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Exploring the binding mechanism of the main proteinase in SARS-associated coronavirus and its implication to anti-SARS drug design

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Abstract—The main proteinase of SARS-associated coronavirus (SARS-CoV) plays an important role in viral transcription and replication, and is an attractive target for anti-SARS drug development. The important thing is to understand its binding mechanism with possible ligands. In this study, we investigated possible noncanonical interactions, potential inhibitors, and binding pockets in the main proteinase of SARS-CoV based on its recently determined crystal structure. These findings provide a wide clue to searching for anti-SARS drug. Interestingly, we found that similar structure patterns exist in SARS-CoV main proteinase with Poliovirus 3c Proteinase, Rhinovirus 3c Protease, Nsp4 Proteinase From Equine Arteritis Virus, Hepatitis C Virus Ns3 Protease, Hepatitis A Virus 3c Protease, and Dengue Virus Ns3 Protease. It suggests that the available drugs in these viruses could be used to fight SARS disease.

Keywords: SARS-CoV; Main proteinase; Noncanonical interactions; Inhibitor; Binding.

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3. Results and discussion

The noncanonical interactions in SARS-CoV main proteinase structures are shown in Figure 1. There are two pairs of main-chain-side chain interactions: Glu288 (donor) and Trp207 (acceptor), Ile152 (donor) and Phe8 (acceptor). There are four pairs of side chain–side chain interactions: Arg40 (donor) and Tyr54 (acceptor), Arg298 (donor) and Phe8 (acceptor), Pro184 (donor) and Phe185 (acceptor), and Tyr126 (donor) and Phe140 (acceptor). These residues are marked as @ in Figure 2.

Among these interactions, Phe8 accepts two N–H···π bonds in a sandwich fashion, one donated by a side-side chain Arg298, and one donated by a main-side chain Ile152, as existed in human rac1.8 Taken together with another N–H···π interaction between Glu288 and Trp207, these noncanonical bindings connected N-terminus and C-terminus of the enzyme together, then fixed to Ile152 (domain II) and Trp207 (domain III), this makes the domain II and III not flexible due to the loop formed by above interactions in the right and a loop already existed in the left (Fig. 1A), that is stabilizes the structure of the protease. Furthermore, we turn to examine the remaining three pairs of interactions: Arg40 and Tyr54, Pro184 and Phe185, and Tyr126 and Phe140. It can be seen from Figure 1B that these noncanonical bindings will be able to stabilize the protein structure around the active center formed by Cys-His catalytic dyad (Cys145 and His41). On the other hand, after binding, the small helix Arg40 and His41 locate will expect to move up, the loop Phe140 and Cys145 locate and the loop Pro184 and Phe185 locate will expect to move down, hence make the active center more open for substrate binding (Fig. 1B). These results can be used for rational design of mutagenesis experiments and analysis of conservation of interactions at functional sites. In recent years, the noncanonical interactions have been shown to be important for the stability of protein structure9–11 and ligand recognition.12

The structurally similar nonhomologous virus proteins identified by VAST and DALI methods focus on 6 proteases: 1CQQ (Rhinovirus 3c Protease With Ag7088 Inhibitor), 1L1N (Poliovirus 3c Proteinase), 1MBM (Nsp4 Proteinase From Equine Arteritis Virus), 1DY8 (Hepatitis C Virus Ns3 Protease with inhibitor FKI), 1QA7 (Hepatitis A Virus 3c Protease with inhibitor NFA) and 1DF9 (Dengue Virus Ns3-Protease Complexed With Mung-Bean Bowman–Birk Inhibitor). The structure-based sequence alignment of the above six proteases with SARS-CoV main protease is shown in Figure 2. The results demonstrate that a number of similar beta sheets (marked by b) exist. In particular, there are three similar structural patterns showing conservative residues (bold representation). The catalytical important residues are included in the first two highly conservative patterns: His-Cys or His-Ser catalytic dyads.2,13–18 In the third pattern, Gly is conservative in 6 proteases except SARS-CoV main protease, the significance of mutation from Gly161 to Tyr161 in SARS is unclear. Anyway, the three structural patterns form the common active center of these seven enzymes, as seen in their superpositions (Fig. 3). Since these nonhomologous virus proteases share the same active center, we can consider the possibility of applying the inhibitors from these enzymes to SARS-CoV main proteinase. In fact, Anand et al.3 has indicated that the inhibitor AG7088 may be modified to make it useful for SARS therapy. Chou et al.7
conducted the docking studies of KZ7088 (a derivative of AG7088) to the SARS-CoV main proteinase (based on a theoretical model). Here, we studied the possibility of other inhibitors in anti-SARS drug development.

Figure 4 shows the structures of docking other inhibitors from HAV, HCV and Dengue virus to SARS-CoV main proteinase, the residues that make contact with these inhibitors are marked as /C212[C213 in Figure 2. Furthermore, the corresponding binding pockets are shown in Table 1. The results reveal that these inhibitors can bind to SARS-CoV main proteinase well.

Thus, the results above suggest that there is a link of SARS-CoV to human rhinovirus, Poliovirus, Arteritis virus, HAV, HCV, and Dengue virus. The inhibitors from these viruses could be modified to make them useful for SARS therapy. In fact, Cinatl et al.19 reported that human interferons, a medicine widely used in HCV patients, was useful for the treatment of SARS. Anyway, whether relationships between SARS-CoV and these pathogens exist or not, the present results provide a wide clue to search for anti-SARS inhibitors.
References and notes

1. Bonanno, J. B.; Fowler, R.; Gupta, S.; hendle, J.; Lorimer, D.; Romero, R.; Sauder, M.; Wei, C. L.; Liu, E. T.; Burley, S. K.; Harris, T. X-ray crystal structure of the SARS coronavirus main protease. (in press) (http://www.rcsb.org/pdb/cgi/explore.cgi?pdbId=1Q2W).

2. Yang, H.; Yang, M.; Ding, Y.; Liu, Y.; Lou, Z.; Zhou, Z.; Sun, L.; Mo, L.; ye, S.; Pang, H.; Gao, G.; Anand, K.; Bartlam, M.; Hilgenfeld, R.; Rao, Z. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. Proc. Natl. Acad. Sci. U.S.A. 2003, 100, 13190–13195.

3. Anand, K.; Ziebuhr, J.; Wadhwani, P.; Mesters, J. R.; Hilgenfeld, R. Coronavirus main proteinase (3CL pro) structure: basis for design of anti-SARS drugs. Science 2003, 300, 1763–1767.

4. Babu, M. M. NCI: a server to identify non-canonical interactions in protein structures. Nucleic Acids Res. 2003, 31, 3345–3348.

5. Shindyalov, I. N.; Bourne, P. E. Protein structure alignment by incremental combinatorial extension (CE) of the optimal path. Protein Eng. 1998, 11, 739–747.

6. Zemla, A. LGA: a method for finding 3D similarities in protein structures. Nucleic Acids Res. 2003, 31, 3370–3374.

7. Chou, K.; Wei, D.; Zhong, W. Binding mechanism of coronavirus main protease with ligands and its implica-

Table 1. Binding pockets of SARS-CoV main proteinase for different inhibitors

| FKI inhibitor from HCV | NFA inhibitor from HAV | Bowman–Birk inhibitor from Dengue virus |
|------------------------|------------------------|----------------------------------------|
| VAL 20 MET 17 GLN 19 PHE 140 |
| THR 25 VAL 18 VAL 20 LEU 141 |
| THR 26 GLN 19 THR 21 ASN 142 |
| LEU 27 VAL 20 CYS 22 GLY 143 |
| ASN 28 THR 21 GLY 23 SER 144 |
| PRO 39 THR 24 THR 24 CYS 145 |
| ARG 40 THR 25 THR 25 GLY 146 |
| HIS 41 THR 26 HR 26 SER 147 |
| VAL 42 LEU 27 LEU 27 TYR 161 |
| CYS 44 ASN 28 ASN 28 MET 162 |
| CYS 117 GLY 29 PRO 39 HIS 163 |
| TYR 118 HIS 41 ARG 40 HIS 164 |
| ASN 119 VAL 42 HIS 41 MET 165 |
| SER 139 GLN 69 VAL 42 GLU 166 |
| PHE 140 ALA 116 ILE 43 LEU 167 |
| LEU 141 CYS 117 CYS 44 PRO 168 |
| ASN 142 TYR 118 MET 49 THR 169 |
| GLY 143 ASN 119 LEU 50 GLY 170 |
| SER 144 GLY 120 ASN 51 VAL 171 |
| CYS 145 SER 121 PRO 52 HIS 172 |
| GLY 146 PRO 122 ASN 53 ALA 173 |
| SER 147 LEU 141 TYR 54 GLY 174 |
| TYR 161 ASN 142 LEU 57 PHE 181 |
| HIS 163 GLY 143 VAL 114 PRO 184 |
| HIS 164 SER 144 CYS 117 PHE 185 |
| MET 165 CYS 145 TYR 118 VAL 186 |
| GLU 166 GLY 146 ASN 119 ASP 187 |
| LEU 167 SER 147 TYR 126 ARG 188 |
| VAL 171 THR 135 GLN 189 |
| HIS 172 ILE 136 THR 190 |
| ALA 173 LYS 137 ALA 191 |
| PHE 181 GLY 138 GLN 192 |
| ASP 187 SER 139 ALA 193 |

Figure 4. The interactions between SARS-CoV main protease (1Q2W, white cartoon) with different inhibitors, binding pockets are represented by blue ball-stick. (A) FKI inhibitor from Hepatitis C Virus Ns3 Protease (yellow spacefill). (B) NFA inhibitor from Hepatitis A Virus 3c Protease (yellow spacefill). (C) Bowman–Birk inhibitor from Dengue Virus Ns3-Protease (yellow stick).
tion to drug design against SARS. *Biochem. Biophys. Res. Commun.* **2003**, *308*, 148–151.

8. Hirshberg, M.; Stockley, R. W.; Dodson, G.; Webb, M. R. The crystal structure of human rac1, a member of the rho-family complexed with a GTP analogue. *Nat. Struct. Biol.* **1997**, *4*, 147–152.

9. Fabiola, G. F.; Krishnaswamy, S.; Nagarajan, V.; Pattabhi, V. C–H···O hydrogen bonds in beta sheets. *Acta Crystallogr., Sect. D.* **1997**, *53*, 316–320.

10. Senes, A.; Ubarretxena-Belandia, I.; Engelman, D. M. The C–H···O hydrogen bond: a determinant of stability and specificity in transmembrane helix interactions. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9056–9061.

11. Babu, M. M.; Singh, S.; Balaram, P. A C–H···O hydrogen bond stabilized polypeptide chain reversal motif at the C terminus of helices in proteins. *J. Mol. Biol.* **2002**, *322*, 871–880.

12. Kryger, G.; Silman, I.; Sussman, J. L. Structure of acetylcholinesterase complexed with E2020: implications for the design of new anti-alzheimer drugs. *Structure** **1999**, *7*, 297–307.

13. Barrette-Ng, I. H.; Ng, K. K.; Mark, B. L.; Van Aken, D.; Cherney, M. M.; Garen, C.; Kolodenko, Y.; Gorbalenya, A. E.; Snijder, E. J.; James, M. N. Structure of arteriviral nsP4. The smallest chymotrypsin-like protease with an alpha/beta C-terminal extension and alternate conformations of the oxyanion hole. *J. Biol. Chem.* **2002**, *277*(42), 39960–39966.

14. Bergmann, E. M.; Cherney, M. M.; McKendrick, J.; Fromann, S.; Luo, C.; Malcolm, B. A.; Vederas, J. C.; James, M. N. Crystal structure of an inhibitor complex of the 3C protease from hepatitis A virus (HAV) and implications for the polyprotein processing in HAV. *Virology* **1999**, *265*(1), 153–163.

15. Di Marco, S.; Rizzi, M.; Volpari, C.; Walsh, M. A.; Narjes, F.; Colarusso, S.; De Francesco, R.; Matassa, V. G.; Sollazzo, M. Inhibition of the hepatitis C virus NS3/4A protease. The crystal structures of two protease-inhibitor complexes. *J. Biol. Chem.* **2000**, *275*(10), 7152–7157.

16. Mosimann, S. C.; Cherney, M. M.; Sia, S.; Plotch, S.; James, M. N. Refined X-ray crystallographic structure of the poliovirus 3C gene product. *J. Mol. Biol.* **1997**, *273*(5), 1032–1047.

17. Murthy, H. M.; Judge, K.; DeLucas, L.; Padmanabhan, R. Crystal structure of Dengue virus NS3 protease in complex with a Bowman–Birk inhibitor: implications for flaviviral polyprotein processing and drug design. *J. Mol. Biol.* **2000**, *301*(4), 759–767.

18. Matthews, D. A.; Dragovich, P. S.; Webber, S. E.; Fuhrman, S. A.; Patick, A. K.; Zalman, L. S.; Hendrickson, T. F.; Love, R. A.; Prins, T. J.; Marakovits, J. T.; Zhou, R.; Tikhe, J.; Ford, C. E.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Brothers, M. A.; DeLisle, D. M.; Worland, S. T. Structure-assisted design of mechanism-based irreversible inhibitors of human rhinovirus 3C protease with potent antiviral activity against multiple rhinovirus serotypes. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*(20), 11000–11007.

19. Cinatl, J.; Morgenstern, B.; Chandra, P.; Rabenau, H.; Doerr, H. W. Treatment of SARS with human interferons. *The Lancet* **2003**, *362*, 293–294.