# The Three-Dimensional Structure of the 15th Domain of the Human Multiple Kazal Type Inhibitor LEKTI


K. Vitzithum, U. C. Marx

Lehrstuhl für Biopolymere, Universität Bayreuth, 95440 Bayreuth, Germany

Universität Bayreuth



## Abstract

We have carried out structural characterisation of the serine proteinase inhibitor "dom15", which is part of the larger precursor protein LEKTI (lympho epithelial Kazal-type related inhibitor) containing 15 potential serine proteinase inhibitory domains. The second and the last domain (dom15) of LEKTI show the typical six-cysteine pattern of 'classical' Kazal-type inhibitors, only differing in the spacing between the first two cysteines (13 and 12 instead of 6 residues). The last domain of LEKTI is of particular interest, because of its partial homology to the only known natural occuring tryptase inhibitor LDTI, as well as the unequivocal correlation between the severe skin disorder disease Netherton Syndrome and defects in the gene encoding LEKTI mainly generating premature termination codons of translation.

Characterization of recombinant dom15 was carried out by amino acid sequencing, mass spectrometry, RP-HPLC, CD- and NMR-spectroscopy. Structural characterisation of the $^{15}$N-labelled protein based on multidimensional NMR studies. As deduced by preliminarily structure calculation dom15 shows a typical Kazal like motif, i.e. a small three-stranded β-sheet and an α-helix, located in a region being consistent with the typical Kazal-type inhibitors pattern. The extra amino acid residues of dom15 compared to classical Kazal-type inhibitors are probably arranged in an additional helical structure in the $NH_2$-terminal part of the protein and indications for an additional helix following the last cysteine are identified.


**Introduction:**

The human precursor protein LEKTI (lympho epithelial Kazal-type related inhibitor) contains 15 potential serine proteinase inhibitory domains (Fig. 1a), two of which (domain 2 and 15) show the typical six-cysteine pattern of 'classical' Kazal-type inhibitors, only differing in the spacing between the first two cysteines (13 and 12 instead of 6 residues), while the other domains lack two of the six cysteins but show analogous disulfide connectivity [1]. Because of the partial identity to the only known natural occuring tryptase inhibitor LDTI (leech derived tryptase inhibitor) of *Hirudo medicinalis* [2], particularly at the proteinase inhibiting region (see Fig. 1b), as well as the unequivocal correlation between the severe skin disorder disease Netherton Syndrome and defects in the gene encoding LEKTI mainly generating premature termination codons of translation [3], the last domain of LEKTI (dom15) is of particular interest. Therefore we carried out structural characterisation of recombinant dom15 comprising the last 76 residues of LEKTI.

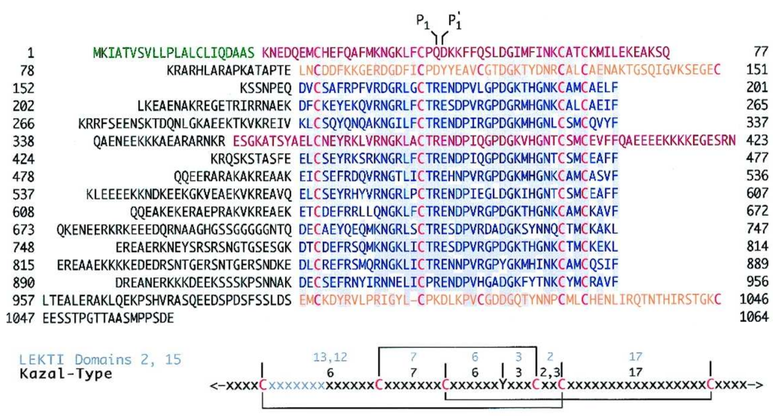


**Fig.1a :** Amino-acid sequence of human LEKTI [secretory signal sequence (green), domain 1 and 6 as isolated of human hemofiltrate (magenta), domain 2 and 15 (orange)] and typical Kazal-type domain in comparison to the cysteine pattern of LEKTI domains 2 and 15.

```
LDTI   KKVCA-----------CPKILKPVCGSDGRTYANSCIARCNGVSIKSEGS--CPTGILN
dom15  SEMCKDYRVLPRIGYLCPKDLKPVCGDDGQTYNNPCML-CHENLIRQTNTHIRSTGKC-
       .::*           *** ******.**:** *.*:  *:   *:. .:   .**
```

**Fig.1b:** Alignment of dom15 and LDTI (by *CLUSTAL W 1.81* )
Identical residues at proteinase inhibiting region (green); cysteines (red); conserved tyrosine (blue)
***=** identical or conserved residues; **:=** indicates conserved subst.; **.=** indicates semi-conserved subst.

**Methods and results:**

**a) Expression and characterization of recombinant dom15**

Recombinant dom15 was expressed and purified from *E. coli* Origami (DE3) cells, similiar as described for the first domain of LEKTI [4]. Isotopic labeled protein was purified from cells grown on M9 minimal media containing $^{15}NH_4Cl$.

Disulfide connectivity consistent with the classical kazal type pattern was verified. NMR samples contained protein at concentrations up to 2.5 mM in 50 mM potassium phospate (10% $D_2O$), pH 6.4 at 298 K.

**b) CD -Spectroscopy**

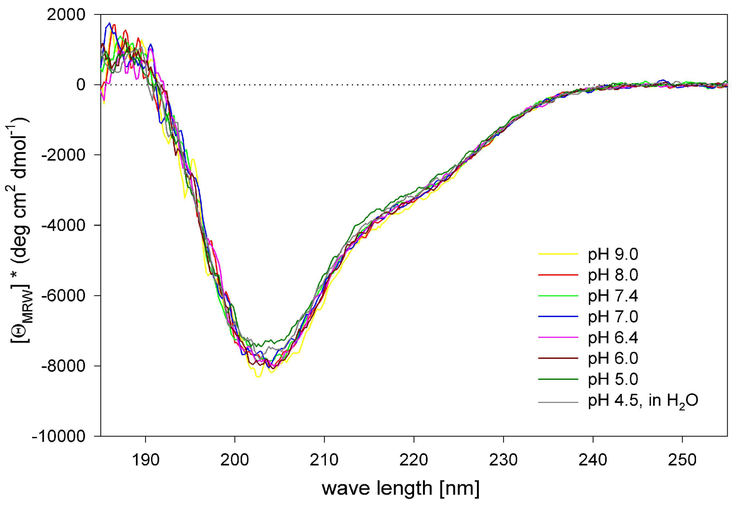


**Fig.2:** Overlay of far-UV-CD-spectra of recombinant dom15 at various pH containing protein at a concentration of 0,035 mM in 10mM potassium phospate at 298 K, values collected by a Jasco J-810 spectrometer

Structural investigations using far-UV-CD-spectroscopy indicate helical and maybe β-sheet structural elements of recombinant dom15 (Fig. 2). No changes in structure were observed over a broad pH range.

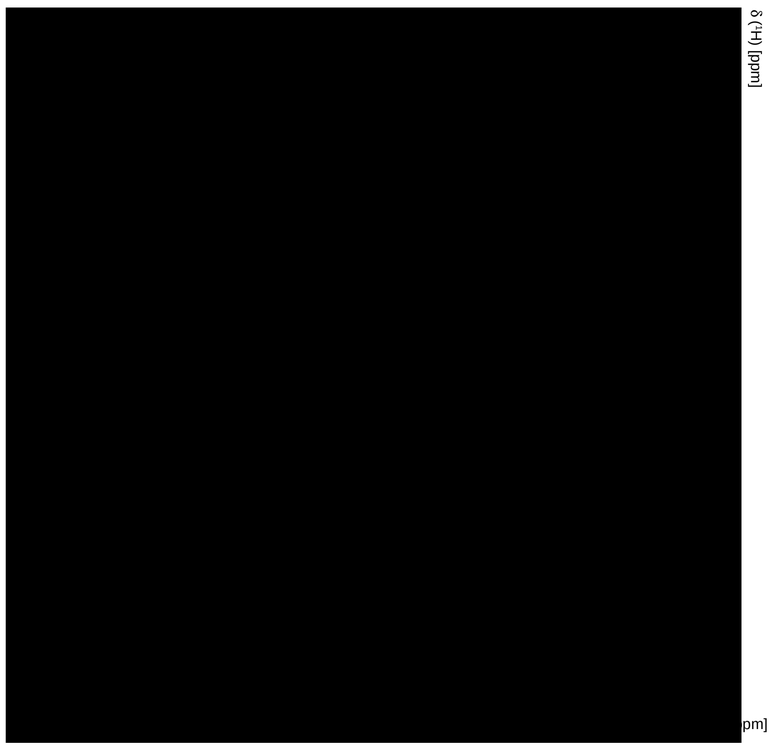


**Fig.3:** $^1$H-$^1$H-NOESY spectrum of recombinant dom15 containing protein at a concentration of 2.5 mM in 50 mM potassium phospate (10% D2O), pH 6.4 at 298 K.

**c) NMR-Spectroscopy**

As homonuclear two-dimensional spectra show lots of overlapping signals (Fig. 3), $^{15}$N-labeling and recording of $^{15}$N-TOCSY-HSQC-, $^{15}$N-NOESY-HSQC-, $^{15}$N-HMQC-NOESY-HSQC-, HNHA- and $^{15}$N-HSQC-spectra (Fig. 4) were necessary for backbone and sidechain assignment of dom15. Aromatic amino acid side chains have been assigned using homonuclear TOCSY-, NOESY- and COSY-experiments. $^1$Hα chemical shifts were taken from HNHA and $^{15}$N-TOCSY-HSQC spectra. Distance restraints for structure calculation were taken from the $^{15}$N-NOESY-HSQC spectrum for NOEs involving amide protons and from 2D-NOESY spectra for NOEs between aliphatic and aromatic protons. $^3J(H^N,H^\alpha)$ coupling constants were obtained by analyzing cross peak to diagonal peak intensity ratios in the HNHA spectrum, corrected by a factor 1.05, as well as by line-shape analysis of the anti-phase cross signal splitting in a high digital resolution 2D-DQF-COSY spectrum using a Lorentzian function for peak fitting. Slow exchanging amide protons were identified from time-dependent $^{15}$N-HSQC experiments in $D_2O$ solution. All NMR spectra were recorded on a Bruker DRX600 spectrometer at 298 K.

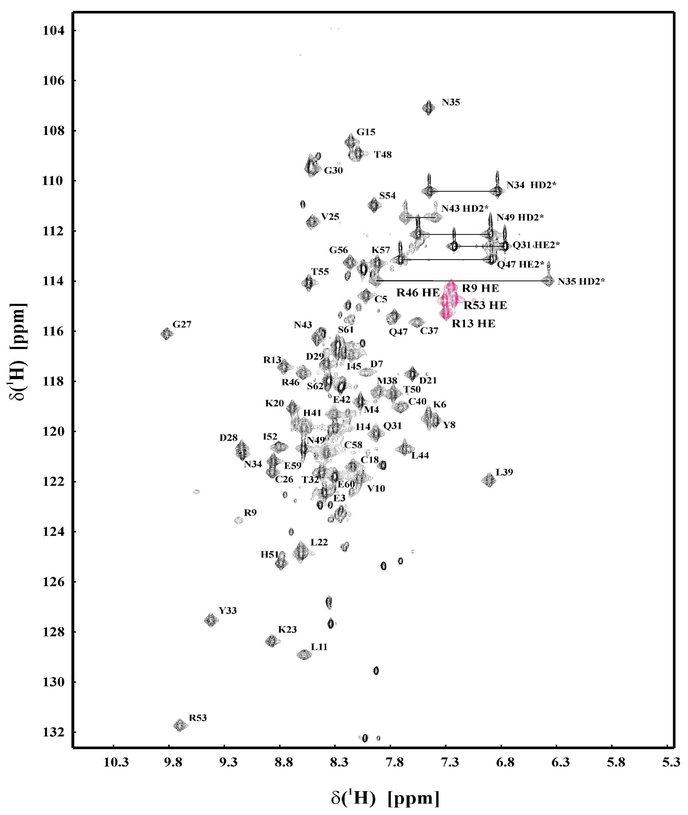


**Fig.4:** $^1$H-$^{15}$N-HSQC spectrum of $^{15}$N-labeled dom15 at 2.0 mM protein concentration in 50 mM potassium phospate (10% $D_2O$) at pH 6.4 and 298 K. Assigned backbone and sidechain amide proton resonances are labeled.

As described by Wishart et al. [5a], four or more sequential upfield shifted $^1$Hα resonances (negative values in Fig. 5) indicate α-helical structure while three or more positive values (downfield shifted $^1$Hα resonances) are typical for β-sheets. Thus, analyzing the $^1$Hα chemical shift of dom15 (Fig.5) indications for two helical regions (C37 to Q47 and K57 to S61) and two sheet elements (L17 to P19 and K23 to C26) can be deduced, whereas the C-terminal part seems to lack any secondary structural elements.

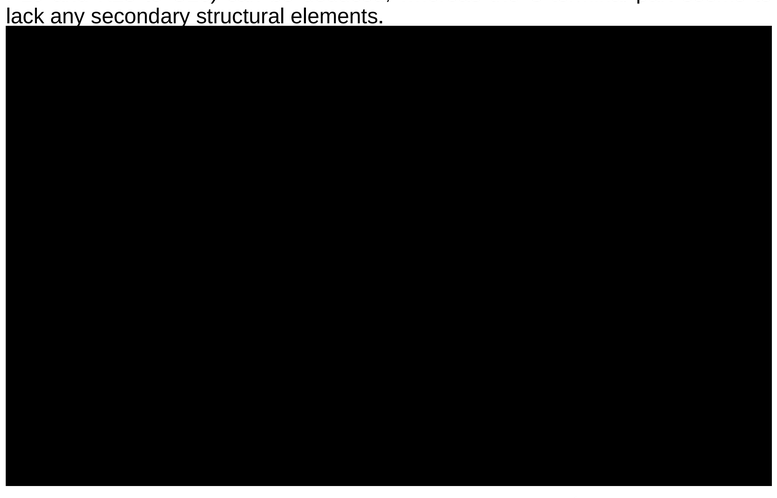

**Fig.5:** Chemical shift analysis of dom15 (difference between obtained $^1$Hα chemical shifts and random coil values [5b]).

**d) Preliminary results of structural calculations**

Structure calculation of dom15 were carried out by XPLOR 3.851 using a modified simulated annealing protocol. 352 distance constraints (209 sequential, 87 long-and 56 medium-range NOEs) obtained from analysis of the NOE connectivities were used to calculate a family of structures. The structure with lowest overall energies and lowest number of violations of experimental data is shown in two orientations in Fig. 6. The heavy side chain atoms of putative P1 and P1' residues (Lys20 and Asp21) are shown (generated with MOLSCRIPT and Raster3D). Sidechains of cysteine residues and disulfide bridges are shown in stick representation (Cys 5 -Cys 40; Cys 18 -Cys 37; Cys 26 - Cys 58).

Although evaluation of experimental data is still in progress a typical Kazal motif for dom15 can already be deduced, i.e. a small three-stranded β-sheet and a α-helix, located in a region of dom15 being consistent with the typical Kazal pattern. The extra amino acid residues of dom15 compared to classical Kazal-type inhibitors are probably arranged in an additional helical structure in the $NH_2$-terminal part of the protein and a further helix following the last cysteine is identified. The putative P1-P1'-residues are located on an exposed loop in agreement with typical serine proteinase inhibitor structures.

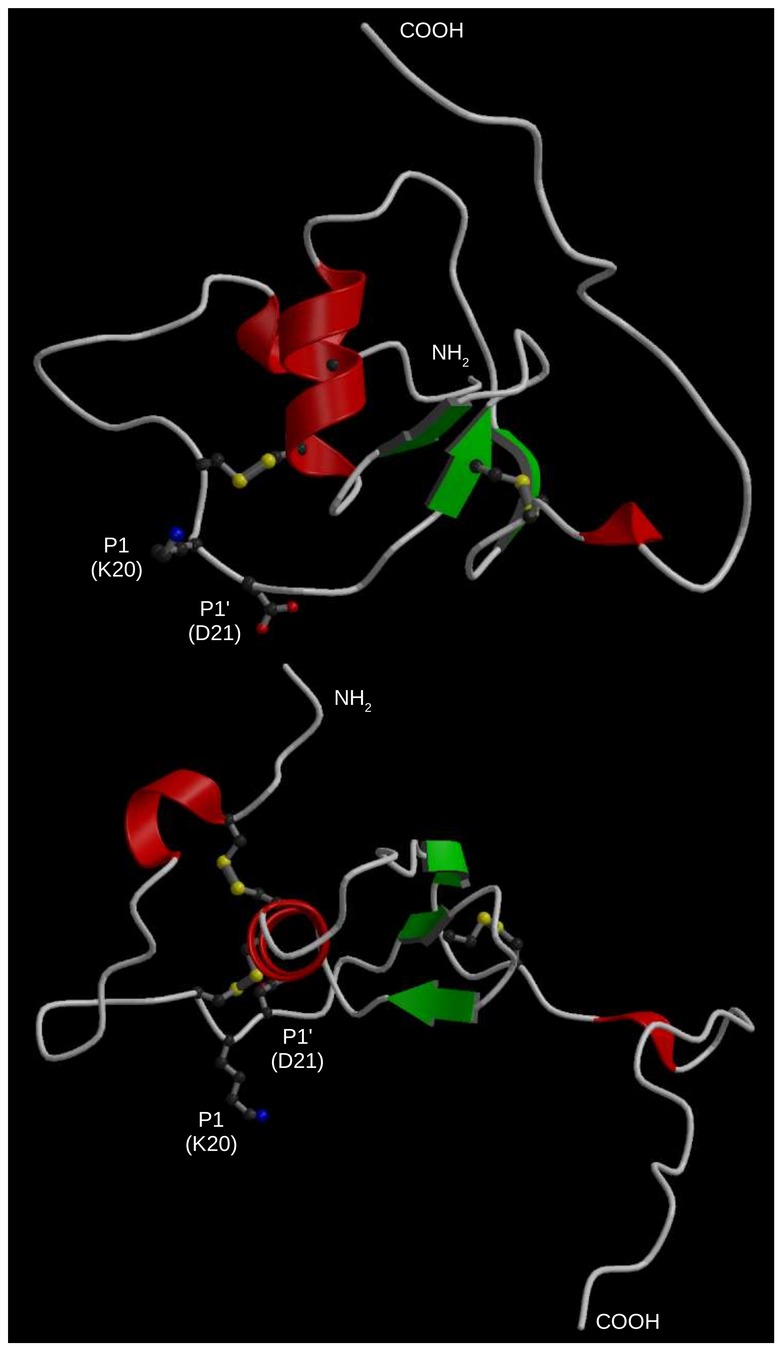


**Fig.6:** Schematic drawing of two orientations of a representative structure of dom15; the heavy side chain atoms of putative P1 and P1' residues (Lys20 and Asp21) are shown (generated with MOLSCRIPT and Raster3D), Sidechains of cysteine residues and disulfide bridges are shown in stick representation (Cys 5 -Cys 40; Cys 18 -Cys 37; Cys 26 - Cys 58), helical elements are displayed in red, β-sheet structure is coloured in green.

## Conclusions

Preliminary structure calculation indicate a typical Kazal motif for the last domain of LEKTI, i.e. a small three-stranded β-sheet and a α-helix, located in a region of dom15 being consistent with the typical Kazal pattern. The extra amino acid residues of dom15 compared to classical Kazal-type inhibitors are probably arranged in an additional helical structure in the NH2-terminal part of the protein and a further helix following the last cysteine is identified.

The putative P1-P1'-residues are located on an exposed loop in agreement with typical serine proteinase inhibitor structures.

**References**


[1] Mägert, H.J., Ständker, L., Kreutzmann, P., Zucht, H.D., Reinecke, M., Sommerhoff, C.P., Fritz, H., Forssman, W.G., (1999), *J. Biol. Chem.* **274**, 21499-21502.

[2] Stubbs, M.T., Morenweiser, R., Stürzebecher, J., Bauer, M., Bode, W., Huber, R., Piechottka, G.P., Matschiner, G., Sommerhoff, C.P., Fritz, H., Auerswald EA, (1997) , *J. Biol. Chem.* **272**, 19931-19937.

[3] Bitoun, E., Chavanas, S., Irvine, A.D., Lonie, L., Bodemer, C., Paradisi, M., Hamel-Teillac, D., Ansai, S., Mitsuhashi, Y., Taieb, A., de Prost, Y., Zambruno, G., Haerper, J.I., Hovnanian, A., (2002), *J. Invest. Dermatol.* **118** 2, 352-61.

[4] Lauber, T., Marx, U.C., Schulz, A., Kreutzmann, P., Rösch, P., Hoffmann, S., (2001 ), *Protein Expr. Purif.* **22** 1, 108-112.

[5a] Wishart, D.S., Sykes, B.D., Richards, F.M., (1992), *Biochemistry* **31**, 1647-1651.

[5b] Wishart, D. S., Bigam, C.G., Holm, A., Hodges, R.S., Sykes, B.D. (1995), *J. Biomol. NMR* **5**, 67-81.