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Dynamics of DDB2-DDB1 complex under different naturally-occurring mutants in Xeroderma Pigmentosum disease

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ABSTRACT

Background: Xeroderma Pigmentosum (XP) is a disease caused by mutations in the nucleotide excision repair (NER) pathway. Patients with XP exhibit a high propensity to skin cancers and some subtypes of XP can even present neurological impairments. During NER, DDB2 (XPE), in complex with DDB1 (DDB-Complex), performs the DNA lesion recognition. However, not much is known about how mutations found in XP patients affect the DDB2 structure and complex assembly. Thus, we searched for structural evidence associated with the role of three naturally occurring mutations found in XPE patients: R273H, K244E, and L350P.

Methods: Each mutant was individually constructed and submitted to multiple molecular dynamics simulations, done in triplicate for each designed system. Additionally, Dynamic Residue Interaction Networks were designed for each system and analyzed parallel with the simulations.

Results: DDB2 mutations promoted loss of flexibility in the overall protein structure, producing a different conformational behavior in comparison to the WT, especially in the region comprising residues 354 to 371. Furthermore, the DDB-complex containing the mutated forms of DDB2 showed distinct behaviors for each mutant: R273H displayed higher structural instability when complexed; L350P affected DDB1 protein-protein binding with DDB2; and K244E, altered the complex binding trough different ways than L350P.

Conclusions: The data gathered throughout the analyses helps to enlighten the structural basis for how naturally occurring mutations found in XPE patients impact on DDB2 and DDB1 function.

General significance: Our data influence not only on the knowledge of XP but on the DNA repair mechanisms of NER itself.

1. Introduction

UV-induced mutations can lead to tumor formation and development of diseases, such as Cockayne Syndrome (CS), Trichothiodystrophy (TTD) and Xeroderma Pigmentosum (XP) [1–5]. In eukaryotes, the nucleotide excision repair (NER) pathway is responsible for removing such DNA lesions and restoring the DNA molecule [1, 6–8]. The NER pathway is majorly coordinated by the XP proteins (XPPs), that range from XPA to XPG [1, 6–8]. Each XPP is engaged to a given role in NER, which are: (i) lesion recognition, promoted by XPC and DDB2, the latter being the product of the XPE gene; (ii) DNA unraveling and opening, promoted by XPB and XPD; (iii) assembly of the

Abbreviations: BPA to BPC, β-Propeller A to C; Co-DDB^{K244E}, Complex DDB1-DDB2^{K244E}; Co-DDB^{L350P}, Complex DDB1-DDB2^{L350P}; Co-DDB^{R273H}, Complex DDB1-DDB2^{R273H}; Co-DDB^{WT}, Complex DDB1-DDB2$^{Wild\text{-}Type}$; Co-DDB1$^{DDB2(K244E)}$, Complex DDB1-DDB2^{K244E}, but with emphasis on DDB1; Co-DDB1$^{DDB2(L350P)}$, Complex DDB1-DDB2^{L350P}, but with emphasis on DDB1; Co-DDB1$^{DDB2(R273H)}$, Complex DDB1-DDB2^{R273H}, but with emphasis on DDB1; Co-DDB1$^{DDB2(WT)}$, Complex DDB1-DDB2$^{Wild\text{-}Type}$, but with emphasis on DDB1; Co-DDB1^{WT}, Complex DDB1-DDB2$^{Wild\text{-}Type}$, but with emphasis on DDB1; Co-DDB2^{K244E}, Complex DDB1-DDB2^{K244E}, but with emphasis on DDB2; Co-DDB2^{L350P}, Complex DDB1-DDB2^{L350P}, but with emphasis on DDB2; Co-DDB2^{R273H}, Complex DDB1-DDB2^{R273H}, but with emphasis on DDB2; Co-DDB2^{WT}, Complex DDB1-DDB2$^{Wild\text{-}Type}$, but with emphasis on DDB2; CPD, Cyclobutane Pyrimidine Dimers; CS, Cockayne Syndrome; DRIN, Dynamic Residue Interaction Networks; NER, Nucleotide Excision Repair; PPI, Protein-Protein Interaction; TTD, Trichothiodystrophy; XP, Xeroderma Pigmentosum; XPE, Xeroderma Pigmentosum, type E; XPPs, Xeroderma Pigmentosum Proteins

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excision machinery, coordinated by XPA and; (iv) excision of the damaged site, promoted by XPF an XPG [6]. Mutation in any XP gene can potentially cause CS, TTD and XP development [1, 7–9].

Although there is a considerable amount of data regarding the structure of XPP from humans and model organisms, such as *Danio rerio*, *Sulfolobus acidocaldarius,* and *Thermoplasma acidophilum,* little is known about how the mutations found in XP patients impact on human XPPs molecular behavior, as well as on their complexes assembly [9]. One particular case is the DDB-Complex, which is composed by the DNA damage-binding protein 2 (DDB2), a DNA-binding protein of 427 amino acid residues, the gene product of XPE and characterized by a single WD β-propeller region; and the DNA damage-binding protein 1 (DDB1), an 1140 residues DNA-binding protein, composed by three WD β-propeller domains [9–11], both being necessary for proper DNA damage recognition during NER. DDB2 is the active damage recognizer, having a higher affinity for cyclobutane pyrimidine dimers (CPD) adducts, and DDB1, although not related to XP development, is necessary for anchoring DDB2 on the DNA molecule for proper recognition [9–11]. XP, type E patients, (individuals caring mutations in the XPE gene) produce a defective DDB2 protein, which compromises DNA damage recognition by the DDB-complex. Although XPE patients do not display neurological impairments, they were reported to have 1000× more probability to develop skin cancers among the XP types, making it understand a relevant subject for the biomedical sciences [7].

Thus, to contribute in the understanding of how naturally occurring mutations found in XPE patients affects the DDB-Complex, three of the four known missense mutations in DDB2 were modeled in the current work: L350P and K244E, related to instability of the DDB-Complex [12, 13], and R273H, described to impair DDB2-DDB1 interaction [13, 14]. Multiple molecular dynamics and dynamic residues interaction networks (DRIN) were performed in triplicates for each system containing DDB2 and its mutant variants, as well for triplicates of systems containing DDB2 complexed with DDB1, and the same complex composed by DDB2 mutants. We observed a wide variety of structural changes caused by the mutant variants, in both DDB2 and DDB1, that could explain the disruption of the DDB-Complex.

2. Materials and methods

2.1. Mutants modeling and validation

The human DDB-Complex, composed by DDB2 and DDB1, is available under the name “Structure of the hsDDB1-hsDDB2 complex” (PDB ID: 3EI4) [15]. Both DDB2 and DDB1 have missing portions of their structures; therefore, the proteins were separated, and these gaps were reconstructed using different software. The *automodel* class from Modeller 9.10 [16], using the native protein sequence as target and the available crystallographic structure as template was employed to fill the missing segments of DDB1, whereas the missing N-terminal portion of DDB2 (residues 1 to 74) was reconstructed using PHYRE 2 [http://www.sbg.bio.ic.ac.uk/phyre2] [17]. The final structures, henceforth known as $DDB2^{WT}$ and $DDB1^{WT}$, were submitted to the server SWISS-MODEL [http://swissmodel.expasy.org/] [18] for validation, using PROCHECK for stereochemical assessment [19], Anolea for non-local atomic interaction energy evaluation [20] and Qmean for combined estimation of local and per-residue quality of the model [21]. The mutant variants of DDB2 ($DDB2^{R273H}$, $DDB2^{K244E}$, and $DDB2^{L350P}$) were individually designed employing PyMOL [https://www.pymol.org/], using $DDB2^{WT}$ as a template. Side-chain rotamers were selected based on similarity to the conformation already available for the native residue in the mutated position. Finally, the reconstructed proteins were reassembled using DeepView [22] and divided into four different complexes: (i) $DDB1^{WT}$ - $DDB2^{WT}$, named Co-DDB^{WT}; (ii) $DDB1^{WT}$ - $DDB2^{R273H}$, termed Co-DDB^{R273H}; (iii) $DDB1^{WT}$ - $DDB2^{K244E}$, termed Co-DDB^{K244E} and; (iv) $DDB1^{WT}$ - $DDB2^{L350P}$, titled Co-DDB^{L350P}.

2.2. Molecular dynamics simulations

For the molecular dynamics simulations, the GROMACS 4.5.1 and GROMACS 4.6.4 [23, 24] simulation suites were employed. All previously mentioned proteins were then inserted in dodecahedral boxes and solvated with SPC [25] water model under periodic boundary conditions. The GROMOS54a7 force field [26] was employed in the simulations, and Na^+ or Cl^- counter-ions were added to neutralize the systems when necessary. Covalent bonds were constrained using the LINCS algorithm [27], and an integration step of 2 fs was applied. The Particle Mesh Ewald [28] method was employed for calculation of electrostatic interactions. The Parrinello-Rahman barostat [29, 30] was employed, with a 2.0 ps coupling constant, while the V-rescale [31], was used with a coupling constant of $\tau = 0.1$. Steepest Descent algorithm was used in the energy minimization step, prior to the simulations. Energy minimization, as well as equilibration steps, were applied to remove any steric clashes on all systems. Three independent simulations of 200 ns were performed for each of the studied systems, generating new velocities at the beginning of each run in an effort to exclude low probability phenomena. All presented results correspond to the mean between the triplicates of each system.

2.3. Dynamic residue interaction networks

The final trajectory of each independent system was then submitted to a Dynamic Residue Interaction Network (DRIN) analysis, where the trajectory files are concatenated by the program CatDCD [http://www.ks.uiuc.edu/Development/MDTools/catdcd/] and later transformed into networks and analyzed in the VMD 1.9.1 platform [32] using the program Carma 0.8 [33]. In this method, alpha carbon atom from each residue is represented by a node, and the motion correlation between the nodes is depicted by an edge. The weight of the motion correlation between nodes during the simulated time is represented by the thickness of the edges, thus, the stronger the motion correlation between residues, the thicker the edge. The analysis also takes into consideration the number of communities (independent groups of residues with strong motion correlation among themselves).

3. Results and discussion

3.1. Loss of stability, flexibility, and molecular motion of DDB2 mutants

The final model for $DDB2^{WT}$ showed 85% residues in most favored regions and 10.6% in additional allowed regions (Supplementary Table.1, see Supplementary Material). In addition, the great majority DDB2 regions were in favorable energy environments and good overall quality (Supplementary Fig. 1, see Supplementary Material). Our model was also compared to another available PDB that contained DDB2-DDB1 structures (PDB: 4E54). As can be seen in Supplementary Table1, our model still reached better results in the Ramachandran plot, and the overall quality of our model was still similar to the other available crystal (Supplementary Figs.3–4, see Supplementary Material), with similar *Z*-score Q-Mean for DDB2 from PDB 4E54 and our model (−2.38 and − 3.10, respectively). The same was seen for DDB1, which displayed a Z-score Q-Mean of −1.09 for PDB 4E54 and − 1.20 for our model, assuring the quality of the final model employed in this work. Finally, for DDB2, as demonstrated in Supplementary Fig.5 (see Supplementary Material) the PHYRE2 server algorithm detected homology for the N-terminal sequence of DDB2 with other crystallographic structures and modeled the N-terminal portion in a similar state as the structure available in PDB 4E54. Please note that the first 20 residues were modeled by ab initio because this portion is still not resolved in any of the structures available. Furthermore, this N-terminal model was able to fit properly in the newly formed complex, as shown in Supplementary Fig. 5 (see Supplementary Material), without causing any clashes and directly influencing the network of interactions