Impact of the N-terminal Finger Domain on the Activity of 3CL-M^{Pro} of SARS-CoV-2

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INTRODUCTION

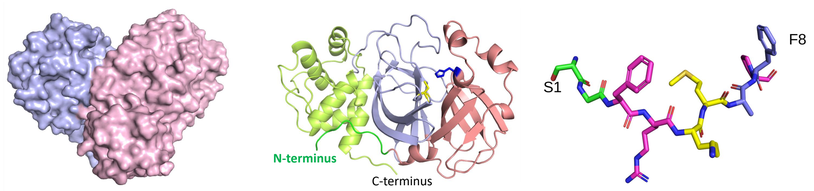
The main protease (3CL-M^{pro}) of SARS-CoV-2 is essential for its viral life cycle by cleaving 12 out of 15 processing sites of the non-structural polyprotein ORF1a,b. It was previously found that 3CL-M^{pro} is enzymatically active in dimeric form and that its stability and activity appears to be ensured by the N-terminal finger domain of the monomers. However, the precise effect of the N-finger domain on the enzyme activity and dimerization remains unclear.

METHODS

Various 3CL-M^{pro} N-finger mutants (1-, 2-, 4-, 6-amino acid deletions and 1-amino acid extension) were expressed and their enzymatic activities, dimerization potentials, and selected 3-D structures were characterized.

RESULTS

1. WT 3CL-M^{pro} and its N-terminal finger domain



3CL-M^{Pro}dimer | 3CL-M^{Pro}monomer | N-finger Domain

2. 3CL-M^{pro} N-finger mutants

1-Add 3CL-M^{pro}

NH_2-ASGFRKMAF	3CL-M^{pro}	GGG-Linker	3X-Flag	Myc-tag	His-tag

WT 3CL-M^{pro}

NH_2-SGFRKMAF	3CL-M^{pro}	GGG-Linker	3X-Flag	Myc-tag	His-tag

1-Del 3CL-M^{pro}

NH_2-GFRKMAF	3CL-M^{pro}	GGG-Linker	3X-Flag	Myc-tag	His-tag

2-Del 3CL-M^{pro}

NH_2-FRKMAF	3CL-M^{pro}	GGG-Linker	3X-Flag	Myc-tag	His-tag

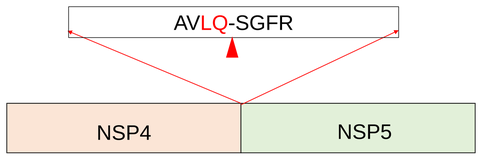
4-Del 3CL-M^{pro}

NH_2-KMAF	3CL-M^{pro}	GGG-Linker	3X-Flag	Myc-tag	His-tag

6-Del 3CL-M^{pro}

NH_2-AF	3CL-M^{pro}	GGG-Linker	3X-Flag	Myc-tag	His-tag

3. Kinetic Analysis of WT-3CL-M^{pro} & N-finger mutants using substrate representing the cleavage site between NSP4 and NSP5 (3CL-M^{pro}) from the replicon polyprotein



Michaelis-Menten kinetic analysis of WT-3CL-M^{pro} variants using a NSP4-NSP5 cleavage site substrate

Enzyme	K_m µM	k_{cat} s^{-1}	k_{cat} / K_m s^{-1} M^{-1}
MCA-AVLQ-SGFR{Lys(Dnp)}RR			
WT-3CL- M^{pro}	15.2 ± 3	0.6 ± 0.05	54,464 ± 9,286
1-Add-3CL- M^{pro}	51 ± 7	0.04 ± 0.005	952 ± 111
1-Del-3CL- M^{pro}	62 ± 10	0.1 ± 0.01	1,799 ± 394
2-Del-3CL- M^{pro}	56 ± 5	0.06 ± 0.01	1,108 ± 239
4-Del-3CL- M^{pro}	N/A	N/A	No activity
6-Del-3CL- M^{pro}	N/A	N/A	No activity

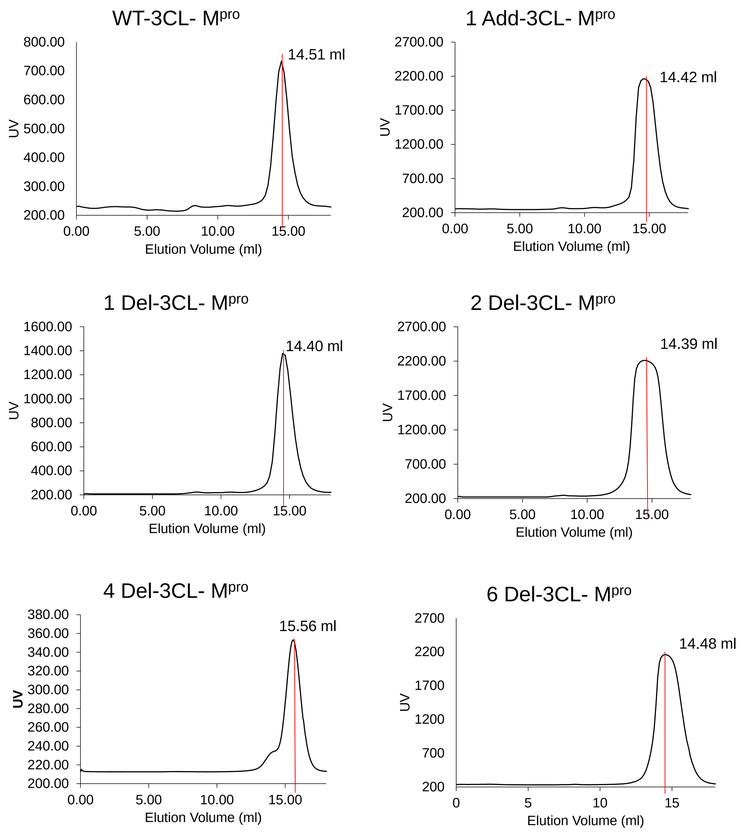
For 1- and 2-amino acid deletin or one amino acid extension N-finger variants:

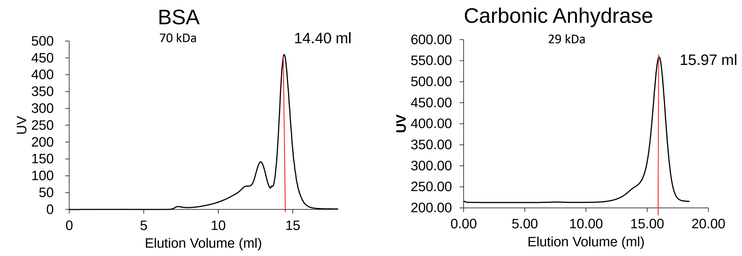
- K_m values increased 3- to 4-fold compared to WT enzyme.
- k_{cat} values decreased 6- to 10-fold compared to WT enzyme.
- k_{cat}/K_m decreased more than 95% compared to WT enzyme.

4- and 6-amino acid deletion variants were inactive

Analysis was done in triplicates.

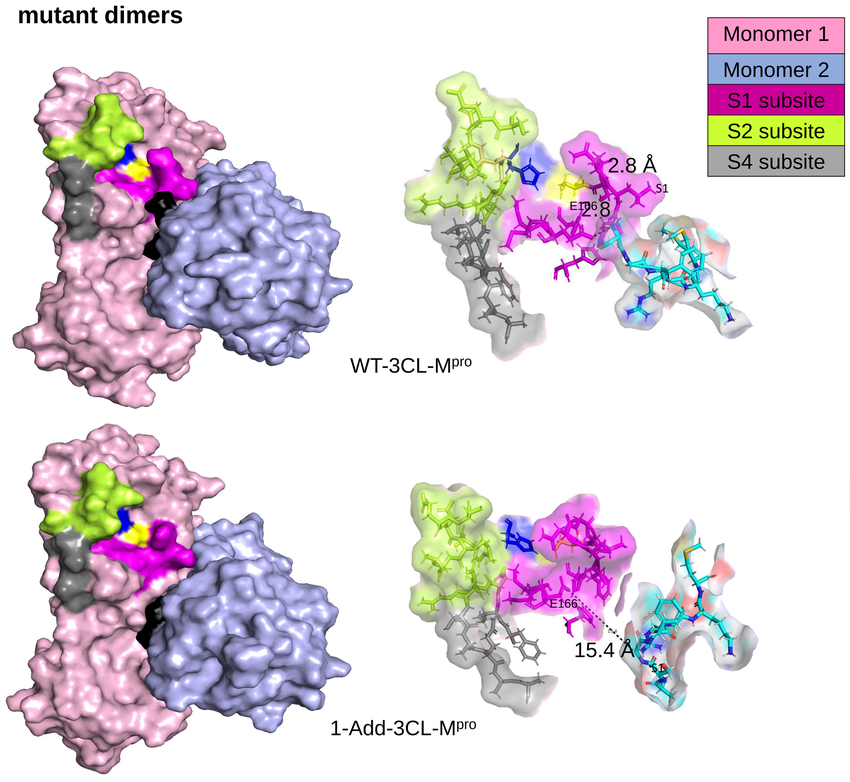
4. SEC profile of WT-3CL-M^{pro} & N-finger mutants

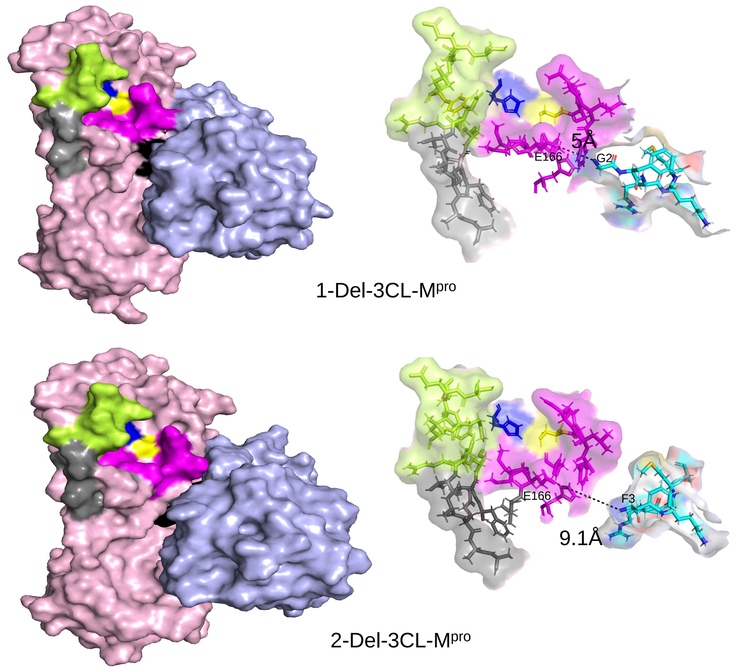




- There were no differences in the elution volume between the WT & N-finger mutants 1-, 2-, 6- Del 3CL-M^{pro} as well as the 1-Add variant, suggesting no effect on the dimerization status.
- For 4-Del 3CL-M^{pro}, the elution volume shifted 1 ml (15.56 ml), compared to WT elution volume (14.51 ml), indicating the presence of monomer conformation.

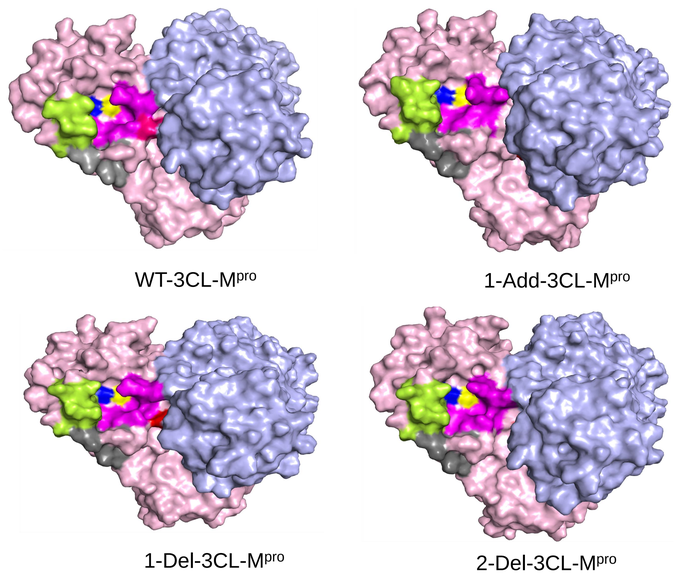
5. Localization of N-termini in Crystal Structure of WT 3CL-M^{pro} & N-finger mutant dimers





- For wild type, N-termini of monomer 2 interact with E166 & F140 of monomer 1 to make a proper active site cleft. Distance between S1 & E166 is **2.8 Å.**
- For 1-Add 3CL-M^{pro} , N-termini of monomer 2 and the E166 of monomer 1 bend away from each other. their distance become **15 Å.**
- For 1- & 2-Del 3CL-M^{pro} altough N-termini & E166 face each other but distance between them got increase to **5 & 9.1 Å**, respectively.
- Modifications of the N-terminus significantly alters the binding site geometry at the S1 site of 3CL-M^{pro} which may prevent the cleavage of the Q-S substrate bond

6. Impact of N-termini on S2 subsite in Crystal Structure of WT 3CL-M^{pro} & N-finger mutant dimers



- N-termini has distal impact on the S2 subsite as well.
- For WT, distance between S46 of S2 subsite & N142 of S1 subunit is 7.2 Å. For 1 Add-3Cl-M^{pro} it becomes 9 Å. For 1 & 2 -3Cl-M^{pro} that distance reduced to 6.9 & 6.8 Å respectively.
- For WT, distance between L50 & Q189 of S2 subsite is 3.1 Å. For 1 Add-3Cl-M^{pro} it becomes 3.6 Å. For 1 & 2 -3Cl-M^{pro} that distance reduced to 3.7 & 4 Å respectively.

CONCLUSIONS

- Deletions of up to 2 amino acids and the extension by 1 amino acid resulted in the loss of more than 95% of the activity, whereas 4 and 6 amino acid deletion resulted in the almost complete loss of the protease activity.
- With the exceptions of the 4-Del 3CL-M^{pro} variant, all other mutant proteins remained as dimers as shown by gel filtration.
- Structural analysis of the 1- and 2-amino acid deletions and the 1-amino acid extension variants showed that the dimer is formed through the same N-terminal-domain interactions as that in the wild type enzyme but significantly alters the S1 subsite

ACKNOWLEDGEMENTS

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