Study of protein ligand interactions by Nuclear Magnetic Resonance

Sylvain PEYRACHE

Introduction

Drug discovery is a particularly long process in the pharmaceutical field; therefore, different strategies aiming to improve the development time and the success of this critical step have been employed. 2 of these strategies, extensive high throughput screenings (HTS), and combinatorial chemical synthesis of compounds, are expensive and time consuming. From a realistic economic context, the fragment-based drug-design is a novel approach that should be considered. This strategy is based on affinity tests with the therapeutic target and simple molecules called fragments. Compared to the conventional HTS, where the screened chemical libraries can contain up to a million compounds, fragment-based libraries are usually limited to a thousand molecules. The concept is rather simple: the fragments having the best affinities are selected and optimised by adding chemical functions or combining fragments together. This analytical technique helps to detect and locate the binding site on a therapeutic macromolecule. Based on this information, the choice of design and modifications, made to increase affinity, can be rationally made.
In this article fragment-based approach was used to design a potent inhibitor of human peroxyredoxin (PRX), an enzyme involved in oxidation/reduction metabolism.

Experimental Conditions

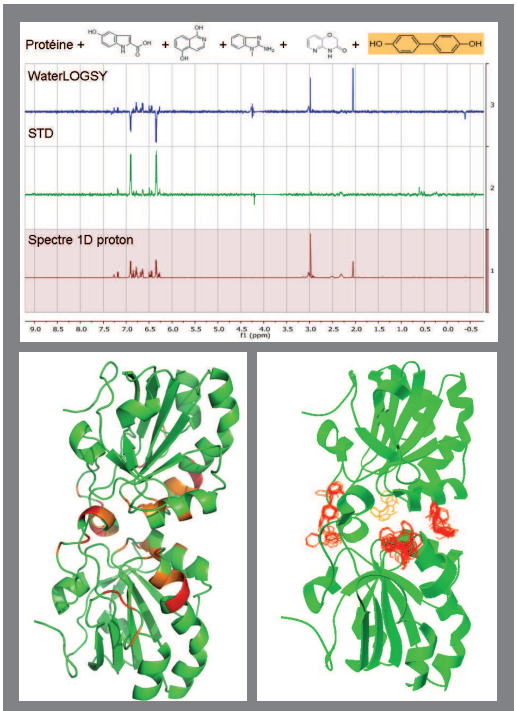
The chemical library was prepared with 183 compounds at 110 mM in deuterated DMSO and screened to ensure that there are no false positive molecules with STD and WaterLOGSY NMR impulsion sequences.
The assays were set up with a mixture of 5 molecules with a final concentration of 0.6mM (100µM of protein in a 50 mM phosphate buffer at pH 7). The samples were then analysed with the same previous STD and WaterLOGSY parameters. The data were then processed using Mestrenova (Mestrelab Research SL). The most positive molecules were analysed at 2 mM with 15N labelled PRX by 2D NMR HSQC 1H-15N, so as to confirm and locate the interacting area of the protein.

Results

From the initial set of 183 molecules tested, only 15 were identified as positive; the 5 molecules, which had the strongest NMR signals, were selected for the HSQC experiment.
Based on these results, interactions were located and modelled using Pymol (Delano Scientific). The possible interaction interface obtained was compared with molecular docking calculations with Autodock4 software.

Discussion

The HSQC experiments highlight a particularly interesting point: the 5 selected compounds seemed to all interact at the active site of the enzyme, and were compatible with calculations performed in Autodock. The binding needed to neutralise the reduced state, which is the active form of PRX.
According to these results the molecules bound tightly the original state and neutralised PRX.



(A) 1D proton, STD et WaterLOGSY spectra of a typical positive sample.
(B) 3D representation of the experimental interacting area (left) and results from docking (right) match the same location.

Conclusion

The fragment-based approach is able to identify potential inhibiting drugs with 3D localisation of molecular interactions. Future works will include: a measure of exact affinity as well as modifications to the molecules, selected to improve their efficiency. Screenings of new compounds are also planned, based on these fragments and their exhibition of polyols similarities and antioxidant properties.

Anabio

Biomolecular NMR Laboratory
UMR 5180
Université Claude Bernard Lyon 1