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EDUCATIONAL BACKGROUND

2013- 2020 — **Ph.D. in Interdisciplinary field of Biosciences**
School of Basic Sciences
Indian Institute of Technology Bhubaneswar, India

Doctoral Dissertation Title: *Role of Various Environmental Stresses and Small Materials on the Structure and Function of Mycobacterium leprae HSP18 - A Biophysical Study*

2010-2012 — **M.Sc. (Microbiology and Microbial Technology)**
(1st Class with 66.79 % Marks)
University of Kalyani, India

2007-2010 — **B.Sc. (Microbiology Honours, Chemistry, Botany)**
(1st Class with 70.1 % Marks)
Kalyani Mahavidyalaya, University of Kalyani, India

2003-2005 — **Higher Secondary (10+2) (1st Class with 69.5 % Marks)**
Kabi Bijoylal H.S. Institute, WBCHSE, West Bengal, India

2003 — **Matriculation (1st Class with 82 % Marks)**
Betai High School, WBBSE, West Bengal, India

RESEARCH/ INDUSTRIAL EXPERIENCE

Nov 2019-Nov 2020 — **Postdoctoral Fellow (NIH Sponsored)**
Duke University, USA

2013-2020 — **Doctoral Student (CSIR-UGC Sponsored)**
Indian Institute of Technology Bhubaneswar
Doctoral Thesis Title: *Role of Various Environmental Stresses and Small Materials on the Structure and Function of Mycobacterium leprae HSP18 - A Biophysical Study*
Supervisor: Dr. Ashis Biswas
Thesis submitted in July 2019

Summer 2011 — **Summer Research Fellow**
Chittaranjan National Cancer Institute, Kolkata
Project Title: *Effect of Doxorubicin on Cancer Cells in In vivo Mice Samples*
Supervisor: Prof. Soumitra Kumar Choudhuri

Summer 2012 — **Summer Industrial Trainee**
East India Pharmaceuticals, Kolkata
Project Title: *Use of Different Microbial Techniques in Pharmaceutical Industries*

TEACHING EXPERIENCE

Jan 2015- Dec 2018 **Teaching Assistant**
Indian Institute of Technology Bhubaneswar
Courses:
Undergraduate Level (B.Tech):
Chemistry Laboratory [CY1P001]
Physical Chemistry (Tutorial)
Postgraduate Level (M.Sc.):
Physical Chemistry Laboratory [CY5P003]
Advanced Instrumentation Laboratory [CY5P004]
Biochemistry [CY7L001]

AWARDS AND ACHIEVEMENTS

- Research is distinguished by Department of Science and Technology (DST), India and highlighted in the newsletter of Vigyan Prasar, DST, India in 2020.
- Senior Research Fellowship from Council of Scientific and Industrial Research and University Grants Commission (CSIR-UGC) in 2016.
- Junior Research Fellowship from Council of Scientific and Industrial Research and University Grants Commission (CSIR-UGC) in 2014 (AIR 71).
- Graduate Aptitude Test for Engineering (GATE) in 2012 (AIR 43).
- Second Rank Holder in Microbiology Honours from University of Kalyani in 2010.

IMPORTANT ROLES

- Actively participated in the procurement of various instruments and establishment of Protein Chemistry Laboratory, Analytical Instrumentation Laboratory-2, Analytical Instrumentation Laboratory-3 (Bio-Instrumentation) and PG Laboratory at IIT Bhubaneswar during PhD tenure.
- Actively participated in the shifting of the above-said laboratories along with the high end instruments, small instruments, chemicals (normal temperature, 4^0 C, -20^0 C, -80^0 C), plasticwares and glasswares from the transit campus to the permanent campus.
- Deeply involved in the rate contract procedure for procurement of chemicals.
- Took leading roles in different institute program such as Open day lab program, Orientation Day program etc.

PAST AND CURRENT RESEARCH STATEMENT

Leprosy is caused by *Mycobacterium leprae*, an obligate intracellular pathogen that is unique in its ability to invade the peripheral nervous system. *M. leprae* is non-culturable in artificial media. It can tolerate and survive under different stress conditions such as low pH, hypoxia and oxidative stress. But, the viability of this pathogen reduces drastically under UV stress. The reasons behind such quick reduction in viability are far from clear. Also, the impact of UV stress/radiation on the antigen(s) which is/are responsible for the survival of this pathogen was still unknown. HSP18, an immunodominant antigen and small heat shock protein, whose chaperone function plays an important role in the growth and survival of this pathogen under various environmental insults. My graduation work represents the effect of different UV stresses/radiations on the structure and chaperone function of HSP18. The chaperone function of HSP18 is decreased significantly with increasing doses of various UV radiations. These different UV irradiations/stresses perturb grossly its tertiary structure and induce tryptophan and tyrosine photo-oxidation to N-formyl kynurenine, kynurenine and dityrosine, respectively. Such photo-oxidation promotes intramolecular subunit cross-linking, lowers the surface hydrophobicity and stability of the protein. All these factors together damage/reduce the chaperone function of HSP18 which may be an important factor behind the rapid death of *M. leprae* under UV stress. I have also revealed some important and unique functionalities of *M. leprae* HSP18. These are (i) HSP18 can exhibit redox scavenging ability and (ii) HSP18 executes chaperone function even under prolonged redox stress conditions. Perhaps, these two important functions of HSP18 possibly help *M. leprae* pathogen to survive under redox stress conditions inside human macrophages. I also assessed/determined the binding parameters and binding modes associated with the interaction of various multidrug therapy (MDT) drug regimen (dapsone, clofazimine and rifampicin) with HSP18. All MDT drugs are found to reduce the stability and chaperone function of HSP18 by modulating its structural conformations. Lowering the chaperone function of HSP18 may be an additional mechanism behind exerting anti-leprotic activity by these MDT drugs. During my doctoral studies, I found evidence of interaction of HSP18 with gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs). Interaction of AuNPs/AgNPs to HSP18 alters the secondary and tertiary structure of HSP18 in a distinctly opposite manner; while HSP18-AuNPs interaction leads to oligomeric association, HSP18-AgNPs interaction results in oligomeric dissociation of the protein. Surface hydrophobicity, thermal stability, chaperone function of HSP18 and survival of thermally stressed *E. coli* harboring HSP18 are enhanced upon AuNPs interaction, while all of them are reduced upon interaction with AgNPs. Altogether, this study evokes the possibility of usage of nanoparticles (especially silver nanoparticles) for the effective treatment of leprosy. Additionally, I have also worked on the effect of different mutations and domains in another small heat shock protein, HSP16.3 from *M. tuberculosis* which lead towards better second generation vaccines against tuberculosis. I was also engaged in several other projects, to investigate the DNA binding modes and cleavage potential of various drug molecules and newly synthesized transition metal complexes which can be potential anti-cancer agents.

Recently after COVID-19 outbreak I am now presently working with various proteins from Kaposi's sarcoma-associated herpesvirus (KSHV), Dengue virus and SARS CoV-2 using theoretical and experimental approaches. Apart from these, I am currently involved in various projects to find out the effect of the various posttranslational modifications on the structure and chaperone function of HSP16.3 and HSP18. I am also engaged in a comparative study of particular domains of small heat shock protein from various species along with domain swapping among different small heat shock proteins.

FUTURE RESEARCH INTERESTS

My future research plan is mainly divided into six categories-

1. Elucidation of structure-function relationships in small heat shock proteins and its importance in human infectious diseases using biophysical and biochemical methods. Small heat shock proteins (sHSPs) exhibit molecular chaperone function and the impairment of molecular chaperone function and structure commonly lead to various diseases. sHSPs responsible for various pathogenic diseases as well as from mammals especially human will also be studied. The co-relation of these sHSPs with diseases, their mechanism and role in pathogenicity will be studied in details.

2. Identifying GroEL inhibitors to combat bacterial antibiotic resistance and multi-drug resistance

The greatest threat of human civilization is increasing incidences of antibiotic-resistant strains of bacteria. To counter this threat, the long-term goal of this research is to develop new drugs that function by targeting bacterial GroEL chaperonin systems. GroEL is a centralized molecular machine that maintains the proper structure and function of hundreds of other proteins. Thus, targeting this one molecular machine will have the cascading effect of inhibiting hundreds of proteins at once, the functional losses of which bacteria will not be able to recover from. A caveat to this strategy is that human cells have a mitochondrial counterpart, called HSP60, and there remains the possibility of inhibitor cross-talk that could lead to toxic effects on host tissues. The central hypothesis is that the structural and functional divergence between bacterial and mammalian chaperonins, as well as other mammalian proteins, will allow the selective targeting of small molecule inhibitors for GroEL and bacteria without toxic side effects to human cells. The overall objective is to identify these unique binding sites, elucidate the structural/functional mechanisms of action for molecules binding to these sites, and define the selectivity profiles for site-specific inhibitors in vitro and in cells. The rationale for the proposed studies is that delineating the precise structural/functional mechanisms of action of chaperonin inhibitors will permit the rational development GroEL-targeting drug candidates.

3. Investigation of small molecules as multi-targeted inhibitors against various infectious viral proteins: A multi-approach study

Proteins from different infectious viruses [Dengue virus, Zika virus, Chikungunya virus, Kaposi's sarcoma-associated herpesvirus (KSHV), SARS CoV-2] play an important role in the viral-life cycle. At present, there is no specific therapy which can effectively block infection of these deadly viruses. Thus, targeting the structural or non-structural proteins by various phytochemicals, bioactive compounds, drug analogous and inorganic compounds as novel therapeutics may retard the functionality of various proteins, thus inhibit the propagation of such viruses. For example, four KSHV proteins such as KSHV protease, vFLIP, LANA1 and vIRF3 (LANA2) play an important role for the infection and viral pathogenesis of KSHV. Multi-targeted small molecule inhibitors of these proteins can be initially screened using in-silico docking and molecular dynamics simulation approaches which can be further validated by various *in-vitro* and *ex-vivo* experimental studies. Likewise, proteases/ proteins such as the main protease (Mpro), papain-like protease (PLpro), RNA dependent RNA polymerase (RdRP), transmembrane protease serine 2 (TMPRSS2) and

angiotensin-converting enzyme 2 (ACE2) from SARS CoV-2 can be initially studied using in-silico docking and molecular dynamics simulation approaches. These theoretical approaches can be further validated by experimental studies using mainly fluorescence based (FRET) assays and other biochemical assays. Some initial studies with the main protease from SARS CoV-2 has already been done using some polyphenols and repurposed drugs which is evident by my recent publication on COVID-19. Further validation of these initial studies can be done by various *in-vitro* and *ex-vivo* experimental studies.

4. Investigation of amyloid fibrillation and their significance in preventing different amyloid diseases. Amyloid fibrillation is associated with misfolding of proteins or intrinsically disordered protein that is responsible for more than 25 diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and type II diabetes. This fibrillation process of amyloids will be investigated along with prevention mechanism of formation of amyloids or reversing back the amyloid formation to prevent/ delay/ cure the onset of different amyloid diseases. Various biophysical and biochemical techniques will be adopted to carry out these studies using insuin, Aβ-peptide, α-Synuclein etc as model proteins.

5. Exploration of therapeutic potential of various peptides. Various peptides are also known to posses anti-oxidant, anti-microbial, anti-cancer and anti-viral activities. Different peptides also tend to bind with many enzymes/proteins to either enhance or destroy their activities which have been important regulator for industry scale use. These peptides can be isolated and recombinantly expressed to study their ability whether they can a) exhibit antioxidant activity by scavenging reactive oxygen species (ROS) and reactive nitrogen species (RNS), b) protect DNA and proteins from ROS/RNS damages, c) exhibit anti-aggregation/refolding activity and to illustrate the molecular basis behind such properties, d) potential as anti-cancer agents and to study possible mechanism behind their anti-cancer activity. Furthermore these peptides can be evaluated to find out their potential as effective anti-microbial agents to combat drug resistance.

6. Assessment of the mechanism of interaction of various drugs & metal complexes with DNA and proteins using biophysical and biochemical techniques. Assessment of the mode of interaction of different drugs and metal complexes provides the pharmacological platform to use these drugs or complexes in different therapy for the treatment of several diseases including cancer. The mechanism of interaction of drugs to DNA and albumin proteins, their toxicity, tolerable dose, nuclease activity and other binding parameters will be studied in details using biochemical and spectroscopic techniques. Transition metal complexes find applications in photodynamic therapy to cure diseases such as cancer etc. The interaction of these complexes with DNA and albumin proteins is vital in order to understand their pharmacological behavior. The photonuclease activity and mechanism of interaction with DNA and albumin proteins for a selected group of newly synthesized copper complexes along with some mycobacterial drugs such as dapsone and rifampicin has recently been demonstrated. I will focus on understanding the strength and the mode of interaction of these complexes with DNA and albumin proteins by various biochemical and spectroscopic techniques.

RESEARCH PUBLICATIONS

1. Rajesh Ghosh, **Ayon Chakraborty**, Ashis Biswas and Snehasis Chowdhuri - “Depicting the inhibitory potential of polyphenols from *Isatis indigotica* root against the main protease of SARS CoV-2 using computational approaches”, ***Journal of Biomolecular Structure and Dynamics***, DOI: 10.1080/07391102.2020.1858164, (2020). **[IF 3.1]**

2. Rajesh Ghosh, **Ayon Chakraborty**, Ashis Biswas and Snehasis Chowdhuri - “Identification of alkaloids from *Justicia adhatoda* as potent SARS CoV-2 main protease inhibitors: An in silico perspective”, ***Journal of Molecular Structure***, DOI: 10.1016/j.molstruc.2020.129489, (2020). **[IF 2.46]**

3. Rajesh Ghosh, **Ayon Chakraborty**, Ashis Biswas and Snehasis Chowdhuri - “Computer Aided Identification of Potential SARS CoV-2 Main Protease Inhibitors from Diterpenoids and Biflavonoids of *Torreya nucifera* Leaves”, ***Journal of Biomolecular Structure and Dynamics***, DOI: 10.1080/07391102.2020.1841680, (2020). **[IF 3.1]**

4. Rajesh Ghosh, **Ayon Chakraborty**, Ashis Biswas and Snehasis Chowdhuri - “Potential therapeutic use of corticosteroids as SARS CoV-2 Main Protease Inhibitors: A computational study”, ***Journal of Biomolecular Structure and Dynamics***, DOI: 10.1080/07391102.2020.1835728, (2020). **[IF 3.1]**

5. Rajesh Ghosh, **Ayon Chakraborty**, Ashis Biswas and Snehasis Chowdhuri - “Identification of Polyphenols from *Broussonetia papyrifera* as SARS CoV-2 Main Protease Inhibitors using *In Silico* Docking and Molecular Dynamics Simulation Approaches”, ***Journal of Biomolecular Structure and Dynamics***, DOI: 10.1080/07391102.2020.1802347, (2020). **[IF 3.1]**

6. Rajesh Ghosh, **Ayon Chakraborty**, Ashis Biswas and Snehasis Chowdhuri - “Evaluation of Green Tea Polyphenols as Novel Corona Virus (SARS CoV-2) Main Protease (Mpro) Inhibitors – An *In Silico* Docking and Molecular Dynamics Simulation Study”, ***Journal of Biomolecular Structure and Dynamics***, DOI: 10.1080/07391102.2020.1779818, (2020). **[IF 3.1]**

7. **Ayon Chakraborty** and Ashis Biswas – “Structure, stability and chaperone function of *Mycobacterium leprae* Heat Shock Protein 18 are differentially affected upon interaction with gold and silver nanoparticles”, ***International Journal of Biological Macromolecules***, 152, 250-260 (2020). **[IF 5.1]**

8. Alok Kumar Panda, **Ayon Chakraborty**, Sandip Kumar Nandi and Ashis Biswas – “The impact of different mutations at arginine141 on the structure, subunit exchange dynamics and chaperone activity of Hsp16. 3” ***Proteins: Structure, Function, and Bioinformatics***, 1-16 (2020) [Selected as cover Image of ***Proteins: Structure, Function, and Bioinformatics***, 88(6), C1 (2020)]. **[IF 2.5]**

9. Sandip Kumar Nandi, **Ayon Chakraborty**, Alok Kumar Panda and Ashis Biswas - “*M. leprae* HSP18 suppresses copper (II) mediated ROS generation: Effect of redox stress on its structure and function” ***International Journal of Biological Macromolecules***, 146, 648-660 (2020). **[IF 5.1]**

10. **Ayon Chakraborty**, Alok Kumar Panda, Rajesh Ghosh and Ashis Biswas – “DNA minor groove binding of a well known anti-mycobacterial drug dapsone: A spectroscopic, viscometric and molecular docking study”, ***Archives of Biochemistry and Biophysics***, 665, 107-113 (2019). **[IF 3.6]**

11. **Ayon Chakraborty**, Alok Kumar Panda, Rajesh Ghosh, Ipsita Roy and Ashis Biswas – "Depicting the DNA binding and photo-nuclease ability of anti-mycobacterial drug rifampicin: A biophysical and molecular docking perspective", ***International Journal of Biological Macromolecules***, 127, 187-196 (2019). **[IF 5.1]**

12. **Ayon Chakraborty**, Sandip Kumar Nandi, Alok Kumar Panda, Pinaki Prasad Mahapatra, Sourav Giri and Ashis Biswas – "Probing the structure-function relationship of *Mycobacterium leprae* HSP18 under different UV radiations", ***International Journal of Biological Macromolecules***, 119, 604-616 (2018). **[IF 5.1]**

13. Sandip Kumar Nandi, **Ayon Chakraborty**, Alok Kumar Panda, Rajiv Kumar Kar, Anirban Bhunia and Ashis Biswas - " Evidences for zinc (II) and copper (II) ion interactions with Mycobacterium leprae HSP18: Effect on its structure and chaperone function", ***Journal of Inorganic Biochemistry***, 188, 62-75 (2018). **[IF 3.2]**

14. Alok Kumar Panda, **Ayon Chakraborty**, Sandip Kumar Nandi, Abhishek Kaushik and Ashis Biswas – "The C-terminal extention of *Mycobacterium tuberculosis* Hsp16.3 regulates its oligomerization, subunit exchange dynamics and chaperone function" ***FEBS J***, 284, 277-300 (2017)**.** **[IF 4.7]**

15. Sandip Kumar Nandi, **Ayon Chakraborty**, Alok Kumar Panda and Ashis Biswas - "Conformational perturbation, hydrophobic interactions and oligomeric association are responsible for the enhanced chaperone function of *Mycobacterium leprae* HSP18 under pre-thermal condition" ***RSC Advances,*** 6, 62146-62156 (2016). **[IF 3.1]**

16. Alok Kumar Panda, Sandip Kumar Nandi, **Ayon Chakraborty**, Ram H. Nagaraj and Ashis Biswas-"Differential role of arginine mutations on the structure and functions of alpha-crystallin", ***Biochimica et Biophysica Acta-General Subjects***, 1860, 199-210 (2016). **[IF 3.7]**

17. Sayed Muktar Hossain, Avinash Lakma, Rabindra Nath Pradhan, **Ayon Chakraborty**, Ashis Biswas and Akhilesh Kumar Singh - "Synthesis and characterization of a novel, ditopic, reversible and highly selective, "turn-on" fluorescent chemosensor for Al^{3+} ion", ***RSC Advances***, 5, 63338-63344 (2015). **[IF 3.1]**

18. Sandip Kumar Nandi, Alok Kumar Panda, **Ayon Chakraborty**, Sougata Sinha Ray and Ashis Biswas - "Role of subunit exchange and electrostatic interactions on the chaperone activity of *Mycobacterium leprae* HSP18", ***PLoS One***, 10(6), e0129734 (2015). **[IF 2.8]**

19. Saswati, **Ayon Chakraborty**, Subhashree P. Dash, Alok Kumar Panda, Rama Acharyya, Ashis Biswas, Subhadip Mukhopadhyay, Sujit K Bhutia, Aurelien Crochet, Yogesh P. Patil, Munirathinam Nethaji and Rupam Dinda - "Synthesis, X-ray structure and in vitro cytotoxicity studies of Cu(I/II) complexes of thiosemicarbazone: special emphasis on their interactions with DNA", ***Dalton Transactions***, 44(13), 6140-6157 (2015). **[IF 4.1]**

20. Sandip Kumar Nandi, **Ayon Chakraborty**, Alok Kumar Panda, Sougata Sinha Ray, Rajiv Kumar Kar, Anirban Bhunia and Ashis Biswas - "Interaction of ATP with a small heat shock protein from *Mycobacterium leprae*: effect on its structure and function", ***PLoS Neglected Tropical Diseases***, 9(3), e0003661 (2015). **[IF 4.5]**

- **Cumulative Impact Factors of Journal Published**: **73.66**
- **Average Impact Factors of Journal Published: 3.683**
- **Total Citations: 235; Average Citations: 11.75; h-index: 8; i10-index: 7**

JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
https://doi.org/10.1080/07391102.2020.1779818

Evaluation of green tea polyphenols as novel corona virus (SARS CoV-2) main protease (Mpro) inhibitors – an *in silico* docking and molecular dynamics simulation study

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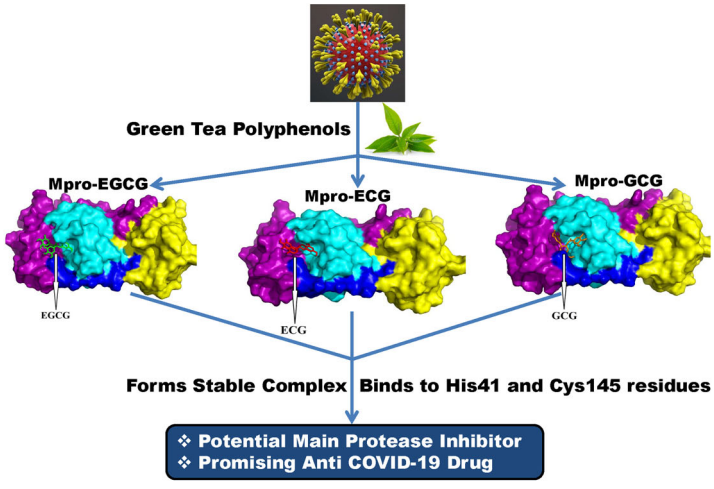
Communicated by Ramaswamy H. Sarma

ABSTRACT

Coronavirus disease 2019 (COVID-19) is a viral respiratory disease which caused global health emergency and announced as pandemic disease by World Health Organization. Lack of specific drug molecules or treatment strategy against this disease makes it more devastating. Thus, there is an urgent need of effective drug molecules to fight against COVID-19. The main protease (Mpro) of SARS CoV-2, a key component of this viral replication, is considered as a prime target for anti-COVID-19 drug development. In order to find potent Mpro inhibitors, we have selected eight polyphenols from green tea, as these are already known to exert antiviral activity against many RNA viruses. We have elucidated the binding affinities and binding modes between these polyphenols including a well-known Mpro inhibitor N3 (having binding affinity −7.0 kcal/mol) and Mpro using molecular docking studies. All eight polyphenols exhibit good binding affinity toward Mpro (−7.1 to −9.0 kcal/mol). However, only three polyphenols (epigallocatechin gallate, epicatechingallate and gallocatechin-3-gallate) interact strongly with one or both catalytic residues (His41 and Cys145) of Mpro. Molecular dynamics simulations (100 ns) on these three Mpro–polyphenol systems further reveal that these complexes are highly stable, experience less conformational fluctuations and share similar degree of compactness. Estimation of total number of intermolecular H-bond and MM-GBSA analysis affirm the stability of these three Mpro–polyphenol complexes. Pharmacokinetic analysis additionally suggested that these polyphenols possess favorable drug-likeness characteristics. Altogether, our study shows that these three polyphenols can be used as potential inhibitors against SARS CoV-2 Mpro and are promising drug candidates for COVID-19 treatment.

GRAPHICAL ABSTRACT



Abbreviations: COVID-19: corona virus disease 2019; SARS CoV-2: severe acute respiratory syndrome corona virus-2; Mpro: main protease; MD: molecular dynamics; RMSD: root mean square deviation; RMSF: root mean square fluctuation; Rg: radius of gyration; SASA: solvent accessible surface area

ARTICLE HISTORY
Received 18 May 2020
Accepted 26 May 2020

KEYWORDS
COVID-19; SARS CoV-2 main protease; docking; molecular dynamics simulation; green tea polyphenols/catechins

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/07391102.2020.1779818.

International Journal of Biological Macromolecules 152 (2020) 250–260

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Structure, stability and chaperone function of *Mycobacterium leprae* Heat Shock Protein 18 are differentially affected upon interaction with gold and silver nanoparticles

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ARTICLE INFO

Article history:
Received 14 January 2020
Received in revised form 15 February 2020
Accepted 16 February 2020
Available online 19 February 2020

Keywords:
Mycobacterium leprae HSP18
Nanoparticles
Molecular chaperone

ABSTRACT

Gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) have several biomedical applications. However, the effective usage of these two nanoparticles is impeded due to limited understanding of their interaction with proteins including small heat shock proteins (sHSPs). Specifically, no evidences of interaction of these two nanoparticles with HSP18 (an antigenic protein) which is an important factor for the growth and survival of *M. leprae* (the causative organism of leprosy) are available in the literature. Here, we report for the first time evidences of "HSP18-AuNPs/AgNPs interaction" and its impact on the structure and chaperone function of HSP18. Interaction of citrate-capped AuNPs/AgNPs (~20 nm diameter) to HSP18 alters the secondary and tertiary structure of HSP18 in a distinctly opposite manner; while "HSP18-AuNPs interaction" leads to oligomeric association, "HSP18-AgNPs interaction" results in oligomeric dissociation of the protein. Surface hydrophobicity, thermal stability, chaperone function of HSP18 and survival of thermally stressed *E. coli* harbouring HSP18 are enhanced upon AuNPs interaction, while all of them are reduced upon interaction with AgNPs. Altogether, our study reveals that HSP18 is an important drug target in leprosy and its chaperone function may possibly plays a vital role in the growth and survival of *M. leprae* pathogen in infected hosts.

1. Introduction

The science, engineering and technology associated with the manipulation of matter at the nanoscale are grouped as nanotechnology. Although considered to be a discovery of modern science, nanotechnology is serving mankind from ancient times e.g. gold nanoparticles were used to tint window glasses ruby red by the medieval glass artisans. Copper and silver metal particles (between 5 and 100 billionth of a metre) were used by the people of Umbria, Italy during the renaissance period (1450–1600 CE) to prepare iridescent ceramics.

The different chemical, optical, electrical and magnetic properties of the nanoparticles compared to their bulk counterparts and their affinity for binding many different (bio)molecules including proteins have made them a subject of great interest in almost every branch of science in recent times [1,2]. Their small size makes them capable of interaction with biological fluids which mainly comprised of proteins and such interaction influence the bio-reactivity and cellular uptake of different nanoparticles [3]. Due to having high surface to volume ratio, nanoparticles are often highly reactive and possess greater adsorption capacity, which enables them to carry or to get bound with other molecules such as proteins, drugs, organic molecules etc [4]. Various types of nanomaterials, either man-made or natural, are available like metal and metal oxides, silicon based semiconductor nanomaterials, carbon based nanomaterials, nanofilms, nanosheets, polymeric nanomaterials etc., which have potential application in biomedical fields [5,6]. Among these nanoparticles, gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) are most studied.

Over the years, many synthetic routes have been adopted to synthesize gold and silver nanoparticles of different sizes [7–10]. Both of them are extensively used in imaging field [11–14]. Beside imaging purposes, AuNPs and AgNPs are extensively used in developing different clinical diagnostic methods for detecting many important diseases including cancer [12,14,15]. These two nanoparticles are also potential candidates for the treatment of diabetic complications and various other diseases [9,12,16–18]. Reports regarding the anti-fungal and anti-viral activities of gold and silver nanomaterials are also available in literature [19–22]. These two nanoparticles also exhibit antibacterial as well as antimicrobial activities [23–26]. The effect of AgNPs on the growth of different *Mycobacterium* species has also been explored to a large extent.

Abbreviations: sHSP, small heat shock protein; AuNPs, gold nanoparticles; AgNPs, silver nanoparticles; DTT, dithiothreitol; IPTG, isopropyl-β-D-thiogalactoside; bis-ANS, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid, dipotassium salt.

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https://doi.org/10.1016/j.ijbiomac.2020.02.182
0141-8130/

International Journal of Biological Macromolecules 127 (2019) 187–196

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Depicting the DNA binding and photo-nuclease ability of anti-mycobacterial drug rifampicin: A biophysical and molecular docking perspective

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ARTICLE INFO

Article history:
Received 10 September 2018
Received in revised form 25 November 2018
Accepted 6 January 2019
Available online 08 January 2019

Keywords:
Drug-DNA interaction
Photo-nuclease activity
Rifampicin

ABSTRACT

Rifampicin, an important member of ansamycin family, exhibits various biological activities. It is frequently used for the treatment of tuberculosis and leprosy. Recently, its interaction with protein is evidenced. But, its interaction with DNA is still unknown. Whether, exhibition of anti-cancer activity of rifampicin is associated with DNA-cleavage activity is also unknown. In this study, an attempt has been taken to understand these two unknown aspects. Spectroscopic studies indicated that rifampicin binds to CT-DNA with a binding constant of ~5.22 $\times 10^5$ M^{-1}. Several independent experiments like CD analysis, competitive displacement experiments and viscosity measurements revealed that rifampicin intercalates into the CT-DNA. Molecular docking studies corroborate this fact and depicted that this drug binds to the GC-rich region of DNA through multiple hydrogen bonding having the relative binding energy of −9.21 kcal mol^{-1}. Besides, DNA binding ability, rifampicin causes the photo-cleavage of pUC19 DNA via singlet oxygen pathway. To the best of our knowledge, we report for the first time the DNA binding and DNA cleavage ability of rifampicin. This study provides a clue behind the execution of the anti-cancer activity of rifampicin. Overall, all these information can be used for further understanding the pharmacological effects of rifampicin.

1. Introduction

The interaction of small molecules with DNA has gained attention in recent years and has become one of the major research subjects. This is primarily due to the ability of different small molecules to interact with regions of DNA resulting in alteration of the functioning of DNA by targeting its specific sequences, thus regulating the protein synthesis, expression of genes and growth of cells [1–3]. Since DNA has been known to be the cellular target for many cytotoxic and therapeutic agents [4], therefore understanding how drug molecules interact with DNA has become an active research area at the interface between chemistry, molecular biology and medicine. The knowledge of interaction studies of small molecules with DNA helps us gain insight into its mode of action at the molecular level, the origin of some diseases and also in screening of drugs as anti-cancer agents [5,6]. Generally, drugs can interact with DNA in a covalent as well as non-covalent manner. Anthramycin, Mitomycin C and Cisplatin covalently bind to the DNA and modulate its function [7]. It is noteworthy to mention here that majority of anti-tumor, anti-cancer, anti-viral and anti-inflammatory drugs bind to DNA via non-covalent interactions [7]. Some of them (Hoechst 33258, Distamycin, Mithramycin etc.) bind to the minor groove of DNA [8–10] and some (Nogalamycin, Ibuprofen, Naproxen etc.) interact with DNA via intercalation [11–13]. All these information are extremely important not only to modify the existing DNA binding drugs for improving their efficacy but also useful to synthesize new drug molecules which can target specific genes. Despite several attempts made to gain knowledge about the interaction between DNA and different drug molecules, investigations pertaining to the interaction between ansamycin antibiotics especially rifampicin and DNA are completely absent in the existing literature.

Ansamycin antibiotics are basically bacterial secondary metabolites which exhibit a wide range of biological activities [14–16]. These antibiotics possess unique chemical structure (Fig. 1). Generally, they contain two aromatic moieties which are connected by a long aliphatic chain [14–16]. Most of the members of this family contain either a naphthalene ring or a naphthoquinone ring [14,15]. Rifampicin is the most important member of this family and has got US Food and Drug

Abbreviations: CT-DNA, calf-thymus DNA; EB, ethidium bromide; DAPI, 4′, 6-diamidino-2-phenylindole; MG, methyl green; CD, circular dichroism.

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https://doi.org/10.1016/j.ijbiomac.2019.01.034
0141-8130/

Archives of Biochemistry and Biophysics 665 (2019) 107–113

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

DNA minor groove binding of a well known anti-mycobacterial drug dapsone: A spectroscopic, viscometric and molecular docking study

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ARTICLE INFO

Keywords:
Dapsone
Drug-DNA interaction
Spectroscopy
Molecular docking
Groove binding

ABSTRACT

Dapsone is a sulfone drug mainly used as anti-microbial and anti-inflammatory agent for the treatment of various diseases including leprosy. Recently, its interaction with protein (bovine serum albumin) is evidenced. But, the binding propensity of this anti-mycobacterial drug towards DNA is still unknown. Also, the mode of dapsone-DNA interaction (if any) is still an unknown quantity. In this study, we have taken a thorough attempt to understand these two unknown aspects using various biophysical and *in silico* molecular docking techniques. Both UV–visible and fluorescence titrimetric studies indicated that dapsone binds to CT-DNA with a binding constant in order of $10^4\ M^{-1}$. Circular dichroism, thermal denaturation and viscosity experiments revealed that dapsone binds to the grooves of CT-DNA. Competitive DNA binding studies clearly indicated the minor groove binding property of this anti-mycobacterial drug. Molecular docking provided detailed information about the formation of hydrogen bonding in the dapsone-DNA complex. This *in silico* study further revealed that dapsone binds to the AT-rich region of the minor groove of DNA having a relative binding energy of $-6.22\ kcal\ mol^{-1}$. Overall, all these findings evolved from this study can be used for better understanding the medicinal importance of dapsone.

1. Introduction

DNA, an important bio-molecule, carries the genetic information. Replication and transcription are the two major functions of DNA and are extremely essential for growth and survival of the cell. Improper transcription and DNA replication can cause several human diseases such as Meier–Gorlin syndrome, Seckel syndrome, cancer, type I auto-immune poly endocrinopathy syndrome, Opitz-Kaveggia syndrome, cohesinopathies, diabetes etc [1,2]. Thus, DNA is one of the potent targets of many drugs. Interacting with DNA, drugs can modulate the transcription and replication process and can eventually control many human diseases [3]. It is well known that effective anti-viral, anti-cancer and antibiotic drugs preferentially interact with genomic DNA [4]. Generally, drugs can interact with DNA in a covalent as well as non-covalent manner. Anthramycin, mitomycin C and cisplatin covalently bind to the DNA and modulate its function [5]. It is noteworthy to mention here that majority of anti-tumor, anti-cancer, anti-viral and anti-inflammatory drugs bind with DNA via non-covalent interactions [5]. Some of them (pirenzepine, capsaicin, sulindac etc.) bind to the minor groove of DNA [6–8] and some (mitoxantrone, ibuprofen, naproxen etc.) interact with DNA via intercalation [9–11]. Scientists often utilize many experimental and theoretical approaches to depict the mode of "drug-DNA interaction". Theoretical studies pin-point the site or location with a drug molecule at which a drug molecule can bind/interact [12–15]. These *in silico* studies also provide knowledge about the forces (electrostatic interaction, Van der Waals interaction, hydrophobic interaction and hydrogen bonding) which actually govern this "drug-DNA interaction" [12–15]. All these information are extremely important not only to modify the existing DNA binding drugs for improving their efficacy but also useful to synthesize new drug molecules which can target specific genes. Even though many attempts have been made to understand the interaction between DNA and drug molecules, we noticed some lacunae. Reports on "anti-mycobacterical drug-DNA interaction" are sparse [16]. Still, the information about the interaction between most of the anti-mycobacterial drugs and DNA is missing in the literature. One such anti-mycobacterial drug is dapsone.

Dapsone (4, 4′-diaminodiphenylsulfone), an aniline derivative, belongs to the class of synthetic sulfones (Fig. 1), is used as both antibiotic

Abbreviations: CT-DNA, calf-thymus DNA; DAPI, 4′, 6-diamidino-2-phenylindole; MG, methyl green; EB, ethidium bromide; CD, circular dichroism

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https://doi.org/10.1016/j.abb.2019.03.001
Received 6 November 2018; Received in revised form 18 February 2019; Accepted 1 March 2019
Available online 06 March 2019
0003-9861/

International Journal of Biological Macromolecules 119 (2018) 604–616

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Probing the structure-function relationship of *Mycobacterium leprae* HSP18 under different UV radiations

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ARTICLE INFO

Article history:
Received 24 March 2018
Received in revised form 1 July 2018
Accepted 23 July 2018
Available online 25 July 2018

Keywords:
Mycobacterium leprae HSP18
Small heat shock protein
UV radiation
Photo-oxidation
Cross-linking
Chaperone function

ABSTRACT

Ultraviolet radiation, an effective sterilizing source, rapidly kills the causative organism (*Mycobacterium leprae*) of leprosy. But, the reasons behind this quick death are not clearly understood. Also, the impact of UV radiation on the antigen(s) which is/are responsible for the survival of this pathogen is still unknown. Many reports have revealed that *M. leprae* secrets a major immunodominant antigen, namely HSP18, whose chaperone function plays an important role in the growth and survival of this pathogen under various environmental insults. However, the effect of UV radiation on its structure and chaperone function is still unclear. Therefore, we have taken a thorough attempt to understand these two aspects of HSP18 under different UV radiations (UVA/UVB/UVC; doses: 1–50 J/cm^2). Our study revealed that its chaperone function is decreased significantly with increasing doses of various UV radiations. These different UV irradiations perturb only its tertiary structure and induce tryptophan and tyrosine photo-oxidation to *N*-formyl kynurenine, kynurenine and dityrosine. Such photo-oxidation promotes the subunit cross-linking within a HSP18 oligomer, lowers the surface hydrophobicity and thermostability of the protein. All these factors together damage/reduce the chaperone function of HSP18 which may be an important factor behind the rapid death of *M. leprae* under UV exposure.

1. Introduction

One major breakthrough of leprosy research has taken place when G. H. Armauer Hansen identified the causative organism (*Mycobacterium leprae*) of this disease in 1873. [1]. Since then, many molecular biological studies have been undertaken so as to 1) identify and characterize the immunodominant antigenic proteins of *M. leprae*, 2) elucidate the whole genome sequence of this organism, 3) understand the immunopathological mechanism of this pathogen and 4) develop suitable vaccine candidates for the effective treatment of leprosy. Presence of several antigenic proteins such as 10 kDa, 15 kDa, 18 kDa and 65 kDa antigen have been evidenced in *M. leprae* [2–4]. All of them contribute to virulence and persistence of *M. leprae* pathogen inside the infected hosts. Among these *M. leprae* antigens, the 18 kDa antigen is found to stimulate both human and murine T-cells [5–7]. Raison and coworkers first showed that this antigen is recognized by pooled sera of human patients with lepromatous leprosy [8]. Subsequently, when the monoclonal antibody of the protein became available, it was evidenced that B-cells were also recognized by the epitopes of this antigen [9]. Like other mycobacterial antigens, 18 kDa protein possesses epitopes that are antigenic to T-cells and are considered to be a potent stimulator of CD4$^+$ T-cell responses [10–12]. It also exhibits MHC class II-restricted cytotoxicity [13]. Mustafa et al. reported that the T-cell clones specific for peptides carrying amino acids 1–38 and 41–55 of *M. leprae* HSP18 were found to be cross-reactive with *M. tuberculosis* complex, *M. avium* and *M. scrofulaceum* heat shock proteins [11].

Basically, this immunodominant 18 kDa antigen is also termed as HSP18 and belongs to class 3 (acr 3) heat shock protein family [14]. HSP18 shares moderate (around 60%) and a high degree of sequence homology with *M. tuberculosis* HSP16.3 and homologs of class 3 sHSPs found in other mycobacterial species, respectively [15, 16]. This mycobacterial sHSP possesses a highly conserved α-crystallin domain which is flanked by poorly conserved N-terminal domain and proceeded by a flexible C-terminal domain (Fig. 1). It is a major β-sheet protein and exists as a large oligomer (29-mer) [17]. Apart from others, our group has convincingly shown that HSP18 can exhibit molecular chaperone function [16–18]. Later, we have elucidated the

Abbreviations: sHSP, small heat shock protein; DTT, dithiothreitol; IPTG, isopropyl-β-D-thiogalactoside; bis-ANS, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid, dipotassium salt; NFK, *N*-formyl kynurenine; Kyn, Kynurenine.

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https://doi.org/10.1016/j.ijbiomac.2018.07.151
0141-8130/