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Ebola Viral Protein 35 N-terminus is a Parallel Tetramer

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Abstract

Members of *Mononegavirales*, the order including non-segmented negative sense RNA viruses (NNSV), encode a small number of multifunctional proteins. In members of the *Filoviridae* family, virus protein 35 (VP35) facilitates immune evasion and functions as an obligatory co-factor for viral RNA-synthesis. VP35 functions in an orthologous manner to phosphoproteins (P proteins) from other NNSVs. Although the critical roles of Ebola viral VP35 (eVP35) in immune evasion and RNA synthesis are well-appreciated, a complete understanding of its organization its role in carrying out its many functions has yet to be fully realized. In particular, we currently lack information on the role of the oligomerization domain within eVP35. To address this limitation, we report here an investigation of the oligomer structure of eVP35 using hybrid methods that include multi-angle light scattering (MALS), small angle X-ray scattering (SAXS), and crosslinking coupled with mass spectrometry (XL-MS) to determine the shape and orientation of the eVP35 oligomer. Our integrative results are consistent with a parallel tetramer, in which the N-terminal regions that are required for RNA synthesis are all oriented in the same direction. Furthermore, these results define a framework to target the symmetric tetramer for structure-based antiviral discovery.

Keywords

Mass spectrometry (MS); cross linking; native mass spectrometry; filoviruses; multi-angle light scattering (MALS); small angle X-ray scattering (SAXS); oligomer structure

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INTRODUCTION

Ebola virus (EBOV) is a representative member of the *Filoviridae* family¹ that can cause sporadic but deadly outbreaks of severe hemorrhagic fever in humans and non-human primates^{2, 3}. The high case fatality rates associated with some outbreaks, as high as 90%, are attributed to uncontrolled viral replication that results in global immunosuppression. Although several therapies, including the use of antibodies are being actively investigated^{4–7}, no safe and efficacious approved vaccine or therapeutics are currently available. Progress in these endeavors requires a better understanding of the viral proteins that inhibit immune responses.

Filoviruses are non-segmented, negative-strand RNA viruses (NNSVs) that belong to the order *Mononegavirales* and contain genomes that encode for the following proteins: nucleoprotein (NP), viral protein 35 (VP35), VP40, glycoproteins (GP and sGP), VP30, VP24, and the RNA-dependent RNA polymerase (Large protein or L)^{3, 8}. Of these, VP35 plays a critical role in viral pathogenesis, both as an inhibitor of innate immune responses and as a critical cofactor for viral replication. EBOVs VP35 (eVP35) functions as an interferon (IFN) antagonist by blocking the RIG-I pathway from detecting viral PAMPs at several points in the pathway. Previous studies showed that VP35 proteins can inhibit RIG-I activity through interactions with viral dsRNA⁹. The X-ray structure of the C-terminal IFN inhibitory domain (IID) shows that eVP35 IID endcaps dsRNA, preventing its detection by RIG-I as well as RIG-I activation and signaling^{10, 11}. In addition, VP35 binds and sequesters PACT, an activator of RIG-I, resulting in attenuated RIG-I signaling and antiviral activity^{12–15}. VP35 also serves as a substrate for IKKe/TBK-1, preventing phosphorylation of transcription factors IRF3/7¹⁶. Collectively, these studies demonstrate how VP35 proteins use multiple mechanisms to counter host antiviral responses.

The eVP35 protein also plays a critical role as a cofactor for viral replication, along with NP and L as well as VP30 for transcription. Although the exact mechanism by which VP35 mediates RNA synthesis is unclear, recent studies demonstrate that a peptide from the N-terminus of eVP35 (NPBP, NP binding peptide) binds to and regulates eNP oligomerization and viral replication^{17, 18}. Studies in Marburg VP35 (mVP35) also revealed similar behavior, where a NPBP peptide derived from mVP35 N-terminus controlled interactions between mNP and ssRNA¹⁹. Whereas the N-terminal NPBP and the C-terminal IID are structurally and functionally well-defined for eVP35 and mVP35, the roles of the intervening regions in VP35 are not well-understood.

Previous work showed that the N-terminal region of eVP35 likely contains a homo-oligomerization domain and that oligomerization may be critical for VP35-mediated functions, including immune evasion and viral replication^{20–22}. Studies of the analogous VP35 proteins in other NNSVs, the phosphoproteins (P), reveal that P forms a tetramer where the coiled-coil structures facilitate oligomerization by adopting either a parallel or antiparallel arrangement^{23–26}. These results suggest orientation of the oligomers likely plays a critical role in defining how viral replication is coordinated by these proteins along the RNA nucleocapsid. Although the recent structure of the Marburg VP35 N-terminus revealed a parallel orientation¹⁹, its trimeric arrangement is inconsistent with the data reported by

other studies^{23–27}. Altogether, these observations indicate that additional characterization of the Ebola VP35 N-terminal oligomerization domain is required.

Here we describe a multidisciplinary approach that includes multi-angle light scattering (MALS), small angle X-ray scattering (SAXS), mass-spectrometry-based crosslinking (XL-MS), and native mass spectrometry to characterize the eVP35 N-terminus to gain insight into the orientation and arrangement of a functionally critical element in VP35.

MATERIALS AND METHODS

Protein expression and purification.

All constructs were expressed in *E. coli* and purified by methods previously described. Briefly, constructs fused to maltose binding protein were expressed in BL21 (DE3) *Escherichia coli* cells from a modified pET15b vector (Novagen/EMD Millipore Headquarters, Bellerica, MA) for 14–16 h at 18 °C, and then centrifuged at 10,500 x *g* for 10 min. The pellet was resuspended in buffer containing 20 mM Tris, pH 8.0, 150 mM NaCl, 5 mM β -mercaptoethanol, and protease inhibitors and lysed with a cell homogenizer (Avestin, Ottawa, ON). The lysate was centrifuged at 47,000 x *g* for 40 min and then applied to a series of chromatographic columns. (GE Healthcare Bio-Sciences, Pittsburgh, PA). Final purity of samples was assessed by Coomassie Blue staining of SDS-PAGE.

Multi-angle light scattering.

Molecular weights of purified proteins (2 mg/mL) were assessed as they eluted (at 0.3 mL/min) from a 24 mL Superdex 200 10/300 GL (GE Healthcare, Chicago, IL) using a Dawn Helios II (Wyatt, Santa Barbara, CA) multi-angle light scattering detector coupled to an Optilab T-rEX (Wyatt, Santa Barbara, CA) refractometer; $dn/dc = 0.185$; Bovine serum albumin (BSA) was used as a standard reference for normalization

Native mass spectrometry.

The protein was concentrated to ~ 50 μ M, captured on a spin column comprised of a 30 kDa molecular weight cut off filter (Sartorius, AG, Goettingen, Germany) and then washed 6X with 500 μ L each of 200 mM ammonium acetate followed by centrifugation. An aliquot of 2 μ L was removed, diluted with 18 μ L of 200 mM ammonium acetate, and submitted to native MS with an Exactive Plus Extended Mass Range (EMR, Thermo Scientific) operated in the positive-ion mode with a capillary voltage of 1.5–1.8 kV and a capillary temperature of 100 °C. The in-source collision voltages were 200 V for desolvation. The spectra were signaled averaged from m/z 500 to 15,000 for time periods of 2–5 min.

Small angle X-ray scattering (SAXS).

Data were collected at the ALS SYBYLS beam line 12.3.1 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory by using protein samples at 2, 3, and 5 mg/mL of protein and matched buffers. Scattering data were collected at three exposures (0.5, 1, and 6 s). Data processing was performed using PRIMUS. Forward scattering $I(0)$ and the radius of gyration R_g were calculated from Guinier analysis. Maximum particle dimension D_{max} was determined using the pairwise distribution function $P(r)$. Molecular

weight was estimated using SAXS MoW2. Ab initio bead models of selected scattering curves were generated using the ATSAS software package. Each model was generated from 10 runs of DAMMIF using P1 symmetry, which were then averaged using DAMAVER.

Protein crosslinking and in-gel digestion.

A sample of cleaved VP35 (lacking MBP fusion) (50 μ g) and 1:1 molar ratio of VP35:LC8 samples were prepared in 20 mM HEPES, pH 8.3 to give a final protein concentration of 0.5–2.0 mg/mL. Freshly prepared BS³ homo-bifunctional crosslinker (available from www.creativemolecules.com) prepared as a 1:1 mixture of “light” and “heavy” (d_{12}) was added to the protein solution to give 1 mM final concentration of BS³. After addition of the crosslinker, the solution was vortexed and incubated for 45 min at 37 °C with mild shaking. The reaction was quenched by adding 1 M ammonium bicarbonate to give a final concentration of 50 mM, and then further incubated for 20 min at 37 °C. The reactants and solvent were evaporated in a vacuum centrifuge. SDS-PAGE was used to monitor, pre-concentrate, and separate the crosslinked products. Monomer, dimer, and tetramer bands from a reaction were excised, de-stained, alkylated, and digested, as described previously.²⁸ Briefly, acetonitrile was used for de-staining, and disulfide reduction and alkylation were done by adding 10 mM DTT and 55 mM iodoacetamide in 100 mM ammonium bicarbonate (in the dark). Gel bands were rehydrated at 4 °C, and a solution of trypsin was added to effect protein digestion (overnight at 37 °C), followed by chymotrypsin digestion. A solution of 0.1% formic acid was used to quench the reactions.

Mass spectrometry and database searching.

Digested samples were acidified with 0.1% formic acid prior to injection into a Thermo LTQ Orbitrap XL LC/MS/MS (Bremen, Germany) interfaced to an Eksigent NanoLC Ultra 2D Plus (AB Sciex, Dublin, CA). Samples were automatically loaded onto a C-18 trap column (Thermo Scientific Acclaim Pepmap 100, C-18, 0.1 mm x 2 cm, 5 μ m, 100 Å) and then eluted to a custom-packed reversed-phase C-18 analytical column (75 μ m x 15 cm, 5 μ m, 100 Å). A typical nanoLC gradient for the proteolytic peptides was 1.5–80% organic solvent over a period of 155 min, followed by 80% organic solvent for 20 min, and then a return of 80–1.5% organic solvent for the final 13 min for equilibrating the LC system. The flow from the column elution (260 nL/min) was sprayed by a custom-pulled tip emitter into the mass spectrometer with an ESI voltage of +2.6 kV. Data acquisition by the LTQ Orbitrap XL first used an FTMS analysis to produce a “survey” scan, followed by six data-dependent MS/MS scans for which six precursor ions (represented by the six most intense peaks under the “mass tag” option) were fragmented to obtain sequence information.²⁹ Precursor ions with charge states of greater than +1, corresponding peak intensities above 500 counts, and presenting as a “light/heavy” pair from the isotope encoding of the crosslinker were chosen. The mass range was 300–2000 m/z .

The MS raw files were processed into mzXML files using MassMatrix MS Data file conversion (Cleveland, OH). Data were searched with xQuest by using the following search parameters: mass measurement accuracy for precursor ions, 15 ppm; mass accuracy for product ions, 0.5 Da; enzyme, trypsin and chymotrypsin_lowspec (FWYML); number of missed cleavages allowed, 2; ion charge_common, 1, 2, 3; ion charge_xlink, 2, 3, 4, 5, 6; and

variable modification, oxidation of methionine. Target-decoy FASTA analysis was used to search for reverse peptide sequences, and the False Discovery Rate (FDR) was set to 5%. The sequences of VP35 and LC8 were manually input to the software. Results of crosslinked, mono-linked, and intra-linked products were also manually validated by insuring the presence of 1:1 intensity-ratio doublets due to deuterium encoding, ppm mass accuracy of precursor ions, and appropriate product-ion spectra (with suitable b- and y-ions).

RESULTS AND DISCUSSION

SEC-MALS and native mass spectrometry analysis show that MBP-VP35 constructs form different oligomeric states, with the predominant species being tetramer.

Previous sequence analysis of eVP35 by our group and others^{20, 21} have suggested that the N-terminus of eVP35 contains a coiled-coil domain, a structural motif involved in oligomerization, encompassed within residues 80–120 (Figure 1a). Further analysis of this sequence using the LOGICOIL algorithm³⁰ revealed a pattern of a heptad repeats (Fig. 1b and Supplementary Fig. 1); this repeat allows most coiled-coil residues at positions spaced at a and d to stabilize the interactions³⁰. In addition, LOGICOIL predicts that the likely oligomeric state of eVP35 is a tetramer (Supplementary Fig. 1).

To test this, we generated three constructs fused to maltose-binding protein (MBP) containing eVP35 residues: 20–150, 50–150 and 70–150. Each of these constructs was subsequently analyzed by using size-exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) to determine the approximate molecular weights of the corresponding proteins. Results from SEC-MALS show that all three eVP35 constructs, MBP-eVP35 20–150, MBP-eVP35 50–150, and MBP-eVP35 70–150, have polydispersity indices ranging from $1.001\text{--}1.003 \pm 0.015$, indicating that these samples are homogenous. Furthermore, all three constructs predominantly form tetramers (Fig. 1c), suggesting that residues 70–150 form a minimal oligomerization interface and that residues 20–50 are not likely to be involved in facilitating the interaction.

To determine the oligomeric state of the protein, we also conducted a native MS experiment. The results conclusively show that the protein exists primarily as a tetramer (yellow shading) with traces of smaller oligomers (blue, green, and orange shading) (Fig. 1d). Although the smaller oligomers may be formed by decomposition accompanying introduction to the mass spectrometer, the solution sample seems to contain these species because they persist at the lowest collision energy in the mass spectrometer and the SEC sometimes was resolved to show lower molecular weight species.

SAXS analysis suggests that the VP35 N-terminus forms an asymmetric multimer.

To obtain insight into the oligomeric nature of VP35, we used SAXS to determine the scattering patterns and behavior of VP35 in solution (Fig. 2; Table 1). Guinier analysis of the experimental scattering curves (Fig. 2; Supplementary Fig. 2) showed that the VP35 N-terminal oligomers, consisting of three different N-terminal truncations, displayed good solution behavior and had no indication of sample aggregation. As a further test, we

observed that the low- q region of the Guinier plot is linear in the range of $q \times R_g$ (radii of gyration) < 1.3 , confirming that there are no concentration-dependent effects, aggregation, or inter-particle interference in the samples. The R_g determined from the Guinier plot and the $P(r)$ are 52.3, 57.7, and 59.1 Å for MBP eVP35 70–150, MBP eVP35 50–150, and MBP eVP35 20–150, respectively. $D_{\max}$ values of 155.2, 184.5, and 205.6 Å were also calculated from the $P(r)$ distribution for each VP35 construct (Fig. 2), indicating that each of these MBP-tagged VP35 constructs form folded, asymmetrical tetramers (Fig. 2).

Ab initio modeling of refined SAXS data of VP35 constructs form envelopes expected for parallel arrangements.

We used DAMMIF and DAMMIN algorithms to generate *ab initio* models from the refined $P(r)$ distribution data generated. The two smaller protein envelopes (MBP-VP35 70–150 and MBP-VP35 50–150) were independently fit within the larger protein envelop (MBP-VP35 20–150) by using Situs SAXS docking software³¹. The models (Supplemental Fig. 3) indicate that both MBP-VP35 70–150 and MBP-VP35 50–150 bead models can be docked within one end of the MBP-VP35 20–150 surface model and that increasing the size of the VP35 construct results in differences only at one region of the envelop. This is consistent with a parallel arrangement where MBP molecules are clustered in the docked region of the envelopes with each larger construct extending from the opposite end. In an antiparallel arrangement, one would expect MBP clusters at two ends that would project away from each other in larger constructs.

Chemical crosslinking of VP35 and LC-MS/MS analysis demonstrate proximity of C-terminal polypeptides.

Crosslinking of proteins was introduced in the 1970s with analysis by gel electrophoresis and then significantly improved starting in the late 1980s with mass spectrometry analysis³². Since then, it has been widely used to identify neighboring proteins in complexes (for recent review, see³³). It accomplishes this by forming covalent bonds between two spatially proximate residues within a single and/or between two polypeptide chains to establish also orientation. Although crosslinking coupled with mass spectrometry is now used to locate protein-protein interactions, there are reports using it, and even fewer in combination with SAXS, to determine orientation of dimers^{34–40}.

We used our two longer VP35 constructs, 20–150 and 50–150, in crosslinking experiments to determine the proximal polypeptides within the oligomer. After crosslinking, we subjected the product mixture to SDS-PAGE to separate monomer, dimer, and tetramer populations and, after digestion in the gel, analyzed the corresponding bands by mass spectrometry (Fig. 3a).

To facilitate identification of crosslinked peptides and ensure that they arise from actual crosslinking, we used an isotopically encoded crosslinker (a mixture of “light” and “heavy”). The separations of 3.0185 m/z of +4-charged peptide (Fig. 3a and Table 1) from the deuterium replacements in crosslinkers (isotope encoding) serve to validate that the peptides contain the crosslinker. In the product-ion spectrum (Fig. 3b), we found appropriate b- and y- ions that localize the lysine residues that are linked (Fig. 3c shows a map of all the

crosslinked peptides at the C-terminus of the VP35 20–150 construct). We detected crosslinked peptides as +4 and +5 ions with the sequences ITSLENGLKPVYDMAK¹²⁶TISSLNR–ITSLENGLK¹¹⁹PVYDMAK, the +5 ion of ITSLENGLKPVYDMAK¹²⁶TISSLNR–ITSLENGLKPVYDMAK¹²⁶, the +5 ion of ITSLENGLK¹¹⁹PVYDMAKTISSLNR–ITSLENGLK¹¹⁹PVYDMAK, and the +2 ion of ITSLENGLK¹¹⁹PVYDMAKTISSLNR–ITSLENGLKPVYDMAK¹²⁶TISSLNR (Fig. 3c, green). These peptides are located near the C-terminal region of VP35 protein. We also found intra-linked peptides (Fig. 3c, red; residues 38–69 and 11–126) and mono-linked peptides (Fig. 3c, purple; residues 38–69, 111–126, and 111–133) with different charge states (both N- and C-terminal peptides). There are no crosslinked peptides detected involving residues 90–110 because there are no lysines in that region (the cross linker used is specific to K residues). The results show that crosslinking involves only C-to-C terminal linked peptides (Fig. 3d), thus identifying the arrangement of the tetramer. The absence of N-to-C terminal crosslinked peptides, even in a manual search, indicates that the arrangement of VP35 tetramer is parallel.

CONCLUSION

Oligomerization through the N-terminus appears to be critical for the roles played by VP35 in viral replication. Although previous biochemical and structural studies characterized the oligomerization region, the results are incompatible and inconclusive. In this study, using a series of eVP35 N-terminal constructs, we define the oligomeric state under physiological buffer conditions. Importantly, the observation of C-to-C and N-to-N crosslinked peptides in our mass spectrometry studies demonstrate that the N-terminal coiled-coil region of the eVP35 tetramer is likely arranged in a parallel orientation. The combination of high mass accuracy (± 5 ppm), isotopically encoded lysine-specific cross linkers, and product-ion spectra (appropriate b- and y- ions) permit confident identification of mono-linked, intra-linked, and crosslinked products. Additional support for the parallel orientation of the tetramer comes from the SAXS studies where the MBP fusion of the tetramer is oriented in one direction as well as SEC-MALS studies.

While this manuscript was in submission, the X-ray crystal structures of the Ebola and Reston VP35 oligomerization domains were published⁴¹. Based on the analyses presented in that manuscript, the structures revealed that the Ebola VP35 oligomerization region forms a parallel trimer via a coiled-coil motif, which dimerizes in an antiparallel arrangement with another trimer. In contrast, the same study revealed that the Reston VP35 oligomerization region forms a parallel tetramer. Consistent with these observations, SEC-MALS data revealed that the Ebola VP35 oligomerization region forms both tetramers and trimers in solution, which is consistent with the findings in our study. Further, VP35 oligomerization domains from Reston virus, Sudan virus, Tai Forest virus, and Bundibugyo virus also behave as tetramers in solution. Although it is not clear if these differences have direct functional consequences, the presence of various multimeric states of VP35 support the potential of viral proteins to act as context dependent regulatory elements or context independent regulatory elements during infections⁴². Nevertheless, a structure of the protein in vitro brings us a step closer to that in vivo.

Our study provides molecular insights into an oligomerization domain of VP35 from Ebola virus, a representative member of the *Filoviridae* family. Because analogous regions exist in P proteins in other NNSVs, it is likely that this aspect of the function is shared. Importantly, loss of oligomerization appears to have deleterious impact of the key functions of VP35, including lower IFN evasion activity and loss of RNA synthesis co-factor activity. Although the functional impact of the parallel/anti-parallel orientation of the VP35/P proteins in the NNSV family remains unclear and studies using life cycle modeling systems⁴³ such as the minigenome⁴⁴ or virus like particle (VLP) formation assays will likely be useful^{43, 45}, this study provides a framework for characterization. We expect that the use of hybrid methods, including XL-MS, SEC-MALS and SAXS, will play important roles in understanding VP35/P proteins and other oligomeric viral and host factors that are significant for viral pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Hydrodynamic analysis support a tetrameric structure of VP35 N-terminus.

(a) Schematic of the domain organization of Ebola VP35. (b) LOGICOIL prediction for the formation of coiled-coil regions. VP35 sequence and register of the heptad repeats are identified. (c) SEC-MALS analysis of MBP-VP35 fusions. The masses of MBP-VP35 70 to 150 (black) and MBP-VP35 20 to 150 (red) were determined to be $1.92 \pm 0.12 \times 10^5$ Da and $2.15 \pm 0.13 \times 10^5$ Da, respectively. Theoretical masses for tetramers are 2.11×10^5 Da and 2.34×10^5 Da, respectively. Experiments were carried out at least in duplicate and representative data are shown here. (d) Native mass spectrometry of VP35 (~5 mM in 200

mM ammonium acetate) showing the protein principally exists as tetramer (yellow) plus some smaller oligomers and monomer.

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Figure 2. Characterization of VP35 N-terminus by SAXS.

(a) Scattering profiles, (b) Guinier plots, and (c) $P(r)$ distributions of MBP eVP35 70–150 (light blue), MBP eVP35 50–150 (medium blue), and MBP eVP35 20–150 (dark blue), respectively, at 3 mg/mL. (d) *Ab initio* models of the above constructs generated by DAMMIF and DAMAVER.

Figure 3. Cross-linking MS studies identify parallel interactions in eVP35 oligomerization domain

(a) Workflow of chemical cross-linking combined with mass spectrometry experiments. (b) Precursor ion spectrum of C-terminal (111–133 VP35 + 111–126 VP35) crosslinked peptides from truncated VP35. The presence of doublet, due to deuterium labeling of BS³ cross linker, ensures the formation of crosslinked, loop-linked, or mono-linked peptides. (c) Product-ion spectrum of the same peptides with some labeled b- and y- ions. Ions labeled in red contain the BS³ cross linker. Ions labeled in blue are common ions without cross linker. (d) Chemical crosslinking map of truncated 20–150 VP35. Crosslinked, mono-linked and intra-linked peptides are labeled in green, purple and red, respectively. Nomenclature is that of Xquest.

Table 1.

List of identified cross-links, and mono-links from VP35 20–150.

<i>m/z</i>	Charge	Peptide structure
900.4811	5	¹¹¹ ITSLENGLKPVYDMA <u>K</u> TISSLNR ¹³³ – ¹¹¹ ITSLENGL <u>K</u> PVYDMAK ¹²⁶ -α126-β119
1121.3429	4	¹¹¹ ITSLENGLKPVYDMA <u>K</u> TISSLNR ¹³³ – ¹¹¹ ITSLENGL <u>K</u> PVYDMAK ¹²⁶ -α126-β119
1054.7641	5	¹¹¹ ITSLENGLKPVYDMA <u>K</u> TISSLNR ¹³³ – ¹¹¹ ITSLENGLKPVYDMA <u>K</u> TISSLNR ¹³³ -α126-β126
900.4759	5	¹¹¹ ITSLENGL <u>K</u> PVYDMAKTISSLNR ¹³³ – ¹¹¹ ITSLENGL <u>K</u> PVYDMAK ¹²⁶ -α119-β119
1054.7614	2	¹¹¹ ITSLENGL <u>K</u> PVYDMAKTISSLNR ¹³³ – ¹¹¹ ITSLENGLKPVYDMA <u>K</u> TISSLNR ¹³³ -α119-β126
1344.7136	2	¹¹¹ ITSLENGL <u>K</u> PVYDMA <u>K</u> TISSLNR ¹³³ -K ¹¹⁹ -K126
896.8149	3	¹¹¹ ITSLENGL <u>K</u> PVYDMA <u>K</u> TISSLNR ¹³³ -K119-K126
902.1450	3	¹¹¹ ITSLENGL <u>K</u> PVYDM(O) <u>A</u> <u>K</u> TISSLNR ¹³³ -K119-K126
969.7149	4	³⁸ IPVSDIFCDIENNPGLCYASQKQQT <u>K</u> PN <u>K</u> TR ⁶⁹ -K63-K67
908.1489	3	¹¹¹ ITSLENGLKPVYDMA <u>K</u> TISSLNR ¹³³ -K126
681.3618	4	¹¹¹ ITSLENGLKPVYDMA <u>K</u> TISSLNR ¹³³ -K126
974.2164	4	³⁸ IPVSDIFCDIENNPGLCYASQKQQT <u>K</u> PN <u>K</u> TR ⁶⁹ -K67
908.1489	3	¹¹¹ ITSLENGL <u>K</u> PVYDMAKTISSLNR ¹³³ -K119
681.3642	4	¹¹¹ ITSLENGL <u>K</u> PVYDMAKTISSLNR ¹³³ -K119
976.0057	3	¹¹¹ ITSLENGL <u>K</u> PVYDMAK ¹²⁶ -K119