P15. SPECTROSCOPIC CHARACTERIZATION OF THE COPROPORPHYRIN III – COPROPORPHYRIN FERROCHELATASE COMPLEX OF LISTERIA MONOCYTOGENES

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The coproporphyrin-dependent (CPD) heme biosynthesis pathway, utilized by monoderm bacteria to produce heme *b*, has been discovered in 2015 [1]. Coproporphyrin III (cpIII) is the substrate of coproporphyrin ferrochelatases (CpfCs) which catalyze the insertion of ferrous iron into the porphyrin ring, producing iron coproporphyrin III (coproheme). In the successive step, coproheme decarboxylases (ChdCs) generate heme *b* by two decarboxylation steps, targeting the propionate groups of coproheme at positions 2 (p2) and 4 (p4), forming vinyl groups. After the cleavage of p2, the transiently formed monovinylmonopropionyl intermediate rotates by 90 degrees inside the protein pocket to bring p4 near the catalytic tyrosine, to allow the decarboxylation of p4 to form heme *b* [2-5].
Recently, crystallographic and spectroscopic studies of wild-type(WT) coproporphyrin ferrochelatase from *Listeria monocytogenes* (*Lm*CpfC)and several variants complexed with coproheme allowed us to conclude that the propionate 7 (p7) is not hydrogen bonded in solution and that the hydrogen bonds with p4 and p2 are important for the stabilization of coproheme inside the protein pocket [6,7].
Furthermore, a comprehensive spectroscopic characterization of proximal variants of coproheme-*Lm*CpfC in solution allowed us to confirm the presence of a proximal Tyr ligand. In addition, the UV-Vis electronic absorption and resonance Raman (RR) studies of the cpIII - WT *Lm*CpfC complex and several variants highlighted the differences between the RR spectra of the cpIII-WT and coproheme-WT complexes, allowing us to compare these two species in terms of the distortion and dimension of the porphyrin rings, and of the hydrogen bonds interactions between the propionates and the conserved polar amino acids of the protein pocket.

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[1] H.A. Dailey, S. Gerdes, T.A. Dailey, J.S. Burch, J.D. Phillips, 2015, *Proc Natl Acad Sci USA*, 112, 2210 – 2215.
[2] B.R. Streit, A. Celis, G.C. Moraski, K.A. Shisler, E.M. Shepard, K.R. Rodgres, G.S. Lukat-Rodgers, J.L. DuBois, 2018, *J. Biol. Chem*, 293, 3989–3999.
[3]L. Milazzo, T.Gabler, D.Pühringer, Z.Jandova, D. Maresch, H.Michlits, V.Pfanzagl, K. Djinović-Carugo, C.Oostenbrink, P.G. Furtmüller, C. Obinger, G. Smulevich, S. Hofbauer, 2019, *ACS Catal*, 9, 6766–6782.
[4] F. Sebastiani, H. Michlits, B. Lier, M. Becucci, P.G. Furtmüller, C. Oostenbrink, C. Obinger, S. Hofbauer, G. Smulevich, 2021, *Biophys. J*, 17, 3600-3614.
[5] F. Sebastiani, R. Risorti, C. Nicoli, H. Michlits, M. Becucci, S. Hofbauer, G. Smulevich, 2022, *J. Inorg. Biochem*, 229, 111718.
[6]S. Hofbauer, J. Helm, C. Obinger, K. Djinović-Carugo, P.G. Furtmüller, 2020, *FEBS J*, 287, 2779-2796

[7] T. Gabler, F. Sebastiani, J. Helm, A. Dali, C. Obinger, P.G. Furtmüller, G. Smulevich, S. Hofbauer, 2022, *FebsJ*, 6, 1680 – 1699.