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Molecular design and validation of halogen bonding orthogonal to hydrogen bonding in breast cancer MDM2–peptide complex

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ABSTRACT

Peptide therapeutics has been raised as an attractive approach for the treatment of breast cancer by targeting the oncogenic protein MDM2 that inactivates p53 tumor suppressor. Here, we performed molecular design of halogen bonding orthogonal to hydrogen bonding at the complex interface of MDM2 protein with its cognate peptide ligand to improve the peptide binding affinity and specificity. Crystal structure analysis, high-level quantum chemistry (QC) calculations and combined quantum mechanics/molecular mechanics (QM/MM) modeling revealed that halogen substitution at position 3 of the benzene moiety of peptide Phe3 residue can constitute a putative halogen bonding, which is shown to be geometrically perpendicular to and energetically independent of a native hydrogen bonding that share a common carbonyl oxygen acceptor. The designed halogen bonding was then validated by surface plasmon resonance (SPR) assays, that is, substitution with bromine at position 3 can considerably improve peptide affinity by ~4-fold, but the peptide binding does not change substantially upon the bromine substitution at other positions of the Phe3 benzene moiety (the negative controls that are theoretically unable to form the halogen bonding), indicating that the orthogonal molecular interaction (OMI) system between the designed halogen bonding and native hydrogen bonding can co-work well at the complex interface of MDM2 protein with its halogenated peptide ligands.

1. Introduction

The MDM2 is an oncoprotein that exerts its oncogenic activity predominantly by inhibiting p53, a tumor suppressor that responds to cellular stresses such as DNA damage and oncogenic activation and is directly or indirectly involved in the development, progression and metastasis of almost all human cancers [1]. Serve as a major regulator, MDM2 is induced by p53 and acts as a feedback inhibitory protein [2]. Over the past decades, the interaction between peptides derived from the p53 and the MDM2 protein has been extensively studied [3–5], and chemical agents have been proposed to abolish this interaction. Over the past decades, a number of small-molecule inhibitors have been successfully developed to target MDM2 protein for cancer therapy [6,7]; idasanutlin (RG7388), an oral potent MDM2 antagonist, for example, is already in a clinical advanced phase for acute myeloid leukemia [8].

Instead of chemical agents, peptide therapeutics has in recent years attracted increased interest for the treatment of breast cancer by disrupting MDM2–p53 interaction [9,10]. Peptide-like drugs exhibit high specificity, low toxicity and satisfactory biocompatibility, which are likely to be more suitable candidates to act as competitive inhibitors of protein–protein interactions, considering their similar binding mode [11]. In order to improve peptide affinity and selectivity, several chemical methods such as hydrocarbon stapling [12] and cyclization [13] have been utilized to modify the natural peptide ligands of MDM2 protein. In the present study, we utilized a new strategy for promoting peptide binding to MDM2 by molecular design of an orthogonal molecular interaction (OMI) between halogen bonding and hydrogen bonding at the complex interface of MDM2 with a p53 peptide [14]. According to a previous report of Voth et al. [15], halogen bonding is shown to be geometrically perpendicular to and energetically independent of hydrogen bonding that share a common carbonyl oxygen acceptor. This orthogonal relationship is accommodated by the in-plane and out-of-plane electronegative potentials of the oxygen, which are differentially populated by halogen bonding and hydrogen bonding. The halogen bonding has been termed as σ-hole that is geometrically and energetically similar to hydrogen bonding [16–18]. Here, an integration of high-level quantum chemistry (QC) calculation, combined quantum mechanics/molecular mechanics (QM/MM) analysis and surface plasmon resonance (SPR) assay was

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employed to design OMI in the context of MDM2–peptide complex crystal structure and to characterize the structural basis and energetic landscape of the designed OMI in small model system and at biological complex interface.

2. Materials, methods, and experiments

2.1. QC analysis of small model systems

The small model systems containing OMIs were analyzed at Møller-Plesset second-order perturbation theory in conjunction with the Dunning's augmented correlation consistent basis set (MP2/aug-cc-pVDZ). The MP2/aug-cc-pVDZ was thought to be adequate for reasonably weak nonbonded interactions and was used in previous theoretical studies of biological adducts involving halogen bonding and hydrogen bonding [19]. Considering that the Dunning's basis set series does not cover iodine, the Lanl2DZ + (df) basis set, which is augmented by a set of *d* and *f* polarization functions (exponents 0.292 and 0.441, respectively) and *s* and *p* diffuse functions (exponents 0.0569 and 0.0330, respectively) from Lanl2DZ basis set [20], was applied for iodine-containing systems. The interaction energy of halogen bonding (ΔE_{xb}) or hydrogen bonding (ΔE_{hb}) was estimated as the difference between the total energy of the bonding adduct and the sum of total energies of the two monomers, and basis set superposition error (BSSE) was corrected by means of the counterpoise strategy [21].

2.2. QM/MM calculation of biological complex systems

A two-layer ONIOM-based QM/MM approach [22] was employed to investigate MDM2–peptide complexes involving OMIs. The high-level QM theory of B3LYP/6-31G(d) was applied to the inner layer that contains the residues that form halogen bonding and hydrogen bonding in the OMIs, while the low-level MM method of UFF force field was used to describe the outer layer that covers rest of the complexes. Hydrogen was used as link atoms to saturate the dangling bonds at partitioning interface and the electrostatic potential between inner and outer layers was treated with mechanical embedding scheme to reduce computational cost. The complex structures were fully minimized with the two-layer QM/MM scheme without constraints [23]. The binding free energy (ΔG) between MDM2 and peptide was calculated *via* supramolecular approach proposed by Zhou et al. [24] based on the minimized structures (Fig. 1).

2.3. SPR assays

The natural peptide and its halogenated versions were synthesized by *F*moc-solid phase synthesis protocol; the *F*moc halogenated amino acids used in the synthesis were obtained from unnatural amino acids library. The N-terminal domain of human MDM2 (residues 23–111) was expressed in *Escherichia coli* BL21-Gold (DE3) with a pDEST-His-MBP vector and then purified by affinity chromatography in nickel-nitrilotriacetic acid Superflow. The MDM2–peptide interaction was measured by SPR using a protocol modified from previous reports [3,4]. Briefly, peptides were immobilized on the surface of sensor chip at room tempature in a BIAcore. A total of 100 nM MDM2 was incubated in 10 mM HEPES buffer (pH 7.4) containing 150 mM NaCl and 0.005% surfactant P20 with varying concentrations of tested peptide on the chip. Nonlinear regression analysis was performed using GraphPad Prism to derive dissociation constants with the equation K_D = [MDM2][peptide]/[MDM2–peptide complex].

3. Results and discussion

The high-resolution crystal structure of human MDM2 in complex with a 12-mer pDI peptide was retrieved from the protein data bank (PDB) database (pdb: 3lnz) [5]. The cocrystallized pDI peptide (LTFEHYWAQLTS) was derived from a phage display library, which exhibited a moderate inhibitory activity against MDM2–p53 interaction (IC_{50} = 550 nM) [5]. By visually dissecting the crystal structure we identified an intramolecular hydrogen bonding (length = 2.05 Å) between the oxygen atom ═O of Gly58 residue and the hydrogen atom —H of Met62 residue in MDM2 protein, while the hydrogen atom —H at position 3 of the benzene moiety of peptide Phe3 residue is close to the oxygen atom ═O of MDM2 Gly58 residue with a spatial distance of 2.75 Å between them. The small distance between MDM2 Gly58 ═O and peptide Phe3 —H suggests that they may form a halogen bonding if the hydrogen atom —H of peptide Phe3 residue is replaced by a halogen atom –X (X = F, Cl, Br or I); the putative halogen bonding seems to be roughly orthogonal to the intramolecular hydrogen bonding Gly58···Met62 of MDM2, and they can co-define an OMI system at the MDM2–pDI complex interface.

In order to examine the hypothesis, we separately modified the —H to –F, —Cl, –Br and –I in the complex crystal structure; each was then subjected to a round of QM/MM minimization with default settings where the MDM2 residues Gly58 and Met62 as well as the modified residue Phe3 (–X) of peptide were included in the high-level QM layer, while rest of the complex was treated with low-level MM approach. The equilibrated OMI systems in QM/MM-minimized complexes of MDM2 protein with different modified versions of pDI peptide are shown in Fig. 2, and their geometric and energetic parameters are listed in Table 1. As can be seen, halogenations can address considerable effects on the geometric profile and energetic property of the OMIs at MDM2–peptide interface. For putative halogen bonding, the equilibrium distance $d_{O\cdots X}$ between the oxygen atom ═O of MDM2 Gly58 residue and the halogen atom –X of peptide Phe3 residue ranges between 3.08 and 3.35 Å, which is in line with the bond length of those biological halogen bonding revealed previously [25]. In addition, the distance is smaller than the sum of the van der Waals radii [26] of respective O and X atoms, indicating that there is an attractive chemical force between the two atoms. The Gly58···Phe3 interaction energy ΔE_{xb} increases significantly from −1.02 (–H) to −2.18 (–Cl), −2.64 (–Br) and −2.31 (–I) kcal/mol upon the halogenations, although the –F substitution (ΔE_{xb} = −1.37 kcal/mol) does not change the energy substantially as compared to the native —H (ΔE_{xb} = −1.02 kcal/mol) –– this is expected because fluorine cannot form halogen bonding in most cases [27]. Overall, it is suggested that halogen bonding is indeed presented at the MDM2–peptide complex interface when the hydrogen atom —H replaced by halogen atom –X. Next, we examined the geometric and energetic effect of halogenations on the hydrogen bonding between the Gly58 oxygen atom ═O and Met62 hydrogen atom —H of MDM2 protein. The native length $d_{O\cdots H}$ and interaction energy ΔE_{hb} of the hydrogen bonding in crystal structure is 2.05 Å and −2.31 kcal/mol, respectively, which are changed modestly by the halogenations, except the substitution by iodine atom –I, which can moderately influence the hydrogen bonding. Moreover, the angle $\theta_{\angle H\cdots O\cdots X}$ is close to 90° ($\theta_{\angle H\cdots O\cdots X}$ = 71.3°, 82.5°, 84.6° and 71.3° for –F, —Cl, –Br and –I, respectively). In this respect, the putative halogen bonding is shown to be geometrically perpendicular to and energetically independent of hydrogen bonding that share a common carbonyl oxygen acceptor; this is consistent with the OMI defined by Voth and coworkers [15].

To examine whether the designed OMI system exists at MDM2–peptide interface, the binding affinities of pDI peptide with –Br substitution at different positions of the benzene moiety of