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Investigation of structural dynamics of Thrombocytopenia Cargeeg mutants of human apoptotic cytochrome *c*: A molecular dynamics simulation approach

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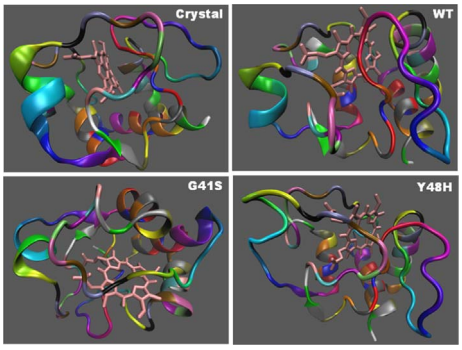
HIGHLIGHTS

- The structural dynamics of G41S and Y48H has been investigated using computational technique.
- We provide new atomistic level molecular mechanism for low platelet formation.
- Conformational switch of α-helix to β-turn should attribute to the loss of H-bonds.
- Essential dynamics unveils that the overall motions are three and two eigenvectors for G41S and Y48H, respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

Naturally occurring mutations to cytochrome *c* (cyt-*c*) have been identified recently in patients with mild autosomal dominant thrombocytopenia (low platelet levels), which yield cyt-*c* mutants with enhanced apoptotic activity. However, the molecular mechanism underlying this low platelet production and enhanced apoptosis remain unclear. Therefore, an attempt is made herein for the first time to investigate the effects of mutations of glycine 41 by serine (G41S) and tyrosine 48 by histidine (Y48H) on the conformational and dynamic changes of apoptotic (Fe^{3+}) cyt-*c* using all atom molecular dynamics (MD) simulations in explicit water solvent. Our 30 ns MD simulations demonstrate considerable structural differences in G41S and Y48H compared to wild type (WT) cyt-*c*, such as increasing distances between the critical electron transfer residues results in open conformation at the heme active site, large fluctuations in β-turns and α-helices. Additionally, although the β-sheets remain mostly unaffected in all the three cyt-*c* simulations, the α-helices undergo conformational switch to β-turns in both the mutant simulations. Importantly, this conformational switch of α-helix to β-turn around heme active site should attributes to the loss of intraprotein H-bonds in the mutant simulations especially between NE2 (His26) and O (Pro44) in agreement with the experimental report. Further, essential dynamics analysis reveals that overall motions of WT cyt-*c* is mainly involved only in the first eigenvector, but in G41S and Y48H the

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overall motions are mainly in three and two eigenvectors respectively. Overall, the detailed atomistic level information provide a unifying description for the molecular mechanism of structural destabilization, disregulation of platelet formation and enhanced peroxidase activity of the mutant cyt-*c*'s in the pathology of intrinsic apoptosis.

1. Introduction

Thrombocytopenia Cargeeg (THC4; OMIM 612004) is a unique autosomal dominant disorder, affecting a seven-generation family, caused by mutations of human cytochrome *c* (cyt-*c*) that dysregulates platelet production [1–3]. The primary life-supporting function of cyt-*c* is control of cellular energetic metabolism as a mobile shuttle in the electron transport chain of mitochondria, where iron of cyt-*c* is in the Fe^{3+} oxidation state (apoptotic). Although, cyt-*c* is an important electron transporter in the mitochondrial respiratory chain and is also an essential mediator of the intrinsic apoptosis pathway. In response to numerous apoptotic stimuli cyt-*c* is released from mitochondria and binds with apoptotic protease activating factor-1 (Apaf-1) in the cytosol. Seven cyt-*c*-Apaf-1 complexes form the apoptosome, a caspase activating platform leading to apoptosis [4–7]. The Thrombocytopenia Cargeeg mutations result in substitution of glycine 41 with serine (G41S) and tyrosine 48 with histidine (Y48H) in human naturally occurring cyt-*c* [8].

Gly41 is conserved in all eukaryotic cyt-*c* and is located at the beginning of Ω-loop (residues 40–57) which plays an important role in cyt-*c* folding and unfolding [8]. A first naturally occurring mutation in human cyt-*c* (G41S), resulting in an enhancement of the ability of cyt-*c* to activate caspases in vitro [1] and human G41S mutant of cyt-*c* exhibits an increased peroxidase activity without prior loss of the Fe-Met80 bond. Although the presence of Ser at residue 41 alters the electronic structure of the heme cofactor and increases the electron-self-exchange rate of the protein [8], the redox potential is not altered and the respiratory activity of cyt-*c* in the mitochondrial electron transport chain is unaffected [1]. The G41S mutation has no impact on the activity of cyt-*c* as a mitochondrial electron carrier but enhances caspase activation and peroxidase activity [1,8,9]. Further, G41S mutant alters the H-bond network in the region comprising residues 40–57, impairing its interactions with the heme center [9]. The respective contributions of the G41S mutant and the presence of cardiolipin-bound cyt-*c* were similar, and not additive, suggesting a shared mechanism for the enhancement of peroxidase activity. In order to explain the pro-apoptotic activity of G41S mutant, it was hypothesized that a more efficient electron transfer would accelerate generation of oxidized species of cyt-*c*, promoting apoptosis [10,11].

Further, tyrosine residues serve as substrates for post-translational modifications and for tuning redox properties and peroxidase activity of cyt-*c* [12]. Mutation of Tyr residues in cyt-*c* has been shown to induce an early alkaline transition with a gain of peroxidase activity [11]. Tyr48 is spatially close to glycine 41 and packs against the loop whose structure is defined by the presence of glycine. Therefore, it is possible that their similar phenotypic effect come from similar phenomena occurring in the protein folding [13]. A second naturally occurring mutation in human cyt-*c* gene was also identified (Y48H), resulting in biological effects similar to those observed in patients carrying the G41S mutation [2]. For both mutations, the detailed atomic level molecular mechanisms leading to alteration of platelet production and apoptosis remain obscure [2].

Since mainly static structural information can be obtained analyzing crystal structures of cyt-*c* mutants if available, molecular dynamics (MD) simulations were used as an alternative tool for probing the structural as well as conformational differences between the two mutants studied herein and the WT. Information about the dynamical properties of these mutants (G41S and Y48H) of cyt-*c* is crucial for understanding their role the protein activity such as peroxidase activities and low platelet formation on a molecular level. As altering one residue to another can create a ripple effect through the protein that eventually affects the overall protein stability and functions [14,15], it is important to probe the perturbations introduced in the protein folding by these new residues on an atomic level using MD simulations. Since horse cyt-*c* behaves very much like its human counterpart [16] and, unlike yeast cyt-*c*, actively participates in cell apoptosis [17], this protein was utilized in the present study. Studies describing the structure and dynamics of cyt-*c* are available [18–23]. In 2009, Raja Singh et al. performed 4 ns MD simulations of the Y67F and F82H mutants of cyt-*c* demonstrating a reduced conformational flexibility of the mutated proteins [24]. Despite the recent advances, however, the detailed microscopic dynamical parts have remained poorly understood in the big picture of cyt-*c* mutants-mediated autosomal dominant thrombocytopenia with enhanced apoptotic activity. We report herein, for the first time, the dominant patterns of structural changes upon mutations of cyt-*c* (G41S and Y48H) from multiple 30 ns MD. The G41S and Y48H mutated cyt-*c* proteins were recently reported as naturally occurring mutants and are associated with a mild autosomal dominant thrombocytopenia caused by disregulation of platelet production [2]. These simulations reveal significant changes in the structure and dynamics of the mutated proteins, which is discussed in terms of secondary structure contents, RMSD, RMSF, H-bonding network, distance between critical residues and essential dynamics.

2. Computational methods

The starting structure of WT cyt-*c* was taken from the 1.94 Å resolution refined structure of the protein horse heart cyt-*c* [25] and deposited in the protein data bank as 1hrc. The Fe of heme is in +3 oxidation state (Fe^{3+}). The scaled Mulliken charges for the heme prosthetic group in the oxidized state were obtained from the theoretical work of Felix Autenrieth et al. [26]. The proteins were simulated at pH 7.0. Each structure was fully solvated with SPC water [27] in a box with sides 3.668 × 3.049 × 3.651 nm with box angles 90° for each side. All these calculations were performed using GROMOS96 [28] force field implemented in GROMACS 4.5.3 [29,30]. The energy minimization was done using the steepest descent algorithm. The maximum step size for energy minimization was 0.01 nm and the tolerance was 6000 kJ/mol/nm. The protein has a net charge of + 8e and was neutralized by introducing eight Cl^- ions by replacing eight solvent molecules that possess the highest electrostatic potential. Before the MD simulation, position restrained MD was performed which involves restraining (partially freezing) the atom positions of the macromolecule, while simultaneously allowing the solvent to move freely during the simulation. In all simulations, the temperature was maintained at 300 K by weak coupling to an external temperature bath [31] with a coupling constant 2 fs, equal to the time step with a dielectric permittivity of $\varepsilon_r = 1$. The protein and the rest of the system were coupled separately to the temperature bath. The LINCS algorithm [32] was used to constrain all bonds. The algorithm is inherently stable, as the constraints themselves are reset instead of derivatives of the constraints, thereby eliminating drift. For the water molecules, the SETTLE algorithm [33] was used. Multiple MD simulations have been performed for 30 ns to reproduce the similar trajectories and the data were collected every 500 ps during the production run. A twin range cut-off was used for the calculation of the non–bonding interactions. The short range cut-off radius was set to 0.9 nm and the long range cut-off radius to 1.4 nm for both Columbic and Lennard-Jones interactions. The