Structural prediction of ensitrelvir-resistant mutants of SARS-CoV-2 main protease

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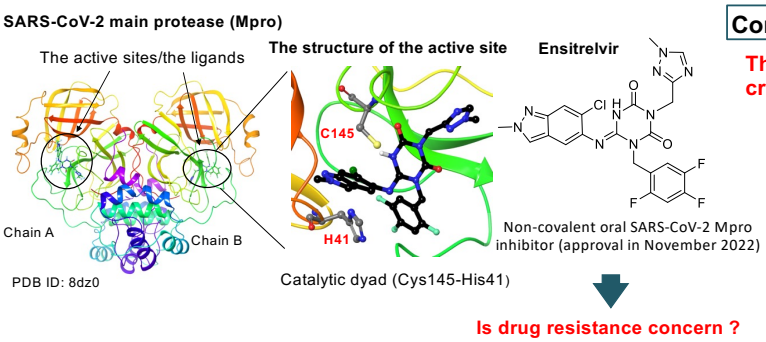
INTRODUCTION



Is drug resistance concern ?

Drug resistance occurs due to single amino acid substitution. Studying effects of amino acid mutations in the conformation of drug target is necessary for anticipating the mechanism underlying drug resistance. Several mutant showing resistance to ensitrelvir have been reported experimentally, but their 3D structures remain unknown, except for M49I (PDB ID: 8dz1).

AIMS

In this study, we predicted the 3D structures of experimentally reported ensitrelvir-resistant mutants using molecular dynamics (MD) simulations and analyzed the mechanisms responsible for the emergence of ensitrelvir resistance.

MATERIALS & METHODS

Setup

- Force field : AMBER ff14SB (for protein)+GAFF2 (for ligand), solvent : TIP3P, NPT (1 bar, 300 K), SHAKE algorithm (for bonds involving hydrogen), periodic boundary conditions with particle mesh Ewald method

Calculations

- MD simulation was performed for the initial structure based on the crystal structure (PDB ID: 8dz0) to construct the wild-type Mpro–inhibitor complex.
- Mutations were introduced into both chains A and B of the wild-type structure, and 100 ns MD simulations were performed to construct mutant Mpro–inhibitor complexes.

Analysis

- The final 10 ns trajectories were used for analysis.
- The extent of structural deviation of the main chain, ligand, and catalytic dyad from the wild type was evaluated using root mean square deviation (RMSD) values.

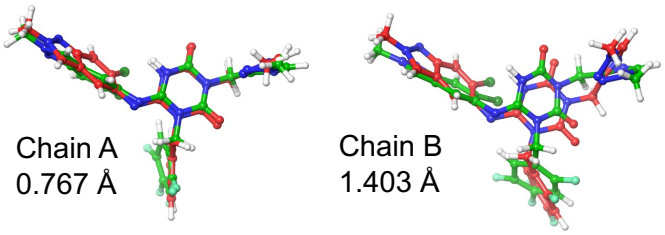
REFERENCE

Unoh Y., et al., *J. Med. Chem.*, **65**, 6499–6512 (2022); Ip J. D., et al., *EBioMedicine*, **91**, 104559 (2023); Noske G. D., et al., *J. Biol. Chem.*, ***299***, 103004 (2023); Mizuno A, et al. *Biol. Pharm. Bull.*, **47**, 967-977 (2024).

RESULTS

Comparison of the artificial mutant with the crystal structure

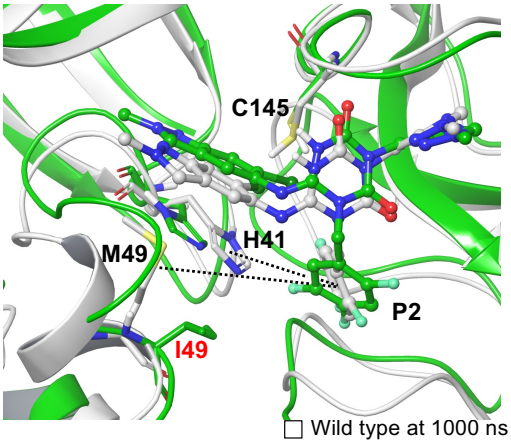
The ligand position in the artificial M49I was similar to that in the crystal structure of M49I (PDB ID: 8dz1).



Ligand of crystal structure M49I (PDB ID: 8dz1)
Ligand of artificial mutant M49I at 100 ns

We considered the methods used in this study to be appropriate.

Comparison of the artificial mutant with the wild type



Wild type at 1000 ns
Artificial mutant M49I at 100 ns

The distance from the side chain of residue 49 to the ring of **P2**: 7.92 Å→5.01 Å

The distance from the center of mass of imidazole group of H41 to the ring of **P2**: 5.13 Å→6.67 Å

In the mutant, the ligand was shifted toward residue 48.

RMSD

	Main Chain	Ligand		Catalytic dyad	
		Chain A	Chain B	Chain A	Chain B
M49I	1.253	1.463	1.536	1.307	2.245

Compared to the RMSD of the ligand, the RMSD of the main chain was small, suggesting that the enzymatic activity is maintained.

CONCLUSIONS

The M49I mutant generated by the method in this study showed a ligand shift similar to that observed in the crystal structure. By analyzing the artificially generated M49I mutant, the mechanism underlying drug resistance was investigated. **Our results suggest that this approach can be applied to analyze the mechanisms of drug resistance in other mutants that have been experimentally confirmed to confer resistance but whose 3D structures remain unknown.**