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Letter to the Editor: Complete ^{1}H and non-carbonylic ^{13}C assignments of native hen egg-white lysozyme

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Biological context

Lysozymes are antibacterial glycosidases that target and cleave the peptido-glycan layer of bacterial cell walls. They are ubiquitous proteins found in birds, mammals and even some bacteriophage. Lysozymes are also among the most intensively studied proteins in all of biology, with more than 10 000 articles having been published on their evolution, structure, function, folding and enzymology over the past 30 years. In purely structural terms, hen egg-white (HEW) lysozyme is perhaps the best characterized of all lysozymes with nearly 150 different X-ray structures deposited in the Protein Data Bank. HEW lysozyme has also been the subject of more than 200 NMR studies since the late 1960s. Partially complete (68%) assignments, including nearly all backbone alpha and amide ^{1}H shifts, have already been reported (Redfield et al., 1988), as have a complete set of backbone ^{15}N assignments (Buck et al., 1995). More recently backbone ^{1}H, ^{13}C and ^{15}N assignments of denatured (8 M urea, pH 2.0) HEW lysozyme have also been presented (Hennig et al., 1999). Here we wish to report the complete ^{1}H and non-carbonylic ^{13}C assignments of native HEW lysozyme as obtained at natural abundance. Our motivation for completing the ^{1}H and ^{13}C assignments of this particular protein arose primarily from our interest in attaining a better understanding of protein chemical shifts. Indeed, HEW lysozyme is a unique protein in that it has some of the best resolved ($<$1.0 Å) X-ray structures collected under perhaps the widest range of conditions (pH, temperature, pressure) of any macromolecule in biology. It also has an unusual abundance of disulfide bonds, a wide range of secondary structures and a large number of strongly ring-current shifted resonances. This combination of unusual properties and abundant structural data makes HEW lysozyme an ideal protein to study the influence of pH, temperature, ring-currents, torsion angles, bond-length distortions and disulfide bond geometry on protein ^{1}H and ^{13}C shifts.

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Methods and results

Hen egg-white lysozyme (99% pure, 3× crystallized) was purchased from Sigma (lot 111H7010) and used without further purification. A 10 mM sample was prepared by dissolving 71.5 mg of solid HEW lysozyme powder in 500 μL double distilled water (80% H_2O/20% D_2O). DSS (2,2-dimethyl 2-silapentane 5-sulfonic acid) was added for direct internal referencing (Wishart et al., 1995). The pH of the sample was 3.6. NMR spectra were recorded at 45 °C on a Varian 800 MHz spectrometer equipped with a 5 mm triple-axis gradient, triple-resonance probe. ^{1}H-TOCSY (mixing time = 60 ms), ^{1}H-NOESY (mixing time = 120 ms), ^{13}C-HSQC and ^{13}C-HSQC-TOCSY (mixing time = 60 ms) experiments were implemented using the Varian Protein Pack pulse sequence library. All spectra were processed and analyzed using the Varian VNMR software package (version 5.1a).

Previously assigned ^{1}H resonances from HEW lysozyme (Redfield et al., 1988; Smith et al., 1993), along with additional 'homologous assignments' from turkey lysozyme (Bartik et al., 1993) and predicted ^{1}H shifts derived from the X-ray structure (3LZT) using TOTAL (Williamson et al., 1995), were used to assign all detectable ^{1}H resonances in the ^{1}H-TOCSY. Ambiguities or uncertainties in the proton assignments were invariably resolved by analyzing the NOESY, ^{13}C-

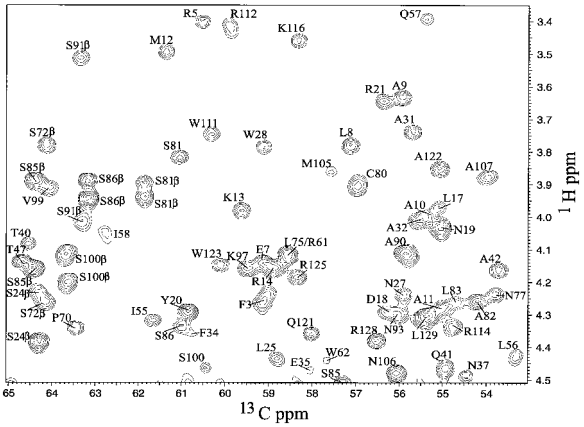

Figure 1. A portion of the ^{13}C-HSQC spectrum (collected at NANUC) of HEW lysozyme showing a number of the assigned backbone alpha ^{13}C resonances.

HSQC or ^{13}C-HSQC-TOCSY spectra. With a nearly complete set of ^{1}H assignments in hand, the ^{13}C assignments were straightforwardly obtained by simple inspection of the ^{13}C-HSQC spectrum (Figure 1). Ambiguities or uncertainties in the initial ^{13}C assignments were resolved by analyzing the ^{13}C-HSQC-TOCSY or by using predicted ^{13}C shifts obtained from an in-house prediction program (Wishart and Nip, 1998). To guard against any assignment bias or potential assignment error, the protein was independently assigned (simultaneously by Y.W. and T.C.B.). The relatively small number of initial assignment conflicts (<1%) was easily resolved and this result gives us some confidence in the correctness of our 'consensus' ^{1}H and ^{13}C assignments.

Extent of assignments and data deposition

The good dispersion and excellent sensitivity offered by an 800 MHz spectrometer, in combination with the high sample temperature and exceptional stability of lysozyme permitted the collection of some very high quality data (see Figure 1). Consequently, more than 98% of all possible ^{1}H assignments, 97% of all possible aliphatic ^{13}C assignments and 100% of all possible aromatic ^{13}C assignments were obtained. The correctness of all previously reported HEW lysozyme assignments (Redfield et al., 1988; Davis, 1989) was also confirmed. In the end, difficulties were only encountered in fully assigning the side chain protons of the lysines and arginines. Solvent overlap also prevented determination of the α^{13}C assignments for S24 and S36. At 14.3 kDa, HEW lysozyme is the largest protein to have had its ^{13}C assignments determined entirely at natural abundance. However, given the high quality of spectra obtained for this protein, we are inclined to believe that under favorable circumstances (i.e. solubility > 5 mM) proteins larger than 15 kDa may be assignable using natural abundance heteronuclear spectroscopy at 800 MHz. The ^{1}H and ^{13}C chemical shifts for HEW lysozyme have been deposited in the BioMagResBank under accession number 4562.

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