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Research article

$279^{Val\rightarrow Phe}$ Polymorphism of lipoprotein-associated phospholipase A_2 resulted in changes of folding kinetics and recognition to substrate

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ABSTRACT

Introduction: PLA2G7 encodes Lp-PLA_2 having role in the formation of atherosclerotic plaques by catalyzing its substrate, phosphatydilcholine (PC), to be pro-inflammatory substances. The increased risk for coronary artery disease (CAD) in Asian population has been related with this enzyme. $279^{Val\rightarrow Phe}$ variant was reported to have a protective role against CAD due to, in part, secretion defect or loss of enzymatic function. Therefore, We study folding kinetics and enzyme-substrate interaction in $279^{Val\rightarrow Phe}$ by using clinical and computational biology approach.
Methods: Polymorphisms were detected by genotyping among 103 acute myocardial infarction patients and 37 controls. Folding Lp-PLA_2 was simulated using GROMACS software by assessing helicity, hydrogen bond formation and stability. The interactions of Lp-PLA_2 and its substrate were simulated using Pyrx software followed by molecular dynamics simulation using YASARA software.
Result: Polymorphism of $279^{Val\rightarrow Phe}$ was represented by the change of nucleotide from G to T of 994th *PLA2G7* gene. The folding simulation suggested a decreased percentage of α-helix, hydrogen bond formation, hydrogen bond stability and hydrophobicity in $279^{Val\rightarrow Phe}$. The PC did not interact with active site of $279^{Val\rightarrow Phe}$ as paradoxically observed in 279 valine. $279^{Val\rightarrow Phe}$ polymorphism is likely to cause unstable binding to the substrate and decrease the enzymatic activity as observed in molecular dynamics simulations. The results of our computational biology study supported a protected effect of $279^{Val\rightarrow Phe}$ Polymorphism showed by the odd ratio for MI of 0.22 (CI 95% 0.035–1.37) in this study.
Conclusion: $279^{Val\rightarrow Phe}$ Polymorphism of Lp-PLA_2 may lead to decrease the enzymatic activity via changes of folding kinetics and recognition to its substrate.

1. Introduction

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), known as platelet activating factor acetyl hydrolase (PAF-AH), is an enzyme associated with cardiovascular disease. Formerly, this enzyme was thought to be a protective enzyme as this enzyme hydrolyzes PAF. However, recent studies weighted its role as pro-atherogenic role (Steen and O'Donoghue, 2013) as hydrolysis of oxidized-LDL (ox-LDL) by this enzyme yields potent inflammatory mediator, lysophosphatidylcholine (lyso-PC) and Nonesterified Fatty Acid (NEFA) (Ferguson et al., 2012). PLA_2 hydrolyzes phosphatidylcholine (PC), phospholipid contained in ox-LDL, yielding Lyso-PC (Gibellini and Smith, 2010; Gonçalves et al., 2012) . In association with disease, previous studies have shown that angina and atherosclerotic event were associated with Lp-PLA_2 level (Liu et al., 2011; Gonçalves et al., 2012; Patil et al., 2007). Our previous study also showed that Lp-PLA_2 activity was increased in subjects with acute myocard infarct (Wahjono et al., 2014).

Lp-PLA_2 is encoded by *PLA2G7* gene having many variants; one of those is $279^{Val\rightarrow Phe}$. This variant has been reported, in Japan and Korea, China, Taiwan, Turkey, Azerbaijan and Kyrgyzstan (Liu et al., 2006; Wang et al., 2006). Four percent of Japanese population having $279^{Val\rightarrow Phe}$ resulted in lost of Lp-PLA_2 function (Samanta and Bahnson, 2008), whereas study conducted in Korea reported that odds ratio of coronary artery disease of subjects having $279^{Val\rightarrow Phe}$ was 0.8, suggesting its protective role (Jang et al., 2011). In addition to loss of its function, $279^{Val\rightarrow Phe}$ variant has a low level of serum Lp-PLA_2, at least in part, possibly due to defect of Lp-PLA_2 secretion (Ishihara et al., 2004). The low concentration of serum Lp-PLA2 in $279^{Val\rightarrow Phe}$ population is probably due to folding defect (Jang et al., 2006).

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Therefore, the aims of this study are to explore the effect of $279^{Val \rightarrow Phe}$ mutation on its folding kinetics and its enzymatic activity at molecular level.

2. Methods

2.1. Study population

A case control study was conducted in Saiful Anwar Hospital in November 2011 to February 2015. One hundred three male patients were included if they had diagnosed MI (Myocard Infarct) and dyslipidemia. Diagnosis of MI was based on the Third Universal Definition of Myocard Infarction (Thygesen et al., 2012). The diagnosis of dyslipidemia met the NCEP ATP III criteria (Sidhu and Naugler, 2002). Patients having orthopaedic problems, weight gain or loss within last six month, and diabetes mellitus were excluded.

Control subjects were 40 outpatients who met the inclusion criteria: age-matched, asymptomatic CAD patients with negative result of treadmill test. On the basis of a medical questionnaire, subjects with a history of atherosclerotic, arterial disease (stroke, myocardial infarction, angina, or peripheral vascular disease), or known malignancy were excluded. All the subjects gave their informed consent, and the study was approved by the local ethics committee.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood leucocytes using *QIAmp DNA Blood Mini Kit Qiagen*. Genotyping for the variant $279^{Val \rightarrow Phe}$ (rs16874954) was performed using PCR/restriction fragment length polymorphism (RFLP)-based technique as previously described (Stafforini et al., 1996). Briefly, Exon 9 of the *PLA2G7* gene was amplified by using the primers 5′-CCCCATGAAATGAACAATTATAT-3′ and 5′-GGGGGCAAAAGAA-TAGCCTTATAA-3′. The 317-bp PCR products were digested with BST4Ci at 65 °C in 3 h. The 148- and 169-bp DNA fragments indicated the presence of the mutation.

2.3. Details of folding simulation

A fully extended Lp-PLA_2 was used as starting structure for all folding simulation both of wild type and $279^{Val \rightarrow Phe}$. Amino acid sequence was retrieved from NCBI FASTA (accession code: Q13093.1). A wildtype Lp-PLA_2 was constructed using Chimera. The Φ and Ψ angle was set to $-135°$ and $135°$ respectively according to (Lei et al., 2008). The $279^{Val \rightarrow Phe}$ starting structure was constructed by replacing valine with phenylalanine.

A total of 160 ns simulation was performed in 16 simulation using GROMACS 5.0.2 software. Each variant was simulated 8 times in 292.39 K, 299.93 K, 307.61 K, 315.43 K, 323.39 K, 331.49 K, 339.75 K, and 348.14 K respectively.

CHARM22-all-atom force field with CMAP correction was used to enhance accuracy of dynamic properties of protein being studied (Lindahl et al., 2010). A 5000-step minimization was done followed by 2 ns equilibration in canonical ensemble. Production run was performed for 10 ns each temperature in canonical ensemble. LINCS algorithm for constrain allowed us to use time step of 2 femtoseconds. Simulation were performed in GBSA as a solvent using dielectric constant of 2 (Wolf and de Leeuw, 2008) and salt concentration of 0.2 M. Both of neighbor searching and GB pairwise summation was performed every 40 steps using cutoff of 6 A.

Data was analyzed using GROMACS analysis tool. Secondary structure prediction used STRIDE (Frishman and Argos, 1995). As the hydrogen bond in protein is categorized as medium hydrogen bond (Minch, 1999), only medium to strong hydrogen bonds were analyzed. Hydrogen bond was defined as the distance of O atom

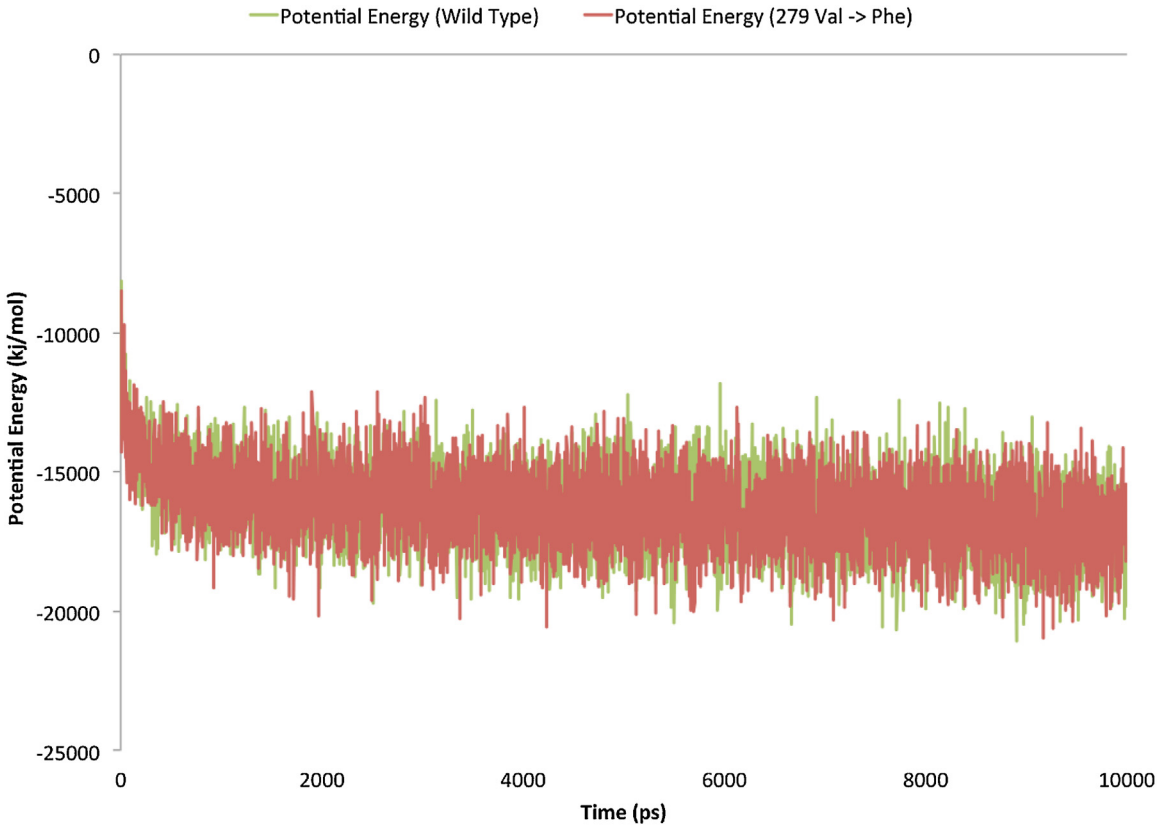

Fig. 1. Potential Energy of Lp-PLA_2 wild type and $279^{Val \rightarrow Phe}$. Both of them were constant. This data suggested that the simulation run well.