English Abstract

Shortly after the identification of HIV as a causative agent of AIDS, an aspartic protease was identified in the viral genetic information. The very same time protease has become one of the dominant therapeutical targets in AIDS therapy. The introduction of protease inhibitors into the antiretroviral therapy has led to a significant improvement in the quality and length of life of HIV patients. However, the virus is still able to effectively prevent the impact of an inhibitor via generating inhibitor-resistant mutated protease variants. Thus, there is a constant need for novel types of inhibitors that would be capable of effectively blocking these resistant variants and simultaneously not supporting the development of novel resistant viral strains. One way to identify such inhibitors could be searching for compounds interacting with the enzyme at different sites than the active cavity, via the mechanisms of noncompetitive or uncompetitive inhibition. The group of compounds called metallacarboranes - inorganic compounds consisting of carbon, boron, hydrogen and metall ion - were shown to exhibit such an activity against HIV-1 protease. However, for further optimization of these inhibitors, detailed biophysical investigation of the enzyme-inhibitor complex is needed. This work focuses on the development of tools for structural characterization of the complex between GB-110 - one of the uncompetitive metallacarborane inhibitors - and HIV-1 protease using EPR spectroscopy linked with site-directed spin labeling as a method of choice. Preparation of the expression constructs encoding HIV proteases modified for spin labeling, protein expression, characterization and optimization of site-directed spin labeling are described in this thesis.

(In Czech)