A STUDY OF THE DYNAMIC STATE OF HISTIDINE RESIDUES IN TRYPTOPHAN SYNTHETASE α SUBUNIT BY ^{13}C NUCLEAR MAGNETIC RESONANCE

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SUMMARY: Tryptophan synthetase α subunit in which the histidine C_2 (ring) positions are enriched in ^{13}C and labeled with deuterium was prepared by incorporation of labeled histidine into protein of *Escherichia coli*. ^{13}C nuclear magnetic resonance studies of the specifically labeled enzyme demonstrate that all four histidine residues of α subunit are highly immobilized within the protein matrix.

Carbon-13 nuclear magnetic resonance has considerable value for the study of macromolecular structure because of the wide chemical shift range of the ^{13}C nucleus[1-3] and its low natural abundance, which permits selective ^{13}C enrichment.[4] We wish to report the results of the first ^{13}C nmr studies on an intact, unmodified enzyme having residues specifically enriched in ^{13}C. These measurements illustrate the potential of ^{13}C nmr, particularly when employed in conjunction with the selective enrichment technique, for providing detailed information concerning the dynamic properties of macromolecules in solution.

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MATERIALS AND METHODS

L-Histidine specifically enriched to 60% with ^{13}C at the C_2 (ring) position and labeled with deuterium at the C_2 carbon[5] was incorporated *in vivo* into the protein of *Escherichia coli* strain B8.[6] Similar *in vivo* incorporation experiments using [2-^{14}C]-L-histidine yielded *E. coli* protein in which only histidine residues were enriched in ^{14}C relative to natural abundance. Deuterium labeling as well as ^{13}C enrichment was employed in preparing samples for nmr measurements because the low magnetogyric ratio of the deuterium nucleus makes it relatively ineffective in causing dipole-dipole relaxation, a process which results in severe broadening and thus overlap of many signals in ^{13}C and ^{1}H spectra of macromolecules. Tryptophan synthetase α subunit was isolated[6,7] from the ^{13}C and ^{2}H labeled protein. The enzyme samples employed for nmr studies were at least 95% homogeneous as judged by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate[8] and had a specific activity of 5,000 units/mg, which corresponds to the highest specific activity previously reported for the enzyme.[7]

Carbon-13 nmr spectra of solutions of the labeled subunit in D_2O (0.1 *M* phosphate buffer, pD = 7.0) were determined at 24 KGauss using a Varian XL-100 spectrometer modified for Fourier transform operation.[9] The T_1 relaxation measurements were done using the standard 180°, τ, 90° pulse sequence.[10]

RESULTS

A portion of a proton noise decoupled ^{13}C nmr spectrum of the labeled α subunit is shown in Figure 1. The five clearly resolved regions of the spectrum may be assigned by comparison with previously reported ^{13}C spectra of amino acids and proteins.[1-4] The signal attributable to the four labeled histidine

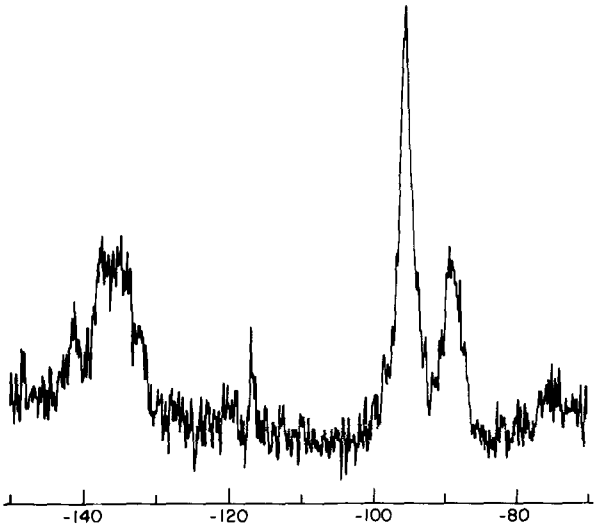

Figure 1. ^{13}C nmr spectrum of labeled tryptophan synthetase α subunit (2.9×10^{-3} *M*) in D_2O at 10°C determined at 24 KGauss under conditions of proton noise decoupling. Scale; ppm relative to glycine α carbon. The 90° rf pulse was applied at 0.51 sec intervals.

C_2 carbons appears at -95 ppm relative to the α carbon of glycine as an intense peak which can easily be distinguished from other nearby, partially overlapping peaks. The width of this signal is 50 ± 5 Hz. The band centered at -89 ppm consists of signals from 82 carbons in aromatic amino acid side chains. The narrow band at -116.5 ppm occurs in the region where signals from the 11 ε carbons of arginine and the six C_4 (ring) carbons of tyrosine are expected to occur. The broad resonance centered at -135 ppm comes from the 290 amide carbonyl carbons; the shoulder at -141 ppm probably arises from the 28 carbonyl carbons of carboxyl groups. The -95 ppm peak presumably overlaps the signals from the 12 quaternary (ring) carbons of phenylalanine.

The signal from the C_2 carbons could be characterized by a single T_1 value of 0.5 sec. Making the reasonable assumptions[11] that T_1 is determined primarily by dipole-dipole interaction with the directly bonded deuterium and that the molecular reorienta-

tion of the protein is approximately isotropic, the correlation time for rotational reorientation, τ_c, of the histidine side chains may be calculated[3,12] to be 2.7×10^{-8} sec. This value is similar to values of rotational correlation times of proteins of comparable molecular weight to α subunit as determined by nanosecond polarization spectroscopy[13] and to the value of τ_c determined by ^{13}C nmr spectroscopy for the α carbons in native ribonuclease.[3]

DISCUSSION

The ^{13}C spectrum shown in Figure 1 convincingly demonstrates the value of the selective enrichment technique in permitting unambiguous assignment and study of resonances which in natural abundance ^{13}C nmr spectra of macromolecules would be weak and would overlap with other nearby signals. This technique can obviously be extended to identification and study of virtually any class of carbon atoms in an enzyme molecule. Such enrichment causes at most a minute perturbation of native structure, whereas many other techniques for introducing reporter groups into enzyme molecules result in potentially substantial structural changes which are difficult to evaluate.

Tryptophan synthetase is an enzyme with a molecular weight of 29,000 containing four histidine residues,[14] one or more of which appear to be located at or near the active site.[15,16] The magnitude of the τ_c value which characterizes the signals from all four histidine C_2 carbons suggests that they are not free to rotate at a rate appreciably greater than the overall rate of rotation of the enzyme molecule. This means that all four histidine residues in native tryptophan synthetase α subunit, including those near the active site, are highly immobilized. Our results thus illustrate the potential of specific ^{13}C enrichment in

allowing ^{13}C nmr studies of particular regions of unmodified macromolecules in solution. This technique clearly has special value in cases where nmr signals from labeled residues overlap with those from unlabeled sites.

Although we were not able to resolve signals from individual C_2 carbons, our results also suggest that selective deuteration will have value in narrowing individual ^{13}C nmr signals from macromolecules. The availability of a τ_c value for the C_2 carbons makes it possible to estimate quantitatively the contribution of various relaxation mechanisms to the width of signals from individual C_2 carbons.[11] We conclude that dipole-dipole interaction with the directly bonded deuterium makes only a small contribution (*ca.* 3 Hz) to observed line width whereas for labeled C_2 carbons directly bonded to *hydrogen*, this contribution would be considerably greater, *i.e.*, approximately 40 Hz. A major reason why the deuterium labeled histidine C_2 carbons in tryptophan synthetase α subunit do not give ^{13}C nmr signals as narrow as might be anticipated is that there is a contribution of approximately 15 Hz[11] to the line widths of individual resonances from scalar coupling of the second kind[12] involving the directly bonded deuterium. It should be possible to eliminate the broadening associated with scalar coupling by use of a deuterium decoupling rf field a factor of 5 or 10 larger than that available on the present Varian XL-100 spectrometer.[17] When it becomes feasible to use such high rf fields, much narrower lines, characteristic of the small ^{13}C-^{2}H dipole-dipole interaction, should be obtained in favorable cases, permitting detection of small chemical shift differences that reflect non-equivalence of side chains and subtle changes in conformation in large proteins.

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