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Prediction of tertiary structure homology between bactericidal/permeability increasing protein of innate immune system and hydrolase enzymes

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Abstract

Bactericidal/permeability increasing protein (BPI) is known as the important arm of the innate immune system that provides the first line of defense against various gram-negative bacterial infections in mammalians. The critical aspect of the antibacterial action of BPI against these bacteria is the binding of BPI to the envelope lipopolysaccharides (LPS). However, there is very rare information about the molecular events following the interaction between BPI and bacterial compounds. As the first ever time report, by using the available bioinformatics and computational tools, BPI protein family was predicted to be structurally and functionally comparable to lipolytic hydrolases particularly including esterase-lipases. This computational identification helped us to propose a potential mode of action for BPI family in mammalian immune system, opposing to the LPS hydrolysis by bacterial phospholipases.

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Introduction

Bactericidal/permeability increasing (BPI) protein is an important arm of innate immune system, providing the first line of defense against gram-negative bacterial infections in mammalians. BPI is about 55 kDa cationic antimicrobial compound that is known to be present on the surface of the polymorphonuclear neutrophil cells and inside the specific granules of eosinophils. This protein possesses high affinity toward the lipid A moiety of the lipopolysaccharides (LPS) in the outer leaflet the gram negative bacterial outer membrane. It has been found that binding of BPI to the LPS molecules exerts its anti-microbial activities through: 1) induction of cytotoxicity via sequential damages to the membrane lipids of bacteria, 2) aggregating and neutralizing the bacterial membranes LPS molecules and 3) opsonization of bacteria and proceeding phagocytosis (Elsbach and Weiss, 1998; Beamer *et al.*, 1998; Reddy *et al.*, 2004; Domingues *et al.*, 2009). BPI is a highly conserved protein that has been well characterized from rabbit (Weiss *et al.*, 1982), humans (Gray *et al.*, 1989), teleost fish (Inagawa *et al.*, 2002), rat (Takahashi *et al.*, 2004), mice (Lennartsson *et al.*, 2005), and invertebrates (Gonzalez *et al.*, 2007).

Bactericidal/permeability increasing protein is known to have a boomerang shaped structure that is composed of two functional domains: 1) the cationic N-terminus region has anti-bacterial activity and lipopolysaccharide neutralizing properties, 2) the anionic C-terminal is required for the opsonization of the bacteria (Domingues *et al.*, 2009). To date, BPI-based strategy is known to be the important potential alternative to conventional therapies against bacterial infections in mamalians. The reports have shown that the 21 kDa recombinant N-terminal fragment of a BPI protein, corresponding to the first 193 amino acids of BPI exhibits highly selective bactericidal and LPS neutralizing effects (Horwitz *et al.*, 2000).

Despite these information, there are no details in the literatures about the molecular events following the interaction between BPI and bacterial outer membrane LPS molecules. Earlier reports suggest that the binding of BPI to live bacteria results in: i) a discrete increase in the permeability of the outer membrane, ii) hydrolysis of envelop LPS by bacterial phospholipase and some host phospholipases A2 of bacterial phospholipids that are refractory to phospholipase action in unperturbed bacteria , and iii) interruption of cell division (Elsbach 1998). A later report suggests that the high-affinity binding property of human BPI to the lipid A moiety of LPS prevents the transfer of the LPS to cellular receptors and facilitates its uptake by macrophages through a phagocytic manner (Iovine *et al.*, 2002). Recently, it has been reported that the interaction between BPI and LPS is followed by internalization and leakage induction through the (hemi) fusion and aggregation of bacterial outer and inner membranes enriched in phosphatidylglycerol, resulting in the effective neutralization of the LPS toxicity (Domingues *et al.*, 2009).

Although, the biological effects of the BPI proteins are known to be linked and initiated with their complex formation with bacterial outer membrane LPS molecules, but our understanding about the molecular mechanism of action of BPI proteins remains obscure, still yet. Considering this in view, we interested and aimed to understand about the direct function of BPI molecules when interacted with the bacterial LPS.

We presently used bioinformatics and computational modeling to investigate and enhance our information regarding the likely structures and the possible mode of actions of the BPI proteins. Usually, the early step towards understanding a protein functions and its mechanisms of action is to identify the potential domains and folds present in its structure. Particularly, the identification of the homologous tertiary structures provides the strong clues. Therefore, as a part of our studies towards learning more about the mode of action of BPI, we utilized the internet-based bioinformatics tools to freshly analyze the 3D structure of BPI that enabled us the potential mechanism of action to be proposed. A molecular mechanism based on lipolytic pathway was likely

predicted and proposed following the interaction between BPI and bacterial outer membrane LPS molecules.

Material and methods

Materials

A BPI protein sequence as an experimental material was randomly searched and extracted from the universal database using NCBI site at www.ncbi.nlm.com.

Methods

Protein Structural analysis was performed by internet-based bioinformatics and computational tools. Prediction of the tertiary structures was carried out by using Geno3D and Phyre 2 servers that are available freely through internet. Conserved domains were identified using NCBI Conserved Domain Architectural Retrieval Tool (CDART). Primary sequence similarities and alignments were generated by BLASTp and CLUSTALW soft wares at NCBI site.

Results and discussion

The binding of BPI proteins to the bacterial envelope LPS is well known as the critical aspect of the antibacterial action of these proteins against gram-negative bacteria. On the other hand, the limitation of BPI cytotoxicity to gram-negative bacteria strongly reflects and certifies the high affinity of BPI for LPS molecules located on the outer leaflet of these bacterial membranes. This interaction is known to be determined by the amino-terminal half of the BPI molecules (Elsbach 1998; Reddy *et al.*, 2004; Domingues *et al.*, 2009). Despite this interactive structural identifications, the molecular events following the formation of complex between BPI and LPS molecules not yet been justified in the literature reviews. We thought that identification of the more detailed structural properties of BPI proteins may help us to speculate the mechanism of its action as an active partner in the complex and to generate the more valuable information regarding the function of each partner as well as the role that they may perform following the complex formation.

Therefore, as a part of the structural studies on BPI proteins, we randomly extracted a BPI sequence (accession number: 1509290A) from publicly available database and then used Geno3D and Phyre 2 servers to predict its likely tertiary structure. Interestingly, the obtained results showed that esterase-type hydrolases are the best match for BPI sequence (fig. 1a, 1b). Our overall comparative results predicted that the regions dominated by the ß-strands might be the homologues parts between BPI and hydrolase enzymes (fig. 1c, 1d). As it has been shown and highlighted on the figure, the comparable ß-stranded regions between BPI molecule and esterase-type hydrolase considerably exhibit the similar patterns and orientations.

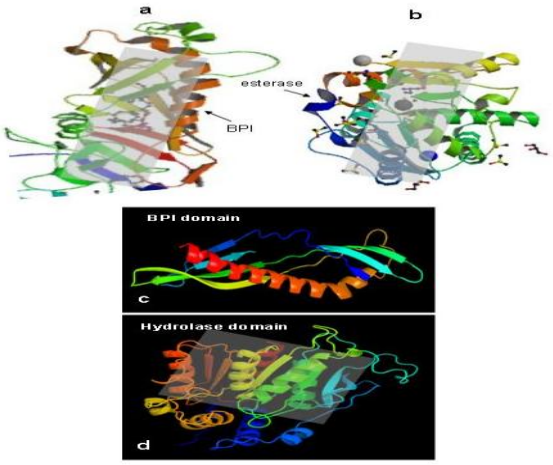

Fig. 1. Prediction and comparison of the BPI and esterase 3D. Three dimensional structure of a BPI (a) (accession no. Q8K4I4) was predicted based on the crystal structure of esterase-type hydrolases (b). The best template PDB was detected to be '3d7rA' using Geno3D online server. BPI (c) and hydrolase (d) domains were predicted by Phyre v 2.0 online sever and compared. The highlighted Beta-stranded regions seem to be considerably similar parts in both prediction modes.

Following this identification, the biologically active forms of BPI and esterase proteins are predicted to be composed of two similar structures by Geno3D. The assembly of N and C-termini in BPI and esterase molecules and the composition of two separate homologue polypeptides form their native structures (fig. 2). The homologous Beta-stranded regions are well detectable on both assemblies.

This was the first ever time report indicating the similarity between the BPI and an esterase-type hydrolase enzyme. Therefore, the more research was necessary and required for more clarification and explanation of this predicted structural similarity. In this regard, we extended our bioinformatics investigations to understand more about the esterase domain. By using CDART protein domain prediction tool at NCBI, it was revealed that the predicted esterase template of BPI from *Staphylococcus aureus* is the homologue of the esterase-lipase hydrolases from different cellular organisms such as fungi and bacteria. The best architectures were detected to be carboxylesterease type B, acyl-peptide hydrolase, entrochelin esterease, and peroxisomal long chain acyl-coA hydrolase. Further analysis result showed that all of these predicted hydrolases use lipids or their derivatives as substrates and hydrolyze ester bonds through the lipolytic pathway. On the other hand, the lipolytic-related structural and functional domains are well presented and shared in all these architectures made (fig. 3).

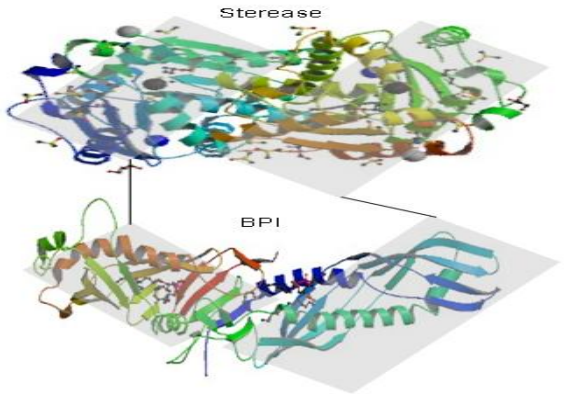

Fig. 2. Assemblies of BPI and esterase domains. The biological active forms of the BPI and esterase-type hydrolase are predicted to be composed of two similar structural / functional domains by using Geno3D server. The highlighted Beta-stranded regions present the possible homologous parts between BPI and esterase molecules.

Our finding shows that the BPI protein family of innate immune system can be structurally and functionally comparable to lipolytic hydrolases particularly including esterase-lipases. This identification presented here helped us to understand and propose a potential mode of action for BPI family in mammalian immune system. Comparison of the BPI and its predicted template esterase structures reveals that these protein families are able to bind lipid molecules or use it as substrate (fig. 3).

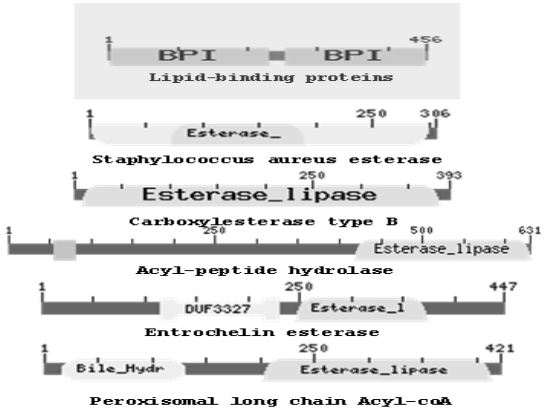

Fig. 3. Prediction of BPI and esterase-lipase domains. BPI and esterase-lipase domains were predicted using CDART protein domain prediction tool at NCBI. The lipid-related structural and functional domains are well presented and shared in all architectures made.

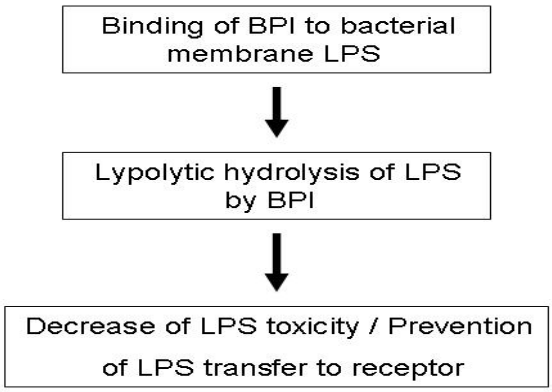

Fig. 4. Schematic representation of the proposed mode of action of BPI. The mechanism of action of the BPI was proposed to be based on the hydrolytic process on Lipopolysaccharides (lipolytic pathway).

Identification of the presence of substrate similarity between BPI and esterase family is the most important outcome of this research, predicting the lipolytic pathway for mechanism of action of bactericidal/permeability increasing protein of innate immune system. As a conclusion, based on our results obtained, we proposed a schematic representative mode of action for BPI proteins as follows: i) BPI

binds to the lipid A moiety of bacterial membrane LPS, ii) Bound BPI protein hydrolyzes the LPS molecules through the enzymatic lipolytic manner, and iii) enzymatic cleavage of LPS causes a considerable decrease in LPS toxicity and or it prevents the transfer of LPS to the cellular receptors, resulting in an effective down regulation of inflammatory response of the innate immune system (fig. 4). This may consistent to the well known binding capacity of BPI to LPS that is explored as a possible means of reducing injury caused by the bacterial infections.

This prediction is opposed to the previously suggested pathway for bacterial LPS degradation following the BPI protein attachment to LPS molecules. Based on the earlier hypothesis, the binding of BPI to gram-negative bacteria increases the permeability of their outer membrane. This is then followed by the hydrolysis of envelop LPS by some bacterial phospholipases (Halsbach 1998; Domingues *et al*., 2009). Based on our computed data It seems that BPI itself act as esterase enzyme and cleave the envelop LPS upon binding to LPS molecules, preventing LPS transfer to specific receptors on host cells. We hope that the present results will provide the new insights into the mode of action of BPI family in immune system of mammals and will be used in medical biotechnology in the future.

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