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Detailed Molecular Modeling of Point Mutations of P53

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Abstract

TP53 encodes p53, a nuclear phosphoprotein with cancer-inhibiting properties. Mutations in the p53 are associated with more than 50% human cancers, and 90% of these affect p53-DNA interactions, resulting in a partial or complete loss of transactivation functions.

In theory, it should be possible to restore at least some functional activity of p53 mutants by enhancing the stability of the protein in its folded state, and/or providing additional DNA contacts. However, so far, the exploitation of the available clinical data has been hampered by our limited understanding of the structural and functional characteristics of the individual p53 mutants.

Accordingly, for the first time we propose a computational study of the p53 core domain and 4 oncogenic mutants involved in different cancer pathologies. Our data, based on accurate molecular modeling techniques, identify different mutant classes distinct from the wild type that correlate with the well-defined region of the core domain structure, and with the relevant thermodynamic stability.

Introduction

It is becoming progressively accepted that the progression of mammalian cells towards malignancy is an evolutionary process that involves an accumulation of mutations on both the molecular and chromosomal level. Inherent in models for malignant progression is the concept that an initial mutation in an important regulatory gene (protein) may be pivotal in this process. Once the initial mutation is introduced, loss of normal gene function or the acquisition of deleterious functions may lead to additional mutations furthering the malignant transformation of the cell.

A candidate for the involvement in this process is the tumor suppressor, p53. The p53 protein provides one of the key regulatory elements monitoring genomic integrity in mammalian cells and is involved in a multiplicity of cellular functions. This ubiquitous factor is kept in a repressed state in normal cells, but is activated by post-translational modifications in response to forms of stress, both genotoxic and non-genotoxic. When active, the p53 protein accumulates to high levels in the nucleus and acts as a multi-functional transcriptional factor to enhance or repress the expression of several genes involved in cell cycle progression, apoptosis, adaptive response to stress, differentiation and DNA repair.

Tumor-specific p53 mutations were first identified in 1989. Loss of p53 function is the most common event in human cancer, with more than half of all invasive tumors involving the decrease or total loss of p53 functions. In contrast to many other tumor suppressors, which are often inactivated by deletion or frameshift mutations, most of mutations in TP53 are point mutations, which are exceptionally diverse in their nature and position.

The open reading frame (ORF) of human p53 codes for 393 amino acids, consisting of three major structural domains: an N-terminal domain, which contains a strong transcription activation signal, a DNA-binding core domain (CD), and a C-terminal domain, which mediates oligomerization (see Figure 1).

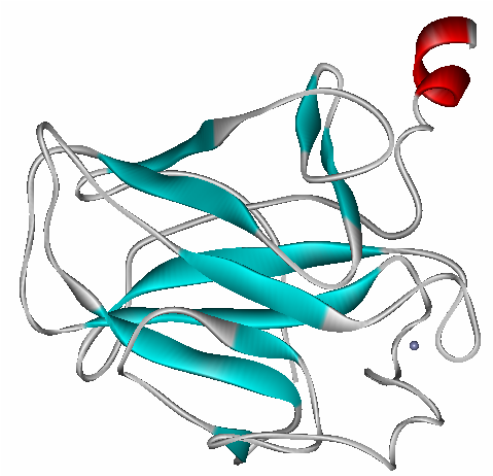

Figure 1. Core domain of the tumor-suppressor protein p53.

A detailed analysis of p53 mutations shows that the vast majority of the mutations of p53 cluster in conserved regions of the CD (residues 96-292), and some 20% of all mutations are concentrated at five "hotspot" codons in the core domain: 175, 245, 248, 249 and 273. The notion that several categories of mutant may exist has received much attention since it was realized that not all mutants are functionally equivalent, and substitutions in p53 CD have been classified in a number of ways. In general, however, the substitutions found in cancer fall into two broad categories: 1) *contact mutations*, located at the p53/DNA interface and breaking crucial contacts between the protein and the nucleic acid, or between p53 and other proteins and 2) *conformational mutations*, located in the protein skeleton and thought to prevent its correct folding, thus thwarting high-affinity DNA binding. Indeed, accurate thermodynamic experimental analyses have revealed that

p53 is only marginally stable at body temperature, so any mutation which further reduces stability is likely to lead to unfolding/misfolding in vivo.

Accordingly, in this study we present for the first time free energy perturbation calculations of the effects of the mutations G245S, R248Q, R249S and R273H on the stability of p53 core domain. The relative calculated free energy differences $\Delta\Delta G_{calc}$ between native and denatured models for WT p53 CD and each mutant are in good agreement with the corresponding experimental values. The decomposition of $\Delta\Delta G_{calc}$ into the contributions of specific interactions, and their relevant analysis have allowed us to draw detailed information, which helped us to understand the mechanism of the stability changes caused by the considered mutations.

Computational details

The 3D molecular model of p53 was obtained starting from the relevant human p53 crystal structure (entry 1TSR in PDB), by applying a relaxation procedure based on AMBER force field and GB/SA continuum solvation model. As expected, no relevant structural changes were observed between the p53 relaxed structure and the original 3D structure, as depicted in Figure 2. The simulations were carried out with the AMBER 6.0 suite of programs at physiological pH. Further assumptions were made concerning protonation state assignments for the ionizable residues. The non-bonded model for the zinc ion was adopted, and to the metal the full normal charge of 2+ and the van der Waals radius taken from the work of Stote and Karplus were assigned.

To let the protein relax in an aqueous environment, the macromolecule was immersed in a sphere of TIP3P-water molecules. The solvent sphere together with the WT p53 CD were minimized with a gradual decrease in the position restraints of the protein atoms.

Further, to achieve electroneutrality, the appropriate number of counterions (Na^+ and Cl^-) were placed next to solvent exposed charge residues.

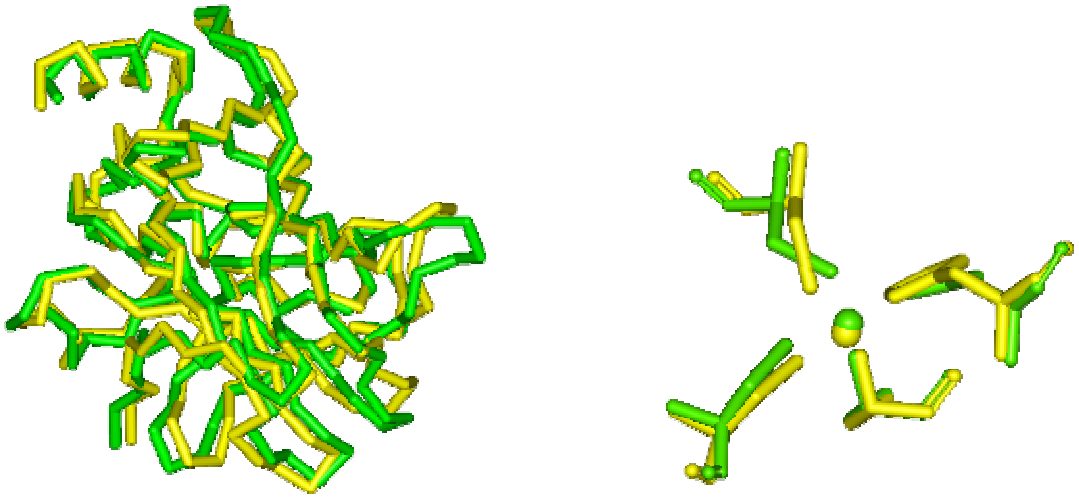

Figure 2. Overlapped images of the starting (green) and energy minimized (yellow) models of the entire CD (left, rmsd = 1.4 Å) and of the zinc-binding region (right, rmsd = 0.4 Å) of p53.

Since X-ray/NMR structures of mutant p53 CD molecules have not been reported yet, the initial structures for the mutant p53 simulations were prepared, relying on experimental evidences that G245S, R248Q, R259S and R273H should adopt overall fold similar to the WT protein, by a side chain rotamer library method.

All MD simulations and energy minimizations were performed with the SANDER module of AMBER 6.0 and the Cornell et al. force field

In the denatured state simulations we assumed a tripeptide model of sequence AXB, in which A and B were the amino acid residues preceding and following the mutating residue X, respectively. Therefore, in the specific application reported here, we used residues 244-246 (G244-G245-M246), 247-249 (N247-R248-R249), 248-250 (R248-R249-P250), and 272-274 (V272-R273-V274) of the WT p53 CD and mutated

G245→S245, R248→Q248, R249→S249 and R273→H273 in solution to determine an approximate value of ΔG_D. Then, the value of ΔG_N was determined in two different simulations that 'alchemically' transformed X→Y and Y→X in the proteins for each mutation. Each tripeptide was blocked by a CH_3 group prior to residue A and a $NHCH_3$ group following residue B.

Finally, the free energy calculations were carried on these systems, and the free energies for both the forward (λ: 0→1) and reverse (λ: 1→0) directions were obtained. The perturbation group was assumed to be the whole mutant residue, but only interactions occurring between this residue and other residues were included in the evaluation of ΔΔG. Thus, we are implicitly assuming that the intragroup energies are similar in the native and denatured protein.

Results and Discussion

Table 1 reports the results obtained from the application of the FE calculations to the p53 WT and its 4 considered mutations.

Mutation	$\Delta\Delta G_{exp.}$ (kcal/mol)	$\Delta\Delta G_{calc.}$ (kcal/mol)
R248Q	1.87 ± 0.09	2.04 ± 0.14
R273H	0.45 ± 0.04	0.22 ± 0.15
G245S	1.21 ± 0.03	1.50 ± 0.19
R249S	1.92 ± 0.04	2.20 ± 0.15

Table 1. Comparison of calculated and experimental energy of denaturation for Wt p53 and its 4 mutations.

From Table 1 we can see that statistical error in the simulations is of the order of a few tenths of kcal/mol; further, he calculated results are of the observed sign (wild type more stable than mutants) and have a magnitude close to the experimental values in all cases. Accordingly, MD/FPE appears sufficiently accurate to be used for obtaining information about the difference in stability of the protein conformations under consideration, and to permit analysis of the origin of these differences.

As an example, we can consider mutation R248Q, for which $\Delta\Delta G$ = 2.04 kcal/mol, and try to find an explanation for the cause of the greater stability of the WT R248 with respect to the MUT Q238. The analysis of the MD trajectories reveals that, in the structure of the native P53, R248 is involved in a persistent, fundamental hydrogen bond (HB) with S240 (average dynamic length = 2.6Å), which lies between the two Zn^{++} ligands, C238 and C242 (see Figure 3). This no longer exists in Q248. The natural question is then: is this HB the key to the greater stability of the WT protein? To answer this question, we mutated the charges of the R248 NH group onto N and H, in the native structure and in the corresponding tripeptide and, as a result, we estimated that the unsatisfied HB can explain 0.8 kcal/mol of the MUT instability ($\Delta\Delta G_{elec}$ = 1.00 kcal/mol). On the other hand, the side truncation of an exposed CH_2 is found to cost 0.8 kcal/mol, since the variation of the van der Waals surface in the mutation, ΔA_{vdW} = 58 Å2 (213 *vs*. 165) and the corresponding variation in the solvent accessible surface is ΔSAS = 16 Å2 (139 *vs*. 123).

Conclusions

We can use MD/FPE method to analyze the effect of protein stability of site-specific mutation. Nonetheless, a number of caveats should be emphasized, such as: (1) the

uncertainty in structure to use to transform the denatured protein into its site-specific mutant; (2) the conformational space of a floppy peptide and how relevant this sample is to the actual denatured protein and (3) the procedure gives un upper bound for the amount of solvent in the denatured protein, so that $\Delta\Delta G$ is slightly overestimated, although error bars do not make this overestimation definitive. Nevertheless, using the slow-growth procedure we can unambiguously compute both the electrostatic and the vdW contribution to $\Delta\Delta G$ and, by resorting to further techniques, such as the zeroing of the charges, we can get new insight into protein stability.

Acknowledgments

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