PIEF-GA-2013-622296, Acronym: Organozymes

Recipient: Carlos Aydillo, Host CECB, Professor Morten Meldal, UCPH

The main goal of the project was the development of new medium sized peptide-transition metal complexes acting as enzyme like catalyst molecules, "organozymes" that promote proteolysis and in particular of Alzheimer's disease related peptide Aβ 1-42. The implementation of catalytic molecules as medicines is a new paradigm in the treatment regime that, in principle, can overcome a number of the difficulties with present drugs. Background observation in host group indicated that metal complexes of *N*-heterocyclic carbenes can present internal proteolytic activity and hydrolyze peptide bonds. With these results in mind, it was envisioned metal complexes of peptidocarbenes could show selectivity and cleave external peptide bonds in pathogen substrates. Such molecules were termed organozymes. Through combined specific molecular recognition and catalytic activity towards the core region of the Aβ 1-42 AD peptide, synthesized as a FRET substrate, it seemed feasible to design suitable catalyst libraries and screen for organozymes capable of performing the desired cleavage of the plaque forming Aβ 1-42 substrate.

New building blocks that would allow the incorporation of dipeptide mimetic carbene precursors into peptides chains were designed and synthesized in preparative amounts. These building blocks also included protected polar side chain functional groups. They were incorporated into model peptides and palladium coordination was established and investigated by high resolution mass spectrometry. Structural analysis of the palladium-peptidocarbene complexes was demonstrated from single beads on PEGA 1900 resin used in subsequent combinatorial analysis. Several catalyst libraries were designed for an enhanced molecular recognition as proposed theoretical calculations (Molecular Dynamics calculations using Molecular Operating Environment). These libraries were synthesized in such a manner that each bead of the library would present the Alzheimer core sequence as a FRET substrate flanked by 3-nitrotyrosine and 2-aminobenzamide and simultaneously a member of a library of palladium peptidocarbene complexes with a high propensity for interaction with the Alzheimer substrate. Several library constructs were tested. Finally, solid phase combinatorial libraries of approximately 300.000 compounds were generated using one-bead-two-compounds strategy. Thus each polymeric bead of this library had a unique Pd complex and in addition the Alzheimer core sequence as FRET substrate. Upon metal coordination, during the screening of these libraries, positive hits could be observed indicating cleavage of substrate by increasing fluorescence. Hits in the form of single fluorescent beads on a large background of non-fluorescent beads were observed and these could be isolated. The catalysts were cleaved off the single hit bead and were identified by MS/MS using a Bruker Solarix Fourier Transform Cyclotron Resonance Mass Spectrometer. Approximately 60 hits were analyzed and showed clear evidence of consensus, as an indication of the specificity of recognition. The most promising catalysts were resynthesized on a larger scale for solution studies. These catalysts were mixed with beads containing the Alzheimer FRET substrate and inspected for cleavage. Although cleavage clearly was observed for the catalysts in the library when linked to the same resin bead, providing really exciting promising screening results, the cleavage could not be reproduced within the time of the project. However, the host laboratory has continued the work of Carlos Aydillo, and recently we demonstrated cleavage of FRET peptide substrates in solution including the Alzheimer substrate. However,

investigations are still required in order to really evaluate the cleavage mechanism and optimize cleavage conditions. The results and data obtained in the project will be published in due course and in collaboration with other coworkers at CECB. The grant recipient is currently drafting the first manuscript.

This project has the bold aim of providing artificial peptide catalysts that act as enzymes and in addition to having a metal associated catalytic activity also provide specific recognition and adhesion to the Alzheimer substrate which represent the core nucleation site of the formation of Alzheimer fibrils characteristic for the plaques in the demented Alzheimer's brain. It is the first time catalysis and recognition of the substrate at the same location is attempted. This is crucial for the organozyme action of these catalysts and their potential use as catalytic drugs. The advanced work performed in this project and the processes created on the way were fundamental for our design and synthesis of organozyme libraries and the results of this project will form the basis of many future projects both within Alzheimer and cancer apoptosis studies.

The project has opened the opportunity to develop a drug that can not only cleave Alzheimer peptide and maybe eventually clear the dement brain from plaques, it also pave the way for new approaches towards other incurable diseases. The socioeconomic burden of Alzheimer's disease and similar currently incurable diseases is huge for the society and devastating for the victims and their families. A drug in this category will also have very significant industrial potential.

The project was hosted at CECB:

Web: http://cecb.ki.ku.dk/,

Contact: Professor Morten Meldal, CECB, Department of Chemistry, Faculty of Science, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark.

Mobile: +45 21308299, meldal@nano.ku.dk