REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training / Research abroad:

1	Name and designation of ICMR-IF	: Dr. Kaushik Ghosh, Associate Professor
2	Address	Department of Chemistry, IIT Roorkee, Roorkee-247667, Uttarakhand
3	Frontline area of research in which training/research was carried out	Bioinorganic chemistry of protein aggregation in Alzheimer's disease
4	Name and address of the Professor of the host institute	Professor Peter Faller
5	Duration of the fellowship	:2 and ½ months
6	Highlights of the work conducted	See Annexure I

Kaushik Ghosh

ICMR Sanction No. Signature of ICMR-IF

INDO/FRC/452/Y-78/2014-15-IHD

Annexure I

(i) Technique/expertise acquired
(ii) Research results, including any paper prepared / submitted for publication
(iii) Proposed utilization of the experience in India

(a) Teaching
(b) Research
(c) Collaboration

(Details are attached in the next page)

Annexure I

Introduction

Protein aggregation through amyloid formation were well accepted for different disease like Alzheimer's disease (AD), Parkinson's disease, Huntinton's disease, type II diabetes etc. Alzheimer's disease (AD) exhibit two important hallmarks: first is the formation of extracellular amyloid plaques and second is the intracellular neurofibrillary tangle formation. There are amyloid precursor proteins (APP) in the brain cells which produce different Aβ peptide fragments. In amyloidogenic pathway $A\beta_{1\text{-}40/42}$ are produced. On the other hand, in non-amyloidogenic pathway $A\beta_{1\text{-}16}$ $A\beta_{17\text{-}40/42}$ and $A\beta_{11\text{-}40/42}$. $A\beta_{1\text{-}40/42}$ and $A\beta_{1\text{-}40/42}$ peptides in a healthy brain Aβ in soluble monomeric form, on the other hand for AD patients aggregation of Aβ and plaques were found in the brain.

Copper and zinc both the metal ions were found in the plaques. Normal copper concentration in the brains are ~ 1μM in the CSF and around 10μM in synaptic cleft. However copper concentrations in the plaque were found to be 400μM which is higher than any one of them. During signal transmission zinc is released in the in the synapse and the concentration is 200-300 μM. But the concentration of zinc in plaque is 1000μM. In fact iron was also found to be present in the plaque and the concentration in the plaque is ~1000μM.

These data clearly indicate that there must be some role of metal ions like copper zinc and iron in the aggregation. It is important to note here that the metal ions like copper and iron is redox active on the other hand zinc is not redox active.

The recent reports not only predict the role of metal ions but also there are several reports which clearly show the role of ROS (reactive oxygen species) generation in amyloidogenic aggregation.

Copper ion has a double deleterious role in the amyloid cascade. After binding to Aβ, copper can either modulate its aggregation or enhance ROS generation.

Hence metal ion could play two different type of roles in protein aggregation. In one hand through chelation it could enhance the process of aggregation. On the other hand through metal ion could also take part in the process of aggregation via reactive oxygen species generation i. e. ROS production.

1. **Experiments pertaining to ROS generation: Ascorbic acid assay**

Reagents needed :

1. Sodium ascorbate
2. Phosphate buffer pH 7.4
3. Copper sulfate solution
4. Chelator/metal complex
5. A β-peptide

Instrument used: UV-Visible spectrophotometer

Reference used: *J. Inorg. Biochem.* ***2012****, 117, 322-325*

Metal ion titration (we need to have a phenol donor to see copper to phenolato charge transfer band) for a ligand

2. **Titration of manganese complex with copper**

Reagents needed

1. Phosphate buffer pH 7.4
2. Copper sulfate solution
3. Manganese complex
4. Ligand (L)

Instrument used: UV-Visible spectrophotometer

Experiments pertaining to ROS generation during HSA aggregation by ascorbic acid assay.

Reagents needed

1. Sodium ascorbate
2. Phosphate buffer pH 7.4
3. Copper sulfate solution
4. Human serum albumin (HSA)
5. Ethanol

Instrument used: UV-Visible spectrophotometer

We tried to understand the change in ROS generation during aggregation.

Reference used: *J. Phy. Chem. B* ***2010****, 114, 10228-233*

(i) Technique/expertise acquired :

This area is one of the frontier areas in biological inorganic chemistry. There are several important techniques I have learnt. First, the most important part was the role of metal ion in ROS (reactive oxygen species) generation in the physiological condition. This ROS generation could be estimated during any kind of metal ligand reaction by ascorbic acid assay.

Second, investigation of generation of hydroxyl radical by the formation of 7-OH-CCA dye from 3-CCA (coumarin -3-carboxylic acid).

This is done in spectrofluorimeter. Hydroxyl radical reacts with 3-CCA and 7-OH-CCA dye is produced which provide fluorescence at 452 nm while the excitation wavelength is 395 nm.

Third, I have learnt to handle AFM (atomic force microscopy) and I learnt how to utilize this technique for protein aggregation studies.

(ii) Research results, including any paper prepared/submitted for publication: The compounds we tested were new and the results are promising.

The results are summarized below:

i	*Titration experiment of manganese complex with copper solution exhibited formation of copper complex in phosphate buffer pH 7.4* $MnL + Cu \rightarrow CuL + Mn$ *(L = ligand/chelator)*
ii	*The ligand could take out copper from Cu-* $A\beta_{1-16}$ *and formation of CuL was observed in physiological condition* *Cu-* $A\beta_{1-16} + L \rightarrow CuL + A\beta_{1-16}$
iii	*Ascorbic acid assay for the determination of ROS indicated that treatment of MnL as well as L provide the formation of CuL and there is no more any redox activity. Hence L is a very good candidate for making Cu-* $A\beta_{1-16}$ *redox inactive.*
iv	*We have examined the generation of hydroxyl radical by fluorescence spectrophotometer. 3-CCA (coumarin-3-carboxylic acid) assay gave rise to fluorescence due to hydroxyl radical generation. The ligand stopped the generation of hydroxyl radical and the data was consistent with ascorbic acid assay.*

However in terms of publication, the results will be verified after few repeats of the same experiments and this work is preliminary and there should be statistical data (with error bars). Moreover we have only used $A\beta_{1-16}$. The whole length amyloid $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides

would be utilized. I definitely contributed in the area of metal chelation and drug discovery for AD and Prof. Peter Faller will provide authorship if there is any manuscript submitted in near future with the work I have done during my stay over here.

(iii)Proposed utilization of the experience in India

Research/collaboration: The assay I have learnt here and the AFM studies for protein aggregation will enrich the research activities of my group in IIT Roorkee. The interaction with different faculty members, scientists and the students who are working here provided several new ideas which I will explore after I go back to India. With the results we obtained here, and the literature survey we are going to submit a project in CEFIPRA. This is the Indo-French forum of Government of India.

Teaching: I teach several courses to B. Tech , M. Tech, M. Sc. and Ph. D. students in IIT Roorkee which is an institute for human resource generation (funded by MHRD ministry) . This experience will not only strengthen the research component of my research lab but also it will enrich the teaching material due to my practical experience regarding metal ion related disease and their prevention. Last but not the least I would like to mention that I teach a course (Course No. CY-912 entitled "Frontiers in Inorganic Biochemistry") to the final year M.Sc. students and pre-Ph.D. students. The results are the experiments will have direct impact on such course.

END

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