BL-5A, AR-NW12A, AR-NE3A/2013G738, 2014G190, 2016G199

Structure-based discovery of inhibitors of human DJ-1, a Parkinson's disease associated protein.

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DJ-1 is a Parkinson's disease associated protein but its exact role in the progression of the disease remains uncertain. We have employed X-ray crystallography to identify the binding mode of low-affinity inhibitors. Based on the structural data, optimized inhibitors displaying high-affinity were obtained.

1 Introduction

Parkinson's disease is a devastating neurodegenerative disorder [1]. The brains in patients with advanced disease are profoundly damaged, and show insufficient levels of the neurotransmitter dopamine. Mutation in the protein DJ-1 produces a condition termed early onset Parkinson's disease [2]. Unfortunately, the molecular mechanism leading to this condition is unknown

Among the reasons hampering the characterization of DJ-1 is the absence of a well-defined inhibitor. The evidence reported so far suggested that an inhibitor binding to the Cys106 may inhibit the biological function of DJ-1. Herein, we have identified and optimized compounds with a well-defined inhibition mechanism against DJ-1. These compounds belong to the isatin family and showed strong inhibitory properties in vitro and in cell. We hope these compounds will contribute to understand the function of DJ-1 and its connection to Parkinsonism.

2 Experimental

Human DJ-1 was expressed in high-levels in *Escherichia coli*, and purified to homogeneity. Crystals of DJ-1 in complex with inhibitors were prepared by the soaking method. Crystals of reduced DJ-1 were obtained by the hanging-drop method by mixing the protein (20 mg mL^{-1}) with a solution containing 100 mM Tris-HCl (pH 8.5), 200 mM sodium citrate, 30% (v/v) PEG-400, and 5 mM DTT. Crystals of DJ-1 were subsequently soaked with compounds 1 ~ 40 mM for 1 ~ 24 hr. The selected crystals were frozen in liquid N_2 until data collection.

Data were collected at BL-5A, AR-NE3A and AR-NW12A of the Photon Factory (Tsukuba, Japan) under cryogenic conditions (100 K). Data were processed with MOSFLM and SCALA. Structures of DJ-1 in complex with various compounds were determined by the method of molecular replacement with PHASER and refined with REFMAC5 and COOT.

3 Results and Discussion

A screening of DJ-1 with a small library of fragment compounds found that the molecule of isatin binds to DJ-1 with significant affinity. This observation was corroborated by other techniques [3]. The crystal structure of DJ-1 in complex with isatin at 1.5 Å resolution revealed a covalent bond between the Cys106 residue and DJ-1 (Fig. 1). This is the first evidence of a small molecule directly bound to the key Cys106 of DJ-1 suggesting that isatin (and isatin-like compounds) may be a promising family of compounds inhibiting the activity of DJ-1.

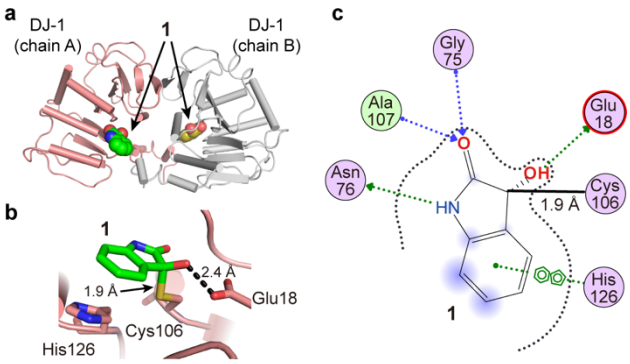

Fig. 1: Structure of isatin (***I***) bound to DJ-1. **(a)** Position of isatin in the dimer. **(b)** Close-up view of the active site. **(c)** Environment of isatin when bound to DJ-1. Adapted from [3].

The analysis of the interactions between isatin and DJ-1 revealed some critical elements necessary for binding, but also regions suitable for optimization. The structural information guided the optimization of isatin to increase the affinity. In particular the addition of a phenyl group and a halogen at specific positions increased the affinity of the compounds for the protein (Fig. 2). These compounds bind in the same conformation as isatin, and also engage in a covalent bond with Cys106.

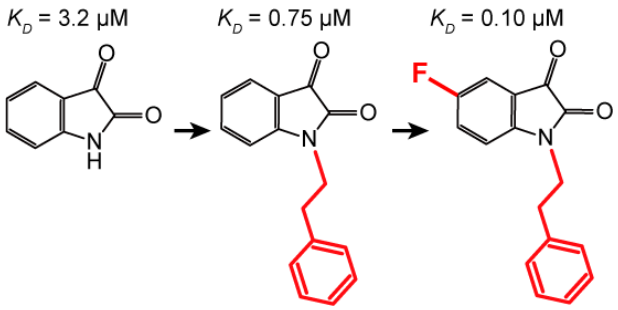

Fig. 2: Optimization of isatins. Affinity expressed as K_D.

Importantly, these compounds do not only bind, but also inhibit the enzymatic activity of DJ-1 (Fig. 3). Because these compounds make a covalent bond with Cys106, it was hypothesized these compounds could behave as inhibitors. Among several biological functions, DJ-1 is an enzyme with glyoxalase and deglycation activity [4]. The enzymatic assays in the presence of several compounds indeed showed robust inhibitory properties, the fluoride/phenyl derivative having the strongest effect (compound 15) (Fig. 3).

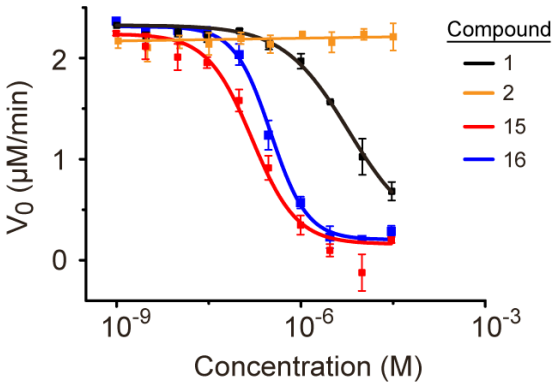

Fig. 3: Enzymatic inhibition by isatins. Compound 2 corresponds to an isatin derivative not binding to DJ-1 because it lacks one of the carbonyl oxygens. Adapted from [3].

In summary, we have determined the crystal structure of isatin bound to human DJ-1. Based on this structure we have increased the affinity of the hit-compound and demonstrated that these compounds also inhibit the enzymatic activity of the protein. We hope these compounds will help to clarify the function of DJ-1 in Parkinsonism and other biological processes.

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