SERCA2a protein purification to study interaction between SERCA2a-PLN complex and new istaroxime follow-on compounds

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Abstract:
Agents that improve intracellular Ca^{2+} dynamics represent a favourable therapeutic approach for heart failure. Istaroxime is a promising agent combining Na^+/K^+ pump inhibition and SERCA2a stimulation. Thanks to a joint lab in BTBS, we developed new istaroxime follow-on compounds able to improve cardiac performance in diseased animals by selectively SERCA2a stimulation.

The aim of the project was to investigate the molecular mechanism of action of these new molecules, analysing their interaction with SERCA2a and/or its physiological inhibitor (PLN) through NMR studies. To do this, we started setting up the best protocol to purify SERCA2a; a synthetic PLN was used.

Preliminary NMR data showed that one of the new follow-on compounds was able to bind only the full length PLN_{1-52} like its parent compound istaroxime. Concerning SERCA2a purification, we started from pig cardiac microsomes (enriched vesicles of sarco-endoplasmic reticulum, ER), but we didn't reach a sufficient SERCA2a purity level. Thus, we moved to SERCA2a production in engineered hSERCA2a-YFP-His_8 *Saccharomyces cerevisiae*. After 20h induction, the yeast effectively expressed SERCA2a and it mirrored the ER-protein localization pattern.

Overall, these data are preliminary to complete ligand-based NMR studies concerning SERCA2a/PLN complex interaction with istaroxime and its follow-on compounds.