ESRF	**Experiment title:** Cambridge MRC Block Allocation Group **The structural basis of specific inhibitors of phosphoinositide 3-kinase**	**Experiment number:** **LS-1525**
Beamline: ID14-2	**Date of experiment:** from: 4 Sep 99 to: 6 Sep 99 from: 13 Nov 99 to: 15 Nov 99	**Date of report:** 17 Feb 2000
Shifts: 3 4	**Local contact(s):** Ed Mitchell Soichi Wakatsuki	*Received at ESRF:*

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Report:

Phosophoinositide 3-kinases (PI3K) act as effector enzymes in many mammalian signal transduction pathways. The PI3Kγ isozyme is regulated by association with heterotrimeric G-protein βγ subunits and Ras. The 2.2 Å X-ray crystallographic structure of PI3Kγ that we have determined from data collected at ESRF ID14-4 shows that it has a modular four domain organisation that is common to all class I PI3Ks. The enzyme consists of a Ras-binding domain (RBD), a C2 domain, a helical domain similar to HEAT-repeat-containing structures, and a catalytic domain related to protein kinases. The structure of the enzyme in a complex with ATP suggested the manner in which the enzyme uses these modules to reversibly interact with its upstream activators and phospholipid membranes.

In the last period, we collected 2.0 Å and 2.4 Å resolution data sets for complexes of porcine PI3Kγ with wortmannin and LY294002, respectively. These compounds can specifically inhibit PI3Ks and have been fundamental to understanding the biology of the

PI3Ks. In order to get a better understanding of the structural features of the active site that may be useful for pharmaceutical design, we also collected 2.0-2.5 Å data for the human PI3Kγ and complexes of it with the broad spectrum protein kinase inhibitor staurosporin. The compounds that we soaked into the crystals are competitive inhibitors of ATP. The structures show that these compounds occupy the ATP binding pocket but make interactions with the enzyme that are only partially analogous to the interactions between the enzyme and ATP. Each of the compounds has an aromatic moiety in the region occupied by adenine in the ATP complex. Wortmannin also interacts with a portion of the ATP-binding pocket that is not occupied by ATP. The wortmannin complex shows that this inhibitor forms a covalent adduct by reacting with the primary amine of Lys 833. The extensive complementarity in shape between wortmannin and the ATP-binding site and the covalent bond account for the high affinity binding of wortmannin. We are currently in the process of comparing the complexes of PI3Kγ with inhibitors in order to formulate principles whereby isozyme-specific PI3K inhibitors can be developed.