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Asp746 to glycine change may have a greater influence than Cys751 to serine change in accounting for ligand selectivity between EGFR and HER-2 at the ATP site

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Summary

We have carried out up to 8.0 ns molecular dynamics simulation on the ATP-bound complexes of EGFR and HER-2 (homology model) receptor kinase domains to explore the possible consequences of amino acid residue changes in or close to the ATP site that might provide insights for selectivity of these kinases towards ATP site inhibitors. The simulation results show the formation of a channel under Thr766 following the movement of the side chain of Gln767 away from the hinge in EGFR. In HER-2, a similar movement of Gln799 occurs, but a simultaneous movement of Arg784 towards the hinge region occurs that tends to close the channel. The movement of Arg784 in HER-2 appears to result from the absence of an anchoring residue like Asp746 in EGFR, which has been changed to Gly778 in HER-2. In EGFR, this Arg784 is held away from the hinge region by interaction with Asp746, thereby leaving the channel open. This might be an important contributory factor to differences in selectivity of the ligands between the two kinases, probably more so than the conservative change of Cys751 of EGFR to serine in HER-2 at the ATP site.

Introduction

The epidermal growth factor receptor kinase (EGFR) and its homolog HER-2, which belong to the erbB/HER receptor tyrosine kinase (RTK) family, have been under investigation as anticancer molecular targets [1]. The overexpression and/or mutation of the above enzymes have been frequently observed in non-small cell lung, breast and ovarian cancers among others [2–4]. HER-2 is frequently amplified in breast cancers that exhibit aggressive growth behavior [5]. Drugs that selectively inhibit HER-2 kinase will be very useful in breast cancer chemotherapy. One of the important groups of 'small molecule' drugs of these receptors is the kinase inhibitor class. These are competitive with ATP [6] binding at the kinase domain. The crystal structure of EGFR as the apoenzyme (pdb code: 1M14) and in complex with the anilinoquinazoline Tarceva (pdb code: 1M17) has been published [7]. Since HER-2 shares 80% homology in the intracellular kinase domain with that of EGFR, we had previously carried out a structural study on its homology model, which was based on the structure of EGFR [8]. An obvious difference in the ATP binding site between EGFR and HER-2 is the replacement of Cys751 in EGFR by Ser783 in HER-2. The selectivity of compounds towards HER-2 relative to EGFR seems to have been achieved by modifying moieties on the compound that do not directly interact with the above-mentioned residue [6]. Hitherto, it has been baffling as to how such a very conservative change

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could lead to such large differences in ligand binding. We are of the opinion that along with the single amino acid change in the ATP site, other changes close to the ATP-site might contribute to the observed selectivity. A closer observation of the ATP site of EGFR from the crystal structure shows three features that prompted us to undertake this study.

1. Connolly surface rendering (Figure 1a) of the ATP site in the complexed form indicates the presence of a narrow channel under Thr766. This channel opens into the ATP site and one of the residues lining its walls is Cys751. This is the residue that is changed to Ser in HER-2. Thr766 is particularly important as it has been proven by mutagenesis that its replacement with hydrophobic residues does not decrease EGFR kinase activity but rather reduces the activity of kinase inhibitors [9]. This implies that while ATP binding is maintained, inhibitor binding may be altered.
2. In the apoenzyme, the NH_2 group of the side chain of Gln767 forms a hydrogen bond with the side chain NH_3 of Lys828 (also an ATP site residue) whereas in the complex with the inhibitor, it is rather the side chain CO of the glutamine that interacts with Lys828 (Figure 1b). This could be a crystallographic artifact, but if not, this could affect channel dimensions since this glutamine is part of the hinge and also lines the channel mentioned above.
3. The side chain of Arg752 interacts with the backbone carbonyl of Val745 and the side chain of Asp746 in the complexed form but loses these interactions in the apoenzyme (Figure 1b). The presence of this interaction is also one of the reasons for the formation of the channel in the complexed form. This interaction would be different in the case of HER-2 where the Asp746 has been replaced by glycine.

We have performed 3.0 ns molecular dynamics simulations of ATP-bound complexes of EGFR and HER-2 kinase domains in explicit water under constant pressure at 300 K to investigate the dynamics of the ATP site and its vicinity in EGFR and HER-2, and gain insights into ATP-site ligand selectivity.

Computational methods

Docking and molecular dynamics

The crystal structure of EGFR kinase domain (pdb code 1M17) and the homology model of HER-2 using the ligand-bound form of EGFR as template [8], were used as the starting coordinates for the enzymes. ATP was docked into its binding site using the GOLD program (version 2.1) [10] from Cambridge Crystallographic Data Center, UK. A cavity detection feature in GOLD was used to restrict the region of interest to concave solvent accessible areas. The ATP site was defined as residues lying within 15.0 Å of Thr766 in EGFR and Thr798 in HER-2. Chemscore in GOLD was used to score the 20 solutions obtained. The top ranked binding conformation of ATP was similar to those in the crystal structures of various other ATP complexes of tyrosine and serine–threonine kinases. This binding conformation of ATP was used as starting conformation in the enzyme-ATP complex. The enzyme-ATP complex was imported into INSIGHT II (Accelrys Software, San Diego) and

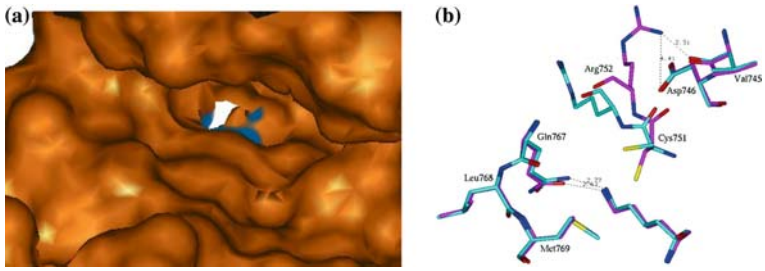

Figure 1. (a) Connolly surface of the EGFR ATP site in the crystal structure of the complexed form with Tarceva removed. The blue color lining the channel represents Cys751. (b) Differences in the orientation of the ATP site residues between the apoenzyme and those in the complexed form of EGFR. The carbon atoms of the apoenzyme are colored cyan while those of the complexed form are colored magenta. Hydrogens are not shown.

centered in a box of dimensions 72.0 × 86.0 × 72.0 Å^3. The 'soak' routine in INSIGHT II was used to solvate the enzyme-ATP complex with water.

The crystal structure of (EGFR or HER-2 kinase) in complex with ATP in explicit water was subjected to molecular dynamics simulations and minimizations using the NAMD program (version 2.5) [11] running on a 5 node Scyld Beowulf linux cluster (Athlon XP 2500 + processor per node). The coordinate and the parameter files (including atomic partial charges) of the complexes for input into the NAMD program were generated by the 'psfgen' utility using the CHARMm PARAM 22 topology file. Each system has about 44,000 atoms and required 4.5 days for 1.0 ns of simulation. The use of the linux cluster enabled us to perform the simulations in a time frame that is 12 times faster than it would take on an SGI R12000 processor. The all atom CHARMM PARAM 22 forcefield [12, 13] was used to describe the potential energy. Newton's equations of motion were integrated using multiple time step velocity Verlet algorithm. The van der Waals interactions were smoothly switched from 8.0 to 10.0 Å. The atom pair list distance was maintained up to a distance of 11.5 Å. The long-range electrostatics was handled by particle mesh Ewald (PME) algorithm using a 68 × 81 × 68 grid and a β-spline interpolation of the 4th order. The van der Waals interactions were calculated every time step whereas the full electrostatics calculation was done every two-time steps. The SHAKE algorithm was used to fix the length of bonds involving hydrogen atoms. The water molecules were described by a TIP3 potential. Periodic boundary conditions were applied to the system. The dielectric constant was set to 1.0. The protocol for the simulation is as follows: The system was first minimized using 10,000 steps of conjugate gradients, followed by 'heating' to 300 K in increments of 25 K every 250 steps. On reaching 300 K, the temperature was maintained by velocity scaling for a further 10 ps. During the above minimizations and dynamics, only the water molecules were free to move. Following this, a harmonic constraint with a force constant of 20.0 Kcal/mol/Å^2 was applied to the enzyme-ATP complex and the dynamics continued for 50 ps. The NVT *ensemble* was employed during the above simulations. Following this, the simulation ensemble was then changed to NPT. The NVT ensemble was used first to prevent instabilities resulting from energy conservation problems stemming from initial bad contacts. The Langevin piston with an oscillation time scale and a damping time scale of 200 fs and 100 fs, respectively, was used to maintain the pressure at 1.01325 bar. The temperature was maintained at 300 K by coupling the system to an external temperature bath, and the simulation continued for 10 ps. The force constant was then reduced to 1.0 Kcal/mol/Å^2 for the enzyme-ATP complex and dynamics continued for another 10 ps. The time step, which was 1.0 fs during all of the above simulations, was then changed to 2.0 fs after the system had stabilized, and the constraining force on the enzyme was removed while the force on ATP was maintained for another 50 ps. The entire system was then allowed to move freely and the trajectory was sampled every 2.0 ps. The trajectory was analyzed using CHARMm program [12].

Results and discussion

The movements of Gln767 and Arg752 in EGFR, and those of the equivalent residues in HER-2, Gln799 and Arg784, respectively, were monitored over the dynamics trajectory for 3 ns, and the plots of the distances monitored during that period are shown in (Figure 2a, b). The directions of the movements are also shown in (Figure 2 c, d). In the case of EGFR, side chain carbonyl of Gln767 was hydrogen bonded to the side chain amino group of Lys828 during the initial 800 ps of simulation. The channel that was seen at the start of the simulation narrowed and eventually closed during this early simulation period. During this period, the side chain of Arg752 interacted mostly with the backbone carbonyl of Val745. After about 1.0 ns of simulation, the side chain of Asp746 began to interact with Arg752, and provided additional binding interactions to hold the side chain of Arg752 away from the hinge. Also, the hydrogen bond between the side chain amide of Gln767 and the amino group of Lys828 was lost, allowing the Gln767 side chain to move, thereby reopening the channel and keeping it open for the rest of the simulation.

In HER-2, Gln799 was shown to be significantly more mobile than its EGFR counterpart Gln767. During the first 1.25 ns of the simulation, the hydrogen bond between Gln799 side chain

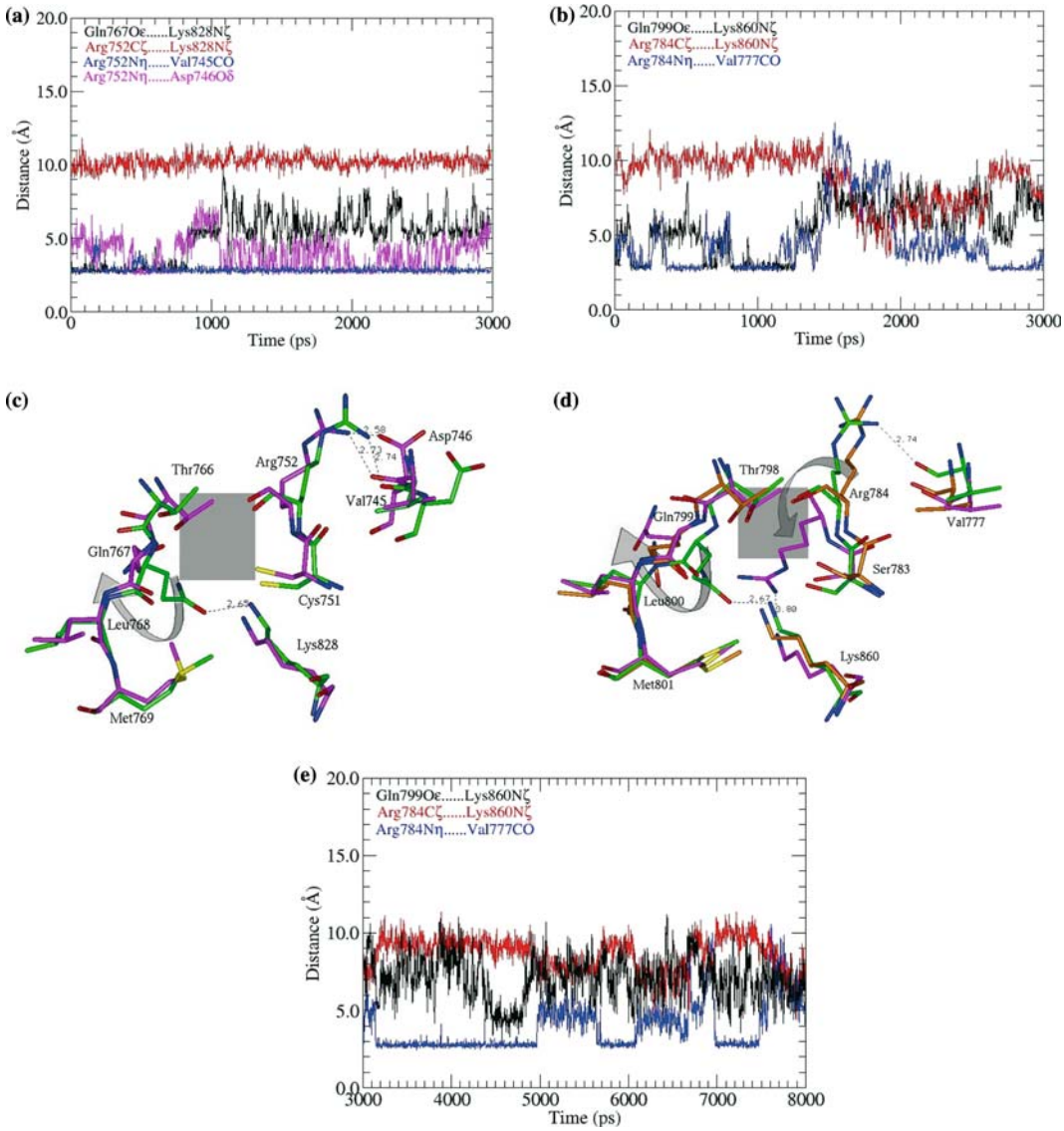

Figure 2. Plots of distances between various residues against simulation time for (a) EGFR and (b) HER-2. The actual movements of the residues during the simulation for EGFR and HER-2 are shown in (c) and (d), respectively. The structures at the start of the simulation are shown in green while those at the end of the simulation are colored magenta. Shown in orange, is an intermediate structure in the HER-2 simulation where the hydrogen bond between Gln799 and Lys860 was broken but the Arg784 had not yet moved down. The grey arrows indicate movement of residues while the grey box shows the approximate location of the channel. Hydrogens and ATP are not shown. The plot of distances between various residues against simulation time for the extended simulation of HER-2 is shown in (e).

carbonyl and Lys860 amino group was in a dynamic state of forming and breaking. The carbonyl moved a distance of about 5.7 Å when the hydrogen bond was absent. During this period, the side chain of Arg784 was held away from the hinge region. When Gln799 interacted with Lys860, the channel closed, but a narrow undulating fissure formed when the hydrogen bond was broken. At 1.5 ns of simulation the side chain of Gln799 moved further away simultaneously as the side chain of Arg784 drifted away from Val777 towards the hinge. This drifting of Arg784 was caused by the loss of the hydrogen bond between it and the backbone carbonyl of Val777. Since Asp746 in EGFR is changed to Gly778 in HER-2, there is one less hydrogen-bond holding Arg784,

thus enabling it to drift away. The hydrocarbon portion of the side chain of Arg784 effectively shuts the channel that may have resulted. This movement of Arg784 causes Ser783 to shift and slightly point away from the ATP site unlike its counterpart Cys751 in EGFR. In the last 500 ps of the simulation, Arg784 moves away from the hinge and re-establishes the hydrogen bond with Val777.

To rule out the possibility that the movements of the residues in the HER-2 simulation were artifactual, we extended the simulation of HER-2 to 8 ns, that is 5 ns following the initial 3 ns described above, and tracked the movement of Arg784 over this additional relatively long period of time (see Figure 2e). Arg784 was held away from the hinge by the hydrogen bond to Val777 for a duration of 2 ns (from time period of 3.0 to 5.0 ns) after which the hydrogen bond breaks again at 6.8 ns, reforms at 7.0 ns, and again breaks at 7.5 ns. Thus, this arginine exhibited a tendency of forming and breaking a hydrogen bond with Val777. Any time the hydrogen bond broke, Arg784 moved towards the hinge and became partially exposed to the ATP site. This indicates that this arginine is mobile and can adopt at least two possible conformational states that would result in opening or shutting of the channel.

The ATP-site channel's opening and shutting would result in different interactions with ATP and inhibitors. This could be a contributing factor to the selectivity of ligands towards one kinase or the other. In EGFR, the channel would cause a portion of the ATP site to be open to external water. In HER-2, this channel may not open readily or is shut, with the possible involvement of Arg784. When hydrocarbon portion of Arg784 plugs the channel it would increase the hydrophobicity of the ATP site of HER-2.

Conclusions

The dynamic effects of the changes of amino acid residues outside the ATP site between EGFR and HER-2 that interact with residues in the ATP site, might explain the differences between the two enzymes at this site. These effects cannot be accounted for by homology models alone, but by dynamics simulation studies like the one reported here. It appears that the consequences of the change of Asp746 in EGFR to Gly778 in HER-2 along with the change of Cys751 to Ser783 in the ATP site account for the selectivity of ligands between EGFR and HER-2. This study suggests that the Asp746 in EGFR to Gly778 in HER-2 introduces large dynamic and conformational changes that would appear to be more significant than just the conservative change of Cys751 to Ser783 in regards to ATP site binding selectivities. The dynamics-generated structures are being used to probe ligand selectivity between the two enzymes, and to guide the design of inhibitors.

Supplementary material

Plots of energy, temperature and pressure over the sampling period for EGFR and HER-2 simulations.

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