Investigation of the binding of p53 and DNA upon DNA-contact mutations (R273C and R273H) and their effect on cancer

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Abstract: In this contribution, we elucidate the structural and functional consequence of p53 proteins upon DNA-contact (R273C & R273H) mutations and their molecular mechanisms at the atomic level by molecular dynamics simulations. Further, we also carried out a docking study to observe the p53-DNA binding pattern upon DNA-contact mutations. In this study we clearly observe that, due to DNA-contact mutations, the p53 structure is damaged and the p53-DNA interaction pattern is lost, which may lead to cancer.

Keywords: P53, rigidity, molecular dynamics simulations, DNA, binding affinity, cancer

1. Introduction

p53 is a tumour suppressor protein which is encoded by the P53 gene. P53 is also called “the guardian of genome” and it plays an essential role in cell cycle regulation (like cell apoptosis). P53 is mainly involved in control and monitoring the cell division. The P53 gene encodes a protein called p53, which is a homo-tetramer, consisting of 393 amino acids. The most frequently mutated region in human cancer is the DNA-binding domain (DBD) [1]. Most of the mutations are missense mutations present frequently in the DBD, hence this leads to loss of target gene transactivation [1]. It is clearly proven that p53 plays a crucial role in cancer progression [2-10] as well as in different physiological [11] and anti-cancer responses [12]. The following six residues, Arg-248, Arg-273, Arg-175, Gly-245, Arg-249 and Arg-282, of p53 are frequently mutated in human cancer [13]. Most of the tumour-related p53 mutations, called hotspot mutations, occur in the DNA-binding core domain of p53. Arg-273 (R273), a DNA-contact amino acid, is one of the most frequently altered amino acid residues in human cancer, and it mutates into histidine (46.6%) and to cysteine (39.1%) [14, 15]. R273 residues play an important role in docking, p53 interaction with the DNA backbone [16]. Substitution of R273 by histidine or cysteine amino acid residues, referred to as R273H and R273C, leads to a dramatic reduction in the DNA binding affinity [17]. In this study, molecular dynamics (MD) simulations and the docking approach are applied to observe the structural and functional behaviour of the p53 protein upon mutations and their binding affinity with DNA at the molecular level.

2. Simulation methods

2.1. Molecular dynamics simulations

The crystal structure of native (PDB ID: 2AC0_A) and mutant (PDB ID: 4IBQ_A & 4IBS_A) p53 and DNA (PDB ID: 4IBY_B) was obtained from the protein data bank (PDB) [18]. We consider a monomer of native and mutant p53 (chain A) and a monomer of DNA (chain B) for our studies. The superimposed structures of native and DNA-contact mutant p53 proteins are shown in Fig. 1.

Fig. 1. Schematic representation of superimposed structure of native and mutant p53 proteins. a) R273C, and b) R273H. The native structure is shown in blue; the mutants in brown (a) and purple (b).

MD simulations were performed using the GROMACS 4.6.1 package [19]. Native and mutant p53 structures were used as input structures for the MD simulations. The systems were solvated in a cubic box with TIP3P water molecules at 10 Å marginal radius. At physiological pH conditions, the structures were found to be positively charged. Hence, we added chloride ions (Cl⁻) to make the system electrically neutral in the simulation box. Initially, the solvent molecules were relaxed, whereas all the solute atoms were harmonically restrained to their original positions with a force constant of 100 kcal/mol for 5000 steps. After this, the whole molecular system was subjected to energy minimization for 5000 iterations by the steepest descent algorithm,
implementing the amber ff99SB-ILDN force field. The Berendsen temperature coupling method is applied to regulate the temperature inside the box. The Particle Mesh Ewald (PME) method was used to treat the long-range electrostatic interactions. The pressure was maintained at 1 atm with an allowed compressibility range of 4.5 x 10^{-5} atm. The LINCS algorithm was used to constrain the bond lengths involving hydrogens, permitting a time step of 2 fs. Van der Waals and Coulomb interactions were truncated at 1.0 nm. The non-bonded pair list was updated every 10 steps and conformations were stored every 0.5 ps. A position restraint simulation for 1000 ps was performed to allow solvent molecules to enter the cavity region of the structure. Finally, the systems were subjected to MD simulations for 85 ns. We then performed root mean square deviations (RMSD), Radius of gyration (Rg) and Solvent accessible surface area (SASA) analysis between native and DNA-contact mutants to examine the structural and functional behaviour of the p53 protein. To further support our MD simulation results, the large scale collective motions of the native and DNA-contact mutants of p53 were conducted by using essential dynamics (ED) analysis. The dynamics of two proteins were best characterized via through their phase space behaviour. The eigenvectors of the covariance matrix were called its principle components. The change of a particular trajectory along each eigenvector was obtained by this projection.

2.2. Protein-DNA interactions

We docked the monomer of p53 (output of MD simulation) with a monomer of DNA by the HADDOCK server (http://www.nmr.chem.uu.nl/haddock). A detailed analysis of the docked complex exposes notable features. Calculation of the HADDOCK score is essential to understand the affinity level between the biological partners. A HADDOCK score is defined to rank the structures after each docking stage. It is a weighted sum of intermolecular electrostatic (Elec), van der Waals (vdw), desolvation (Dsolv), AIR energies and a buried surface area (BSA) [20, 21].

3. Results and discussion

3.1. Molecular dynamics simulations

We performed RMSD, Rg, SASA and principle component analysis (PCA) between the native and mutant (R273C and R273H) p53 protein structures. The RMSD for all Cα-atoms from the starting structure was analyzed to study the convergence of the protein system. In the RMSD plot (Fig. 2a), from initial to ~ 2500 ps, both mutant (R273C and R273H) structures show less deviations than the native structure. After 2500 ps, both native and mutant structures show almost a similar way of deviations till 23000 ps. After that, again both mutant structures show a more abrupt decrease in RMSD value than the native structure till the end of the simulation. The average RMSD values of the native and mutant structures (R273C and R273H) were signified in Table 1. It clearly indicates that, due to the mutations, p53 loses its stability and this affects the structural orientation of the p53 protein.

Fig. 2. RMSD, Rg and SASA of native and mutant (R273C AND R273H) p53 proteins versus time at 300 K.

Table 1. Average value of RMSD, Rg, SASA and co-variance matrix of native and mutant (R273C and R273H) structures.

<table>
<thead>
<tr>
<th></th>
<th>Native</th>
<th>R273C</th>
<th>R273H</th>
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<tbody>
<tr>
<td>RMSD (nm)</td>
<td>0.23 ± 0.03</td>
<td>0.18 ± 0.02</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>Rg (nm)</td>
<td>1.68 ± 0.01</td>
<td>1.64 ± 0.01</td>
<td>1.65 ± 0.01</td>
</tr>
<tr>
<td>SASA (nm)</td>
<td>39.9 ± 1.1</td>
<td>36.4 ± 0.9</td>
<td>36.8 ± 0.9</td>
</tr>
<tr>
<td>Co-variance (nm²)</td>
<td>113.24</td>
<td>76.73</td>
<td>73.43</td>
</tr>
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</table>

The conformational alteration of the p53 protein upon DNA-contact mutations (R273C and R273H) is further supported by the Rg, SASA and PCA analysis. The Rg parameter provides an indicative level of compaction in the protein structure. It is defined as the mass-weighted root mean square distance of the collection of atom from their common centre of mass. In the Rg plot (Fig. 2b), the native structure shows a higher Rg value from the start to the end of the simulation than both mutant structures. The average Rg value of native and both mutant structures is listed in Table 1.

The change of SASA of the native and mutant (R273C and R273H) proteins with time is shown in Fig. 2c. The native and mutant structures (R273C and R273H) show a similar way of deviation till ~ 15,000 ps. After that, both
mutant structures show a lower value of SASA with time than the native p53. The average value of SASA during the simulation time period in native and mutant (R273C and R273H) structures is signified in Table 1.

The lower fluctuation in the Rg in both mutant structures indicates that the protein might be undergoing a significant structural transition. This is further supported by the fluctuations in SASA. A lower value of SASA in both mutant structures (R273C and R273H) the p53 protein loses its flexibility conformation and becomes more rigid in nature. This is further supported by PCA analysis. The spectrum of the corresponding eigenvalues indicates that the fluctuation of the system is basically restricted within the first two eigenvectors. The projection of trajectories obtained at 300K onto the first two principle components (PC1, PC2) shows the motion of native and DNA-contact mutant proteins in phase space, as shown in Fig 3.

![Projection of the motion of the protein in phase space along the first two principal eigenvectors at 300 K.](image)
a) native and R273C; b) native R273H.

Both DNA-contact mutants (R273C and R273H) cover a shorter region of phase space than the native structure. The overall flexibility of native and mutant (R273C and R273H) structures is calculated by a trace of diagonalized covariance matrix. We obtain the following values for native and mutant (R273C and R273H) structures: 113.68 nm$^2$, 76.73 nm$^2$, and 73.43 nm$^2$, respectively (Table 1), and again it confirm that the mutant structures lose their overall flexibility and become more rigid in nature. These changes might disturb the functional behaviour of the p53 protein and lead to cancer.

### 3.2. Protein-DNA interactions

From the MD approach we conclude that, due to DNA-contact mutations (R273C and R273H) the p53 protein loses its stability and becomes more rigid in nature. And this rigid confirmation can alter the binding phenomenon of protein and DNA molecule. To validate this, we apply the docking approach to evaluate the binding pattern between the native and mutant p53 proteins with DNA by the HADDOCK server.

Calculation of the HADDOCK score is essential to understand the binding affinity between the biological partners. The overall Haddock score of the native-DNA complex shows a value of $-72.9 \pm 6.9$, whereas R273C-DNA and R273H-DNA complexes show a score of $-64.0 \pm 5.9$ and $-63.3 \pm 4.7$, respectively (Table 2). A higher negative value of the HADDOCK score for the native-DNA complex indicates a higher binding affinity between the biological partners when compared to R273C-DNA and R273H-DNA complexes. The buried surface area (BSA) is used to quantify the protein surface which is not exposed to water. A higher BSA value enables a close proximity between the biomolecules. The desolvation energy, restraints violation energy and BSA have a good correlation with the docking score of the complex during docking. From Table 2, it is clearly indicated that the DNA-contact mutations (R273C and R273H) lose their interaction with DNA.

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Haddock score</th>
<th>Van der Waals energy (Kcal mol$^{-1}$)</th>
<th>Electrostatic energy (Kcal mol$^{-1}$)</th>
<th>Desolvation energy (Kcal mol$^{-1}$)</th>
<th>Restraints violation energy (Kcal mol$^{-1}$)</th>
<th>Buried surface area (Å$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>$-72.9 \pm 6.9$</td>
<td>$-65.5 \pm 5.8$</td>
<td>$-174.4 \pm 18.8$</td>
<td>$65.7 \pm 8.8$</td>
<td>$72.4 \pm 42.09$</td>
<td>$2173.1 \pm 93.9$</td>
</tr>
<tr>
<td>R273C-DNA</td>
<td>$-64.0 \pm 5.9$</td>
<td>$-62.6 \pm 1.6$</td>
<td>$-138.7 \pm 24.4$</td>
<td>$46.7 \pm 4.8$</td>
<td>$69.2 \pm 65.62$</td>
<td>$2175.1 \pm 173$</td>
</tr>
<tr>
<td>R273H-DNA</td>
<td>$-63.3 \pm 4.7$</td>
<td>$-65.6 \pm 8.1$</td>
<td>$-176.6 \pm 41.8$</td>
<td>$81.1 \pm 8.6$</td>
<td>$65.4 \pm 65.36$</td>
<td>$1888.6 \pm 80.7$</td>
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Hydrogen bonds are by far the most important specific interactions in biological recognition processes and particularly essential in determining the binding specificity [22, 23]. The intermolecular hydrogen bonds can provide favourable free energy to the binding [24, 25]. The number of intermolecular hydrogen bonds was calculated for native and mutants (R273C and R273H) – DNA complex and are shown in Table 3. The native p53-DNA complex shows a total of 16 hydrogen bond formations between the complexes whereas R273C-DNA and R273H-DNA complexes show 6 hydrogen bonds (Table 3). Protein-DNA docking analysis and intermolecular hydrogen bonding patterns confirm that due to DNA-contact mutations (R273C and R273H) the p53 protein has severe interaction losses. Therefore, this functional loss might be the reason to suppress the cancer activity in p53 protein.
Table 3. Number of hydrogen bonds of native and mutant complex.

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Number of Hydrogen Bonds</th>
</tr>
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<tbody>
<tr>
<td>Native-DNA</td>
<td>16</td>
</tr>
<tr>
<td>R273C-DNA</td>
<td>6</td>
</tr>
<tr>
<td>R273H-DNA</td>
<td>6</td>
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</tbody>
</table>

4. Conclusion
In this study, we highlighted the structural and functional significance of p53 proteins upon DNA-contact (R273C & R273H) mutations and their binding pattern with a DNA molecule. Due to DNA-contact mutations (R273C and R273H), the p53 protein loses its stability and becomes more rigid, which is well supported by RMSD, Rg, SASA and PCA analysis. This alteration might change the structural conformation and binding of the p53 protein. From the docking approach we clearly notice that the DNA-contact mutations (R273C and R273H) in the p53 protein might disturb the binding affinity with DNA, which is sufficient to inhibit the suppression of cancer activity. This insight might help scientists to develop a potential drug target for p53 cancer associated diseases.

5. References