Carbon-13 Nuclear Magnetic Resonance Study of Tyrosine Titrations

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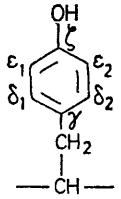
Summary The ionization of the phenolic hydroxy-groups of tyrosines in peptides and proteins in alkaline solutions produces appreciable shifts of the C^{ζ}, C^{ε}, and C^{γ} (**1**) carbon-13 nuclear magnetic resonances, from which the apparent pK values of tyrosine residues can be obtained.

IN the titration of the histidine residues of proteins, ^1H n.m.r. spectroscopy has been shown to be particularly useful, because the C-2 protons are well separated from other resonances and changes in chemical shifts resultant on ionization are relatively large.[1] By contrast, the observation of the proton resonances of tyrosine residues in proteins is much more difficult, due to spin–spin coupling of the tyrosine aromatic protons, their relatively small chemical

shift change on titration and overlap with resonances from other aromatic residues.[2] However the ^{13}C n.m.r. spectrum of tyrosine shows some aromatic carbon resonances which are well separated from other resonances in the ^{13}C n.m.r. spectra of proteins.[3,4] Here we examine the use of ^{13}C n.m.r. spectroscopy in the titration of tyrosine residues in peptides and proteins.

All ^{13}C n.m.r. spectra were obtained with a Brüker HX-90 spectrometer operated at 22·63 MHz for ^{13}C as described previously.[4] Because of the low solubility of L-tyrosine below about pH 9, two small tyrosine peptides glycyl-L-tyrosyl amide and glycyl-L-tyrosyl-glycine were studied. Their purity was checked by ^{13}C n.m.r. spectroscopy, which also showed no differences between the spectra of the two peptides in the aromatic region. From the pH dependence of the chemical shifts as shown in the Figure it is possible to calculate apparent pK values from the C^{ζ}, $C^{\varepsilon 1,\varepsilon 2}$, and C^{γ} curves of 9·77, 9·75, and 9·75 respectively. These are in the range expected for the phenolic hydroxy-group of tyrosine in peptides.[2,5] The total shifts caused by the ionization of the phenolic hydroxy group are C^{ζ} −10·4 p.p.m., $C^{\varepsilon 1,\varepsilon 2}$ −3·3 p.p.m., $C^{\delta 1,\delta 2}$ +0·1 p.p.m. and C^{γ} +6·2 p.p.m., where a negative sign indicates a downfield shift. Qualitatively similar changes in chemical shift have been observed for phenol in a range of organic solvents of increasing basicity.[6] A number of factors including inductive[7] and resonance[6] effects (through bond) and electric field[7] and π-polarisation[8] effects (through space) have contributed to the observed shifts. For our purposes the large shift of the C^{ζ} resonance on ionization is particularly noteworthy although the C^{γ} and $C^{\varepsilon 1,\varepsilon 2}$ resonances also give useful shifts.



From the ^{13}C n.m.r. spectrum of the carbonyl and aromatic regions of denatured lysozyme it can be seen that the resonances are normalised as a result of unfolding.[4] The region of the spectrum near the tyrosine C^{ζ} resonance is clear except for the arginine C^{ζ} resonance. The intensity of this tyrosine non-protonated resonance is less than one half that of the tyrosine $C^{\delta 1,\delta 2}$ and tyrosine $C^{\varepsilon 1,\varepsilon 2}$ resonances because of a longer spin–lattice relaxation time compared with the protonated carbon atoms and also a possible reduction in nuclear Overhauser enhancement due to contributions from nondipolar relaxation mechanisms. This effect is likely to be less important in studies of native proteins, where most carbon atoms experience little or no nuclear Overhauser enhancement, due to long rotational correlation times and where the relaxation times of even unprotonated carbon atoms are relatively small.[9]

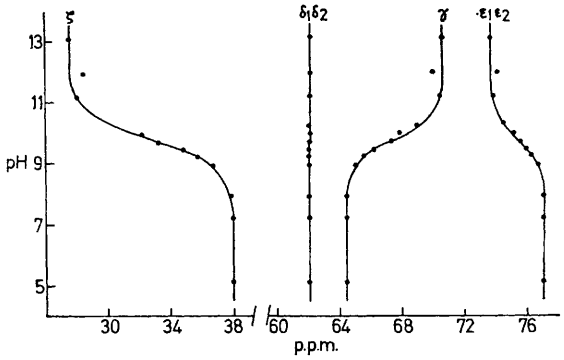


FIGURE. Chemical shifts in p.p.m. upfield from CS_2 of the aromatic carbon resonances of tyrosine (**1**) in glycyl-L-tyrosyl-amide (pH ⩽ 11·2) and glycyl-L-tyrosylglycine (pH ⩾ 11·9) as a function of pH in water.

We are currently studying the ^{13}C n.m.r. resonances of tyrosine in ribonuclease-A and have chosen this protein for two reasons. Firstly tryptophan is absent, which simplifies the spectrum in the region of the tyrosine C^{γ} and C^{ε} resonances. Secondly, the six tyrosine residues in this protein are known to exist in a variety of environments,[10] with differing pK values.[5] Preliminary results indicate that this is indeed the case, and further work is in progress to elucidate the detailed behaviour of the resonances. It is clear that with the use of improved procedures for increasing further the intensities of ^{13}C n.m.r. resonances,[11] it should be possible to follow the behaviour of single tyrosine residues in proteins much more readily using ^{13}C n.m.r. spectroscopy than using ^{1}H n.m.r. spectroscopy.[2]

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