-retinal, also have dipole moments identical with that of *all-trans*-retinal. In addition, it is found that the dipole moments of analogous aldehydes are strongly correlated with the number of the conjugated π-electrons.

Introduction

Rhodopsin, the visual pigment of rod outer segments, consists of 11-*cis*-retinal covalently bound to the protein, opsin, via a Schiff base linkage with an ϵ-amino group of lysine.[1,2] Light absorption causes photoisomerization to *all-trans*-retinal in the primary photochemical steps of the visual process.[3] There have been extensive conformational studies of the retinal isomers both in crystalline form[4,5] and in solution.[6-9] In crystalline form, 11-*cis*-retinal assumes a distorted 12-s-cis conformation[4] in which the polyene chain largely deviates from a planar structure. In solution, however, a strong temperature effect of the absorption spectra,[8-11] NMR experiments,[12] and theoretical considerations[13] suggest that a distorted 12-s-cis conformation coexists in thermodynamic equilibrium with a distorted 12-s-trans conformation. Little is known regarding the relative amounts of both conformers. The calculated potential energy curve for twisting about the 12-s bond yields the lowest energy minimum for a distorted 12-s-cis conforma-