ESRF	**Experiment title:** Structural Studies on cyclin-dependent kinase 2 (CDK2) in complex with specific CDK inhibitors	**Experiment number:** LS-647
Beamline: D2/AM	**Date of experiment:** from: 1 l-3-97 to: 12-3-97	**Date of report:** 25-8-97
Shifts: 3	**Local contact(s):Dr.** Jean-Luc Ferrer	*Received at ESRF:* 2 9 AOUT 1997

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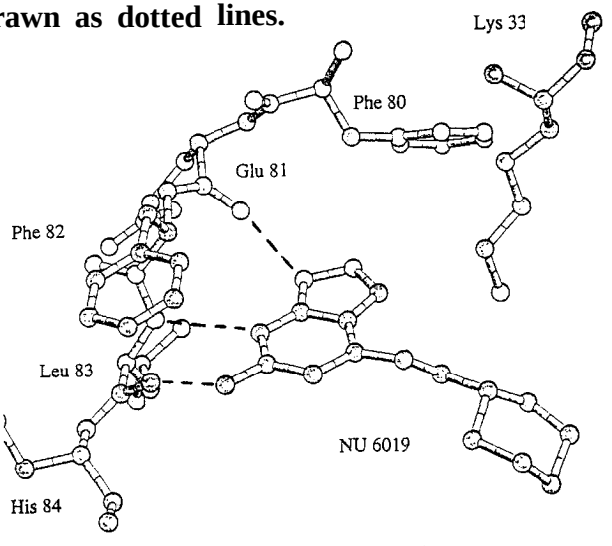
Report: Sequential activation of the CDKs directs progress through the eukaryotic cell cycle (1). Loss of CDK regulation has been genetically linked to the development of human cancers. There is a strong interest in the design of potent and specific CDK inhibitors for use in cancer treatment. Specific CDK inhibitors will be important tools to probe the roles of this kinase family in cell cycle control and apoptosis. A novel series of purine-based CDK inhibitors with IC_{50} values in the low μM range have been identified through the Anticancer Drug Discovery Initiative, (ADDI), at Newcastle University (unpublished results). We have previously determined structures for two of these compounds, 06-cyclohexylmethyl guanine and 06-cyclohex-3-enylmethylguanine bound to CDK2. Knowledge of the binding mode of these two compounds and of the microbial alkaloid staurosporine (a potent but non-specific protein kinase inhibitor) within the CDK2 ATP-binding site (2) has informed another round of compound synthesis. Three compounds were tested in the present beamtime allocation. Following a soak of 28hrs at 5mM compound in mother liquor, compound NU6019 (tetrahydropyranylmethylguanine) was shown to bind to CDK2. Compounds NU6018 (naphthylmethylguanine) and NU 6017

(galactosylguanine) were also tested using similar soak conditions, but difference Fourier maps showed that they had not bound. The CDK2-NU6019 structure is being refined at 2.1 Å resolution. The model currently includes CDK2 residues l-43,46-298, and has RJR, = 27.9/32.9 respectively.

Figure 1: Schematic drawing of compound NU6019 bound to CDK2. Hydrogen bonds are drawn as dotted lines.



NU6019 N9 acts as a H-bond donor to the carbonyl group of E81, the NH group of L83 to N3, and the NH, group attached to NU6019 C2 to the carbonyl group of L83. The binding of the guanine ring is in a different orientation and not co-planar with that of the adenine ring in the ATP structure (3). The tetrahydropyranyl ring occupies a position close to where the ribose ring of ATP binds (3) but does not mimic the H-bonding interactions of the ribose 2' and 3' hydroxyl groups. There is an ordering of the activation loop in its inactive conformation reflected as a marked decrease in the average B factors for residues in this region. Additional interactions occur between amino acid residues in the glycine-rich loop and the activation loop. Further compounds are now being designed for crystallographic studies and to determine using biochemical methods the effects of compound binding on CDK2-cyclin association and activation of kinase activity.

References

1. Morgan, D.O., (1995), Nature 374: 131-134.
2. Lawrie, A.M., et al., (1997), Nature Struct. Biol. 4: (in press)
3. De Bondt, H.L., et al., (1993), Nature 363: 592-602.