

Crystal structure of human Fyn tyrosine kinase complexed with staurosporine

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Introduction

Human Fyn is a member of the Src family of non-receptor type tyrosine kinases that collectively are involved in the cytoplasmic signal transduction cascade for a variety of membrane receptors. Besides the role of Fyn in T-cell development and activation, it is involved in myelination of neurons and in disorders of the central nervous system. Fyn induces synaptic and cognitive impairments in a transgenic mouse model of Alzheimer's disease and is essential for haloperidol-induced catalepsy, which resembles Parkinson's disease, in mice. Therefore, selective inhibition of Fyn kinase may help counteract these disorders without immunosuppressive side effects.

We believe the present study to be the first report of the structure of the Fyn kinase domain complexed with staurosporine.

Manuscript preparation

A truncated version of the Fyn kinase domain (residues 260–537) was inserted into the baculovirus expression vector pFastBac1, incorporating a C-terminal hexahistidine purification tag. The enzyme was overexpressed in the baculovirus expression system using *Sf21* insect cells. Protein purification was performed using His Trap HP column, Superdex 200 column and Mono-Q column (GE Healthcare) using the AKTA prime system.

The Fyn–staurosporine complex solution was prepared by direct suspension of the inhibitor into the protein solution, and incubated at 4°C until the crystallization experiments. Hexagonal-plate crystals of the complex were obtained at 4°C using a reservoir solution of 1.15 M ammonium phosphate dibasic, 0.2 M NaCl and 0.1 M imidazole buffer, pH 8.0. After dipping into Paratone-N oil (Hampton Research), the crystals were frozen using a nitrogen gas stream at 100 K. Diffraction data were collected at a wavelength of 1.0 Å using the synchrotron radiation at Photon factory beamline NW12A and a CCD detector Quantum 210 (ADSC) with an exposure time of 10 s per image at a crystal to detector distance of 250 mm. X-ray diffraction data consisting of the 360 images were processed and scaled using the program HKL2000 (HKL).

The Fyn kinase domain conserved typical kinase folding, which had a structural feature by which the hinge region linked the β -strand-rich N-lobe and the α -helix-rich C-lobe (Figure 1). The C-terminal 20 amino residues of the crystallized protein, Phe524–Leu537 and a following hexahistidine-tag, were omitted from the final model since the electron density discontinued at Phe524 and the corresponding density to these residues was not found within the vacant space in the crystal lattice.

We have reported the structure of the Fyn kinase domain and elucidated the structural basis for staurosporine binding to three protein tyrosine kinases, Fyn, Lck, and Csk. The structural studies may help in the development of new selective Fyn kinase inhibitors [1].

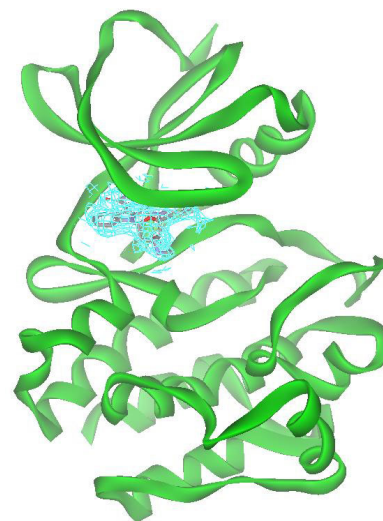


Figure 1 Crystal structure of human Fyn kinase domain

Reference

[1]T. Kinoshita et al., *Biochem. Biophys. Res. Commun.* 346, 840 (2006).

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