Intitulé du Sujet de Thèse : Exploration of cytochrome P450 reductase dynamics by EPR modern approaches

Laboratoire : Bioénergétique et Ingénierie des Protéines, BIP-UMR7281

Equipe : Biophysique des métalloprotéines

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Contexte de l'étude. Obtaining structural insights of proteins is highly important to understand chemical mechanisms in biological process. It is then crucial for pharmaceutical and biotechnical applications. Fluctuation of protein conformation is recognized as one of the fundamental properties with major consequences for their functions. Indeed, it can be very efficient to modulate and/or regulate the catalytic activity of enzymes and to bind to other molecules (substrates, cofactors ..). Unraveling the close link between protein flexibility and function during all steps of catalytic cycles is a current challenge of high impact in the field of redox enzymes in which electron transfer (ET) are highly sensitive to relative distances or orientations of redox centers. This project is about the cytochrome P450 reductase (CPR), a membrane flavoenzyme. It transfers the electrons from NADPH to acceptors as P450s for their catalytic reactions. It has a major role in metabolism of steroids or fat acids.[1] CPR has two domains for FAD and FMN binding and a flexible hinge that links the previous domains.[2] ET goes from NADPH (hydride transfer) to CYPs through the flavines sites. For efficient ET, FAD and FMN domains need to be dose (closed conformation of CPR), but inter-protein ET (FMN to CYPs) is possible if CPR adopts a more open conformation where CPR and CYPs get closer. This two conformation model is quite simple ("closed" vs. "open"), and is based on crystallographic and *in silico* studies. Biophysical studies suggest that, in solution, CPR exists as a continuum of populations.[2]

Descriptif du projet

The PhD project is focused on the relation between domain motion and CPR activity via ET. Site Directed Spin Labeling (SDS coupled to EPR (Electron Paramagnetic Resonance) approach will be used.[3, 4] One or two paramagnetic probes (nitroxides) will be introduced at chosen positions in the protein. Usually, these probes are cysteine specific, but in CPR, natural cysteines are involved in catalytic process. Non natural amino acid (nnaa) incorporation and their labeling turned out to be a powerful alternative.[5] It is also possible to incorporate two nnaa on the same protein to measure distances between paramagnetic centers. These approaches based on the reactivity of chemical groups on nnaa to introduce biophysical probes (fluorescence, NMR or EPR), via bioorthogonal reactions, "click chemistry" or photo activation have been developed in recent years.[6-10] We will incorporate one or two nnaa on CPR. Distance measurements will be done by pulsed EPR. We will also use the flavines prepared in specific redox states (semi-quinones) as endogenous probes to distance measurements. Purification protocols, labeling experiments and yields will have to be optimized. Domain motion in relation with the ET in CPR will be studied, in presence of its partner as well. This interdisciplinary project will be done in collaboration with Gilles Truan and Phillippe Urban in Toulouse. It is at the interface of chemistry, biochemistry and EPR spectroscopy. The research work will be helped by the presence of the EPR facility of Aix-Marseille at the BIP lab, and by the Protein Expression Facility in collaboration with Deborah Byrne.

The applicant must have a Master degree in chemistry, preferentially with a chemistry/biology background. He/she must have interest to research at the interface of chemistry/biology and spectroscopy. Highly motivated, independent and dynamic, he/she should be able to work in a multidisciplinary team. Please send applications including (i) a detailed CV, (ii) official transcripts of master and undergraduate studies, (iii) an application and motivation letter, and (iv) a recommendation letter by e-mail at marlene.martinho@univ-amu.fr by May, the 18th 2021.

Références Bibliographiques

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