Ph.D. thesis abstract

Overcoming drug resistance : The discovery, design and characterization of new nonpeptidic inhibitors of HIV-1 protease

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Abstract

HIV-1 protease is an aspartic protease which plays an essential role in the life cycle of HIV virus. It is responsible for the cleavage of the viral polyproteins into the structural and functional proteins during viral maturation. The efficient inhibition of the protease thus leads to the formation of immature and non-infectious viral particles. The introduction of protease inhibitors dramatically changed the treatment of retroviral infection. The viral replication was reduced to undetectable level and the rate of disease progression was significantly lowered. However, resistance to the inhibitors was observed. The first inhibitors had limited bioavailability, caused severe side effects and easily developed resistance. To combat these negative factors, second-generation inhibitors have been developed. Understanding the mechanisms of resistance toward inhibitors is critical for the optimal use of antiretrovirotics in clinical practice. There is still need for new nonpeptidomimetic inhibitors with unique resistance profiles, different modes of action and novel structures.

This thesis gives an overview of the development of different classes of new, unusual protease inhibitors. In addition, it presents data on the resistance development during antiretroviral treatment with a protease inhibitor called nelfinavir. The main part of the thesis is devoted to the design and characterization of metallacarboranes, novel nonpeptidic HIV protease inhibitors that represent attractive pharmacophores with therapeutic potential.

The aims of the thesis

The first aim of the thesis was to prepare, enzymologically, structurally and thermodynamically characterize recombinant HIV-1 proteases bearing mutations responsible for the resistance to nelfinavir. The main purpose of this project was to understand how single mutations contribute to the affinity of protease to nelfinavir and how the additional mutations can modulate their effect. The understanding of the effects of individual mutations on inhibitor binding determined by enzymological assay, X-ray crystallography, and isothermal titration calorimetry is important for the design of next generation HIV protease inhibitors.

During random testing of different inorganic and organic compounds, we identified metallacarboranes as promising inhibitors of HIV-1 protease. The second aim of the thesis was to determine inhibition characteristics, selectivity, specificity, and resistance profile of novel non-peptidic HIV protease inhibitors containing metallacarborane clusters.

Conclusions

In this thesis, I analyzed the mechanisms of resistance toward one first generation inhibitor nelfinavir. In cooperation with the laboratory of Dr. Mammano within the 5th EU framework, I prepared HIV-1 proteases bearing the single mutations D30N, L90M, N88D and A71V and their combinations, which were identified as main contributors in resistance development to nelfinavir. Many scientific groups imputed the affect of D30N mutation on resistance to the loss of hydrogen bonding between asparagine at position 30 and the hydroxyl group of nelfinavir. Using structural and thermodynamical analysis, we found that although this bond is slightly weakened, it is still maintained. The kinetic analysis showed high level of resistance for mutant D30N/N88D that is a rather frequent combination found in patients. The main difference in the binding of nelfinavir to the active site of this protease variant was identified and explained by distinct contact between mutated residues mediated by water molecules. Thermodynamical analysis revealed unfavorable change both in enthalpic and in entropic contribution. Similar analyses were carried out for other mutant proteases as well. Molecular analysis finally allowed to interpret the experimental data and showed that the entropy of the binding play significant role in the development of nelfinavir resistance.

Seven years ago, during random testing of hundreds of inorganic and organic compounds for their possibility to inhibit HV-1 protease, we identified metallacarboranes as potent inhibitors of the enzyme. First, we aimed to characterize them by enzymological essay to determine the binding characteristics. We found some structure activity relationship and identified metallacarboranes as nanomolar non-peptide inhibitors of HIV protease. Further, the structure of parental metallacarborane bound to the wild-type HIV-1 protease was determined which allowed us to identify the binding mode of these inorganic compounds into the active site of the enzyme. This was the first 3-D structure of a carborane-protein complex ever solved.

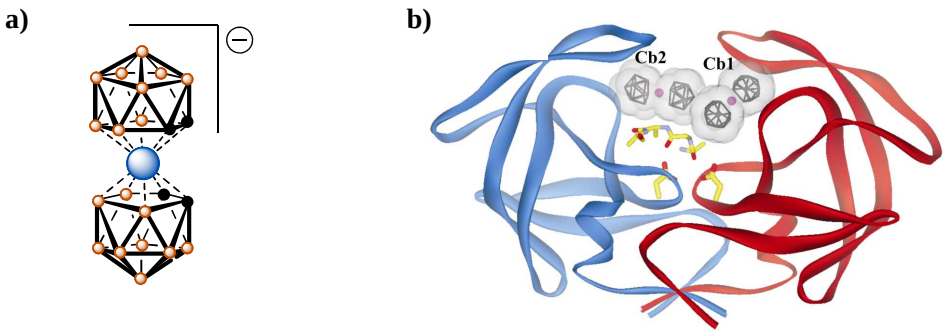

Figure 1. (a) Chemical formula of parental metallacarborane GB-18. BH groups are depicted as orange balls, CH groups as black balls, and cobalt ion as blue ball. (b) The crystal structure of the HIV-1 protease: GB-18 complex. Two molecules of GB-18 are represented by sphere model and called as Cb1 and Cb2. The structure was solved in the laboratory of Dr. Řezáčová from IOCB AS CR.

We knew from the history of the development of other inhibitors how important their specificity towards their target was. We decided to test it and found out that metallacarboranes inhibit also homologous protease from HIV-2 virus and another retroviral protease from endogenous virus MIA14, but less tightly. They weakly inhibit other tested aspartic proteases (pepsin and cathepsin D) and did not inhibit other enzymes analyzed. The important requirement, especially during development of second generation HIV-1 protease inhibitors, is the inhibition effectivity against multi-drug resistant strains. Tested metallacarboranes kept their potency and were effective inhibitors of various resistant proteases even derived from patients with a long treatment history, in which the antiretroviral therapy failed. We invested much effort to the structural analysis of these compounds. After many unsuccessful attempts to get the crystal, Dr. Řezáčová luckily solved the structure of an inhibitor containing two metallacarborane clusters exhibiting low nanomolar inhibition potency. The strategy of the connection of two clusters with amino group containing linker resulted in the formation of the most effective inhibitors. However, the position of the linker was not visible in the map of electron densities due to its high flexibility. Molecular modeling and calculations were used to predict possible linker conformers.

a)

PR1	D30N/N88D
PR2	M46I/A71V/V82T/I84V
PR3	A71V/V82T/I84V
PR4	V32I/I47A
PR5	L10I/I15V/E35D/N37S/R41K/I62V/ L63P/A71V/G73S/L90M
PR6	L10I/L24I/L33F/M46L/I54V/L63P/ A71V/V82A/I84V
PR7	L10F/L19I/K20R/L33F/E35D/M36I/ R41K/F53L/I54V/L63P/H69K/A71V/ T74P/I84V/L89M/L90M/I93L

b)

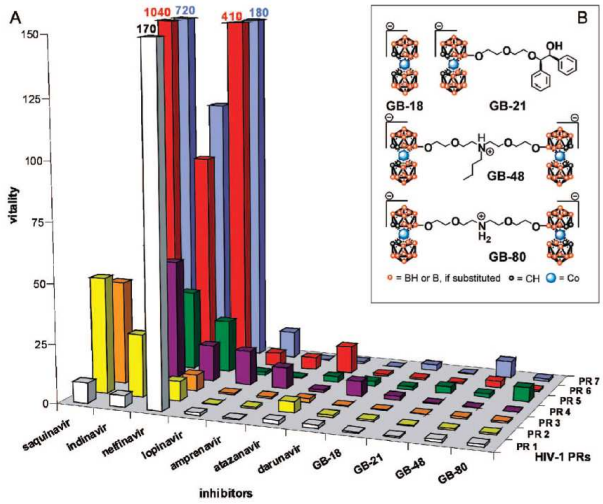

Figure 2. (a) The list of mutations of recombinant HIV-1 proteases used in study. (b) Vitalities of seven clinically used protease inhibitors and metallacarboranes with the panel of protease mutants. Chemical formulas of metallacarboranes are shown.