NH resonances have been assigned at this pH.[17] Performance of the multiple-quantum correlation experiment at this pH allows completion of the amide ^{15}N assignments.

Our study demonstrates that all the amide ^{15}N resonances of Bleo can be detected and assigned in aqueous solution at natural abundance within a reasonable amount of time by the two-dimensional multiple-quantum method. We are currently employing this method to characterize complexes of this antibiotic with metals and nucleic acids.

Acknowledgment. We thank Dr. W. T. Bradner of Bristol Laboratories for the Blenoxane employed in this study and Dr. Sethulakshmi Pillai for technical assistance. This investigation was supported by Grant CA-39958 from the National Institutes of Health (J.D.G.).

Registry No. Bleo A_2, 11116-31-7.

Assignment of Proton Amide Resonances of T4 Lysozyme by ^{13}C and ^{15}N Multiple Isotopic Labeling

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Received April 3, 1986

The unambiguous resolution and assignment of resonances from specific protons is the major limitation in ^1H NMR studies of proteins.[1] Heteronuclear double-resonance spectroscopy of samples labeled with stable isotopes such as ^{13}C and ^{15}N offers one solution to the problem.[2-4] This methodology has been used to identify the signals from specific imino protons in transfer RNA and amide protons in peptides. When introduced into proteins, a heteroatomic label can be used to edit a complex proton NMR spectrum into a subset of resonances from a particular functional group.[5,6] We have observed the peaks from the amide protons of the five phenylalanines in T4 lysozyme labeled with (^{15}N)-phenylalanine but could not assign the signals to specific amino acids based solely on the chemical shifts.[5,7]

We now demonstrate a general method which permits the observation and assignment of the ^1H, ^{13}C, and ^{15}N signals from any amide unit. It is possible to uniquely ^{13}C–^{15}N co-label specific peptide bonds of the sequence AB by biosynthetically incorporating a (^{13}C)carbonyl-labeled amino acid A and an (^{15}N)amino-labeled amino acid B into a protein.[8] Often, only one such sequence will occur in the protein. The assignments of the doubly labeled peptide

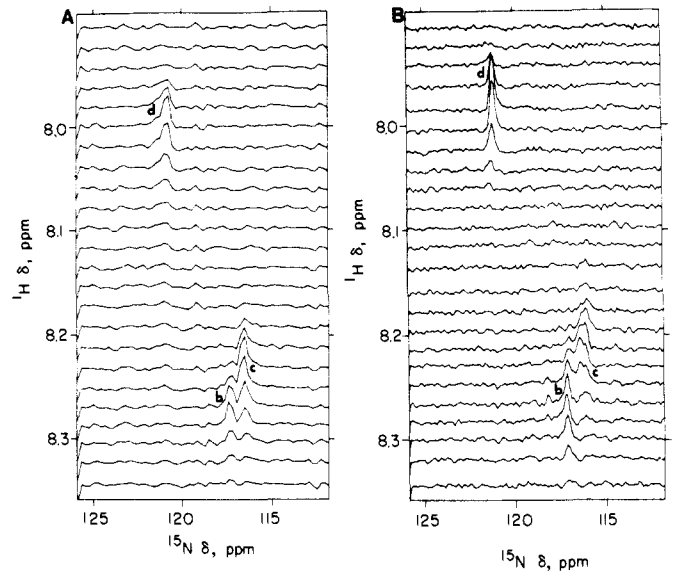

Figure 1. Forbidden echo map[5] of the correlated ^1H and ^{15}N chemical shifts for (A) (^{15}N)phenylalanine-enriched T4 lysozyme and (B) (1-^{13}C)leucine/(^{15}N)phenylalanine T4 lysozyme. Samples contained 30–40 mg of protein mL^{-1} in 100 mM sodium phosphate buffer, pH 6.5, with 500 mM NaCl, 1 mM $MgSO_4$, 1 mM 2-mercaptoethanol, and 10% deuterium oxide. The spectra were recorded with a 500-MHz spectrometer equipped with a probe (Cryomagnetics, Inc., Indianapolis, IN) tuned to both ^1H and ^{15}N. The jump–return pulse sequence was used for selective 90° and 180° pulses.[10] A total of 832 and 800 scans, respectively, were acquired for 128 values of t_1. The values of t_1 were incremented in 600- and 1200-μs steps, giving ^{15}N sweep widths of 1666 and 833 Hz, respectively. Sets of 1000 × 128 points were transformed and the section of the maps near 8.3 ppm (^1H) are presented. The preparation periods were 3.5 ms. The temperature was maintained at 15 ± 1 °C for both spectra.

Table I. Correlated Proton and Nitrogen Chemical Shifts with Proton Relaxation Times for Phenylalanine Amide Protons in T4 Lysozyme

amide	peak[a]	exchange kinetics[b]	^1H, ppm[c]	^{15}N, ppm[d]	^1H T_1, ms
Val 103–Phe 104	a	slow	9.35	121.2	300 ± 30
Thr 152–Phe 153	b	slow	8.29	117.3	290 ± 40[e]
Leu 66–Phe 67	c	slow	8.27	116.3	290 ± 40[e]
Gly 113–Phe 114	d	fast	8.07	121.1	290 ± 30
Ile 3–Phe 4	e	fast	7.74	120.4	420 ± 50

[a] The peak designation is that from ref 5. [b] The H/D exchange kinetics were measured at 4 °C and pD 5.5. Fast refers to exchange half-times of hours and slow refers to times of weeks.[5] [c] ±0.02 ppm. [d] ±0.2 ppm; referenced to ammonia at 25 °C. [e] The T_1 relaxation times could not be distinguished clearly for these overlapping signals.

^1H and ^{15}N resonances are accomplished by observation of the ^{13}C–^{15}N scalar coupling using detection of proton resonances by ^1H–^{15}N forbidden echo spectroscopy.[5,7,9] Assignment of the ^{13}C resonance can be accomplished by direct observation of the ^{13}C–^{15}N scalar coupling in the ^{13}C NMR spectrum of the same labeled protein.

We have applied this approach to three (^{15}N)phenylalanine-labeled T4 lysozyme samples which also contained (^{13}C)-carbonyl-labeled leucine, valine, or glycine. This permitted us to unambiguously assign the signals from three of the five phenylalanine amide protons. We have assigned the remaining two peptide ^1H–^{15}N resonances on the basis of their hydrogen exchange properties.

Samples of T4 lysozyme containing the ^{15}N–^{13}C double label were produced from a high expression plasmid in a derivative of the *E. coli* strain RR1 auxotropic for phenylalanine (PheA), leucine, and valine (IlvC) grown on a defined medium including

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(1) Abreviations: NMR, nuclear magnetic resonance; 2D, two dimensional.

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(^{15}N)phenylalanine and the appropriate ^{13}C-enriched amino acid.[5] On the basis of ^{13}C NMR measurements, approximately 60% of the phenylalanine amides were ^{15}N labeled (data not shown). The loss of the ^{15}N label is attributed to the action of transaminases. Proton and nitrogen chemical shifts were correlated with forbidden echo spectroscopy as described previously.[5]

Figure 1A shows a portion of the ^{1}H–^{15}N forbidden echo spectrum of (^{15}N)phenylalanine-labeled T4 lysozyme. This region of the two-dimensional spectrum has three peaks, corresponding to three phenylalanine amide protons and labeled peaks d, b, and c as described in our earlier work.[5] Figure 1B shows the same region of the ^{1}H–^{15}N two-dimensional spectrum of a sample labeled with (^{15}N)phenylalanine and (1-^{13}C)leucine. Although the line width and ^{13}C–^{15}N spin–spin coupling are of the same order of magnitude (~17 Hz), one can readily see that peak c appears as a poorly resolved doublet in consecutive traces. The four remaining Phe amide resonances are unchanged. On this basis we assign peak c to amide resonance of the Leu 66-Phe 67 peptide.

We have introduced two other ^{13}C labels along with (^{15}N)-phenylalanine into the T4 lysozyme and the results are summarized in Table I. Incorporation of (1-^{13}C)valine affects signal a corresponding to Phe 104, which is situated adjacent to a valine residue. Similarly, Phe 114 adjoins Gly 113, and insertion of (1-^{13}C)glycine into the protein alters the resonance d.

Peaks e and b are unaffected by any of the ^{13}C-enriched amino acids and hence correspond to the resonances of Phe 4 and Phe 153. These can be assigned on the basis of their solvent exchange properties and the known secondary and tertiary structure at these positions as established by X-ray crystallography. Phe4 is in a solvent-exposed region of the protein while Phe 153 is found in an α-helical region. As demonstrated in our earlier work, peak e exchanges rapidly with D_2O ($t_{1/2}$ ~ 1 h, pD 5.5, 4 °C) and is assigned to phenylalanine 4. In contrast, peak b exchanges slowly ($t_{1/2}$ ~ 1 week) and is assigned to Phe 153. The other slowly exchanging amides (Phe67 and Phe104) are also in α-helical regions while the other fast exchanging amide at residue 114 is in a solvent-exposed region.

Heteronuclear difference decoupling can be added to the standard saturation–recovery experiment for the determination of longitudinal (T_1) relaxation rates. Subtraction of T_1 data sets with on- and off-resonance ^{15}N decoupling produces difference spectra which contain only the signals from protons bonded to the uncommon isotope. The T_1 values for the amide protons of the phenylalanine residues are listed in Table I. The values for Phe 67, Phe 104, Phe 114, and Phe 153 are determined to be 300 ± 30 ms. The signal at 7.74 ppm assigned to Phe4 has a slower rate of 420 ± 50 ms, indicative of a location in a region of unique flexibility or dipolar environment. This is consistent with the three-dimensional structure of T4 lysozyme as deduced by X-ray methods, where the atoms of Phe4 exhibit greater disorder than any of the other phenylalanine sites.[11]

These studies demonstrate that selective ^{15}N and ^{13}C labeling of T4 lysozyme permits the observation and unambiguous assignment of the ^{1}H and ^{15}N NMR resonances of specific amide units. However, since the line widths and ^{13}C–^{15}N couplings are similar in magnitude, it is unlikely that this approach will be useful for proteins much larger than lysozyme (18.7 kdaltons). Armed with these assignments, the structure and dynamics of T4 lysozyme can be probed by using NMR resonance lifetime and solvent exchange measurements.

Acknowledgment. We thank Sara Kunz for technical assistance and the National Science Foundation for support (FWD, PCM 8304174). A.G.R. was funded through U.S.P.H.S. Grant GM 20168, and R.H.G. was supported by U.S.P.H.S. Fellowship GM 09700. This is contribution number 1595 from the Brandeis University Biochemistry Department. L.P.M. is a recipient of a Natural Sciences and Engineering Research Council of Canada 1967 Science Scholarship and an Alberta Heritage Foundation for Medical Research Independent Studentship Award.

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Synthesis and Characterization of the Phosphinidene Borate Complexes $[Li(Et_2O)_2PRBMes_2]$ and $[Li(12\text{-crown-}4)_2][RPBMes_2]\cdot THF$, R = Ph, C_6H_{11}, or Mes (Mes = 2,4,6-$Me_3C_6H_2$): The First Structurally Characterized Boron–Phosphorus Multiple Bonds

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Although multiple bonding between boron and nitrogen[2] (and to a lesser degree carbon)[3] has been well established, there are no structurally characterized compounds in which boron is multiply bonded to a heavier main-group element. In this paper we report on the synthesis and characterization of a number of compounds in which boron is multiply bonded to phosphorus. These are the novel complexes $[Li(Et_2O)_2RPBMes_2]$ (Mes = 2,4,6-$Me_3C_6H_2$; R = Ph, **1a**; R = C_6H_{11}, **1b**; R = Mes, **1c**) and the ion pairs $[Li(12\text{-crown-}4)_2][RPBMes_2]\cdot THF$ (R = Ph, **2a**; R = C_6H_{11}, **2b**; R = Mes, **2c**).

The synthesis of the title compounds involved the treatment of an Et_2O solution of Mes_2BPHR at 0 °C with 1 equiv of *t*-BuLi to give a solution of $[Li(Et_2O)_2RPBMes_2]$. Reduction of the volume and cooling afforded crystals of **1a**, **1b**, or **1c** in ca. 60% yield. Addition of 2 equiv of 12-crown-4 to **1a**, **1b**, or **1c** in ether gave yellow precipitates which were recrystallized from THF/Et_2O to give **2a**, **2b**, or **2c** in ca. 65% yield. Full synthetic details are given in the supplementary material.

The structures of three of the title compounds **1b**, **1c**, and **2c** were determined by X-ray crystallography.[4] The structures of **1c** (**1b** is very similar) and the anion of **2c** are illustrated in Figures 1 and 2. They show several interesting features. They are the first X-ray structures of monomeric compounds having a bond between planar boron and planar phosphorus centers. Furthermore, in **1b** and **1c**, these two planes C(1)C(10)BP and LiPBC(19) are almost coincident, having twist angles of 11.7° (**1b**) and 3.8° in (**1c**). For **2c** the atoms C(1)C(10)BPC(19) are virtually coplanar. The planarity of the phosphorus center, instead of the expected pyramidicity,[5] indicates the presence of only one stereochemically active lone pair at this site. The other available electron pair is presumably involved in multiple bonding to boron as indicated by the small twist angles. The eclipsed configuration in all three compounds is, of course, contrary to what is expected on steric grounds.

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(4) Crystal data at 130 K with Mo Kα (λ = 0.71069 Å) radiation: **1b**, monoclinic, $P2_1/c$, Z = 4, a = 16.512 (5) Å, b = 10.247 (3) Å, c = 20.489 (6) Å, β = 109.35 (2)°, R = 0.065, 364 parameters, 2475 unique observed data; **1c**, monoclinic, Cc, Z = 4, a = 21.717 (8) Å, b = 7.972 (3) Å, c = 23.683 (8) Å, β = 122.06 (2)°, R = 0.084, 359 parameters, 2320 unique observed data; **2c**, monoclinic, $C2/c$, 2 = 8, a = 35.408 (10) Å, b = 13.398 (4) Å, c = 20.697 (7) Å, β = 106.4 (2)°, R = 0.126, 337 parameters, 3183 unique observed data. The somewhat high R value for **2c** was due mainly to the disorder commonly found in the cations $[Li(12\text{-crown-}4)_2]^+$. This resulted in a significant reduction in the intensity of the data at 2θ values greater than ca. 40°. However, the anion $[MesPBMes_2]^-$ is not disordered and the bond distances and angles are normal and close to those seen in **1b** and **1c**.

(5) Compounds **1b** and **1c** could also be considered as lithium phosphides $LiPX_2$ with the expectation that these, as well as their As–Bi analogues, should be planar like their $LiNX_2$ counterparts. However, the only examples of monomeric heavier pnictide complexes of this type are $[Li(THF)_3P(H)Mes]$ and $[Li(1,4\text{-dioxane})_3AsPh_2]$ and these are both *pyramidal* at the pnictogen. Thus the structures of both **1b** and **1c** are contrary to what is expected unless P–B multiple bonding is assumed: Bartlett, R. A.; Dias, H. V. R.; Hope, H.; Murray, B. D.; Olmstead, M. M.; Power, P. P. *J. Am. Chem. Soc.*, in press. Bartlett, R. A.; Olmstead, M. M.; Power, P. P.; Sigel, G., unpublished results.

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