Preliminary X-ray crystallographic analysis on the F112L Sorcin mutant.

Introduction

Sorcin, (soluble resistance-related calcium binding protein) is a 22-kDa Ca^{2+}-binding protein, expressed in several cell types including cardiomyocytes. It belongs to the recently identified penta-EF-hand (PEF) family. As all members of the PEF family, sorcin is homodimeric and each monomer is formed by two different domain: a flexible glycine reach N-terminal domain and a C-terminal domain which contains five EF hands. As shown by the X-ray structures of the PEF proteins solved so far, stabilization of dimeric protein is achieved by pairing of the uneven EF-hand, EF5.

Like all members of PEF family sorcin undergoes a calcium dependent translocation from cytosol to the membrane where it binds its molecular targets. In vitro, sorcin binds to cardiac RyRs (ryanodine receptors) and L-type Ca^{2+} channels. Thus, it has been hypothesized that sorcin may play a role in excitation-contraction coupling in the heart. Recently, the spontaneous mutation F112L, within the D helix, has been identified in sorcin . This naturally occurring sorcin missense mutation (F112L) is associated with hypertrophic cardiomyopathy, hypertension, and impaired modulation of cardiac ryanodine receptor and is thought to account for an inherited form of hypertrophic cardiomyopathy and hypertension.

The structure of the hamster and human SCBD (Soluble Calcium Binding Domain) have been solved (PDB codes 1GJY and 1JUO) but the structure of the whole protein is still unknown. The aim of our project is to solve the structure of the whole F112L in order to understand how the N and C terminal domain interact with each other and to understand how a single mutation may cause the change of the interaction with the RyR and hence hypertrophic cardio-myopathy and hypertension .

Results

Crystallization of F112L sorcin mutant was achieved at 293 K by the hanging drop vapour-diffusion method. A volume of 2 ul of the protein sample was mixed with an equal amount resercoir solution containing 12 % v/v dioxane, ammonium sulfate at 1.0 M at pH values between 5.5-6.5. Good data have been collected as 0.3 oscillation frames at the ID-14-1 beamline , at a wavelength of 0.933 Å. Data were collected at 100 K using 25 % (v/v) glycerol as cryoprotectant to a resolution of 2.7 Å. Data analysis, performed with DENZO,

indicates that the crystal are hexagonal $P6_222$ with unit cell dimensions of a=b=64.389Å, c=314.864 Å, == 90°, =120°. The data, scaled using SCALEPACK, have an R_{merge} =0.074 and a χ^2=1.2 , with a completeness of 99.7 %.

Unfortunately all the crystals from other projects tested during the scheduled shifts have been unsuccessful either due to poor diffraction or to very high decay under X-ray.

During the shifts we experienced discrepancies between what inputted in the proDC program and what written on the marccd image, but this software discrepancy has been very successfully solved by people at 2525!