CARBON-13 MAGNETIC RESONANCE STUDIES OF CARBON-13 ENRICHED AMINO ACIDS

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Abstract:

Carbon-13 magnetic resonance data is presented for carbon-13 enriched L-amino acids. The results are compared to the corresponding proton chemical shifts and discussed in terms of their potential applications to peptides and proteins.

Carbon-13 enriched amino acids were obtained from algae grown on enriched carbon-13 CO_2 as the sole carbon source. The algae were hydrolysed to a protein fraction, and then to an amino acid mixture (4.9 g) which was separated by ion-exchange chromatography. Details of the procedure will be presented elsewhere[1]. The products (70% yield) were characterized for purity and amino acid content using thin layer chromatography and combined gas liquid chromatography-mass spectrometry[2]. The carbon-13 isotopic content determined in this way was 14.6 ± 1.5%.

The carbon-13 high resolution magnetic resonance studies were carried out using a home-built instrument operating at 15 MHz, with both continuous wave and pulsed modes. Details of this instrument will be presented elsewhere[3]. A 60 MHz noise generator decoupled all protons simultaneously[4,5].

Carbon-13 chemical shifts with respect to the α-carbon atoms of glycine are shown in Table 1. These were determined using saturated solutions of the

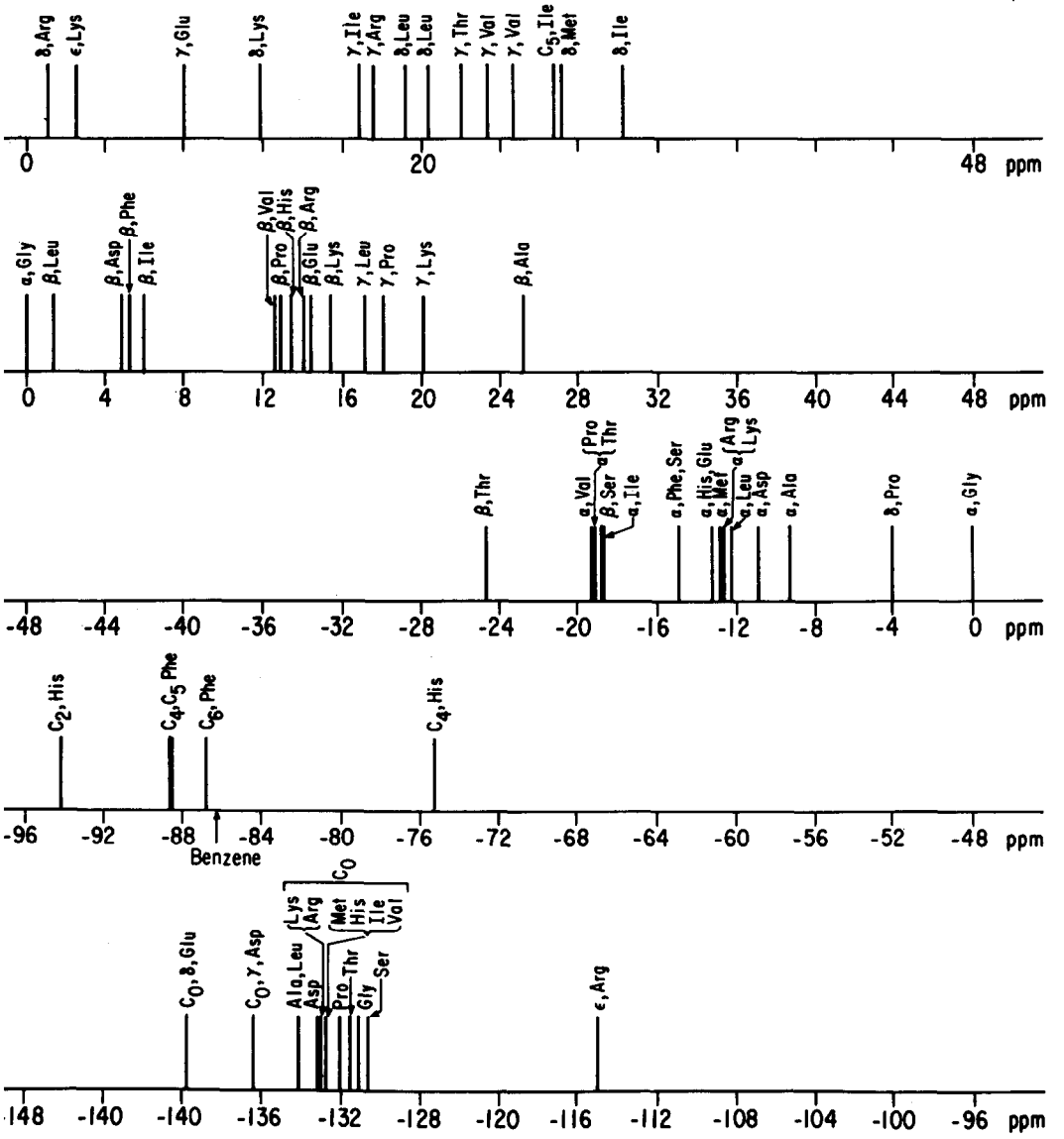

Figure 1 The observed C-13 shifts of the amino acids, neutral pD, relative to the observed glycine α carbon (86.5 ppm upfield of benzene).

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individual amino acids at netural pD and 25°C. Solutions were in D_2O and the deuterium resonance was used as a lock signal. Small corrections (<0.1 ppm) were determined for variations in this solvent resonance.

Carbon-13 enrichment was preferred to the utilization of natural abundance

for ease in finding the resonances and for reduction in the time required to accumulate the spectra using a time-averaging computer. Nevertheless, intermediate passage conditions had to be employed, and this led to some broadening of the resonances, (by 2 - 3 Hz). However, spectra derived from the Fourier transform of the free induction decay[6] in the pulse mode gave truer linewidths. In some cases such as the C_3 of Phe and C_5 of His, no resonances could be observed probably due to very long T_1 relaxation times, since these carbon atoms have no protons which can act as dipolar relaxers.

The carbon-13 chemical shifts could be predicted with considerable accuracy using the empirical rules and refinements derived by Grant and Paul[7] and Horsley and Sternlicht[8]. The shift parameters for different substituent groups were to a first approximation additive.

It is of interest to compare the carbon-13 with the proton chemical shifts of the amino acids which are shown in Table 2 and are derived from the work of McDonald and Phillips[9]. It is apparent that while the proton chemical shifts are an order of magnitude smaller than the carbon shifts the patterns are superficially similar. However, accurate predictions of the proton shifts based on empirical parameters or charge density calculations[10] are not as straightforward as for carbon-13. Stothers and Lauterbur[11] and Marciel[12] have shown that to a first approximation the chemical shifts of the carboxyl carbon correlates with its electronic charge density. The carboxyl resonances of the amino acids were observed to fall in a narrow range, with the γ and δ carboxyl resonances of aspartic and glutamic acids respectively being well resolved with a separation of 3.4 ppm. These resonances shift downfield with increasing pD, with a total titration shift of ca 6 ppm over the range pD 1 - 9. Furthermore, large solvent induced pertubations of carboxyl carbon-13 resonances of ketones and esters have been described by Marciel and Natterstad[13]

The present study was undertaken to provide a basis for further potential studies on peptides and possibly carbon-13 enriched proteins. Previous

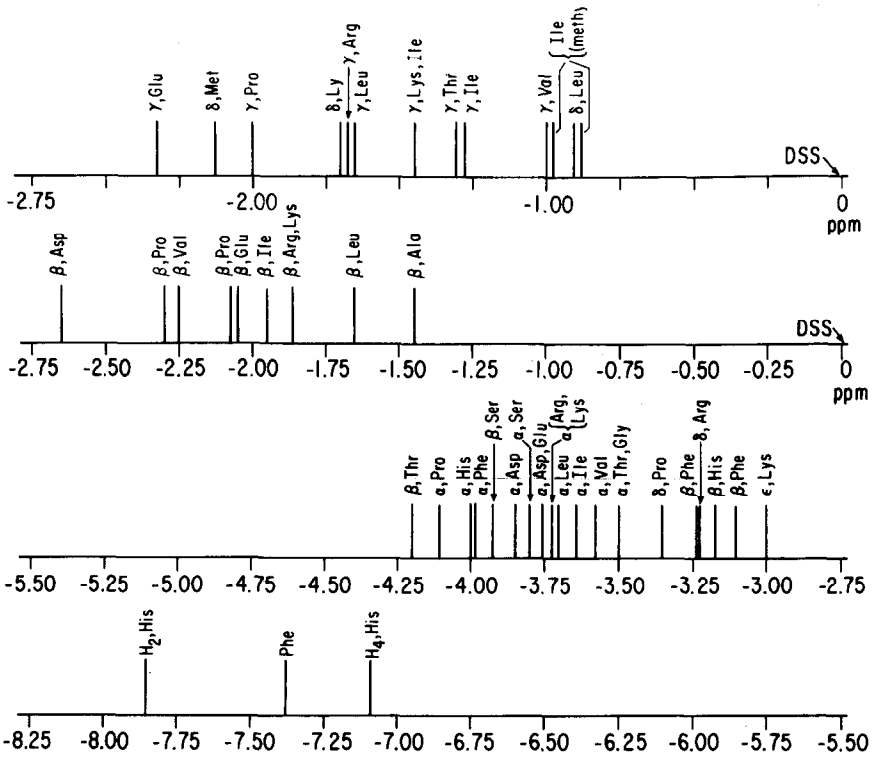

Figure 2 The observed proton chemical shifts of the amino acids at neutral pD. Proton spin-spin couplings are not included.

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work[8] indicates that the effect of peptide bond formation on the α-carbon will be relatively small. However, compared to the similar sequence-induced proton shifts[14,15] they may be adequate to provide useful information on peptide structure and conformation.

Deuterium isotopic substitution has been introduced[16,17] as a means to simplify the proton magnetic resonance spectra of proteins[18,19]. In this way studies have been carried out by Jardetzky and coworkers[16,20] with the enzyme staphylococcal nuclease which would not otherwise have been possible. It would appear that carbon-13 labelling of proteins may offer an alternate or complimentary approach, which is 'positive' in the sense that its own resonance will be observed.

References

1. J. S. Cohen, I. Putter and O. Jardetzky; in preparation.
2. W. VandenHeuvel and J.S. Cohen; in preparation.
3. W. Horseley, D. Jones, S. Smiriga, H. Sternlicht and D. Wilkinson; in preparation.
4. R. Ernst, J. Chem. Phys., 45, 3845 (1966).
5. F.J. Weigert, M. Jautelat and J.D. Roberts; Proc. Natl. Acad. Sci., 60, 1152 (1968).
6. R. Ernst; Rev. Sci. Instr., 37, 93 (1966).
7. D.M. Grant and E.G. Paul; J. Am. Chem. Soc., 86, 2984 (1964).
8. W. Horsley and H. Sternlicht; J. Am. Chem. Soc., 90, 3738 (1968).
9. C.C. McDonald and W.D. Phillips; J. Am. Chem. Soc., 91, 1513 (1969).
10. G. DelRe, B. Pullmann and T. Yonezawa; Biochem. Biophys. Res. Comm., 75, 153 (1963).
11. J.B. Stothers and P.C. Lauterbur; Can. J. Chem., 42, 1563 (1964).
12. G. Marciel; J. Chem. Phys., 42, 2746 (1965).
13. G. Marciel and J. Natterstad; J. Chem. Phys., 42, 2572 (1965).
14. M. Sheinblatt; J. Am. Chem. Soc., 88, 2123, 2845 (1966).
15. A. Nakamura and O. Jardetzky; Proc. Natl. Acad. Sci., 58, 2212 (1967).
16. J.L. Markley, I. Putter and O. Jardetzky; Science, 161, 1249 (1968); Z. Anal. Chem., 243, 367 (1968).
17. H.L. Crespi, R.M. Rosenberg, and J.J. Katz; Science, 161, 795 (1968).
18. C.C. McDonald and W.D. Phillips, J. Am. Chem. Soc., 89, 6332 (1967).
19. J.S. Cohen and O. Jardetzky; Proc. Natl. Acad. Sci., 60, 92 (1968).
20. J.L. Markley, I. Putter, J.S. Cohen and O. Jardetzky; unpublished results