Mini Review

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Studies on crystallization of peptidyl-prolyl cis-trans isomerase and AreB from *Aspergillus flavus*

Abstract

Aspergillus flavus is a disease causative agent of many agriculture plants, common hosts are cereal grains, legumes, and tree nuts. Many strains produce a significant quantity of toxins named as mycotoxins. *A. flavus* is also an opportunistic pathogen of human and animals, that cause aspergillosis in immunocompromised individuals. *A. flavus* has Peptidyl-prolyl cis-trans isomerases (PPIase), which catalyze the isomerization of peptide bonds preceding proline residues, a process of high valuable for correct folding. PPIase belongs to the superfamily of a protein named as cyclophilin, which found in eukaryotic, bacterial and archaea. AreB hypothetical protein is similar to GATA transcription factors maintain transcription during growth and differentiation by identifying distinct GATA sites with a tandem of two conserved zinc fingers. Both zinc fingers wrap around a palindromic GATA site. This study will examine the protein structure from the X-ray crystals of PPIase and AreB and the structure determination of PPIase and AreB among the different species of *A. flavus*.

Keywords: *aspergillus flavus*, immunocompromised, peptidyl-prolyl cis-trans isomerases, cyclophilins, AreB, x-ray crystallization, GATA transcription factors

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Introduction

Peptidyl-prolyl cis-trans isomerases (PPIase) is also known as rotamase or cyclophilins,[1] catalyze cis-trans isomerization of peptide bonds, preferring those preceding proline residues.[2] The enzyme can be found in a large variety of organisms such as bacteria, plants, and mammals, sometimes as single domain proteins, but also as components in larger complexes.[3] PPIase accelerate protein folding both *in vivo* and *in-vitro*. They bind to and mediate the biological effects of the immunosuppressive agent cyclosporine A,[4] they participate in cell surface recognition[5] and heat-shock response.[6] Cyclophilins are a diverse family regarding function and have incriminated in protein folding processes which rely on catalytic/chaperone-like activities.[7] Biological processes that reliable on the action of proteins and their domains, for example, folding collaborator proteins assist protein folding as disulfide isomerases or peptidyl-prolyl cis/trans isomerases.[8] These proteins have evolved to recognize precise signatures of protein sequences and manage *in vivo* protein folding.[9] A considerable number of proteins have acknowledged holding peptidyl-prolyl cis/trans isomerase a domain that has been constituting another specific protein recognition unit.[10] The peptidyl-prolyl cis/trans isomerases are enzymes that gather speed the slow cis/trans isomerization of peptidyl-prolyl bonds in different folding states of an intention protein. PPIase-catalysed protein conformational changes happened during the refolding of denatured proteins,[11] de novo protein synthesis and the arrangement of biologically active conformations of polypeptides,[12] Based on drug specificity and primary sequence homology. PPIases have been separated into three distinct families:[13] a) the cyclosporin A (CsA)-binding proteins, cyclophilins,) the FK506 and rapamycin binding proteins, FKBPs,). The parvulins, do not bind immunosuppressant drugs.[14] Even though cyclophilins and FKBPs were identified for some decades, the cellular purpose of these enzymes is not yet absolutely understood. They concerned in the folding of newly synthesized proteins, transport and gathering of fundamental cellular protein complexes,[15] In contrast, the purpose of a part of the third PPIase family, Pin1 could expose in much more details, and a significant task in the cell cycle mechanism in eukaryotes was proved.[16]

The GATA-binding proteins are a collection of structurally associated transcription factors that bind to the DNA compromise sequence GATA.[17] Members of the GATA protein family (GATA1-6) purpose as lineage-specific transcription factors for a digit of cell types in the hematopoietic system.[18] For example, GATA1 is basic for erythroid and megakaryocytic development.[18] GATA2 plays a key role in regulating the transcription of genes involved in the improvement and proliferation of hematopoietic[19] and the endocrine cell lineages GATA3 is a significant regulator of T cell improvement, including Th2,[20] and regulatory T cells,[21] and plays a major role in endothelial cell biology and GATA4 regulates genes occupied in embryogenesis, and myocardial separation and function.[22] The function of GATA proteins depends critically on two highly conserved zinc fingers and nearby basic regions.[23] The C-terminal "C-finger" and its adjacent basic region is necessary and sufficient for GATA to bind its cognate sequence, WGATAR (W=A/T, R=A/G.[24] The N-terminal "N-finger" also bind DNA independently but has a preference for GATC core motifs.[25] Both fingers participate in binding the palindromic GATA motif ATCWGATA (W=A/T), resulting in markedly increased affinity,[26] GATA proteins give to transcriptional regulation by facilitating chromosome looping, thereby mediating long-range manage of gene appearance in the nucleus. For example, GATA1 and its cofactor FOG-1 openly engage looped enhancers and mark gene promoters at the b-globin locus.[27] However, the molecular mechanism by which GATA and Friend of GATA (FOG) proteins mediate loop formation remains unclear. Structural studies based on both nuclear magnetic resonances (NMR) spectroscopy and X-ray crystallography[28] has characterized the DNA-binding mechanisms of the GATA C-finger.[29] In addition, the structure of the GATA1 N-finger bound to the FOG-1 zinc finger 1 has been distinguished by NMR.[30]

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Crystallization

For a protein to crystallize, it must reach the thermodynamically unstable state of supersaturation.[31] Crystallization experiments are carried out by the sitting-drop method at 16°C using 96-well plates, common precipitants are salts, like ammonium sulfate, organic solvents, including ethylene glycol and polyethylene glycol (PEG) 200, or different polymers, represented mostly by larger PEGs. The commercial crystallization kits Index, PEG/Ion, PEG/Ion2, Crystal Screen1, Crystal Screen 2 and Natrix is used to find the initial conditions for crystal growth. There are certain parameters affecting crystallization like temperature, pH and protein concentration.[32]

X-ray crystallography, data collection & data processing

When accelerated electrons hit metal, energy is released in the form of X-ray photons.[33] The X-rays can be looked upon as particles,[34] but also as waves having a specific amplitude and phase. During data collection, the crystal exposed to X-rays of a specific wavelength,[35] ranging from 0.5-1.6Å for a typical experiment. When the X-rays hit the crystal, most of them are straight through, but some are hit the atoms in the crystal, causing them to oscillate. The X-rays is remitted with the same amplitude and phase as before but in a different angle. Before data collection, crystals are soaked in a cryo protectant solution.[36]

Solve phase problem

More than a few methods can be used to resolve the phase problem like single isomorphous replacement method (SIR),[37] Multiple Isomorphous Replacement,[37] (MIR), single wavelength anomalous dispersion (SAD),[38] and multiple wavelengths anomalous dispersion (MAD),[37] One can also combine the two techniques, use single or multiple isomorphous replacements together with anomalous dispersion, referred to as SIRAS and MIRAS, respectively. After crystallographic data collection, all data sets are indexed and integrated with MOSFLM.[37]

Conclusion

Analysis of the published data shows that PPIase and AreB hypothetical protein similar to GATA transcription factor from *A. flavus* are not expressed, purified and crystallized as well their structures are not determined. But their relative solved structures are found in Protein Data Bank (PDB) from different species. Crystallization is necessary to get the three-dimensional structure of nucleic acids and proteins; it often represents the bottleneck in structure determination. Our assimilation of crystallization mechanisms regarding PPIase and AreB is still incomplete. In this review, we emphasize essential feature of the crystallization process.

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Conflict of interest

The author declares no conflict of interest.

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