EME PhD SUBJECT

TITLE: Novel Chemical Approaches to Ultratrace Selenoproteomics

ABSTRACT:
Selenium is an essential trace element known for its antioxidant activities. The physiological role of selenium is principally awarded to its co-translational incorporation into selenoproteins as selenocysteine (SeCys), referred to as the 21st amino acid. Twenty-five selenoproteins have been predicted by bio-informatics and constitute the human selenoproteome. One third of these proteins have never been identified in vivo and their functions are unknown. The understanding, on the molecular level, of the function and regulation of selenoproteins, often evoked in the context of cardiomyopathy, thyroid function, cancer, fertility and aging, is critically dependent on the availability of adequate analytical methodology. It should allow the comprehensive qualitative and quantitative analysis for the full set of selenoproteins (selenoproteome) at concentrations down to 1 ng/mL (as Se). Selenoproteins are low abundant (atto- to femtomolar level; selenoprotein/total protein ratio <10^{-5}) and only for few of them antibodies are available and often with low affinity.

This project proposes the development of novel chemical approaches for the characterization of selenoproteome. This strategy will be based on the synthesis of new solid supported reagents able to selectively bind Seleno Cysteines (SeCys) of selenoproteins in Cys-containing proteins. Starting from biological matrices, our tools will allow for selective recovery and preconcentration of selenoproteins for proteomic characterization and quantification. This approach focuses on the understanding of the SeCys chemistry in order to develop new “fishing tools” that would offer an opportunity for the proteomic analysis of selenoproteins (evoked in the context of cardiomyopathy, cancer, fertility, aging...) using cutting-edge proteomics approaches.

Keywords: Selenoproteins, selenopeptides, proteomics, mass spectrometry, chemical synthesis, organic/analytical chemistry.

CONDITIONS D’EXERCICE / WORKING CONDITIONS

Laboratory: IPREM, UPPA/CNRS  
web site : https://iprem.univ-pau.fr/fr/index.html

PhD Director: R. Lobinski
PhD co-Director: L. Ronga
In Collaboration with : Laboratoire ARNA, Université de Bordeaux

Place: IPREM, UMR 5254
Start: October 2018  
Duration: 3 years

Employeur (employer): Université de Pau et des Pays de l’Adour (UPPA)
monthly salary before taxes: 1768 €
SAVOIR-FAIRE DU LABORATOIRE / HOST LABORATORY PROFILE

The Institute of Analytical Sciences and Physico-Chemistry for Environment and Materials (IPREM) is a Joint Research Unit CNRS / UPPA (UMR 5254). IPREM members are interested in the development of fundamental knowledge in physico-chemistry, analytical chemistry and microbiology, in relation to applications concerning the structure of the living, the management of the environment and the functional properties of different classes of materials. Their skills are based on analytical strategies, modeling, physico-chemical approaches, fine studies of structures and reactivity, development, characterization and implementation at different scales. They make it possible to display an original position in the field of applications in many industrial sectors both at national and international level.

The PhD student will have access to the largest platform in France of the couplings of separation techniques with ICP-MS which has recently been completed by the latest nanoHPCL–Orbitrap technology (Fusion Lumos, Tribrid MS) for sensitive proteomic analysis.

MISSION - ACTIVITES PRINCIPALES / MISSION – PRINCIPAL ACTIVITIES

I. Le contexte scientifique Scientific Context

Selenium is an essential trace element known for its antioxidant activities. In mammals, selenium deficiency has been associated with muscular, neurological and immune disorders, and also with an increase in cancer incidence and mortality [1,2]. The vital role of selenium has been widely recognized by selenium supplementation of the diet [3]. The physiological role of selenium is principally awarded to its co-translational incorporation into selenoproteins as selenocysteine (SeCys), referred to as the 21st amino acid [4]. Twenty-five selenoproteins have been identified and constitute the human selenoproteome [5]. Out of them, the most widely studied have been the families of glutathione peroxidases (GPx), essential for fundamental defense mechanisms against oxidative stress, and thioredoxin reductases (TR) where selenium is crucial for catalytic activity. One third of the putative human selenoproteins has never been identified in vivo and their functions are unknown.

The understanding, on the molecular level, of the function and regulation of selenoproteins often evoked in the context of cardiomyopathy, thyroid function, cancer, fertility and aging (which are all important societal concerns) is critically dependent on the availability of adequate analytical methodology. It should allow the comprehensive qualitative and quantitative analysis for the full set of selenoproteins (selenoproteome) at concentrations down to 1 ng/mL (as Se). Selenoproteins are low abundant (atto- to femtomolar level; selenoprotein/total protein ratio <10^{-5}) and only for few of them antibodies are available and often with low affinity.

In a previous study, our laboratory brought a significant progress (decrease of detection limits of 2 orders of magnitude) in the detection of selenoproteins in cytoplasmic cell extracts without the use of radioactive isotopes owing to the development of a combination of isoelectrofocusing selenoprotein separation combined with nano- and femtosecond laser ablation inductively coupled plasma mass spectrometry (ICP MS) [6]. The number of detectable selenoproteins in human cell lines and serum samples increased from 2-3 to 10 which still does not cover the integrality of the selenoproteome.

Another “quantum leap” is necessary to gain at least one order of magnitude more in terms of detection limits which demands changing the paradigm in the approach as the limits of the cutting-edge instrumentation have apparently been reached. Also, a serious limitation to be overcome remains the formal identification of the detected selenoproteins which is currently impossible by canonical proteomics at such low levels in samples of such complexity. Moreover, most selenoproteins
have close Cys- and Ser-containing homologs, their complete proteomic characterization requires a specific selenium based approach and their quantification needs the use of selenopeptides.

II. Objectives
This project focuses on the understanding of the selenocysteine chemistry in order to develop new preconcentration methods that would offer an opportunity for the proteomic analysis of the purified selenoproteome using cutting-edge proteomics approaches.

This research program will be structured in three main tasks:
1) Development of chemical approaches able to selectively extract selenoproteins from biological matrices: synthesis of new solid supported reagents.
2) SelenoProteomics: development of top-down and bottom-up approaches targeting the 25 human selenoproteins using nanoHPLC-Advion-Orbitrap (Fusion Lumos) Tribrid MS.
3) Quantification of selenoproteins: synthesis of enriched stable isotope labelled selenopeptides and development of methods for the isotope dilution HPLC/nanoHPLC – ICP MS of selenoproteins.

III. Literature References

COMPETENCES REQUISES / REQUIRED COMPETENCES

Master’s degree in the field of chemistry of biomolecules (synthesis, characterization, activity evaluation). Experience in synthetic chemistry or mass spectrometry is an advantage. Great motivation for scientific research. Good knowledge of English or French (speaking and writing).

CRITÈRES D’ÉVALUATION DE LA CANDIDATURE / CRITERIA USED TO SELECT CANDIDATE

Application file assessment: Selection committee.
Candidates will first be selected based on their application file.
Those selected after this first step, will then be interviewed.
Application will be evaluated based on the following criteria:
- Grades and ranking during her/his Master degree, steadiness in academic background
- English language proficiency
- Candidate’s ability to present her/his work and results, candidate's motivation, scientific maturity and curiosity
- Work experience similar to an internship in a laboratory – or likewise; previously achieved research work (reports, publications).

CONSTITUTION DU DOSSIER DE CANDIDATURE, DATE LIMITE DE DEPOT / REQUIRED DOSSIER, DATE
Please send an e-mail with your candidature containing:

- CV
- cover letter detailing candidate's motivations
- candidate's MSc marks and ranking
- letters of recommendation
- contact details of two referees

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<th>DATE LIMITE DE DEPOT DU DOSSIER:</th>
<th>End July 2018</th>
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**CONTACTS**

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