STUDY OF THE TITRATION BEHAVIOR OF N-TERMINAL GLYCINE RESIDUES OF MYOGLOBINS BY NATURAL ABUNDANCE ^{13}C NMR SPECTROSCOPY

David J. WILBUR and Adam ALLERHAND
Department of Chemistry, Indiana University, Bloomington, Indiana 47401, USA

Received 22 April 1977

1. Introduction

Numerous narrow individual-carbon resonances* have been observed in the aromatic region of natural-abundance ^{13}C NMR spectra of small native proteins [1–4]. In contrast, few of the aliphatic and carbonyl carbons have yielded resolved individual-carbon resonances, even at a magnetic field strength as high as 63 kG [5–7]. In the case of the carbonyl groups, individual-carbon resonances of some carboxylate groups (of hen egg-white lysozyme) and of N-terminal glycine residues (of horse myoglobin and of the γ-chains of human fetal hemoglobin) have been observed at the downfield edge [7] and upfield edge [3,8], respectively, of the carbonyl region of the spectrum. In this report we show that natural-abundance ^{13}C NMR spectroscopy can be used for measuring the pK_a values of N-terminal glycine residues of proteins. We report the pK_a value of Gly-1 of horse cyanoferrimyoglobin. With the use of much higher magnetic field strengths than our 14.2 kG, it should be possible to determine pK_a values of N-terminal residues other than glycine.

2. Materials and methods

The preparation of samples has been described [9]. ^{13}C NMR Spectra were obtained at 15.18 MHz, essentially as already described [9]. Chemical shifts, reported in parts per million downfield from the ^{13}C resonance of Me_4Si, were measured digitally with respect to a trace of internal dioxane (at 67.86 ppm). Estimated accuracy is ±0.1 ppm.

Abbreviation: Me_4Si, tetramethylsilane

* We use the term 'individual-carbon resonance' to designate a peak that arises either from a single carbon of a protein or from two or more equivalent carbons (such as the two δ-carbons of a phenylalanine residue which is undergoing fast internal rotation about the $C^{\beta}-C^{\gamma}$ bond)

3. Results and discussion

Figure 1 shows the effect of pH on the carbonyl region of the proton-decoupled natural-abundance ^{13}C NMR spectrum of horse cyanoferrimyoglobin (in H_2O, 0.1 M KCl, 39°C). At pH values lower than about 7.5, the carbonyl resonance of Gly-1 is situated upfield of all other carbonyl resonances of myoglobins [3], so that its titration behavior can be readily followed. Above pH 7.5, this resonance merges with the main carbonyl bands, and becomes relatively difficult to identify (fig.1). Nevertheless, with an appropriate choice of numerous pH values, it is possible to follow the complete titration of the carbonyl resonance of Gly-1, even at our low magnetic field strength of 14.2 kG (figs 1 and 2). Application of the usual Henderson-Hasselbalch treatment to the experimental chemical shifts of horse cyanoferrimyoglobin (given in fig.2) yields a pK_a of 7.81 ± 0.05. The limiting chemical shifts at low and high pH are 167.9 ppm and 176.2 ppm, respectively. These values are similar to those reported for Gly-1 of small peptides [10] and of a modified sperm-whale ferrimyoglobin in which Val-1 was replaced by a ^{13}C-enriched glycine residue [11].

We also examined the titration behavior of the carbonyl resonance of Gly-1 of horse ferrimyoglobin

 North-Holland Publishing Company – Amsterdam

(in the pH range 5.1–7.7) and kangaroo cyanoferrimyoglobin (in the pH range 4.9–7.5). Within experimental error, these proteins yielded the same pK_a

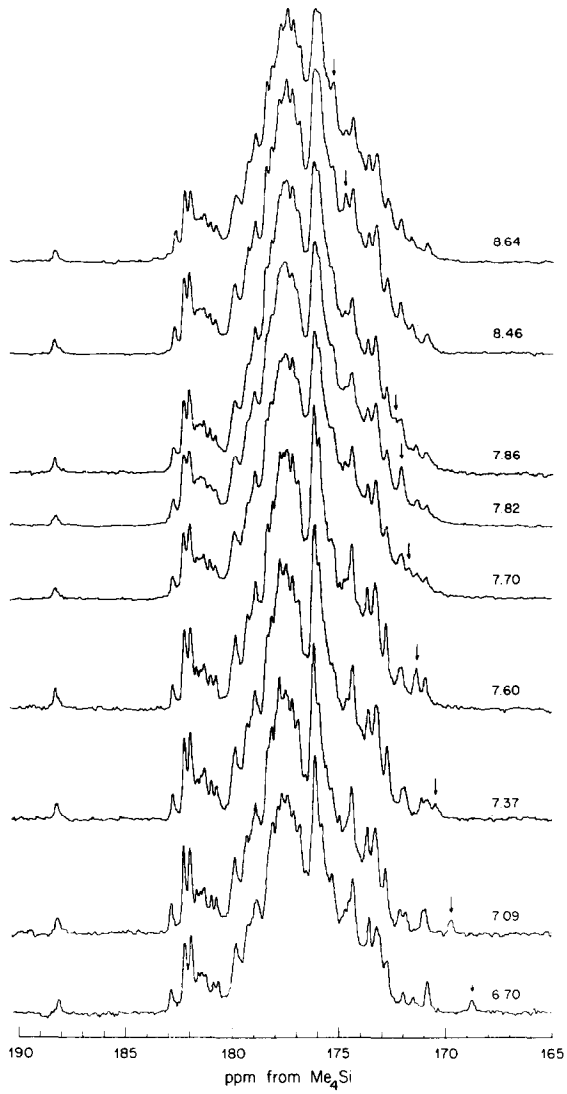

Fig.1. Effect of pH on the region of carbonyl resonances in proton-decoupled natural-abundance ^{13}C NMR spectra (at 15.18 MHz) of horse cyanoferrimyoglobin (9–13 mM protein in H_2O, 0.1 M KCl, 39°C). Each spectrum was obtained with the use of 32 768 accumulations, a recycle time of 1.1 s (10 h total time) and a digital broadening of 0.6 Hz. In each spectrum, an arrow indicates the position of the carbonyl resonance of Gly-1. Numbers on the right-hand side are pH values. Spectra recorded at 20 additional pH values (see fig.2) are not shown).

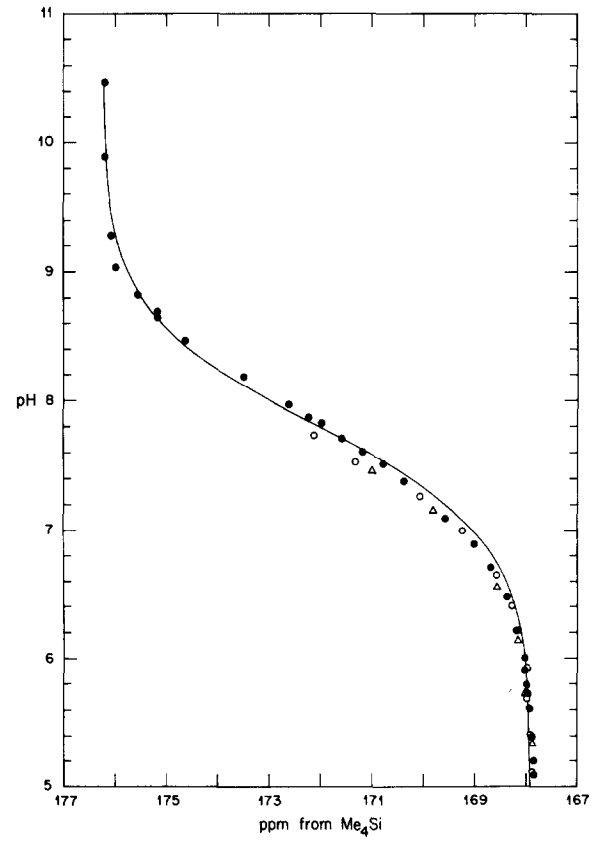

Fig.2. Effect of pH on the chemical shift of the carbonyl resonance of Gly-1 of horse cyanoferrimyoglobin at 39°C (closed circles), horse ferrimyoglobin at 36°C (open circles) and red-kangaroo cyanoferrimyoglobin at 36°C (open trinagles). The solid line is the best-fit theoretical titration curve (assuming a single pK_a) for horse cyanoferrimyoglobin.

(and limiting chemical shift at low pH) as that of horse cyanoferrimyoglobin (see fig.2).

Garner et al. [12] used the kinetics of the reaction of cyanate with the N-terminal amino groups of various myoglobins (at 25°C) to determine the pK_a values of these groups. They reported pK_a values of 7.7 and 8.0 for two myoglobins with Val-1, and pK_a values of 7.2, 7.4, and 7.7 for three myoglobins with Gly-1. Thus, it appeared that the pK_a value of a Gly-1 is generally lower than that of a Val-1 residue [12]. However, we observe a pK_a of 7.8 for Gly-1 of the myoglobins from horse and kangaroo.

It is apparent from fig.1 that (at pH values near or above the pK_a) the identification of the carbonyl

resonance of Gly-1 of a protein would be greatly facilitated by the increased spectral resolution available at higher magnetic field strengths than 14 kG [5]. Also, when dealing with an N-terminal residue such as valine, whose carbonyl resonance overlaps with the main carbonyl band even at low pH [3], the increased resolution of a magnetic field strength such as 40 kG or 60 kG [5] may be essential for the observation of the titration behavior by means of natural-abundance ^{13}C NMR.

Acknowledgements

This work was supported by the United States Public Health Service (Grant GM 22620) and the National Science Foundation (Grants MPS 73-04969 and CHE 76-17572).

References

[1] Allerhand, A., Childers, R. F. and Oldfield, E. (1973) Biochemistry 12, 1335–1341.
[2] Oldfield, E., Norton, R. S. and Allerhand, A. (1975) J. Biol. Chem. 250, 6368–6380.
[3] Oldfield, E., Norton, R. S. and Allerhand, A. (1975) J. Biol. Chem. 250, 6381–6402.
[4] Dill, K. and Allerhand, A. (1977) submitted.
[5] Norton, R. S., Clouse, A. O., Addleman, R. and Allerhand, A. (1977) J. Am. Chem. Soc. 99, 79–83.
[6] Wilbur, D. J. and Allerhand, A. (1977) FEBS Lett. 74, 272–274.
[7] Shindo, H. and Cohen, J. S. (1976) Proc. Natl. Acad. Sci. USA 73, 1979–1983.
[8] Oldfield, E. and Allerhand, A. (1975) J. Biol. Chem. 250, 6403–6407.
[9] Wilbur, D. J. and Allerhand, A. (1976) J. Biol. Chem. 251, 5187–5194.
[10] Keim, P., Vigna, R. A., Marshall, R. C. and Gurd, F. R. N. (1973) J. Biol. Chem. 248, 6104–6113.
[11] Garner, W. H. (1974) PhD thesis, Indiana University.
[12] Garner, M. H., Garner, W. H. and Gurd, F. R. N. (1973) J. Biol. Chem. 248, 5451–5455.