(Tyr-103 and Tyr-146), 7 phenylalanines, 11 histidines, and 2 tryptophans of this protein[17] contain 30 nonprotonated side-chain carbons, all of which yield observable resonances in spectra of the cyanoferrimyoglobin.[8,9] Clearly, peaks 4 and 27 arise from C^{ζ} and C^{γ}, respectively, of a tyrosine residue.[8] We can infer that the titratable tyrosine is Tyr-103 and not Tyr-146 from the fact that spectra of kangaroo myoglobin (a protein with a single tyrosine at position 146[17]) do not yield any titratable tyrosine residue.[8]

Proton NMR spectroscopy has been the favorite method for measuring the ionization behavior of histidine residues of proteins, because the resonances of H^{ϵ_1} (and sometimes H^{δ_2}) can often be observed as resolved single-hydrogen peaks.[28] Carbon-13 NMR is an attractive alternative, especially when dealing with a protein which contains many histidines.[9] The effect of pH on the chemical shift of C^{γ} of a histidine residue not only yields the pK value but also indicates which is the predominant tautomeric state of the imidazole form of the residue: If the imidazolium form of a histidine (Figure 1) deprotonates at N^{δ_1} to yield the "common" N^{ϵ_2}–H tautomer,[9,29] then the C^{γ} resonance should move about 6 ppm downfield.[29] If deprotonation yields the "uncommon" N^{δ_1}–H tautomer,[10] the C^{γ} resonance should move about 2 ppm upfield.[29] The C^{γ} resonances of 8 of the 11 histidines of horse cyanoferrimyoglobin exhibit titration behavior (peaks 11, 14–16, and 18–21 of Figure 8).[9] The direction of the titration shifts indicates that the imidazole form of each titrating histidine is predominantly in the N^{ϵ_2}–H tautomeric state. The effect of pH on the chemical shifts of the nonprotonated aromatic carbons of *P. aeruginosa* azurin (see Figure 2 of ref 10) indicates that only two of the four histidine residues titrate (peaks 11 and 14 of Figure 7A).[10] Peak 14 (Figure 7A) moves about 2 ppm *upfield* when going to high pH (with a pK of 7.5) and must therefore arise from a histidine residue whose imidazole form exists mainly in the "uncommon" N^{δ_1}–H tautomeric state.[10]

In favorable cases, ^{13}C NMR can be used to determine nondestructively the positions in the sequence of chemically modified aromatic amino acid residues, the amount of unreacted protein, and the nature of the chemical modifications.[19,20] For example, a comparison of the spectrum of intact hen egg-white lysozyme (Figure 4A) with the spectrum of one fraction obtained from the reaction mixture after treatment with iodine[24] (Figure 4B) indicates that Trp-108 is the only aromatic residue which has been altered (because peaks 13, 16, and 22 of the intact protein are missing from Figure 4B) and that the altered residue is not oxindolealanine but δ_1-hydroxytryptophan or an ester thereof (based on the chemical shifts of peaks x, y, and z of Figure 4B).[19] Figure 4B is a spectrum of chromatographically fractionated [(RCOO)Trp-108]lysozyme (see preceding section).[24] Actually, more detailed conclusions were obtained from spectra of *unfractionated* reaction mixtures of lysozyme with iodine.[19]

Application of ^{13}C NMR spectroscopy to the study of the reaction of equimolar amounts of *N*-bromosuccinimide and hen egg-white lysozyme (a known procedure for the specific conversion of Trp-62 into oxindolealanine[30]) revealed the complexities of a chemical modification which was previously thought to be very simple.[20]

Carbon-13 NMR can be used to study changes in the properties of a protein upon chemical modification of a specific residue. For example, ^{13}C NMR was used to study the effects of chemical modifications at Trp-108 of lysozyme on the self-association and lanthanide ion binding properties of the protein.[24] Both [(RCOO)-Trp-108]lysozyme and [oxindolealanine-108]lysozyme were studied.[24] Consider the spectra of [(RCOO)-Trp-108]lysozyme shown in Figure 4. The chemical shift of C^{γ} of Trp-62 (peak 25) does not change appreciably when going from pH 4 to pH 7. This fact, taken together with information presented in ref 27, indicates that [(RCOO)Trp-108]lysozyme does not self-associate significantly at neutral pH[24] (as has also been determined by other methods[26]), in contrast to the behavior of the intact protein under similar sample conditions.[27]

I thank the graduate students and postdoctoral research associates, whose names appear in the references, for carrying out the work described here. I thank Ms. Nancy Koehler for technical assistance. Financial support from the National Institutes of Health and the National Science Foundation is gratefully acknowledged.

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Additions and Corrections

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Page 371. Figure 3 is inaccurate as presented. The correct sequence, reading from top to bottom, should be Photosynthetic Membrane, Vesicle, Micelle, and Sensitized Semiconductor.