Crystal Structure of Human Leukocyte Cell-derived Chemotaxin 2 (LECT2) Reveals a Mechanistic Basis of Functional Evolution in a Mammalian Protein with an M23 Metalloendopeptidase Fold*

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Human leukocyte cell-derived chemotaxin 2 (LECT2), which is predominantly expressed in the liver, is a multifunctional protein. LECT2 is becoming a potential therapeutic target for several diseases of worldwide concern such as rheumatoid arthritis, hepatocellular carcinoma, and obesity. Here, we present the crystal structure of LECT2, the first mammalian protein whose structure contains an M23 metalloendopeptidase fold. The LECT2 structure adopts a conserved Zn(II) coordination configuration but lacks a proposed catalytic histidine residue, and its potential substrate-binding groove is blocked in the vicinity of the Zn(II)-binding site by an additional intrachain loop at the N terminus. Consistent with these structural features, LECT2 was found to be catalytically inactive as a metalloendopeptidase against various types of peptide sequences, including pentaglycine. In addition, a surface plasmon resonance analysis demonstrated that LECT2 bound to the c-Met receptor with micromolar affinity. These results indicate that LECT2 likely plays its critical roles by acting as a ligand for the corresponding protein receptors rather than as an enzymatically active peptidase. The intrachain loop together with the pseudoactive site groove in LECT2 structure may be specific for interactions between LECT2 and receptors. Our study reveals a mechanistic basis for the functional evolution of a mammalian protein with an M23 metalloendopeptidase fold and potentially broadens the implications for the biological importance of noncatalytic peptidases in the M23 family.

Leukocyte cell-derived chemotaxin 2 (LECT2)2 is a secretory protein originally identified as a chemotactic factor for neutrophils (1). LECT2 is predominantly expressed in the liver and is a direct target gene of Wnt/β-catenin signaling (2–6). Accumulating evidence shows that mammalian LECT2 is a multifunctional protein that is closely associated with several diseases of worldwide concern, including hepatitis (7), rheumatoid arthritis (8–10), hepatocellular carcinoma (HCC) (11, 12), obesity (13, 14), and renal and hepatic amyloidosis (15–17). It has been reported that concanavalin A-induced hepatitis and collagen antibody-induced arthritides were suppressed by LECT2 (7, 8). In addition, LECT2 expression inhibited the migration and invasion of human HCC cells in vitro and was negatively correlated with vascular invasion and tumor recurrence in HCC patients (11). In contrast, serum LECT2 levels were positively correlated with the severity of obesity and fatty liver in humans, and overproduction of LECT2 caused the development of obesity-associated insulin resistance (13, 14). These findings suggest that LECT2 may be a candidate prognostic marker and a potential therapeutic target for these diseases. Despite the importance of LECT2 functions, the underlying mechanisms remain largely unclear.

The mature human LECT2 is a basic protein consisting of 133 amino acids. Sequence similarity searches using BLAST have shown that LECT2 has a putative peptidase-M23 (PF01551) domain located between amino acids 33 and 129 (18, 19). M23 family members are typically zinc-dependent metalloendopeptidases that cleave the peptide cross-bridges in the peptidoglycans in cell walls and have a specificity that targets glycylglycine peptide bonds (20–23). They are divided into two subclasses based on sequence homology: the M23A family, which includes the β-lytic endopeptidase from Lysobacter enzymogenes and LasA from Pseudomonas aeruginosa, and the M23B family, which consists of the majority of the family members, including LytM from Staphylococcus aureus (22, 24). The M23A endopeptidases possess broader substrate specificities than those of the M23B endopeptidases (22, 24). Proteins of the M23 family are distinguished by conserved Zn(II)-binding res-

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functional evolution of a mammalian protein with an M23 metalloendopeptidase fold and provides a better understanding of the activity against various peptide sequences, including pentaglycine cross-links present in the substrate, resulting in cleavage of the peptide bond (24, 29).

The known M23 family members are all present in bacteriophages and bacteria (19). The peptidoglycan hydrolase activities are required for their physiological roles, including cell division, cell elongation, and bacteriophage infection (29–31). However, human LECT2 is the sole mammalian protein in the M23 family. Although the sequence of LECT2 contains conserved motifs (HXXXD and HXH), it is unclear whether LECT2 has a metalloendopeptidase activity. Moreover, there is no evidence that the functions of LECT2 require enzymatic catalysis. Thus, it remains unclear whether a peptidase activity is required for the roles of LECT2 in various diseases. Most recently, it was reported that LECT2 can bind to the receptor tyrosine kinase human c-mesenchymal epithelial transition factor (c-Met). This binding is antagonistic to the c-Met receptor activation, and LECT2 acts as a tumor suppressor in HCC (32). This finding suggests an alternative function in which LECT2 is involved in protein-protein interaction.

Here, we present the crystal structure of human LECT2 at 1.94-Å resolution. This is the first mammalian protein whose structure has been confirmed to contain an M23 metalloendopeptidase fold. The LECT2 structure contains the conserved Zn(II) coordination geometry that uses the HXXXD and HXH motifs but lacks a proposed catalytic histidine residue (residue 86 is a Tyr instead of a His). Furthermore, a unique N-terminal intrachain (IC) loop blocks the potential substrate-binding groove in the vicinity of the Zn(II)-binding site, which may hinder the suitable positioning of the substrate for hydrolysis. Our proteolytic assays show that LECT2 lacks endopeptidase activity against various peptide sequences, including pentaglycine, which is consistent with its structural features. In addition, our study reveals the possible receptor-binding sites in the LECT2 structure and provides a better understanding of the functional evolution of a mammalian protein with an M23 metalloendopeptidase fold.

Results

Overall Structure of LECT2—The crystal structure of LECT2 was determined at 1.94-Å resolution. There were two molecules in the asymmetric unit, and each chain was composed of residues 1–133 (numbering is given for the mature LECT2). The root mean square deviation (r.m.s.d.) value was 0.34 Å for the aligned Cα atoms between two chains in an asymmetric unit, which shows that there was no significant structural difference between the chains. Because the electron density in chain B was clearer than that of chain A, the following results for chain B are described. The LECT2 structure possesses a large groove with a Zn(II) at the bottom (Fig. 1). The floor of this groove is formed by a central, six-stranded antiparallel β-sheet (β1, β9, β5, β4, β3, and β7), and its walls consist of four loops: loop 1 (Gly1–Gly26, located at the N terminus), loop 2 (Glu60–Asn71, which connects β3 and β4), loop 3 (Leu106–Ser117, which connects β8 and β9), and loop 4 (Cys124–Pro129, located at the C terminus). Loops 3 and 4 formed a partial α-helix (α1) and a 310-helix (η1), respectively. In addition to the central β-sheet, there is a second, much smaller three-strand β-sheet (β2, β8, and β6), which runs parallel to the central β-sheet and is positioned on the lower face of the central β-sheet. In addition, the structure includes three disulfide bonds, Cys7–Cys92, Cys18–Cys23, and Cys81–Cys124, which is consistent with previous assignments of disulfide linkages using mass spectrometry (18).

A database search using the Dali server showed the close structural similarity of LECT2 to the M23 metalloendopeptidases, including the catalytic domains of the M23B endopeptidases LytM (Protein Data Bank code 2B13; Z score, 12.6; sequence identity, 22%; r.m.s.d. for 111 Cα atoms, 2.5 Å) and lysostaphin (Protein Data Bank code 4QP5; Z score, 12.5; sequence identity, 19%; r.m.s.d. for 109 Cα atoms, 2.2 Å) and the M23A endopeptidase LasA (Protein Data Bank code 3IT7; Z score, 9.0; sequence identity, 16%; r.m.s.d. for 109 Cα atoms, 3.4 Å) (Fig. 2A) (24, 26, 28). These three classical M23 family members all specifically target the pentaglycine cross-links present in S. aureus peptidoglycan. Furthermore, LasA can cleave a wider range of glycosylated peptides with aromatic or branched amino acids at the P1’ position such as Pro-Gly-Gly-Val-Tyr-Gly and Leu-Gly-Gly-Val-Ala-Gly (J denotes the cleavage site) (24, 26, 28, 33). A superimposition of the structures of LECT2 and LasA revealed that the central β-sheet of LECT2 was similar to that of LytM, which consists of six antiparallel β-strands with a conserved topology, but loops 1–4 in the two structures showed significant differences in the lengths and conformations (Fig. 2B). A similar variability of loop regions is also found.
in other structural homologs (Fig. 2A). This variability likely reflects the different substrate specificities as indicated by the broader specificity of LasA than that of LytM or lysostaphin (24).

Additionally, a notable difference in the LECT2 structure is an insertion of a protruding IC loop region formed by the Cys18-Cys23 disulfide bond, which immediately precedes loop 1 (Fig. 2B). This N-terminal IC loop lies at one end of the groove of LECT2 and is stabilized by several interactions (Figs. 2, C and D). First, there is a hydrogen bond between the side chains of Arg16 on the IC loop and Asp39 on strand 1. Second, the main chain of Thr17 on the IC loop forms a hydrogen bond with the side chain of Gln116 on loop 3, and the side chain of Asp19 on the IC loop makes a hydrogen bond with the main chain of Gln116 on loop 3. Third, the side chain of His21 forms a hydrogen bond with the main chain of Pro113 on loop 3. Fourth, the main chain of Cys23 on the IC loop makes two hydrogen bonds with the side chain of Arg30 on loop 1.

**Zn(II) Coordination Geometry**—In the LECT2 structure, Zn(II) is coordinated by His35 (Zn(II)–N\_ε\_2, 2.00 Å) of the HXXD motif, His120 (Zn(II)–N\_ε\_1, 2.09 Å) (the second histidine of the HXH motif), and a water molecule (2.77 Å) (Fig. 3A). The distance between Zn(II) and the water molecule (2.77 Å) is longer than the typical Zn(II)-ligand distance. Therefore, there may also be other Zn(II)-binding water molecules, but they are not defined in our structure due to the poor electron density. The four Zn(II) ligands as described above are fixed in space by hydrogen bonds to other amino acid residues (Fig. 3A). Because the N\_ε\_2 of His35 directly contacts the Zn(II), the N\_ε\_1 is protonated and donates a hydrogen bond to the backbone carbonyl oxygen of Gln25 (2.83 Å). This pattern of interaction is known as an elec-His-Zn motif (34) and is believed to make the histidine imidazole ring more basic. Thus, His35 acts as a stronger ligand to the Zn(II). The O\_ε\_2 of Asp39 accepts a hydrogen bond from the guanidino nitrogen of Arg16 (2.88 Å) to stabilize the IC loop as mentioned above. The N\_ε\_2 of His120 donates a hydrogen bond to the O\_ε\_2 of Gln122 (2.66 Å) to form the other elec-His-Zn motif. In addition, the water molecule donates hydrogen bonds to the N\_ε\_2 of...
His\textsuperscript{118} (2.86 Å), which is the first histidine residue of the H\textsubscript{X}H motif, and the hydroxy oxygen of Tyr\textsuperscript{86} (2.95 Å). The superimposition shows that the Zn(II)-binding site of LECT2 closely resembles those of three other structural homologs (Fig. 3B).

The three Zn(II) amino acid ligands as well as one of the two residues involved in acid-base catalysis (His\textsuperscript{118} for LECT2) have almost the same spatial location and orientation in the four structures. However, the position of other proposed catalytic histidine residue, for example His\textsuperscript{260} of LytM, is replaced by a tyrosine residue (Tyr\textsuperscript{86}) in LECT2, although this conserved histidine and LECT2 Tyr\textsuperscript{86} have different sequence alignments (Fig. 3B and C). Such a variation has not been previously observed in M23 metalloendopeptidases.

**Absence of M23 Metalloendopeptidase Activity in LECT2**—LECT2 contains a unique IC loop and lacks one of the two proposed catalytic histidine residues (Tyr\textsuperscript{86} replaces the conserved His). The two catalytic histidines present unprotonated N\textsubscript{ε2} atoms toward the Zn(II)-binding water molecule and are proposed to play alternate roles, either activating the water molecule as a proton acceptor to perform the nucleophilic attack upon the substrate carbonyl carbon or stabilizing the oxyanion intermediate (24, 29). To clarify whether LECT2 functions as an M23 metalloendopeptidase and whether the lack of the conserved histidine residue causes the loss of the M23 metalloendopeptidase activity, we investigated hydrolysis of the typical pentaglycine substrate by both the wild-type and mutant forms of LECT2.
LECT2 and the Y86H mutant using the thin-layer chromatography (TLC) method (Fig. 4A). Lysozyme was chosen as a positive control because it is commercially available. Lysozyme cleaved pentaglycine into di- and triglycine. In contrast, LECT2 did not cause cleavage of pentaglycine, although Tyr86 is likely to have the same ability as the histidine residue based on the chemical properties of the aromatic hydroxy group. Furthermore, the Y86H mutant was also not able to cleave the pentaglycine substrate.

To evaluate further endopeptidase activities of LECT2 and its Y86H mutant, we used various types of proteins (azocasein, myosin, β-galactosidase, phosphorylase b, serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, lysozyme, and aprotinin) as substrates. These proteins were incubated with various concentrations (1, 2, and 5 μM) of the LECT2 WT or Y86H mutant protein at 37 °C for 24 h. C, endopeptidase assay against nine proteins (myosin, β-galactosidase, phosphorylase b, serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, lysozyme, and aprotinin) as substrates. These proteins were incubated with various concentrations (1, 2, and 5 μM) of the LECT2 WT or Y86H mutant protein at 37 °C for 24 h. D, interactions of the tartrate molecule with the active sites of M23 metalloendopeptidases. Left, the tartrate binding in the LytM active site (orange) and superimposition onto the putative active site of LECT2 (cyan). Right, the tartrate binding in the LasA active site (pink). The interactions made by bound tartrate (yellow) are represented by dashed lines. The residues on loop 3 that interact with the tartrate molecule are labeled. E, surface representation of LECT2 showing the steric hindrance with the tartrate molecule. The three residues Ile115, Arg16, and Arg30 in the vicinity of Zn(II) are colored in magenta and shown as stick models. The tartrate molecule from LytM is shown as yellow sticks with the oxygen atoms colored red.

We tried to explain the lack of LECT2 endopeptidase activity against pentaglycine and various other peptide sequences on the basis of the structural features of LECT2. In the structures of LytM and LasA, the binding patterns of tartrate molecules at the active sites are proposed to resemble those of the hydrolyzed products such as glycyglycine (24, 26). We therefore superimposed the structures of LECT2 and LytM to examine the tartrate binding in the groove of LECT2 (Fig. 4D, left). For LytM and LasA, the tartrate molecules make hydrogen bonds to Asn286 and Thr288 on loop 3 of LytM and to Ser116 and Thr117 on loop 3 of LasA. These results indicate that loop 3 is important for the proper positioning of substrates in the active site (Fig. 4D). In contrast, loop 3 of LECT2 projects away from the tartrate molecule (Fig. 4D, left). This loop conformation may be unfavorable for binding of substrates such as pentaglycine. More importantly, the tartrate molecule in the superimposed structures shows steric interference with Arg16 and Ile115 on the IC loop and loop 3 of LECT2 (Fig. 4E). The wall composed of the three residues Ile115, Arg16, and Arg80 makes the space in the vicinity of the LECT2 Zn(II)-binding site too narrow to accommodate the small tartrate molecule that is bound in the active site of LytM, clearly indicating that the groove of LECT2 may sterically hinder the binding of the amino acid, at least at the P2 site of a peptide substrate. For the M23 metalloendopeptidases, both sites P2 and P’2 in a short peptide (P3-P2-P1 ↓ P’1-P’2-P’3) must be occupied for hydrolysis to occur. This is most likely because the conformation...
of a peptide substrate is stabilized by a hydrogen bond between P1 and P4 residues (33). Conversely, this structure suggests that LECT2 could accept the substrate into the Zn(II)-binding site to exert exopeptidase activity because such activity does not require the P2 or P2′ site. However, no exopeptidase activity of LECT2 was observed when pentaglycine was used as a substrate (Fig. 4A).

**Binding of LECT2 to c-Met Extracellular Domain (ECD)—**

Although LECT2 did not cause the cleavage of pentaglycine, LECT2 had the capacity for protein-protein interaction. The interaction between LECT2 and c-Met ECD has been demonstrated by coimmunoprecipitation experiments. The LECT2 binding caused an antagonistic effect on the c-Met receptor activation (32). In our study, the kinetic measurements of the interaction between these proteins were performed for the first time using surface plasmon resonance methods. Sensorgrams of the LECT2 binding to c-Met ECD were used to calculate the dissociation constant (Kₐ). The results showed that LECT2 bound to c-Met ECD with a Kₐ value of 2.4 ± 0.3 µM (Fig. 5). Normally, the c-Met signaling pathway is driven by its cognate ligand, hepatocyte growth factor (35). The receptor tyrosine kinase c-Met has been identified as a receptor tyrosine kinase that is coupled to the c-Met ECD by coimmunoprecipitation experiments. The LECT2-binding protein, and LECT2 binding suppresses the c-Met phosphorylation through the recruitment of protein-tyrosine phosphatase 1B. This result is in the dissociation of the adaptor proteins, such as Gab1, Grb2, and Src, which then blocks the Raf1/ERK signaling (32). Therefore, LECT2 plays a suppressive role in vascular invasion and metastasis in HCC by acting as a ligand for c-Met but not as an endopeptidase. Interestingly, similarly to LECT2, the β-chain of hepatocyte growth factor, a cognate ligand of c-Met, has a chymotrypsin-like serine protease fold but lacks the hydrolytic activity due to the absence of certain catalytic residues (35). The complex structure reveals that hepatocyte growth factor β binds to c-Met by utilizing its pseudo-active site region (35). In addition to LECT2, other noncatalytic bacterial proteins with an M23 metalloendopeptidase fold have been found to engage in protein-protein interactions. For example, the extracellular LytM-like domain of the forespore-expressed membrane protein SpoIIQ from Bacillus subtilis has no metalloendopeptidase activity because it lacks certain active site residues and a catalytic metal ion. In addition, the substrate-binding groove is closed off by its additional N-terminal β-hairpin region. However, SpoIIQ has developed a novel function for mediating protein-protein interactions that are specifically mediated by this additional region (36, 37). Another example is the Escherichia coli cell division protein EnvC. Although the C-terminal LytM domain of EnvC also lacks conserved Zn(II)-coordinating and catalytic residues and is not capable of functioning as a metalloendopeptidase, it can activate amidas that can hydrolyze peptidoglycan. A mutational analysis and molecular modeling studies have suggested that EnvC might use the catalytically inactive groove to bind the amidas autoinhibitory α-helix (38, 39). Based on the examples of SpoIIQ and EnvC, we suspect that the nonconserved N-terminal IC loop together with the central groove of LECT2 may be specific for interactions between LECT2 and its partners. Interestingly, some mult-
multiple domain proteins of the M23 family adopt an autoinhibited state as the result of interactions between two domains. This observation suggests possible binding modes for the protein-protein interactions that occur in those noncatalytic homologs. For instance, in the structure of full-length LytM, a loop from the N-terminal domain binds within the active site groove of the C-terminal catalytic domain, and an asparagine residue on this inhibitory loop occupies one of the ligand sites of Zn(II) (25). Another example is the structure of NMB0315 from Neisseria meningitides. The N-terminal short β3-β4 loop stretches into the active site groove of the C-terminal catalytic domain but is not involved in the Zn(II) coordination (40). These inhibitory segments do not fit into the active site such as a substrate but occupy the substrate-binding groove by tight interactions.

Our study of the structure of LECT2 combined with the findings for other noncatalytic proteins with an M23 metalloendopeptidase fold that we discussed above reveals that these proteins share some common features. Specifically, they lack residues that are important for peptidase activity and/or show variations in the N-terminal region. These variations suggest an efficient mechanism for the evolution of new functions. Although our data do not exclude the possibility that LECT2 has a peptidase activity against a specific peptide sequence, human LECT2 has evolved from a catalytically competent ancestor to a receptor-binding protein that is involved in an important signaling pathway. This function may require the peptidase-inhibitor binding mode for the protein-protein interactions.

**Experimental Procedures**

**Protein Preparation**—The human LECT2 was expressed and purified as described previously (41, 42). Briefly, a DNA sequence encoding the mature human LECT2 was amplified by PCR and cloned into the SmaI/BamHI site of the pET-48b(+) expression plasmid (Novagen). LECT2 with the N-terminal thioredoxin-hexahistidine (His6) tag was overexpressed in E. coli strain Rosetta-gami 2 (DE3) (Novagen). The structure determination was performed using selenomethionine-substituted LECT2 (LECT2SeMet) that was produced in M9 medium containing various amino acids (100 mg/liter l-lysine, l-phenylalanine, and l-threonyl and 50 mg/liter l-isoleucine, l-leucine, l-valine, and l-selenomethionine). After expression of the protein, the harvested cells were disrupted by sonication, and centrifugation was used to separate the insoluble inclusion bodies from the soluble proteins. The suspension of LECT2 inclusion bodies was adjusted to a protein concentration of 0.5 mg/ml in the refolding buffer (50 mM Tris-HCl (pH 8.0) and 500 mM l-arginine) and subjected to high hydrostatic pressure (200 megapascals) in a PreEMT-E150 pressure chamber (BaroFold Inc.) overnight at room temperature. The solution containing the refolded LECT2 was dialyzed to remove l-arginine before purification. The expressed soluble LECT2 and the refolded protein were separately applied to a nickel-nitritiocetic acid Superflow (Qiagen) column. The protein was released by addition of HRV3C protease to the column to remove the N-terminal tag, and the eluted protein was further purified using cation exchange chromatography on a Resource S column (GE Healthcare).

**Crystallization and X-ray Data Collection**—The refolded LECT2SeMet was concentrated to 10 mg/ml in a final buffer containing 10 mM Tris-HCl (pH 7.0), 1 mM iminodiacetic acid, and 50 μM ZnCl2 and was then crystallized using the sitting drop vapor diffusion method (42). The best crystals were obtained by mixing 1.0 μl of the protein solution with 1.0 μl of the reservoir solution, which consisted of 0.2 M ammonium sulfate, 0.1 M HEPES (pH 7.5), and 25% (w/v) PEG 8000 at 20 °C. The x-ray diffraction data were collected using the BL-5A beamline at the Photon Factory (Tsukuba, Japan) with an ADSC Quantum 210r CCD detector. A crystal of LECT2SeMet was used to collect a single wavelength anomalous dispersion data set at the selenium peak wavelength of 0.9792 Å. The data set was indexed and integrated using the HKL2000 program suite (43). Table 1 summarizes the data collection and data processing statistics.

**Structure Determination and Refinement**—The selenium positions in the protein crystal were determined with SHELXD (44). Heavy atom refinement and phase calculations were performed with SHARP (45). An initial model was automatically built with the ARP/wARP package from the CCP4 suite (46). The missing amino acid residues were manually added using Coot (47). The obtained model was refined using Refmac5 (48) from the CCP4 program suite. Iterative rounds of refinements were continued with Coot and Refmac5. The crystallographic water molecules were automatically introduced using ARP/wARP with subsequent manual modification. The quality of the model was verified using PROCHECK (49). The final crystal structure was refined to Rwork and Rfree values of 17.7 and 22.3%, respectively. The refinement statistics are summarized in Table 1. The visualization and superimposition of the protein structures were achieved using PyMOL, and the r.m.s.d. values for superimposed structures were calculated using the Dali server (50).

**Site-directed Mutagenesis**—The LECT2 Y86H mutant was generated with the PCR-based site-directed mutagenesis method using a QuickChange site-directed mutagenesis kit (Stratagene) (51) with the pET48b-LECT2 plasmid as a template. The primers were purchased from Operon Technologies Inc. and designed as follows: 5’-GGGAAGGTTTTTTGTTTCAAAAATGTTTCTACCATAGCCAAATTAG-3’ and 5’-CCTTATGGCTTAAATTGTCACACTTTTGGACACAAAAACCTCTTCCC-3’. The underlined characters in the primer sequences are the altered nucleobases. The mutation was verified by the DNA sequencing service of FASMAC. The Y86H mutant was expressed in soluble form and purified according to the method described for the wild-type LECT2.

**M23 Metalloendopeptidase Activity Assay**—The assay was performed according to a previous report with some modifications (25, 26). The soluble form of the expressed LECT2 was concentrated to 5 mg/ml in a buffer containing 10 mM Tris-HCl (pH 7.0) and 50 mM ZnCl2. Subsequently, 0.2 mg/ml LECT2 or lysostaphin was incubated with 1 mM pentaglycine as the substrate (Bachem AG) for 24 h at 37 °C in 20 mM Tris-HCl (pH 7.5) with a final Zn(II) concentration of 2 μM. The mature recombinant lysostaphin from Staphylococcus simulans biobar staphyloyticus (Wako) was used as a positive control in the assay system. After the reaction, the enzyme activity was mea-
Lectin 2 (LECT2), a member of the C-type lectin superfamily, is known for its role in the recruitment of neutrophils. In a recent study, the authors investigated the endopeptidase activity of LECT2 and its interaction with the c-Met receptor. They used a combination of biochemical and biophysical techniques to characterize these interactions.

**Data collection and refinement statistics**

| Parameter | Value |
|-----------|-------|
| Resolution (Å) | 1.94 |
| No. of unique reflections | 18,525 |
| r.m.s.d. | 1.77/22.3 |
| Intensities | 95.04, 3.82, 1.15 |
| Bond lengths (Å) | 0.019 |
| Bond angles (°) | 1.745 |
| Ramachandran plot | Favorable, allowed, disallowed (%) 95.04, 3.82, 1.15 |

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