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Letter to the Editor: Assignment of ^{1}H and ^{15}N resonances of mouse lysozyme M

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Biological context

Lyosozyme is a carbohydrate hydrolase that catalyses the hydrolysis of the β-1,4 glycosidic bonds of polysaccharides (Imoto et al., 1972), and several research groups have vigorously investigated the structure–function relationships. Lysozyme plays a first step in host defenses, and can be found in most tissues in animals. In house mouse, the closely related mouse lysozymes M and P tend to be expressed in different tissues; mouse lysozyme M is strongly expressed in both macrophages and macrophage-rich tissues, while mouse lysozyme P is mainly expressed in small intestine (Cross et al., 1988).

Mouse lysozyme M, which comprises 130 amino acids and is a 15 kDa monomeric protein, belongs to mammalian members of chicken-type lysozyme and has higher lytic activity against *Micrococcus luteus* than hen lysozyme (HEL; 129 amino acids). The two mammalian members of lysozyme, mouse lysozyme M and human lysozyme (HUL; 130 amino acids), differ from HEL by insertion of a glycine at position 48, and contain two prolines in the same sequence positions, one of which differs from HEL. The sequence similarities of mouse lysozyme M to HEL and HUL are 56.9% and 77.7%, respectively. The ^{1}H assignments of HEL (Redfield et al., 1988) and HUL (Redfield et al., 1990) have been published and their three-dimensional structures have already been determined, but that of mouse lysozyme M has not yet been investigated by using either X-ray crystallography or NMR.

The NMR method has played important roles in characterizing the internal mobilities of proteins. Recent study of the mobilities of HEL and mutants suggested that the dynamics played an important role in its activity (Mine et al., 1999). The present study was undertaken to determine the solution structure and dynamics of mouse lysozyme M in the free form and in complex with substrates. Here we report the complete ^{1}H and ^{15}N backbone resonance assignment of mouse lysozyme M.

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Methods and results

Mouse lysozyme M was obtained from the *Pichia pastoris* expression system constructed in our group. Briefly, the ^{15}N-labeled protein was expressed in FM22-glycerol minimal medium (100 ml of 10× glycerol, 1 ml PTM1, 6 ml of 250× biotin, 6 ml 10 M KOH were added to 900 ml FM22) containing $(^{15}NH_4)_2SO_4$ as the sole nitrogen source. The protein was purified by cation exchange (CM-Toyopearl 650M) chromatography. The yield of purified protein was about 15 mg per liter of culture. The NMR samples (1.0 mM) of purified unlabeled and uniformly ^{15}N-labeled mouse lysozyme M were prepared in H_2O/D_2O (90%/10%, v/v), and the pH was adjusted to 3.8.

All NMR experiments were performed at 35 °C on Varian INOVA 600 MHz spectrometers equipped with a pulsed-field-gradient unit and triple resonance probe with actively shielded Z-gradients. 3D ^{15}N-edited NOESY (120 ms mixing time), ^{15}N-edited TOCSY (45 ms mixing time), 2D ^{1}H-^{15}N HSQC, ^{1}H COSY, ^{1}H NOESY (80 and 120 ms mixing time), and ^{1}H TOCSY (45 ms mixing time) were used for assignments. All spectra were processed with the NMRPipe package (Delaglio et al., 1995) on Sun Microsystems and Silicon Graphics workstations.

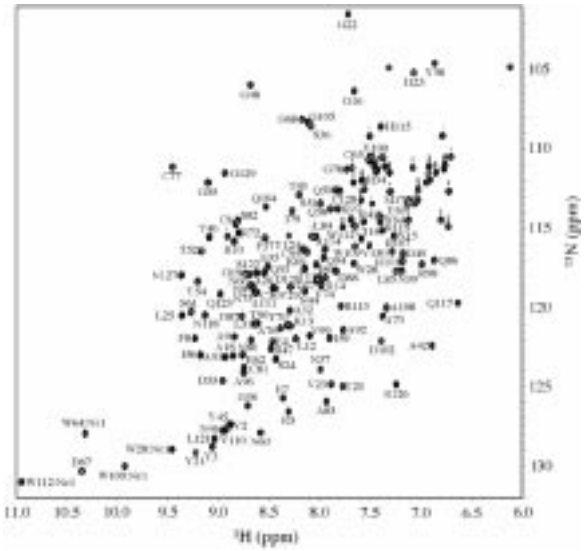

Figure 1. ^{1}H-^{15}N HSQC spectrum of 1.0 mM mouse lysozyme M in H_2O/D_2O (90%/10%, v/v), at pH 3.8 and 35 °C. Backbone NH resonances are labeled with their single letter code and residue number.

The resonance assignments of mouse lysozyme M were successfully performed by employing the 2D- and 3D-NMR spectra using the standard strategy (Wüthrich, 1986). The ^{1}H chemical shifts were directly referenced to that of DSS, and ^{15}N chemical shifts were indirectly referenced (Wishart et al., 1995).

Extent of assignments and data deposition

The ^{1}H-^{15}N NMR spectra of mouse lysozyme M were well dispersed, and all ^{1}H and ^{15}N polypeptide backbone resonances were assigned, except Lys^{1}, Pro^{71} and Pro^{103}. The assignment accounts for all of the main-chain amide cross peaks in the ^{1}H-^{15}N HSQC spectrum (Figure 1). Nearly complete ^{1}H side chain assignments have been made, but some distal side chain ^{1}H resonances in arginines and lysines and side chain NH_2 resonances of asparagines and glutamines have not been assigned. The ^{1}H-^{15}N HSQC spectrum of mouse lysozyme M shows many similarities to that of HUL (Ohkubo et al., 1991). The data from the backbone amide H-D exchange experiment and sequential NOE data from the 3D ^{15}N-edited NOESY showed that the secondary structure of mouse lysozyme M is quite similar to HEL and HUL. Detailed analysis of internal motions and 3D structure calculations for mouse lysozyme M are in progress. The ^{1}H and ^{15}N chemical shift assignments of mouse lysozyme M have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4751.

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