ABSTRACT

Recently, non-cellular viral agents became the focus of a large number of scientific groups. A prominent and widespread group of these viruses are flaviviruses, which include, for example, Zika virus, Dengue fever virus, tick-borne encephalitis virus and West Nile virus. There is a considerable diversity among these viruses, however, highly conserved proteins can be found throughout this viral genus. The largest and most conserved protein encoded by flaviviruses is the nonstructural NS5 protein. Its N-terminal domain bears the methyltransferase (MTase) activity. Thanks to the methylation of its genome, it allows the virus to initiate translation and at the same time mask it from the host's immune system. By blocking the active site of this enzyme with a small molecule, viral infection could be stopped not only in one flavivirus, but, due to the high conservation of MTases, in all other flaviviruses.

This diploma thesis deals with the aforementioned MTase domain of the NS5 protein, specifically of the West Nile virus (WNV). After designing an insert encoding the WNV MTase domain, amplifying it and ligating it into the vector, the MTase domain was prepared by a recombinant expression, followed by purification. Subsequently, complexes of the protein with small molecules (MTase ligands) were formed, in which the temperature stabilities were measured and compared. Furthermore, crystallization of a complex of WNV MTase with its inhibitor sinefungin was performed and subsequent crystallization optimization was conducted. In the future, the protocol presented here could be used to test various small molecules that could inhibit flaviviral MTases, thereby stopping the infection caused by these viruses.