Oral Contributions

[MS7 - 04] Crystallization of Erg11p – the cytochrome P450 target of triazole antifungals

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Lanosterol 14-demethylase (Erg11p or Cyp51p) is the target of triazole antifungals, a class of drugs commonly used to treat life-threatening infections. Erg11p is a membrane-anchored cytochrome P450 enzyme in the biosynthetic pathway of ergosterol, a component of the fungal cell membrane required for cell growth. Crystal structures of the soluble Cyp51p from *Mycobacterium tuberculosis* and N-terminal truncated catalytic domains from mammalian cytochrome P450s are unsuitable for modelling azole-target interactions in fungi. The aim of this study was the functional and structural characterisation of Erg11p in order to establish a platform for the development of improved antifungal drugs less susceptible to the development of resistance.

Full-length membrane-anchored CaErg11p from fungal pathogen *Candida albicans* was expressed in *Saccharomyces cerevisiae*. Milligram quantities of stable and monodisperse CaErg11p suitable for crystallography were obtained by solubilization in n-decyl-β-D-maltopyranoside, Ni-NTA affinity chromatography and size exclusion chromatography. The same strategy was used to prepare Erg11p from the fungal pathogen *Candida glabrata* (CgErg11p). Inhibitor binding assays were performed for CaErg11p and CgErg11p by measuring the wavelength shift in the heme peak absorbance. Adaption of the purification protocol to *S. cerevisiae* enabled the production of large, diffracting crystals of ScErg11p. Data collection was carried out at the Advanced Light Source at the Lawrence Berkeley National Laboratory, California and several ScErg11p structures were solved. The 1.9-2.8 Å crystal structures of empty and ligand-bound ScErg11p are the first crystal structures for any full-length cytochrome P450 enzyme showing resolution of the membrane spanning helix and give insight into the binding of lanosterol and triazole drugs.

The detergent extraction and purification protocol has produced quantities of stable membrane-anchored CaErg11p and CgErg11p for crystallography and yielded diffracting ScErg11p crystals reproducibly. The ScErg11p crystal structure may enable the development of improved antifungals via structure-based drug design.

Keywords: lanosterol 14-demethylase; Cyp51; antifungals