Journal of Molecular Graphics and Modelling 81 (2018) 68–76

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

In silico investigation of the molecular effects caused by R123H variant in secretory phospholipase A2-IIA associated with ARDS

Benedetta Righino [a], Angelo Minucci [a, b], Davide Pirolli [e], Ettore Capoluongo [a, b], Giorgio Conti [c], Daniele De Luca [c, d], Maria Cristina De Rosa [e, *]

[a] Institute of Biochemistry and Clinical Biochemistry, Catholic University of the Sacred Heart, L.go F. Vito 1, 00168 Rome, Italy
[b] Department of Laboratory Medicine, "Policlinico Gemelli" Foundation, L.go A. Gemelli 1, 00168 Rome, Italy
[c] PICU, Department of Anesthesiology and Critical Care, Catholic University of the Sacred Heart, L.go F. Vito 1, 00168 Rome, Italy
[d] APHP, South Paris University Hospitals, Medical Center "A. Beclere", Paris, France
[e] Institute of Chemistry of Molecular Recognition - CNR, c/o Catholic University of the Sacred Heart, L.go F. Vito 1, 00168 Rome, Italy

ARTICLE INFO

Article history:
Received 6 November 2017
Received in revised form
26 January 2018
Accepted 18 February 2018
Available online 6 March 2018

Keywords:
Molecular dynamics simulation
sPLA2
PLA2G2A gene
Molecular modelling
Electrostatic potential

ABSTRACT

Phospholipase A2-IIA catalyzes the hydrolysis of the sn-2 ester of glycerophospholipids. A rare c.428G > A (p.Arg143His) variant in PLA2G2A gene was found in two infants affected by acute respiratory distress syndrome (ARDS) by whole coding region and exon/intron boundaries sequencing. To obtain insights into the possible molecular effects of the rare R123H mutation in secretory PLA2-IIA (sPLA2-IIA), molecular modelling, molecular dynamics (MD) using principal component analysis (PCA) and continuum electrostatic calculations were conducted on the crystal structure of the wild type protein and on a generated model structure of the R123H mutant. Analysis of MD trajectories indicate that the overall stability of the protein is not affected by this mutation but nevertheless the catalytically crucial H-bond between Tyr51 and Asp91 as well as main electrostatic interactions in the region close to the mutation site are altered. PCA results indicate that the R123H replacement alter the internal molecular motions of the enzyme and that collective motions are increased. Electrostatic surface potential studies suggest that after mutation the interfacial binding to anionic phospholipid membranes and anionic proteins may be changed. The strengthening of electrostatic interactions may be propagated into the active site region thus potentially affecting the substrate recognition and enzymatic activity. Our findings provide the basis for further investigation and advances our understanding of the effects of mutations on sPLA2 structure and function.

1. Introduction

Phospholipases A2 (phosphatide 2-acylhydrolase, EC 3.1.1.4) are a widely distributed group of enzymes that catalyse the hydrolysis of ester bonds at the *sn*-2 position of phospholipids [1]. Secretory phospholipases A2 (sPLA2) represent the extracellular forms secreted by several cell types; over 10 distinct sPLA2 isotypes have been described in mammalians and the -IIA (sPLA2-IIA) is the main pulmonary isotype, secreted into the alveoli by alveolar macrophages [2].

sPLA2s are relevant in lung physiopathology, since they may contribute to the production of inflammatory mediators and are responsible for surfactant phospholipids catabolism. These two processes are typical of acute respiratory distress syndrome (ARDS), which is a life-threatening condition characterized by high lung tissue inflammation and surfactant dysfunction [1,3]. sPLA2-IIA is increased in broncho-alveolar lavages (BAL) from ARDS animal models [4,5]; moreover BAL levels of sPLA2-IIA are increased in ARDS adult patients and correlate with clinical severity [6,7]. Given the role of sPLA2 in ARDS in adult patients, we set up an international network to investigate the role of sPLA2 in various pediatric critical respiratory conditions, designing a multi-step translational project whose plan has been described elsewhere [8]. We demonstrated that sPLA2-IIA is a main enzyme isotype in infants with post-neonatal ARDS: moreover, in these patients, sPLA2 activity correlates with clinical severity and also with mortality, length of pediatric intensive care (PICU) stay and respiratory outcomes [9,10]. Pediatric ARDS is defined as an acute-onset

* Corresponding author.
E-mail address: mariacristina.derosa@icrm.cnr.it (M.C. De Rosa).

https://doi.org/10.1016/j.jmgm.2018.02.014
1093-3263/

multifactorial condition needing peculiar triggering factors and an underlying given degree of predisposition [11]. In fact, polymorphisms of the NFκB gene, which is the sPLA2-IIA transcription factor, are associated with the risk to develop ARDS and its outcomes [12,13]. It is known that a sPLA2 gene polymorphism is associated with complications of chronic obstructive pulmonary disease, however data about sPLA2 genetics and ARDS are lacking [14]. Within our multi-step project we also meant to study sPLA2 genetics, thus we sequenced sPLA2-IIA gene (PLA2G2A) in infants with ARDS [8].

sPLA2-IIA protein has been described in detail [1]. It is characterized by a low molecular weight (14 kDa), seven conserved disulfide bonds and His/Asp dyad for the catalytic mechanism and Ca^{2+} bound in the active site. The active site possesses a hydrophobic channel, which is formed by Leu2, Val3, Phe5, His6, and Ile9 on the N-terminal α1 helix (2–13) and Alal7 and Ala18 on the short α2 helix (16–24). The calcium ion, which is essential for the sPLA2 -IIA activity is bound to the main chain carbonyl oxygen atoms of residues His28, Gly30, and Gly32 and to the side chain oxygen atoms of Asp48 at the active site which includes the catalytic His47 and Asp91. The catalytic mechanism for hydrolysis of ester bonds at the sn-2 position of phospholipids is shown in Fig. 1.

The crystal structure and surface electrostatic potential calculation of sPLA2-IIA have shown that this highly cationic protein contains a large number of cationic patches on its molecular surface [16–18]. Some cationic patches are essential for interfacial binding, whereas Arg123 belongs to a cluster of basic residues (cluster A, including Lys52, Arg53, Lys56 and Arg57) which is involved in heparinoid binding [19,20].

Here, we report the discovery of a rare variant in the PLA2G2A gene of two pediatric patients, identical twins, affected by ARDS. Predicting how this mutation alters the structure and function of sPLA2-IIA may be helpful for the identification of the role of sPLA2s in the mechanism underlying the disease. Molecular dynamics simulation has been widely used to study the effect of mutations on the structure and function of proteins [21–24]. Here we have used molecular modelling, molecular dynamics and electrostatic potential calculation to understand the conformational changes and dynamics of sPLA2 under the effect of R123H mutation. Our investigation provides detailed atomistic insights into the structural features of this rare variant.

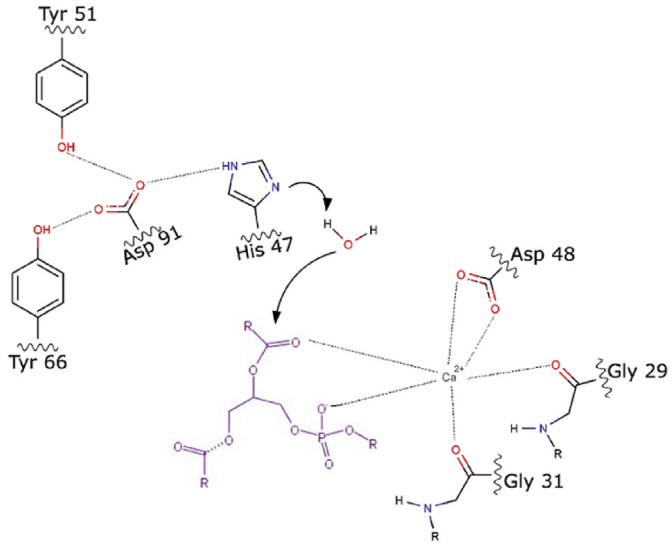

Fig. 1. Schematic representation of the catalytic mechanism of sPLA. The catalytic site consists essentially of His47, Asp91, a calcium ion (cofactor), and a water molecule that acts as the nucleophile. The hydrogen atom of the water molecule is transferred to His47 and the calcium ion stabilizes the oxyanion derived from the substrate's carbonyl oxygen. The histidine imidazole ring becomes positively charged and the protonated His47 is stabilized by Asp91, which hydrogen-bonds to Tyr51 and Tyr66. Residue numbering is based on the PDB file 1DB5 [15]. Substrate is shown in magenta.

2. Materials and methods

2.1. Population

Twenty-four infants diagnosed with ARDS according to the American-European Consensus Criteria were recruited [25]. These were patients previously enrolled in our earlier investigation step about biological and clinical effect of sPLA2 during pediatric ARDS and basic population details have been described elsewhere [10]. These patients were subjected to broncho-alveolar lavage and sPLA2 activity was measured in their epithelial lining fluids (ELF). Details about lavage procedure, sPLA2 activity assay and ELF estimation have been published elsewhere [10]. We also enrolled as controls 25 healthy age matched (within 2 months) infants admitted to our pediatric intensive care unit (PICU) in the month immediately before or after the ARDS infants. These controls were all admitted for postsurgical care, they had no lung disease in the previous three months and they have never been diagnosed with ARDS before. During hospitalization they had a normal lung imaging and clinical examination and never needed any supplemental oxygen.

Finally, 50 non-smoker adults, never diagnosed with ARDS and with no lung disease in the previous three months, have been recruited as further control. These were outpatients seen for glucose-6-phosphate dehydrogenase deficiency genetic test. In all these control subjects 1 mL whole blood was drawn into EDTA tubes at the moment of blood drawings for clinical routine tests and kept at 4 °C. No venepuncture was performed solely for study purposes and the participation to the study did not change patients' care; institutional review board approved the study and informed consent was asked to patients or parents/guardians.

2.2. Molecular testing of the PLA2G2A gene

Genomic DNA was isolated from peripheral blood by the MagCore® Genomic DNA Whole Blood Kit, according to the manufacturer's procedures and using the MagCore® Automated Nucleic Acid Extractor (Diatech Lab Line srl. Jesi, Italy). The published genomic sequence of the PLA2G2A gene and the NCBI Reference Sequence NG_012928.1 were used to design primers for PCR amplification of four genomic coding fragments also covering the exon-intron junctions: fragments 1, 2, 3 (primers: 1AF-1AR: 5′-AAGTTGAGACCACCCAGCAG-3′, 5′-TTTTCCCCCTGAGAGAGGAT-3’; 2AF-2AR: 5′-CCATTTGGGAGGAAGGAGA-3′, 5′-AGGAACCGGCACTGTCTTT-3’; 3AF-3AR: 5′-CTGGAGCTGTGGGACAAGA-3′, 5′-CACAGTCCCCAGCACTGTCTA-3′) encompassing exons 2, 3, 4 and fragment 4 (primers: 4AF-4AR: 5′-CCCACAAGAAGCCACTGAAT-3′, 5′-AATTCAGCACTGGGTGGAAG-3′) encompassing exon 5, were generated [26]. PCR products were sequenced using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA, http://www.appliedbiosystems.com/absite/us/en/home.html) in an automated sequencer ABI Prism 3500 Genetic Analyzer (Applied Biosystems). The same primers were used for PCR amplification and direct sequencing. Using the SeqScape® Software v2.5, sequences were aligned to the reference sequence NG_012928.1. Allelic frequencies were compared with Fisher exact test.