Gene 591 (2016) 14–20

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

NQO1 rs1800566 polymorph is more prone to NOx induced lung injury: Endorsing deleterious functionality through informatics approach

Zarrin Basharat [a,*], Abdelmonaem Messaoudi [b], Sehrish Ruba [c], Azra Yasmin [a]

[a] Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi 46000, Pakistan
[b] Higher Institute of Biotechnology of Beja, Jendouba University, Habib Bourguiba Street, 9000 Beja, Tunisia
[c] Department of Environmental Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi 46000, Pakistan

ARTICLE INFO

Article history:
Received 14 May 2016
Received in revised form 12 June 2016
Accepted 23 June 2016
Available online 25 June 2016

Keywords:
NQO1
NOx
rs1800566
Functional analysis
Bioinformatics

ABSTRACT

Gene-environment interaction studies have led to the identification of genetic mutations in individuals with increased susceptibility to pollution related diseases. rs1800566 polymorphism of NQO1, leading to P187S missense mutation in the transcribed antioxidant protein, causes individuals carrying this mutation more prone to NO_2 induced lung inflammatory injury. Here, we report significant structural and functional changes incurred by NQO1 antioxidant protein as a result of alteration in its nucleotide (C609T) and hence, protein sequence. Detailed insights were obtained regarding the prospective impact of this mutation on the structural stability of normal and mutated NQO1 protein, using a myriad of bioinformatic tools and webservers. Structure analysis showed no significant change at secondary level. A change in the native backbone conformation was observed due to formation of a hydrogen bond. Hydrophobicity and phosphorylation properties, decisive factors for functioning and stability of NQO1 were considerably influenced by P187S mutation. Computational study of the properties of a polymorph linked with NOx induced lung injury sheds light on the molecular basis of this polymorphism and endorses previous findings, reported by the scientists working in this domain.

1. Introduction

Air pollution is a leading cause of decline in human health, especially of decreased functionality or injury of the respiratory system (Minelli et al., 2011). Anti-oxidant gene interaction studies with the gaseous and particulate matter air pollutants have been linked with lung inflammatory diseases on exposure beyond a certain limit. Inbred mouse research has demonstrated that interaction between antioxidant genes and air pollution leads to certain diseases. Genetic variation plays a considerable role in modulating susceptibility to the respiratory effects of air pollutants (Yang et al., 2007; Polonikov et al., 2014; Zhang et al., 2016) and is an emerging area of interest. Pollutant inhalation initiates pathologic progressions like airway inflammation leading to the airways disease exacerbation and pathogenesis. It is imperative to understand exogenous and endogenous oxidant structure and interaction mechanisms with molecules in the lung epithelial lining fluid, cells as well as tissues (Ciencewicki et al., 2008; Enami et al., 2015). All pollutants primarily act by inducing oxidative stress (Kelly et al., 2003) in genes leading to mutated protein product formation. Study and comparison of mutated vs wild type (WT) candidate protein can thus be a good approach for investigating the possible effects of gene-environment interactions.

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a flavoprotein that protects cells against radiation and chemically induced oxidative stress (Faig et al., 2000; Xu and Jaiswal, 2012). Disruption of NQO1 gene in humans leads to increased susceptibility to various diseases like hematological malignancy (Rothman et al., 1997; Nagata et al., 2013), lung cancer (Lin et al., 2003), bladder cancer (Hung et al., 2004; Gong et al., 2013; Cuff et al., 2015), nephropathy (Toncheva et al., 2004; Sharma et al., 2016) etc. The mutation of interest in the present study in NQO1 was 'Pro' to 'Ser' (Fig. 1) at position 187 reported to causes serious lung injury as a result of exposure to NO_2. The WT residue is a proline, recognized as a rigid residue capable of inducing unique backbone conformation which might be essential at this position. The mutation is supposed to perturb this particular conformation, leading to an increase in mutant protein instability.

Gene polymorphisms for the phase-II xenobiotic metabolizing enzyme NQO1 have been allied to pulmonary and epithelial impairments in response to O_3 in the exercising subjects (Bergamaschi et al., 2001;

Abbreviations: WT, Wild type; NQO1, NAD(P)H:quinone oxidoreductase 1; RMSD, Root mean square deviation; CUPSAT, Cologne University Protein Stability Analysis Tool; ΔΔG, unfolding energy; PDB, Protein data bank; MOE, Molecular Operating Environment; NPA, Nosé-Poincaré-Anderson; NVT, N–number of atoms, V–volume, T–temperature; MD, molecular dynamics; ps, picosecond; SPDViewer, Swiss PDB viewer.

* Corresponding author.
E-mail address: zarrin.iiui@gmail.com (Z. Basharat).

http://dx.doi.org/10.1016/j.gene.2016.06.048
0378-1119/

Kummarapurugu et al., 2013). NQO1 Pro187Ser polymorphism was evaluated in a study on nitrogen dioxide and its effects on asthma, which showed a recessive effect for the 187 Pro/Pro genotype (Castro-Giner et al., 2009). To assess the functional significance and potential impact of this mutation on the structure of NQO1 protein, a bioinformatics based approach was pursued to perform the sequence and structural analysis of this protein. The primary goal was to characterize the potential impact of this mutation on the 3D structure of this multi-functional protein responsible for NOx mediated lung injury.

Computational exploration of different mutated residue types at marked positions in a protein structure is a concise and inexpensive process to assess functional changes rising due to genetic sequence alteration. 3D protein structure modeling of side-chains enclosing concerned mutations can assist such an investigation (Xiang and Honig, 2001; Feyfant et al., 2007). Numerous softwares and webservers are available for assessing changes in the properties of mutant sequence and structure of proteins. Impact of mutations on the molecular characteristics of a protein via computational workflow have been implemented previously for cancer (Hussain et al., 2012; Kumar et al., 2013; Doss et al., 2014; Kumar and Purohit, 2014; Ozbabacan et al., 2014; Abdulazeez and Borgio, 2016; Basharat and Yasmin, 2016a; Singh and Mistry, 2016), neurodegenerative disorders (Masoodi et al., 2012; Kamaraj et al., 2015; Qian et al., 2015), cardiovascular disease (Ramesh et al., 2013), dengue shock syndrome (Taqi et al., 2016), inflammatory disorders (Dabhi and Mistry, 2014; Ozbabacan et al., 2014) etc. Computational analysis aids in understanding the molecular mechanism behind pathogenic nsSNPs in correlation to its functional and structural damaging properties. Optimized bioinformatics approach was therefore, used for the targeting/ swift identification of potential impacts of mutated residues on protein stability, phosphorylation, and hydrophobicity properties by comparing mutated and normal protein. This NQO1 mutant vs WT structure and sequence based comparative study is the first of its kind, documenting computational approach for predicting molecular changes in a polymorph responsible for gene environment interaction, leading to deleteriousness. It could operate as a foundation for other environmentally induced disease studies (of not only lung but other organs as well). This study can also be used for accelerated understanding of key underlying processes of epigenetics in various diseases and for inferring mechanistic outcomes for the epistatic relationships among genes and environment via in silico approach.

2. Material and methods

2.1. Sequence data and initial analysis

The amino acid sequence of human NAD(P)H dehydrogenase, quinone 1 (NQO1) protein was obtained from Uniprot with accession no: P15559. The FASTA sequence of human NQO1 containing the P187S mutation was generated by replacing the amino acid proline to serine at position 187. Physicochemical characteristics of WT and mutated sequences were analyzed by Expasy webserver online 'protparam' and 'protscale' tool (Wilkins et al., 1999). Multiple sequence alignment was carried out using ClustalW (Larkin et al., 2007) and analyzed further. Location of mutated residue on the domain surface was found using Hope server (Venselaar et al., 2010)

2.2. Phosphorylation and hydrophobicity profile

NetPhos 2.0 (Blom et al., 1999) was used to predict the phosphorylation potential of WT and mutated protein. Potential changes in overall hydrophobicity due to P187S were calculated by the Kyte and Doolittle (1982) method via 'protparam' and 'protscale' tool at Expasy webserver. Hydrophobicity of the residues was also predicted using Hope server (Venselaar et al., 2010) with DAS server at the backend.

2.3. 3D structural and dynamics simulation analysis

3D structure of NQO1 protein mutant with serine at position 187 has been recently solved by Lienhart et al. (2014) (PDB ID: 4CET). Ser187 was mutated to proline in SPDViewer and RMSD calculation among the WT and mutated structure was carried out using Superpose (Maiti et al., 2004), which works on the principle of a modified quaternion approach. Calculations were based on default similarity cutoff of 2 Å, dissimilarity cut off 3 Å and dissimilar subdomain of 7 residues. DelPhi (Li et al., 2012; Smith et al., 2012) was used to calculate the total difference in energy at solvated condition of WT and mutated polymorph. Profix and TINKER program were run to fix structural defects such as missing atoms. The PDB model structure of both WT and mutant was used as input to obtain the grid and coulombic energies (Biswas et al., 2009; AbdulAzeez and Borgio, 2016). Linear solver was used for calculating Poisson-Boltzmann equation. Other parameters were: Forcefield: Amber, pH: 7, Exterior (solvent) dielectric constant: 80.00, Percent fill: 80.00, Grid scale: 2.00, Salt concentration: 0.10, Probe radius: 1.40, Boundary conditions: 2.

Cursory molecular dynamics (MD) simulation was carried out using Molecular Operating Environment (MOE) according to a previously described protocol (De Martino et al., 2006; Basharat et al., 2016b) with slight modifications. NPA algorithm (Bond et al., 1999) with NVT ensemble environment was chosen for a timescale of 1000 ps and constant temperature of 300 K. Gromos force field with a time step of 2 fs was used for discretizing the equation of motion. Temperature relaxation time of 0.2 ps and a Tol value of $1e^{-012}$ was chosen to solve the holonomic constraints as small value satisfies the constraint better.

2.4. Stability tradeoff in human NQO1

In order to assess the impact of P187S, the sequence and structural analysis were employed. For this purpose, two different algorithms I-mutant2.0 (Capriotti et al., 2005) and CUPSAT were used to calculate protein stability based on protein sequence and to calculate changes based on structure respectively (Parthiban et al., 2006). Results were analyzed further.

3. Results and discussion

Each amino acid has its own specific size, charge, and hydrophobicity value. However, the respective properties of the original WT residue and newly introduced mutant residue frequently fluctuate. At the juncture, the mutant residue serine is smaller than the WT residue proline. The focus of the present study was to determine how this missense mutation contributes to maladies as a result of decreased structural stability of the NQO1 protein. Initially multiple sequence alignment (MSA) was performed between closely related species in order to check if Pro187 is a conserved residue. MSA analysis showed that proline at the 187th position is conserved among all the closely related organisms, whereas neither of them contained a serine at this position (Fig. 2).

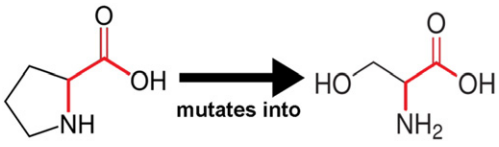

Fig. 1. The schematic structures of the original proline (left) and the mutant serine (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.