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Letter to the Editor: ^{1}H, ^{13}C, ^{15}N resonance assignments of the cytokine LECT2

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Biological context

Human LECT2 (leukocyte cell-derived chemotaxin 2) is a 16-kDa chemotactic protein consisting of 133 amino acids and three intramolecular disulfide bonds. The protein was first purified from the culture fluid of phytohemagglutinin-activated human T-cell leukemia SKW-3 cells as a chemotactic factor to human neutrophils (Yamagoe et al., 1996) and its cDNAs were cloned from cDNA libraries of human, bovine, and murine livers (Yamagoe et al., 1998a,b). LECT2 is identical to chondromodulin-II (Hiraki et al., 1996), a bovine protein that stimulates the proliferation of chondrocytes and osteoblasts (Shukunami et al., 1999). A point mutation in LECT2 (Val58 to Ile58) is associated with the severity of rheumatoid arthritis (RA) (Kameoka et al., 2000). No tertiary structure has been solved so far for LECT2 and its related proteins. In order to reveal the three-dimensional structure of LECT2 and the effect of the point mutation on its conformation, we are doing NMR structural analysis of human LECT2. Here we report the ^{1}H, ^{15}N, and ^{13}C resonance assignments.

Methods and experiments

Human LECT2 (the 133-amino acid mature form, residue numbers 19–151) with an N-terminal His_6-tag was produced in *E. coli* as inclusion bodies, and renatured *in vitro* by a three-step refolding procedure (Ito et al., 2003). To prepare stable isotope-labeled protein, $^{15}NH_4Cl$ (> 99% ^{15}N) and ^{13}C-glucose (> 99% ^{13}C) were used as the sole nitrogen and carbon sources, respectively. The samples used for NMR measurements were 1 mM ^{15}N-labeled and ^{13}C/^{15}N-labeled $(His)_6$-LECT2 dissolved in 50 mM Na_2SO_4 in 85% H_2O/10% D_2O/5% glycerol (pH 6.0, direct meter reading). NMR spectra were recorded at 298 K on Varian Unity Inova NMR spectrometers operated at ^{1}H frequencies of 500- and 750-MHz and equipped with triple-resonance z-gradient probes.

Sequence-specific backbone assignments were elucidated from 3D data of HN(CO)CA, HNCA, CBCA(CO)NH, HNCACB, CBCANH, HNCO, and (HCA)CO(CA)NH. C(CO)NH was used to confirm amino-acid types. For side-chain ^{1}H assignments, H(CCO)NH, HCCH-TOCSY, HCCH-COSY, ^{15}N-edited TOCSY, ^{15}N-edited NOESY, and 2D NOESY were used. ^{1}H chemical shifts were directly referenced to the resonance of 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), while ^{13}C and ^{15}N chemical shifts were referenced indirectly to DSS (Wishart et al., 1995). NMR data were processed using NMRPipe/NMRDraw (Delaglio et al., 1996). Visualization of transformed data and peak-picking were carried out using Sparky (http://www.cgl.ucsf.edu/home/sparky/). Secondary structure was predicted using CSI (Wishart and Sykes, 1994).

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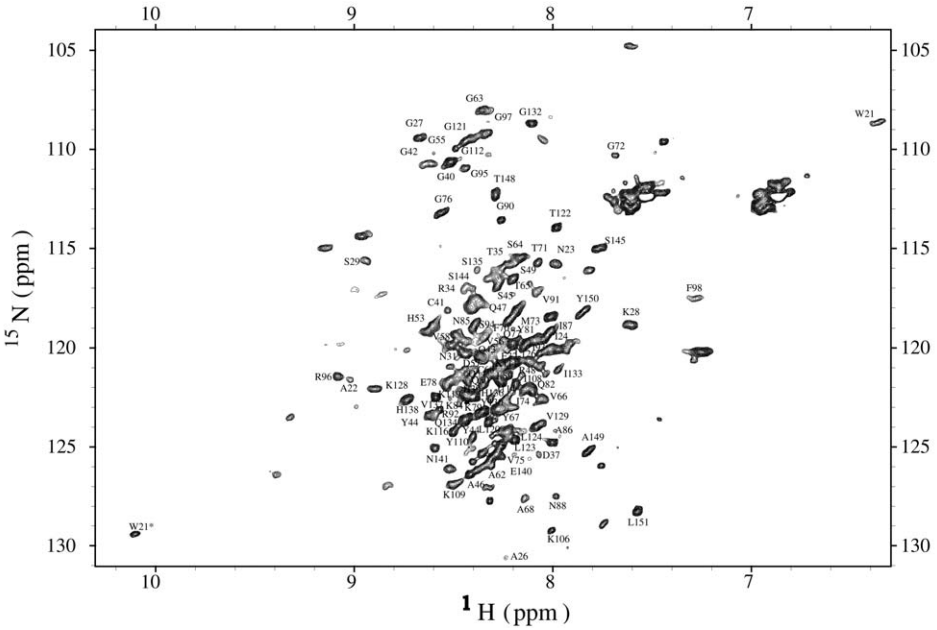

Figure 1. ^{1}H-^{15}N HSQC spectrum of $(His)_6$-LECT2 at 298 K. Amino acid labels were omitted from the middle of the HSQC for clarity. *indicates Trp side chain.

Extent of assignments and data deposition

Most of backbone resonances (90% of ^{15}N, 90% of H^N, 92% of C^α, 76% of H^α, 92% of C^β, and 79% of C′) and a part of aliphatic side-chain resonances have been assigned and deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under an accession number of 6025.

Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of ^{15}N/^{13}C-labeled $(His)_6$-LECT2. A six-residue segment ranging from 100 to 105 and six other residues at positions 19, 24, 25, 38, 51 and 143 remain unassigned as well as the N-terminal His_6-tag. The assignments of these residues have been hampered due to severe overlaps of NMR signals and possible fast exchanges of H^N involved.

The secondary structure prediction by CSI indicates that LECT2 contains several β-strands but no α-helix, which is consistent with the far-UV CD (circular dichroism) data (Ito et al., 2003).

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References

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Hiraki, Y., Inoue, H., Kondo, J., Kamizono, A., Yoshitake, Y., Shukunami, C. and Suzuki, F. (1996) *J. Biol. Chem.*, **271**, 22657–22662.

Ito, M., Nagata, K., Kato, Y. Oda, Y., Yamagoe, S., Suzuki, K. and Tanokura, M. (2003) *Protein Expr. Purif.*, **27**, 272–278.

Kameoka, Y., Yamagoe, S., Hatano, Y., Kasama, T. and Suzuki, K. (2000) *Arthritis Rheumatism*, **43**, 1419–1420.

Shukunami, C., Kondo, J., Wakai, H., Takahashi, K., Inoue, H., Kamizono, A. and Hiraki, Y. (1999) *J. Biochem.*, **125**, 436–442.

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.

Wishart, D.S., Bigam, C.G., Yao, J., Abildaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995) *J. Biomol. NMR*, **6**, 135–140.

Yamagoe, S., Mizuno, S. and Suzuki, K. (1998a) *Biochim. Biophys. Acta*, **1396**, 105–113.

Yamagoe, S., Watanabe, T., Mizuno, S. and Suzuki, K. (1998b) *Gene*, **216**, 171–178.

Yamagoe, S., Yamakawa, Y., Matsuo, Y., Minowada, J., Mizuno, S. and Suzuki, K. (1996) *Immunol. Lett.*, **52**, 9–13.