Molecular Modeling Studies of Peptide Drug Candidates against SARS

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Abstract: Flexible alignment and docking studies were conducted for the three octapeptides, ATLQANEV, AVLQSGFR, and ATLQAIAS, that were cleavable by SARS-CoV M^{pro}. It has been observed that all pharmacophores of the three peptides overlap very well, and that ATLQANEV binds best with the receptor, followed by AVLQSGFR, and then ATLQAIAS. During the process of docking the octapeptides to the SARS enzyme, the residues of the catalytic dyad, i.e., His-41 and Cys-145 are actively involved in forming the hydrogen bonds, so is the center residue (Gln) of all the three octapeptides. The findings are fully consistent with experimental observations. The present studies suggest that the octapeptides ATLQANEV and ATLQAIAS, like AVLQSGFR, might also be the good starting points for designing potential drugs against SARS.

Key Words: SARS, CoV M^{pro}, protease inhibitor, cleavable peptides, autodock, distorted key theory, flexible alignment, binding free energy.

I. INTRODUCTION

The recently found virus, called SARS-coronavirus, is the leading hypothesis for the cause of SARS (Severe Acute Respiratory Syndrome) [1,2]. It is also known that the process of cleaving the SARS-CoV polyproteins by a special proteinase, the so-called SARS-coronavirus main proteinase (CoV M^{pro}), is the key step for the transcription and replication of SARS-CoV [3-7]. The functional importance of the M^{pro} in the viral life cycle has made it an attractive target in developing drugs for SARS therapy [3,4,7]. Anand *et al*. [4] suggested that the rhinovirus M^{pro} inhibitor AG7088 might serve as a starting point for anti-SARS drug based on the theoretical homology model of SARS CoV M^{pro}. Chou *et al*. [3,7] found the fitting problem of AG7088 to the binding pocket of SARS CoV M^{pro}, and they suggested its derivative KZ7088 as a better starting point.

Since peptide drugs are of low toxicity to human body than organic compounds, many efforts have been made to develop peptide inhibitors against the M^{pro}. Chou *et al*. [3,7] suggested the octapeptide, NH2-AVLQSGFR-COOH, as an efficient inhibitor based on the knowledge of catalytic site of the protease and the "distorted key theory" [8,9]. Du *et al*. [6] carried out a bioinformatics analysis of the M^{pro} substrate specificity and polyprotein cleavage sites, and confirmed that the aforementioned peptide proposed by Chou [3,7] was truly a good starting point for drug deign against SARS. Subsequently, two other octapeptides were found that have higher statistical probabilities on the specific cleavage positions of SARS-CoV M^{pro} [10]. These two octapeptides, NH2-ATLQAIAS-COOH and NH2-ATLQANEV-COOH, might be two of the best candidates for inhibiting the SARS enzyme. Meanwhile, the AVLQSGFR originally proposed by Chou *et al*. [3] was synthesized and its antiviral potential against SARS coronavirus was detected [11]. The results indicate that, compared with other compounds reported so far, AVLQSGFR is the most active in inhibiting replication of the SARS coronavirus [EC_{50} =2.7 x 10^{-2} (mg/L)], and that no detectable toxicity is observed on Vero cells under the condition of experimental concentration. Stimulated by the encouraging results, the present study was initiated in an attempt to further study the other two peptides [10], particularly their binding potentials with the active site of the protease.

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II. MATERIALS AND METHODS

Knowledge derived from structure and kinetics modeling, as well as bioinformatics and various theoretical approaches, can timely provide many useful insights, direct or indirect, for drug discovery, significantly expediting its process or stimulating novel strategies and approaches (see, e.g., [3,6,12-71] as well as some recent comprehensive reviews [7,72]). Here we would like to apply the technique of computer modeling and docking technique to find peptide drug candidates for SARS therapy.

1. Alignment of the Three Octapeptides

To approach the problem, let us first make an alignment of the two octapeptides [10] with the original one [3]. The purpose of the alignment is to determine what common features they bear. This can be realized by aligning the three peptides and maximizing their overlap over a set of pre-determined features. Two peptides are considered to have a good alignment if they both have a small strain energy and they have a good match in a series of key features, such as aromatic groups, hydrogen bond donors and acceptors, acidic groups and basic groups, logP(octanol/water) values, as well as geometric shape.

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To realize this, a program was developed to compute a collection of alignments. Each alignment was given a score to quantify the quality of the alignment in terms of both internal strain and overlap of molecular features. Such alignments of putative ligands can be used to deduce structural requirements for biological activity. Furthermore, by adapting the strategy of pharmacophore elucidation, several ligands were aligned and a collection of essential molecular features required for biological activity was derived from the alignment [73-75].

2. Docking Operation

Autodock [73,76,77] was the main software employed to make the docking calculations. The SARS M^{pro} was downloaded from RCSB (http:// www.rcsb.org/) with PDB code 1UJ1. The docking search algorithm used here was the Metropolis method, also known as Monte Carlo simulated annealing. The principle can be briefed as follows. With the protein being stationary throughout the simulation process, the substrate molecule underwent a random walk in the space around the protein. At each step in the simulation, a small random displacement was applied to each of the degrees of freedom of the substrate: translation of its center of gravity; change of orientation; and rotation around each of its flexible internal dihedral angles. The displacement resulted in a new configuration, whose energy was evaluated using the grid interpolation procedure described above. The new energy thus obtained was compared to the energy of the preceding step. If the new energy was lower, the new configuration was immediately accepted; otherwise, the configuration would be accepted or rejected based upon a probability expression depending on the temperature *T* defined by a user. If the temperature was sufficiently high, almost all steps would be accepted. For the case of low temperatures, however, very few steps of higher energy would be accepted. For a detailed description about the simulated annealing approach, see, e.g., [78,79].

The substrate or ligand started moving from its initial position and state, i.e., the energy-minimized conformation. The potential in the docking box was pre-calculated using the grid method. The number of runs was set at 200 in order to explore configuration space large enough for finding the global minimum. After operating the docking by using the grid-based method in which potential energy did not include the solvation term, we re-conducted the docking with the approach that incorporated the solvation term for energy calculation. The latter was much more time-consuming but the results thus obtained would be closer to the real condition where the binding reaction took place. For this procedure the total number of runs was set at 50.

In the grid generation procedure, N-H, O-H and S-H hydrogen bonds were modeled by choosing the proper 12-10 Lennard-Jones parameters in the frame work of Amber. The grid box was centered at the catalytic active region between domain I and II of the M^{pro} according to [3,5]. The spacing between grid points was 0.375 Å. During the docking process, the GA-LS search algorithm (Larmarckian Genetic Algorithm) [50] was chosen to search for the best conformers. A maximum of 200 conformers was considered for each octapeptide. As many as 800 generations were implemented so as to make the energy converge to a global minimum. The ligand was translated 1 Å per step in the box and the rotable bonds were rotated 10 deg per step. After docking the peptides through the aforementioned method, each of them was inverted in orientation, followed by re-docking with Tabu Search [80,81] without grid. The docking computation was performed on the Sun Enterprise 10000 Server with 16 CPUs and 64GB memory.

III. RESULTS AND DISCUSSIONS

1. Alignment of Three Octapeptides

As shown in Fig. **1a**, the three peptides are indeed very similar, especially for the view when they are colored according to the pharmacophores (Fig. **1b**). Virtually all the pharmacophores are nicely aligned with each other, the hydrophobic side chains also have a good match. It has been shown [3,11] that AVLQSGFR is an effective inhibitor against SARS enzyme, implying that the other two might be the same, as will be further confirmed by the docking studies below.

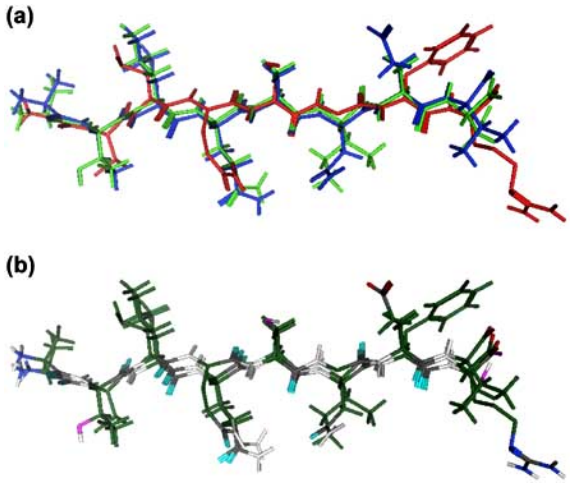

Fig. (1). Alignment of three octapeptides: (a) colored according to different peptides, where red, green and blue represents AVLQSGFR, ATLQAIAS and ATLQANEV, respectively; (b) colored according to different pharmacophores, where purple, turquoise, blue, red, and green represent hydrogen bond donors, hydrogen bond acceptors, cations (excess positive charge), anions (excess negative charge), and hydrophobic areas/aromatic centers, respectively.
(For Interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

2. Docking Results

For convenience, hereafter we use OP, OP1 and OP2 to denote NH2-AVLQSGFR-COOH, NH2-ATLQAIAS-COOH and NH2-ATLQANEV-COOH, respectively. The results by Autodock are shown in Table **1**, where the top 10 low energy conformers for each of the three octapeptides are listed. There are five different types of energy. The torsional free energy is related to the number of total rotable bonds. The binding free energy includes the contributions from the electrostatic interaction, van der Waals interaction, hydrogen bonds, and de-solvation effect. The affinity between ligand

Table 1. Ranking of the Top 10 Conformers Among 200 of Each Octapeptides Generated by Autodock, Where the Unit of All Forms of Free Energy is in kcal/mol

Octapeptides	Rank	Intermolecular contribution	Docking free energy	Binding free energy	Torsional free energy
OP2	1	-17.09	-17.66	-6.51	+10.58
	2	-17.60	-16.71	-7.02	+10.58
	3	-18.02	-16.24	-7.43	+10.58
	4	-18.99	-16.03	-8.40	+10.58
	5	-18.80	-16.02	-8.22	+10.58
	6	-14.82	-15.87	-4.24	+10.58
	7	-15.61	-15.41	-5.02	+10.58
	8	-14.04	-15.29	-3.46	+10.58
	9	-13.89	-15.22	-3.31	+10.58
	10	-16.71	-15.10	-6.12	+10.58
OP	1	-16.54	-16.60	-5.64	+10.90
	2	-16.59	-16.46	-5.70	+10.90
	3	-17.96	-16.17	-7.07	+10.90
	4	-15.65	-15.98	-4.75	+10.90
	5	-16.26	-15.93	-5.36	+10.90
	6	-15.17	-15.33	-4.27	+10.90
	7	-17.43	-15.14	-6.54	+10.90
	8	-16.26	-14.76	-5.36	+10.90
	9	-15.53	-14.58	-4.64	+10.90
	10	-13.67	-14.24	-2.78	+10.90
OP1	1	-15.12	-14.95	-5.47	+9.65
	2	-15.26	-14.67	-5.61	+9.65
	3	-16.52	-14.53	-6.87	+9.65
	4	-14.05	-13.54	-4.40	+9.65
	5	-13.63	-13.41	-3.98	+9.65
	6	-14.68	-13.13	-5.03	+9.65
	7	-14.55	-13.05	-4.90	+9.65
	8	-12.43	-12.63	-2.78	+9.65
	9	-12.52	-12.47	-2.87	+9.65
	10	-13.11	-12.27	-3.46	+9.65

and receptor is measured by the intermolecular free energy that is a dominant factor in the scoring function. The ligand also has the self-energy, i.e., its own conformational energy, which is usually used to score conformers of each ligand. The docking free energy is the sum of the binding free energy and the ligand self-energy. The top 10 conformers were selected and ranked according to its docking free energy. However, the docking free energy may not be applicable in ranking the interactions for different ligands because of the difference in the self-energy. In that case, the binding free energy would be a good measure, which is the sum of the intermolecular energy and torsional free energy. As we can see from the Table **1**, OP2 possesses more conformers with relatively lower free energy, suggesting that it is easier for OP2 to find its stable conformation upon binding with the receptor. Also, the torsional energy of OP is slightly larger than those of the other two, as shown in Table **1**.

Table 2. A Summary of All the Hydrogen Bonds Formed Between the Octapeptides and the M^{pro}, in Which O Denotes the Oxygen Connected with the Carbon in Peptide Bond, N Stands for Nitrogen in Peptide Bond and HN for the Hydrogen Connected with the Nitrogen in Peptide Bond. D, E and G Denote (δ,ε,γ) Delta, Epsilon and Gamma, Respectively. Thus OE Means the Oxygen on the Epsilon Position and HD Means the Hydrogen Connected with an Atom on the Delta Position. The Symbols of "acc" and "don" Denote Hydrogen Bond Accepter and Donor, Respectively

Octapeptides	Number of hydrogen bonds	Involved atom of peptide and its function	Involved atom of M^{pro} and its function
OP	5	HN of ARG-8 (don)	O of ALA-173 (acc)
		HE of GLN-4 (don)	OD of ASP-187 (acc)
		HN of GLN-4 (don)	OD of ASP-187 (acc)
		O of LEU-3 (acc)	HE of HIS-41 (don)
		O of ALA-1 (acc)	HN of CYS-145 (don)
OP1	7	OG of THR-2 (acc)	HN of VAL-186 (don)
		O of ALA-1 (acc)	HG of THR-175 (don)
		O of ALA-1 (acc)	HN of THR-175 (don)
		HN1 of ALA-1 (don)	O of GLY-174 (acc)
		HN2 of ALA-1 (don)	O of GLY-174 (acc)
		O of THR-2 (acc)	HE of HIS-164 (don)
		HE of GLN-4 (don)	O of VAL-184 (acc)
OP2	6	OE of GLN-4 (acc)	HN of ARG-188 (don)
		OG of THR-2 (acc)	HN of VAL-186 (don)
		O of ALA-1 (acc)	HN of THR-175 (don)
		O of ALA-1 (acc)	HE of HIS-164 (don)
		OE of GLU-7 (acc)	HD of HIS-41 (don)
		OE of GLU-7 (acc)	HN of ARG-40 (don)

3. Hydrogen Bonds Between the Ligand and Receptor

Hydrogen bonds make important contributions to the interactions between the ligand and receptor. The hydrogen bonds formed between M^{pro} and the best conformers of the octapeptides are given in Table **2**. As shown in the table, His-41, Cys-145, and His-164 play an important role in forming hydrogen bonds with the ligands. His-41 resides very close to the center of the octapeptides and has two positively-charged hydrogen atoms connected with nitrogen on the imidazole ring. Actually, the catalytic dyad is formed by His-41 and Cys-145, which, along with HIS-164, are frequently involved in forming hydrogen bonds with the ligand in the low energy conformation. Such a spatial arrangement gives them more opportunities to participate in the catalytic reaction. Two other residues, namely, Val-186 and Thr-175, also bear higher frequency than others in this regard. The positions of these 5 residues (His-41, Cys-145, HIS-164, Val-186 and Thr-175) are shown in Fig. **2**; the space defined by them is where the octapeptide is docked to.

As for the octapeptide side, Gln-4 is located in center of the octapeptide that occurs in all the 3 cases, and hence should play an important role in the cleaving process [3,5,6,8,9]. Other residues, such as Ala-1 and Leu-3 of OP, Ala-1 of OP1, and Ala-1 and Glu-7 of OP2 are frequent participants in forming the hydrogen bonding.

It can be seen from Table **2** that OP1 forms the most hydrogen bonds with the receptor. However, OP1 has the highest free energy, i.e., unfavorable in binding with the receptor. The hydrogen bond interaction is just one of the contributions to the free energy. Other interactions, i.e., electrostatic, van de Waals, and solvation, are more important here.

After re-docking each of the 3 octapeptides with its orientation inverted, we found that the free energy of each peptide increased significantly. This was expected because the peptide would not be cleavable by the M^{pro} if its sequence order with respect to the receptor was changed, as reflected by the unfavorable binding energy.

CONCLUSION

Like the octapeptide OP [3], OP1 and OP2 are also cleavable by the SARS M^{pro}. According to the "destroyed

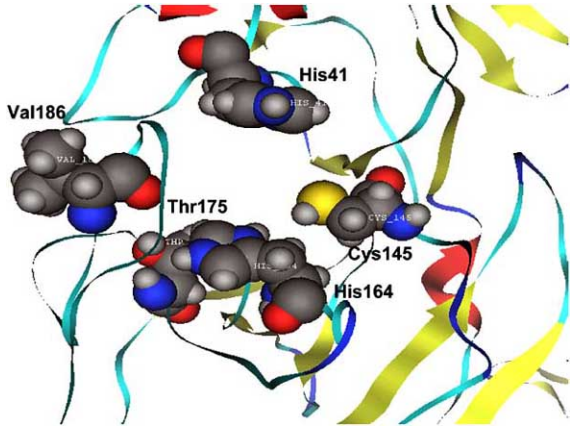

Fig. (2). The locations of His-41, Cys-145, His-164, Val-186 and Thr-175 in M^{pro}. These 5 residues are frequently involved in hydrogen bonding with the peptides. The secondary structure is rendered by flat ribbon and the residues are rendered by space-filling, where red, blue, grey and light-grey represents oxygen, nitrogen, carbon and hydrogen, respectively.

key theory" [9], the two octapeptides may also serve as a good start point for finding drugs for SARS therapy.

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