論 文 の 内 容 の 要 旨

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論文題目

The analysis of the structure and function of human leukocyte cell-derived chemotaxin 2 (LECT2)

（好中球走化性因子 LECT2 の構造･機能解析）

Introduction

Human leukocyte cell-derived chemotaxin 2 (LECT2) was first purified from the culture supernatants of phytohaemagglutinin-activated (PHA) human T-cell leukemia SKW-3 cells as a chemotactic factor to human neutrophils. LECT2 is preferentially expressed in the adult and fetal livers and in the human hepatoma cell lines. Although LECT2 was originally demonstrated *in vitro* to have chemotactic activity, accumulating evidences suggest that LECT2 is involved in a more multifunctional role in the liver, extending from cell growth, differentiation, damage/repair process and carcinogenesis to autoimmune diseases. Moreover, the polymorphism of human LECT2 at V58I is

associated with the severity of rheumatoid arthritis in the Japanese population. Recently, LECT2 has also been identified as a protein associated with human systemic amyloidosis. In addition, proteins homologous to human LECT2 have been isolated in many other vertebrates, such as bovine (*Bos taurus*) and chicken (*Gallus gallus*). Bovine LECT2 is identical to bovine chondromodulin-II, which stimulates the proliferation of chondrocytes and osteoblasts.

The diverse biological functions of human LECT2 are presumably related to its biochemical properties. Human LECT2 is a 16-kDa secreted protein consisting of 133 amino acids and three intramolecular disulfide bonds. Database searches using Basic Local Alignment Search Tool (BLAST) have indicated that human LECT2 belongs to the zinc metallopeptidase family M23. Members of this family have a preference for peptides containing polyglycine residues, especially Gly-Gly-Xaa, where Xaa is any aliphatic hydrophobic residue. However, the actual biochemical properties of LECT2 and the structural basis for its biochemistry are still unknown. Therefore, the present study aimed to clarify the biochemical and structural characteristics of human LECT2.

Expression, high-pressure refolding and purification of human LECT2

In this study, soluble LECT2 was expressed in *E.coli* for the first time. However, the amount of soluble expression was very low and large amounts of LECT2 were found in insoluble inclusion bodies (IBs) (Fig. 1). Therefore, high hydrostatic pressure (HHP), which has been widely used as a powerful tool for refolding protein from IBs, was used to refold LECT2 in order to obtain more soluble protein. The structural features of the refolded LECT2 were then evaluated by CD and NMR. The results clearly demonstrated that LECT2 could be refolded into active form using HHP. The tertiary structure (Fig. 2A) and chemotactic activity (Fig. 2B) of the refolded LECT2 were indistinguishable from those of LECT2 which had been solubly expressed and purified. Therefore, refolded LECT2 was used in this study to investigate the structure and function of the LECT2 protein.

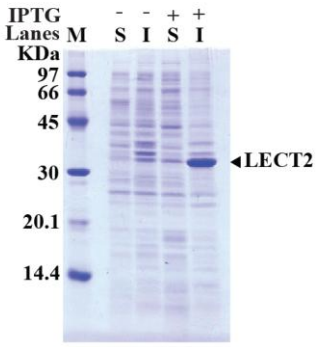

Fig. 1. SDS-PAGE analysis of Trx-His$_6$-LECT2 expressed in *E.coli*. S and I mean soluble fraction and insoluble fraction.

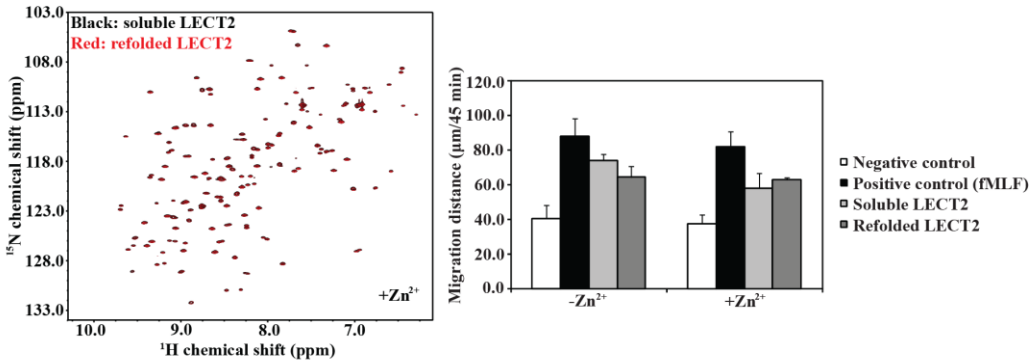

Fig. 2. The $^{1}H-^{15}N$ HSQC NMR spectrum (A) and the chemotactic activity (B) of soluble and refolded LECT2.

The crystal structure of human LECT2

Seleno-L-methionine (SeMet)-labeled LECT2 was crystallized. The optimal crystal was obtained using a reservoir solution consisting of 0.2 M ammonium sulfate, 0.1 M HEPES (pH 7.5) and 25% (w/v) polyethylene glycol 8,000 at 293 K. An X-ray diffraction data set was collected to 1.94 Å using a synchrotron X-ray source (PF BL-5A). The space group of the crystal was $P2_12_12_1$, with unit-cell parameters a=59.4 Å, b=63.5 Å and c=64.0 Å. The asymmetric unit contains two molecules of LECT2. After phase determination and refinement, the structure of LECT2 was resolved. In this structure, LECT2 is bound to one zinc ion (Zn^{2+}), which is four-coordinated by His53, Asp57, His138 and a water molecule (Fig. 3). The structure showed that LECT2 has a fold similar to lysostaphin-type metalloendopeptidase LytM, which is present in bacteriophases and in bacteria, and also shares the conserved sequence motifs HXXXD and HXH.

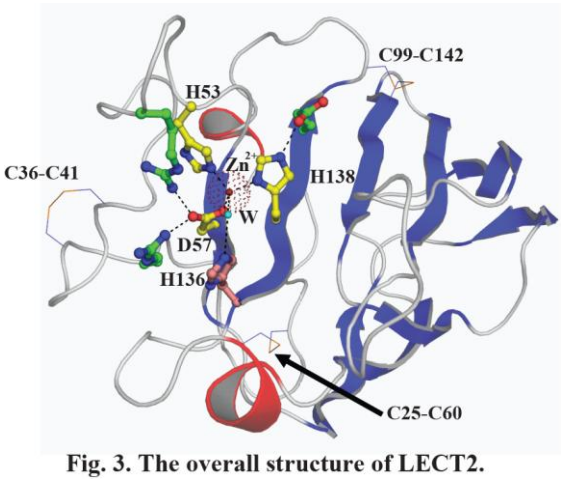

Fig. 3. The overall structure of LECT2.

The metalloendopeptidase activity of LECT2 and the selection of the substrate peptides of LECT2 by phage display screening

Since LECT2 has a structure and active site similar to metalloendopeptidase LytM, it

was hypothesized that LECT2 may also have metalloendopeptidase activity. In this study, azocasein and pentaglycine were used as substrates to determine the proteolytic activity and enzymatic specificity of LECT2. However, it was found that LECT2 could not hydrolyze azocasein or pentaglycine, possibly because the positions and lengths of the four loops in the LECT2 structure are somewhat different from that of LytM so that their enzymatic specificities are different.

In order to clarify whether LECT2 could bind certain peptides or proteins and has metalloendopeptidase activity, the Ph.D.-12 phage display peptide library was used to select the peptides that bind to LECT2. The results showed that the binding peptides contain a short consensus sequence of GYPD. Sequence alignment by BLAST revealed that some immune proteins (*Homo sapiens*) such as T-cell receptor delta chain, immunoglobulin (Ig) heavy chain and C-type lectin domain family 17 (member A, CLEC17A) contain the GYPD motif. Therefore, these proteins are possible receptors of LECT2. The binding of these receptors to LECT2 may be responsible for the chemotactic activity and other biological functions of LECT2.

The comparison of the crystal structure and NMR dynamics of the wild type and mutant LECT2

The polymorphism of human LECT2 at V58I is associated with the severity of rheumatoid arthritis in the Japanese population. In order to investigate this, the LECT2 V58I mutant was expressed and crystallized. However, structural analysis showed that the V58I mutant has the same structure as wild type LECT2.

NMR dynamics of the wild type and mutant LECT2 were also compared. Standard pulse sequences with minimal water suppression were used to record the T_1 and T_2 relaxation times and the $\{^1H\}$-^{15}N heteronuclear NOEs of the wild type and mutant LECT2. By calculating the R_1 and R_2 relaxation rates and the NOE values, it was found that the LECT2 V58I mutant is different from the wild type LECT2, especially in the R_2 and NOEs. The residues related with these differences are mainly located in the vicinity of the zinc-binding site or the V58I mutation. These results suggest that the V58I mutant is different from the wild type LECT2 in its dynamics, which may be associated with the ability of wild type and LECT2 V58I mutant to induce different severities of rheumatoid arthritis.