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Letter to the Editor: Assignments of ^{1}H, ^{13}C, and ^{15}N resonances of human lysozyme at 4 °C

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Biological context

To clarify the low-temperature-induced change of the tertiary structure and dynamics of an enzyme is essential for modifications of the enzyme so as to maintain or maximize its function under low-temperature environment. Our previous NMR study using a model protein (Tsuda et al., 1999) revealed that lowering of the temperature from 30 °C to 4 °C specifically alters the structure of an active site, which accompanies a local structural change of the hinge-residues and decrease in the cavity volume of this protein. The low-temperature-induced change in the cavity volume has been identified for several other model proteins by adiabatic compressibility experiments (Gekko and Hasegawa, 1989). The specification of the low-temperature-sensitive residues of an enzyme presumably allows to examine whether the mutations of the residues alter the function of the enzyme under low-temperature. Here we choose human lysozyme (130 residues, M.w. = 14 300) as a model enzyme to monitor the low-temperature-induced change using multidimensional NMR spectroscopy. The expression system of this enzyme was established (Oka et al., 1999) and temperature dependence of its enzymatic activity can be examined using several substrate analogues. Hence, human lysozyme is thought to be suitable for monitoring the NMR-based low-temperature-induced change at per-residue level and examining the mutation effects on its function under low-temperature. For human lysozyme, we can utilize the X-ray crystal structures (Artymiuk and Blake, 1981; Song et al., 1994) and almost complete assignments of the ^{1}H resonances at 35 °C (Redfield and Dobson et al., 1990) and those of the ^{15}N resonances at 40 °C (Ohkubo et al. 1991). We have been performed the complete assignments of the ^{1}H, ^{13}C, and ^{15}N resonances of human lysozyme at 4 °C. The complete assignments at 35 °C were also performed for confirmation of the ^{1}H and ^{15}N assignments and for new additions of the $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ chemical shifts. The final assignments at 4 °C and 35 °C are currently used for the structure and dynamics determinations of human lysozyme at these two temperatures.

Methods and experimental

The human lysozyme was expressed under ^{15}N- and $^{13}C/^{15}N$-labeled cultures using the expression system described in (Oka et al., 1999). After purification, the ^{15}N- and $^{13}C/^{15}N$-labeled protein samples were dissolved in H_2O containing 10% of D_2O, whose pHs were adjusted to 3.8 by additions of DCl and KOD. For the assignments of ^{1}H, ^{13}C, and ^{15}N resonances, the following 2D- and 3D-NMR experiments were carried out at both 35 °C and 4 °C: ^{15}N-HSQC, ^{13}C-HSQC, CBCA(CO)NNH, HNCACB, C(CO)NH, HCCONH, ^{15}N-edited NOESY, ^{15}N-edited TOCSY, and ^{13}C-edited NOESY. All the experiments were performed on a Varian Unity Inova 500 MHz spectrome-

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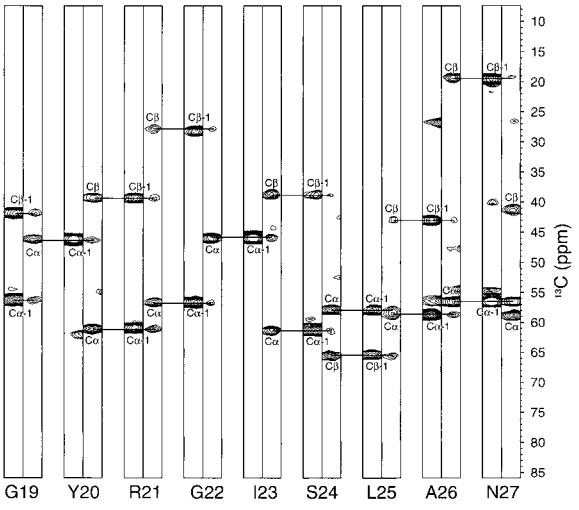

Figure 1. An example of the assignments of ^{13}C-resonances at 4 °C using strips derived from CBCA(CO)NH and HNCACB spectra of residues Gly^{19} through Asn^{27}. Plots are provided as pairs strips from CBCA(CO)NH (on the left) and HNCACB (on the right) spectra for the given residue. CBCA(CO)NH strips indicate C_α^{-1} and C_β^{-1} chemical shifts and HNCACB strips indicate C_α, C_β, C_α^{-1}, and C_β^{-1} chemical shifts.

ter equipped with triple-resonance probe heads and x, y, z-axis pulsed field gradients. The ^{1}H chemical shifts were referenced to the internal standard 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS). The ^{13}C and ^{15}N chemical shifts were indirectly referenced to DSS by using the frequency ratios: 0.251449539 for ^{13}C/^{1}H and 0.101329118 for ^{15}N/^{1}H (Wishart et al., 1995). Spectral assignments and peak-pickings were performed using NMRPipe and PIPP software.

Extent of assignment and data deposition

The backbone ^{1}HN, ^{15}N, $^{13}C_\alpha$, and $^1H_\alpha$ resonances and the side chain $^{13}C_\beta$ and $^1H_\beta$ resonances of human lysozyme were unambiguously assigned at 4 °C and at 35 °C. Figure 1 shows an example of the assignments of ^{13}C-resonances at 4 °C using strips derived from CBCA(CO)NH and HNCACB spectra of residues Gly^{19} through Asn^{27}. Only C_β resonance of Ser^{82} was not assigned since it is disappeared at both temperatures, which was also reported in the case of canine milk lysozyme (Kobashigawa et al., 2001). It appeared that the assignments of $^1H_\alpha$ and $^1H_\beta$ resonances at 35 °C are in accordance with the previous assignments by Redfield and Dobson (1990) within ± 0.2 ppm. In addition, ^{15}N-NMR chemical shifts of human lysozyme are also well in accordance with those reported for hen-egg white lysozyme (Schwalbe et al., 2001) and canine milk lysozyme (Kobashigawa et al., 2001) with regard to their conserved residues. It is noted that our chemical shifts of ^{15}N-NMR are entirely different ($\Delta = -2.1$ ppm) from the reported values by Ohkubo et al. (1991). This discrepancy presumably arises from a difference in referencing but not from an error in their assignment. From comparison of the chemical shift data between 4 °C and 35 °C, it appeared that most of the peaks shift to lower field in the ^{1}H and ^{15}N dimensions with decreasing temperature, as similarly observed in (Tsuda et al., 1999). Each ^{1}H and ^{15}N resonance shows a different degree of movement, which is similarly identified for the $^{13}C_\alpha$, $^{13}C_\beta$, and $^1H_\alpha$ resonances of human lysozyme. These data imply that it occurs a structural change in this molecule with decreasing temperature from 35 °C to 4 °C. The final chemical shift assignments of human lysozyme have been deposited in the BioMagResBank database with accession number of 5130 for 35 °C and that of 5142 for 4 °C.

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