Abstract

Phosphatidylinositol 4-kinases (PI4K/PI4-kinases) catalyse the production of phosphatidylinositol 4-phosphate (PtdIns4P), the first step in the generation of higher phosphoinositides. PtdIns4P is an essential precursor in the production of second messengers, Ins(1,4,5)P_3 and diacylglycerol, in a receptor activated phospholipase C signalling pathway. Moreover, PtdIns4P itself regulates conserved compartment-specific biological processes, mainly via recruiting a broad spectra of effector proteins. Because PI4-kinases have a central position in PtdIns4P synthesis on a surface of intracellular membranes, they are implicated in a wide range of PtdIns4P-induced processes such as lipid transport and metabolism, intracellular trafficking processes and cargo sorting, membrane and cytoskeleton remodelling events, signal transduction and many others. In mammals, two types of PI4-kinases were identified: type II and type III. Both types do not bear high sequence similarity to each other and, therefore, they possess diverse biochemical properties. In order to elucidate their structural relationship to other lipid kinases, structural analysis is highly demanded. The structural characterisation of individual PI4-kinases could also clarify the catalytic mechanism of PtdIns4P synthesis. Furthermore, information about the architecture of the active site could provide the basis for isoform-specific inhibitor design that would be potentially important in human medicine since the selective blocking of enzymatic activity would eliminate phagocytic engulfment of several pathogenic bacteria or block replication of plus sense single strand RNA viruses (+RNA). Altogether, the structural characterisation of each of individual isoforms, preferably in complex with a regulatory molecule, was still not achieved. However, such structural information would help to elucidate structural aspects of fundamental biological processes and structural requirements for viral and pathogen replication.

The studies reported in this thesis provide the structural and functional characterisation of both type II PI4-kinases and PI4K IIIβ. The high-resolution crystal structures of individual isoforms revealed their overall fold and domain organisation and confirmed a bi-lobal character of the C-terminally localised catalytic domain in all three variants. Co-crystallization of PI4-kinases with physiological substrates defined the ATP binding pocket but no crystals were obtained in complex with inositol or inositol 1-phosphate. However, the binding cavity for phosphatidylinositol (PtdIns) was identified based on docking and modelling. Interestingly, a new lateral hydrophobic pocked was found as a putative regulatory site in the case of the type II PI4-kinases. The overall fold of these proteins also confirmed the structural diversity between type II and III PI4-kinases. Whilst type III PI4-kinases are rather

more similar to PI3-lipid kinases, type II PI4-kinases share higher similarity with protein kinases. The crystal structures in complex with potent isoform-specific inhibitors were also obtained. The structural details of the active site in complex with isoform-specific inhibitors helped us to fully understand the inhibition mechanism and provided information needed for specific drug design.