Intitulé du Sujet de Thèse : Study of the membrane cytochrome P450 reductase protein using biostructural EPR .....

Laboratoire : Bioénergétique et Ingénierie des Protéines, BIP-UMR7281.....

Equipe : Biophysique des métalloprotéines et des systèmes dynamiques ...

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Descriptif du projet

Microsomal cytochrome P450 enzymes (CYPs) play a central role in human physiology, contributing to the metabolism of approximately 70% of commonly prescribed drugs, as well as fatty acids and steroids.[1,2] These enzymes rely on a continuous supply of electrons delivered by their redox partner, NADPH cytochrome P450 reductase (CPR), a diflavin-containing protein essential for catalytic activity. Electron transfer (ET) is initiated by the nicotinamide adenine dinucleotide phosphate (NADPH) cofactor, which donates a hydride to CPR. Electrons are then relayed sequentially through CPR's flavin cofactors—flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)—before reaching the terminal acceptor, the heme iron of CYPs. The efficiency of this process is tightly coupled to the conformational dynamics of CPR. In solution, CPR interconverts between "locked/closed" and "unlocked/open" states, and this equilibrium is critical for regulating electron transfer. While these dynamics have been investigated using spectroscopic and structural methods, most studies have relied on truncated or mutated protein variants.[3–7] Consequently, the molecular determinants governing the conformational landscape of the full-length protein remain poorly defined. Although mutations within the flavin domain (FD) and small-molecule binding have been shown to perturb this equilibrium,[2,8] these studies did not simultaneously track flavin redox states, leaving a key aspect of CPR regulation unresolved.

To address this limitation, we will investigate full-length human CPR using Site-Directed Spin Labelling (SDSL) in combination with Electron Paramagnetic Resonance (EPR) spectroscopy.[9] By incorporating non-canonical amino acids at strategically selected positions, followed by site-specific nitroxide labelling, we will probe the conformational dynamics of CPR across its redox cycle. Double Electron–Electron Resonance (DEER) experiments will provide distance constraints through semiquinone/nitroxide and nitroxide/nitroxide spin pairs, enabling structural characterization of distinct conformational states. Importantly, we will examine CPR in a native-like environment and in functional interaction with CYPs, assessing the effects of membrane association[10–13] and redox chemistry on CPR structural dynamics. Complementary biophysical approaches, including cryo-electron microscopy (cryo-EM, in collaboration), will further strengthen the structural framework providing an integrated view of CPR dynamics, modulated by redox conditions to enable efficient electron transfer to CYPs.

Références Bibliographiques

[1] J. Hakkola, J. Hukkanen, M. Turpeinen, O. Pelkonen, *Arch. Toxicol.* **2020**, 94, 3671–3722. [2] F. Esteves, P. Urban, J. Rueff, G. Truan, M. Kranendonk, *Int. J. Mol. Sci.* **2020**, 21, 6669. [3] L. Aigrain, F. Fatemi, O. Frances, E. Lescop, G. Truan, *Int. J. Mol. Sci.* **2012**, 13, 15012–15041. [4] D. Hamdane, C. Xia, S.-C. Im, H. Zhang, J.-J. P. Kim, L. Waskell, *J. Biol. Chem.* **2009**, 284, 11374–11384. [5] T. M. Hedison, S. Hay, N. S. Scrutton, *FEBS J.* **2015**, 282, 4357–4375. [6] O. Frances, F. Fatemi, D. Pompon, E. Guittet, C. Sizun, J. Pérez, E. Lescop, G. Truan, *Biophys. J.* **2015**, 108, 1527–1536. [7] R. B. Quast, F. Fatemi, M. Kranendonk, E. Margeat, G. Truan, *ChemBioChem* **2019**, 20, 659–666. [8] F. Esteves, C. M. M. Almeida, S. Silva, I. Saldanha, P. Urban, J. Rueff, D. Pompon, G. Truan, M. Kranendonk, *Biomolecules* **2023**, 13, 1083. [9] M. Bizet, D. Byrne, F. Biaso, G. Gerbaud, E. Etienne, G. Briola, B. Guigliarelli, P. Urban, P. Dorlet, T. Kalai, G. Truan, M. Martinho, *Chem. – Eur. J.* **2024**, e202304307. [10] K. Yamamoto, U. H. N. Dürr, J. Xu, S.-C. Im, L. Waskell, A. Ramamoorthy, *Sci. Rep.* **2013**, 3, 2538. [11] R. Huang, K. Yamamoto, M. Zhang, N. Popovych, I. Hung, S.-C. Im, Z. Gan, L. Waskell, A. Ramamoorthy, *Biophys. J.* **2014**, 106, 2126–2133. [12] K. A. Gentry, G. M. Anantharamaiah, A. Ramamoorthy, *Chem. Commun.* **2019**, 55, 13422–13425. [13] C. Barnaba, E. Taylor, J. A. Brozik, *J. Am. Chem. Soc.* **2017**, 139, 17923–17934.