Prediction of the three-dimensional structures of histone deacetylase 1 complexed with romidepsin and FK-A5

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Abstract. Romidepsin is an anticancer drug that inhibits histone deacetylase (HDAC) in human cells. Recently, we found that romidepsin and its analog, FK-A5, inhibit phosphoinositide 3-kinase. Thus, romidepsin and FK-A5 are expected to be promising HDAC/PI3K dual inhibitors for anticancer therapy. In this study, the HDAC1-inhibitor complex structures were predicted by using computational docking and molecular dynamics (MD) simulations. Because romidepsin and FK-A5 are large cyclic molecules, large numbers of conformers can be obtained for these molecules by computational chemical treatment. The docking poses were extracted by comparing romidepsin and FK-A5 because similar compounds are recognized by proteins in similar binding modes. MD simulations were conducted for the selected docking poses, and the protein–ligand interactions were analyzed. The computational results are expected to be useful for the rational drug design of HDAC inhibitors.

1. Introduction
In current cancer treatment, molecular-targeted drugs play important roles [1, 2]. Such drugs are compounds designed to bind to specific molecular drug targets and not to any other biomolecules. Therefore, molecular-targeted drugs generally have fewer side effects and greater efficacy than conventional drugs. Especially, dual (or multiple) inhibitors, which inhibit two (or more) drug target biomolecules simultaneously, have been developed. Recently, some multiple inhibitors have been marketed. For example, the breast cancer drug lapatinib is a dual inhibitor that inhibits epidermal growth factor receptor and HER2 [3]. Many of multiple inhibitors are kinase inhibitors. The active sites of kinases are called “kinase pockets.” The similarity of kinase pockets plays an important role in the multiple inhibitor functions of these drugs. As an alternative to the kinase inhibitors, we developed dual inhibitors that inhibit both a kinase and non-kinase, namely, phosphoinositide 3-kinase (PI3K) and histone deacetylase (HDAC) [4, 5].
PI3K is an enzyme that phosphorylates phosphatidylinositol 4,5-bisphosphate in the cell membrane to generate phosphatidylinositol 3,4,5-trisphosphate [6], and PI3K is one of the most promising drug targets for cancer treatment. On the other hand, HDACs deacetylate the acetylated lysine located in the histone tail [2, 7]. HDACs are important enzymes for transcriptional regulation, and 11 types of HDACs (and 7 types of sirtuins) in humans have been reported [8, 9]. The human HDACs and sirtuins are classified into four classes (classes I to IV). Class I HDACs are distributed throughout the entire body, and HDAC1 in particular is an important enzyme involved in more than half the activity of HDACs. HDACs are known as molecular targets for anticancer drugs. In fact, the inhibitors of class I HDACs are marketed as anticancer drugs: HDACs are thought to suppress the transcription of cancer suppressor genes and HDAC inhibitors are considered to activate these genes[9].

Previously, we constructed an assay system to evaluate the inhibitory activities of PI3K inhibitors [4]. Using our assay system, we screened the chemical library of the Screening Committee of Anticancer Drugs supported by a Grant-in-Aid for Scientific Research on Innovative Areas and Scientific Support Programs for Cancer Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan [4, 5]. Via our screening, the HDAC inhibitor romidepsin and its analogs were identified as candidate compounds with PI3K inhibitory activities. Romidepsin is a natural bicyclic depsipeptide produced by Chromobacterium violaceum, and is known as a class I HDAC inhibitor. It was approved by the FDA for the treatment of cutaneous T-cell lymphoma and peripheral T-cell lymphoma. When a PI3K inhibitor was used in combination with an HDAC inhibitor, the cytotoxic effect was enhanced [10]. Therefore, HDAC/PI3K dual inhibitors are promising compounds for the treatment of intractable cancer. Figure 1 depicts HDAC/PI3K dual inhibitor candidates found by our team. FK-A5 is the romidepsin analog that has the highest inhibitory activity for PI3K.

The 3D structures of the PI3K–romidepsin and PI3K–FK-A5 complexes have been predicted [11]. Although computational docking [12] is generally used for predicting the structures of protein–ligand complexes, appropriate docking poses cannot be obtained by simple docking studies for romidepsin and FK-A5 because of their macrocyclic structures. Therefore, we introduced the concept of ligand-based drug design (LBDD) [13] to select appropriate docking poses. Reasonable complex structures that were consistent with the experimental inhibitory activities were predicted. In this study, 3D structures of HDAC1 in complex with romidepsin or FK-A5 were predicted. Recently, in silico drug design trials of HDAC inhibitors have been reported [14, 15]. In these studies, the computational results were consistent with the experimental investigations, and the development of new HDAC inhibitors and detection of important substructures of HDAC can be performed. Therefore, in silico drug design techniques, such as docking and MD simulations, are expected to be useful for the investigations of the complex structures between HDAC and inhibitors. The predicted structures obtained by this study will be useful for the further modification of the candidate compounds.

Figure 1. HDAC/PI3K dual inhibitors: (a) romidepsin, (b) FK-A5, (c) active form of romidepsin, and (d) active form of FK-A5.

2. Computational methods
2.1. Protein and ligand structures
The 3D structure of HDAC1 was retrieved from the Protein Data Bank (PDB) (PDB ID: 4BKX) [16]. Because the entry 4BKX includes HDAC1 and metastasis-associated protein MTA1, MTA1 was excluded from the PDB structure and only HDAC1 coordinates were extracted from 4BKX. The hydrogen atoms and missing heavy atoms were complemented by leap module of AmberTools12 and AMBER12 [17]. In this study, romidepsin and FK-A5 were used as ligands. Romidepsin is a prodrug that is activated in the human body by reduction of its disulfide bonds. The longer thiol moiety of the active form is coordinated to zinc in the active site of HDAC by forming a thiolate ion, and bioreductively activated romidepsin inhibits HDAC. In this study, the inhibitory mechanism of FK-A5 was considered to be the same as that of romidepsin. The active forms of romidepsin and FK-A5 are shown in Figure 1c and d, respectively.

2.2. Computational docking
For computational docking, the GOLD program [18] was used. Because the conformational search for ligand molecules in GOLD is conducted by an empirical procedure, conformational searches for romidepsin and FK-A5 including macrocyclic moieties by GOLD were insufficient. In fact, GOLD trials for the docking of romidepsin and FK-A5 into HDAC1 without any preliminary processing cannot generate appropriate docking poses. Therefore, preliminary conformational searches using LMOD [19] based on normal mode analyses were performed in this study, and the conformers generated by LMOD were used for GOLD trials. LMOD searches for possible conformations of molecules according to physicochemical theory. This method is quite different from the conformational search procedure in GOLD. Using LMOD, 12 and 15 conformers were generated for romidepsin and FK-A5 respectively. All of these conformers were used for GOLD. The ligand-binding pocket of HDAC1 was detected by HBOP and HBSITE [20, 21]. In addition, a distance restriction of 1.5–3.5 Å was adopted between zinc of HDAC1 and the sulfur of thiolate.

Many possible docking poses were obtained by computational docking trials. From these probable candidates needed to be selected. The inhibitory activities of several romidepsin analogs for HDAC1 have been experimentally assessed. Similar binding modes were expected to be observed in HDAC1–romidepsin and HDAC1–FK-A5 complexes, because the interactions between HDAC1 and romidepsin were expected to be similar to those between HDAC1 and FK-A5. Although computational docking is one of the structure-based drug design (SBDD) methods, the basic concept of LBDD, in which similar ligands are recognized by a target protein in similar binding modes, was used here. Thus, docking poses with similar binding modes between romidepsin and FK-A5 were extracted.

2.3. Molecular dynamics simulation
The docking poses generally include some structural distortion because a certain amount of tolerance is considered in computational docking. MD simulations of predicted protein–ligand complexes can relax the distorted structures, and refined structures can be obtained. In this study, MD simulations of HDAC1–inhibitor complexes were performed using the AMBER12 program [17]. The ligand topologies were constructed by antechamber [22] in AmberTools. The cyclic boundary box including TIP3P explicit water was spaced with at least 8-Å margins from the protein surface, and the cut-off distance for van der Waals interactions was set at 10-Å. The AMBER ff12SB [23] and general AMBER force fields [24] were used for the protein (including zinc ion) and ligands, respectively. Before MD simulations, 1000-step structural minimizations of water and counter ion (Cl⁻) were performed, and 2500-step minimizations of whole systems were carried out. Using the minimized structures, temperature-increasing MD simulations were performed for 20 ps, with the temperature raised from 0 to 300 K. Equilibrating MD simulations were then performed for 20 ns at 300 K with a time step of 2 fs. After the MD simulations, 2500-step minimizations of the whole systems were conducted. The minimized structures were used for the illustration of the complex structures, and the data analyses were mainly performed by using MD trajectories.
3. Results and discussion

3.1. Docking poses
In total, 74 and 87 docking poses were obtained by the computational docking trials for romidepsin and FK-A5 respectively. As mentioned above, probable poses were selected by the similarity of protein–ligand interaction modes based on the LBDD concept. In the obtained docking poses, similar hydrogen bonding patterns were observed in HDAC1–romidepsin and HDAC1–FK-A5 complexes. The hydrogen-bonding pattern included two hydrogen bonds: one between Asp99 of HDAC1 and hydrogen in ligands, and the other between His178 and oxygen in ligands. The numbers of poses including the common hydrogen-bonding pattern were 21 and 11 for romidepsin and FK-A5, respectively. From these docking poses, two representative poses were selected respectively for romidepsin and FK-A5: poses 1 and 2 for romidepsin and poses I and II for FK-A5. 21 and 11 poses for romidepsin and FK-A5 were clustered into two clusters each by the orientation of macrocycles. For each cluster, the pose with the best GOLD score was selected as the representative pose. These poses are shown in Figure 2. HDAC1 is represented by ribbons, and the residues that form hydrogen bonds with ligands are illustrated by sticks. The sphere represents the zinc atom in the active site of HDAC1. The Zn–S distances were 2.49 and 2.06 Å for romidepsin poses 1 and 2 respectively. For FK-A5, the Zn–S distances were 2.44 and 2.50 Å for poses I and II respectively. Therefore, the sulfurs atoms of ligands were located deep in the active site of HDAC1 in all of these representative poses.

3.2. Molecular dynamics simulations
Because two candidate poses were selected from the GOLD results of each inhibitor, MD simulations were performed to elucidate which candidate is appropriate. The convergences of MD simulations were evaluated by root mean square deviations (RMSDs). For the main chain RMSD calculations, the initial structures of MD simulations were used as references, and the coordinates of the main chain heavy atoms were used. Figure 3 shows the main chain RMSDs for MD trajectories of four complexes.

**Figure 3.** RMSDs of main chain: (a) romidepsin 1, (b) romidepsin 2, (c) FK-A5 I, (d) FK-A5 II.

**Figure 4.** RMSDs of ligands: (a) romidepsin 1, (b) romidepsin 2, (c) FK-A5 I, (d) FK-A5 II.
On the other hand, for the ligand RMSD, the 3D structures at 10 ns were used as references, and the last 10 ns trajectories were used to reduce the bias of the initial structures. In Figure 4, ligand RMSDs are illustrated. As shown in this figure, RMSDs indicate that the MD simulations of these four complexes converged in the last part of trajectories, and the analyses of MD trajectories can be performed. Comparisons of the 3D structures between the initial and final structures of 20-ns MD simulations are illustrated in Figure 5. The initial and final structures are superposed. HDAC1 chains and ligands before and after MD simulations are shown as light blue and brown respectively. Zinc ions before and after MD simulations are illustrated as purple and orange spheres. These results indicate that the docking results have a low accuracy, and the structural refinements of protein–ligand complexes can be performed by MD simulations. Although the orientations of the ligands were changed by structural relaxations, the configurations of the ligands in the binding pocket of HDAC1, such as the sulfur located close to zinc atom, were nearly conserved.

Figure 5. 3D structures of HDAC1–inhibitor complexes before and after MD simulations: (a) romidepsin 1, (b) romidepsin 2, (c) FK-A5 I, and (d) FK-A5 II. Light blue and brown represent HDAC1 before and after MD simulations respectively. HDAC1 and ligands are illustrated by ribbon and ball-and-stick models, respectively.
3.3. Hydrogen bonds between HDAC1 and ligands

For the MD trajectories, the rates of occurrence of hydrogen bonds were analyzed. To avoid any bias from the initial structures, the last half of the MD trajectories were used for the analyses. For the definition of the hydrogen bonds, the default setting of ptraj module of AmberTools was used (distance < 3.5 Å). In Tables 1 and 2, the rates of occurrence of the hydrogen bonds greater than 10% are shown. The starting and ending points of the arrows indicate the hydrogen donors and acceptors respectively. The atom numbers of ligands are illustrated in Figure 2c and 2d. The common hydrogen bonds in HDAC1–romidepsin and HDAC1–FK-A5 complexes mentioned in subsection 3.1 (hydrogen bonds related to Asp99 and His178) are underlined in Tables 1 and 2. There were no MD trajectories in which both of two common hydrogen bonds (hydrogen bonds related to Asp99 and His178) were conserved. The His178 hydrogen bond was conserved in the MD simulation of romidepsin pose 1, and the Asp99 hydrogen bond remained in the MD simulation of romidepsin pose 2. For the MD simulation of FK-A5 pose I, the rates of occurrence of almost all hydrogen bonds were considerably reduced, and the maximum value of the rate of occurrence was only 5.8%. In contrast, many hydrogen bonds were observed in the MD trajectory of FK-A5 pose II and the common hydrogen bond Asp99 O\(_{\delta2}\) ← (N\(_{28}\))H was conserved. These results suggest that FK-A5 pose II can form hydrogen bonds more stable than FK-A5 pose I. For the ligand recognition of a protein, hydrogen bonds play important roles in determination of the orientation and configuration of a ligand. Thus, FK-A5 pose I, in which the hydrogen bonds between HDAC1 and ligand were hardly observed, appears to be inappropriate for the predicted complex structure, whereas FK-A5 pose II is plausible. Because the binding modes of romidepsin analogs for HDAC1 are assumed to be similar according to the experimental inhibitory activities [4, 5], the hydrogen bond observed in FK-A5 pose II is also considered to be important for

| Table 1. Hydrogen bonds between HDAC1 and romidepsin after MD simulations. |
|-------------------------------|-------------------|-------------------|
|                               | Romidepsin pose 1 | Romidepsin pose 2 |
| Hydrogen bond                  | Occurrence rate   | Hydrogen bond      | Occurrence rate |
| His178 H\(_{\epsilon2}\) → O\(_{33}\) | 66.7%             | Asp99 O\(_{\delta2}\) ← (N\(_{28}\))H | 82.4% |
| Phe205 O ← (N\(_{27}\))H       | 34.4%             | Asp99 O\(_{\delta2}\) ← (N\(_{27}\))H | 75.6% |

| Table 2. Hydrogen bonds between HDAC1 and FK-A5 after MD simulations. |
|-------------------------------|-------------------|-------------------|
|                               | FK-A5 pose I      | FK-A5 pose II     |
| Hydrogen bond\(^{a}\)         | Occurrence rate   | Hydrogen bond      | Occurrence rate |
| No hydrogen bonds\(^{a}\)     |                   | Asp99 O\(_{\delta2}\) ← (N\(_{30}\))H | 70.5% |
|                               |                   | His178 H\(_{\epsilon2}\) → O\(_{31}\) | 64.8% |
|                               |                   | Asp99 O\(_{\delta2}\) ← (N\(_{28}\))H | 64.7% |
|                               |                   | Asp99 O\(_{\delta1}\) ← (N\(_{29}\))H | 61.2% |
|                               |                   | Asp99 O\(_{\delta1}\) ← (N\(_{27}\))H | 49.9% |
|                               |                   | Asp99 O\(_{\delta2}\) ← (N\(_{29}\))H | 37.3% |
|                               |                   | Asp99 O\(_{\delta1}\) ← (N\(_{28}\))H | 31.6% |
|                               |                   | Asp99 O\(_{\delta2}\) ← (N\(_{27}\))H | 23.6% |
|                               |                   | Asp99 O\(_{\delta1}\) ← (N\(_{30}\))H | 20.6% |
|                               |                   | Tyr204 OH → O\(_{31}\) | 15.2% |

\(^{a}\) No hydrogen bonds with a rate of occurrence > 10% were observed in pose I.
the romidepsin complex. Because the hydrogen bond related to Asp99, which was observed in FK-A5 pose II, was detected only in romidepsin pose 2, pose 2 is more reasonable than pose 1. Therefore, the MD simulations suggest that romidepsin pose 2 and FK-A5 pose II are the assumed complex structures, which are consistent with the experimental results.

3.4. Other interactions

The average of Zn–S distances for the last 10 ns trajectories of MD simulations were 1.98, 1.95, 1.95, and 1.97 Å for romidepsin poses 1 and 2, and FK-A5 poses I and II respectively. These distances converged by the 20 ns MD simulations. The crystal structure of the complex between HDAC8 and largazole, which is one of the depsipeptide drugs the same as romidepsin, has been reported (PDB ID: 3RQD) [25]. In the HDAC8–largazole crystal structure, the Zn–S distance was 2.3 Å. Because the distances between the coordinating sulfur of ligand and the zinc atom of proteins are reported to be generally 2.0–2.3 Å [26], Zn–S distances observed for HDAC1–romidepsin and HDAC1–FK-A5 complexes in this study seem to be reasonable. In this study, AMBER ff12SB parameters for zinc were used, and special treatment for coordinate bond was not applied. Although the classical MD simulations cannot investigate the electron state related to the coordinate bond between the metal ion and the ligands, the results of MD simulations indicate that the structural predictions can be performed by the classical approximation of protein–ligand complexes.

In addition to the hydrogen bonds and Zn–S, tentative π–π interaction between the imidazole ring of His28 in HDAC1 and the benzene ring of FK-A5 was observed in FK-A5 pose II. Because classical MD simulations using AMBER ff12SB force field cannot evaluate π–π interaction, the tentative π–π interaction was presumed from the positional relation between aromatic rings in 3D structures obtained by MD simulations. Because the benzyl group is included only in FK-A5, the tentative π–π interaction was one of the distinct differences between HDAC1–romidepsin and HDAC1–FK-A5 complexes. Figures 6 and 7 illustrate interactions between HDAC1 and the ligand at the active site for HDAC1–romidepsin and HDAC1–FK-A5 respectively. In these figures, hydrogen bonds with rates of occurrence <50% are omitted. The experimentally determined inhibitory activity of FK-A5 for HDAC1 was higher than that of romidepsin, which may have been caused by the tentative π–π interaction. Around the benzyl group of FK-A5 in the predicted complex structure, additional space was observed and the introduction of substitution groups to this benzyl group for further drug design may be possible. According to the results of our previous study [11], we assumed that this benzyl group also plays an important role in PI3K inhibition because of large hydrophobic effects. Thus, modification of the benzyl group is expected to improve the inhibitory activities for both PI3K and HDAC1, and better HDAC/PI3K dual inhibitors can be developed.

Figure 6. Interactions between romidepsin and HDAC1.
As mentioned above, romidepsin pose 2 and FK-A5 pose II seem to be appropriate for complex structures because the hydrogen bond related to Asp99 was shared between them. In Figure 8, these structures are superimposed. As shown in this figure, not only the hydrogen bond but also the locations of ligands in the binding pocket of HDAC1 were shared for romidepsin and FK-A5. In addition, the orientations of macrocyclic structures were roughly consistent between romidepsin and FK-A5. Although the similar docking poses were selected for romidepsin and FK-A5, large structural relaxations for ligands were occurred by MD simulations (shown in Figure 5). The results of MD simulations suggest that the binding modes of the two inhibitors were similar even though the large structural relaxations. This supports our predictions of the structures of HDAC1–romidepsin and HDAC1–FK-A5 complexes.

Figure 7. Interactions between FK-A5 and HDAC1.

Figure 8. Superposition of 3D structures of HDAC1–romidepsin and HDAC1–FK-A5 complexes obtained by MD simulations. Dark gray and magenta represent romidepsin and FK-A5 respectively.
3.5. Comparison with HDAC8

Cole et al. reported the crystallographic structure of the HDAC8–largazole complex, which is one of the depsipeptide compounds (PDB ID: 3RQD) [25]. HDAC8, like HDAC1, is a member of class I HDACs. The calculated structures for HDAC1–inhibitor complexes predicted in this study were similar to the crystal structure of HDAC8–largazole complex (Figure 9). In the crystal structure of the HDAC8–largazole complex, a hydrogen bond between Asp101 of HDAC8 and the NH moiety of largazole was observed (Figure 9b). Because Asp101 of HDAC8 corresponds to Asp99 in HDAC1, the binding mode observed in our predicted structures, namely, Asp99 of HDAC1 being bound to the NH of ligands, is consistent with the crystal structure of the HDAC8–largazole complex. Although the sequence homologies are not very high in the HDAC family, the Asp residue located in this position (Asp99 in HDAC1 and Asp101 in HDAC8) is highly conserved. Thus, some researchers believe that the Asp residue in this position plays an important role in substrate/inhibitor recognition of HDAC [27]. In this study, because the importance of Asp99 in HDAC1 was clarified by docking and MD simulations and was consistent with experimental data, the predicted structures of HDAC1–romidepsin and HDAC1–FK-A5 complexes obtained in this study appear to be appropriate.

Figure 9. Experimental structure of complex between HDAC8 and largazole (PDB ID: 3RQD): (a) whole complex, (b) ligand binding pocket.

4. Conclusion

In this study, the 3D structures of HDAC1 complexed with romidepsin and FK-A5, which were developed by our team as HDAC/PI3K dual inhibitors, were predicted by using computational docking and MD simulations. Because multiple docking poses are generally constructed by computational docking trials, many possible docking poses were generated for romidepsin and FK-A5, which include macrocyclic structures. For the pose selection, the concept of LBDD (similar compounds are recognized by a target protein in similar binding modes) was used, and the docking poses with common binding modes were selected for romidepsin and FK-A5. The selected docking poses were validated by MD simulations, and the most likely complex structures between HDAC1 and inhibitors were predicted. Therefore, reasonable predictions of protein–ligand complexes can be obtained by the combined use of SBDD and LBDD, even for relatively large ligand molecules such as macrocyclic compounds.

The experimentally determined HDAC1 inhibitory activities IC₅₀ of romidepsin and FK-A5 were 3.6 and 2.4 nM, respectively [4]. In the complex structures predicted in this study, the number of hydrogen bonds for HDAC1–FK-A5 was greater than that for HDAC1–romidepsin, and the π–π interaction between the aromatic ring of HDAC1 and the ligand was only observed in HDAC1–FK-A5 (between the imidazole ring of His28 and the benzyl group). These interactions seem to influence the
higher binding affinity of FK-A5 for HDAC1. The benzyl group of FK-A5 is also considered to play an important role in PI3K–FK-A5 complex. This indicates that benzyl is a key moiety for further compound design. Therefore, the results of this study will be useful for rational drug design and the development of HDAC/PI3K dual inhibitors.

Acknowledgments
This work was supported by Grants-in-Aid for Scientific Research (15H01064 and 17K08257) from the Japan Society for the Promotion of Science.

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