UNIWERSYTET GDAŃSKI

Summary of the doctoral dissertation of Natalia Karska, M.Sc. tilted: „ Synthesis and conformational research of fragments of homologues glycoprotein N fragments of bovine herpesvirus 1 (BHV-1) and varicella zoster virus (VZV)". The dissertation was prepared under the supervision of dr hab. Sylwia, prof. UG and dr hab. Emilia Sikorska (auxiliary supervisor).

The study of my dissertation was focused on the UL49.5 protein of the BHV-1 and the VZV viruses. They both belong to the same taxonomic unit: family *Herpesviridae*, subfamily *Alphaherpesvirinae* and kind *Varicellovirus*. UL49.5 is a transmembrane protein (type I) which crosses the cytoplasmic membrane of the host cell. Structurally we can distinguish three biologically important fragments: domain on the side of the endoplasmic reticulum (N-terminal domain), transmembrane area and the cytoplasmic domain (C-terminal domain). This protein is known to be involved in the immune system by inhibiting the antigen presentation pathway via major histocompatibility (MHC) class I molecules. Consequently, immune response of the infected organism is impaired. However, the detailed mechanism of infection is not known, mainly by the lack of structural-activity study. So far, most of studies were based only on the analysis of biological activity of mutant protein variants without correlation with their spatial structure. The action of the UL49.5 protein is most likely due to "freezing" TAP transporter in a particular conformation, but the model needs further investigation. Due to the structural motifs/mutations of UL49.5 fragments may determine whether a protein will act as TAP transporter inhibitor or bind its molecular target and devoid of biological activity [4]. However, the UL49.5 bovine herpesvirus type 1 protein mostly acts as inhibitor of TAP transportet, but surprisingly not in the case of varicella zoster virus [1, 2]. That is why the structural comparison study of UL49.5 from both viruses can expalin why homologous BHV-1 and VZV proteins differ in their biological activity. So far, the genetic engineering techniques failed to obtain the native form of UL49.5 protein. Probably to the fact that UL49.5 is a membrane protein and undergoes strong aggregation *in vitro* and is stable only in the presence of components of the peptide loading complex [3].

The goal of my dissertation was to determine the properties of conformation of UL49.5 protein bovine herpesvirus type 1 and mutants thereof and protein UL49.5 virus varicella zoster. Specific objectives of the work were:

1. The design fragments of UL9.5 BHV-1 protein virus and VZV for synthesis.

2. Design mutants of C-terminal fragment and N- terminal fragment of protein UL49.5 of BHV-1 virus for synthesis.
3. Chemical synthesis of peptides both proteins and their analogs and their purification.
4. Preliminary conformational research of peptides synthesized using techniques CD.
5. Determination of the spatial structure of fragments of the native UL49.5 protein of BHV-1 virus and its mutants in SDS micelles and DPC by using NMR techniques.
6. Determination of spatial structure of fragments of protein UL49.5 of VZV virus in DPC micelles by using NMR techniques.
7. Determination of the spatial structure of the native UL49.5 protein of BHV-1 virus in the membrane POPC.
8. Determination of spatial structure of the mutants (the N-terminal) of UL49.5 protein of BHV-1 virus in the membrane POPC.
9. Understanding the dependence between modification the spatial structure and biological activity of UL49.5 protein of BHV-1 virus.
10. Comparison of the theoretical structure of UL49.5 protein BHV-1 virus with the experimental structure the same protein.

In the experimental part I designed and received fragments of UL49.5 protein. All the analogs of fragments of protein UL49.5 were synthesized using the standard solid-phase synthesis technique. Peptides were synthesized using Fmoc-chemistry. Synthetic peptides were purified using reverse-phase high performance liquid chromatography (RP-HPLC). The purity of all peptides was analyzed using reverse-phase RP-HPLC chromatography. Identifity of peptides were confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and electrospray ionization mass spectrometry (ESI-MS). CD studies were carried out at micellar and nonmicellar concentrations of DPC, where the NMR experiments were performed only at micellar concentrations. The MD stimulations were performed in an explicit water/DPC or water/SDS as micelle environment. The NMR data were used as a set of restraints for a simulated annealing protocol and during the entire NTp MD simulations, that generated three-dimensional structures of the peptide in DPC or SDS micelles. The three-dimensional model of UL49.5 was built using the obtained NMR coordinates and molecular simulation was carried out in POPC membrane using Amber 14 (Case et al., 2015).

Results of conformational studies carried out under this work have shown that the UL49.5 protein of BHV-1 virus has a structure of α-helices in a fragment of the *N*-terminal (extracellular) and the transmembrane and C-terminal (intracellular) fragment form a structure disorder. Introduced mutations of motif RRE(9-11) in the N-terminal protein fragment had to abolish the α-helix structure, with a consequent reduction the activity of the protein in compared to the wild type. In the case of a

mutant RRE(9-11)GGG structure of α-helix was aborted. Mutations of RRE(9-11)AAA and RRE(9-11)RRG have stabilized α-helix structure. Studies of the biological activity of the above mutants changing slightly the biological activity of the protein. The hypothesis of reducing activity of the protein as a result of changes the spatial structure of motif RRE (9-11) has not confirmed. The second study by my was motif PPQ(31-33) in the N-terminal fragment of the protein. They were introduced mutations in the protein: PPQ(31-33)GGG, PPQ(31-33)AAA, PPQ(31-33)GGQ and PPQ(31-33)AAQ. Conformational studies of mutant PPQ (31-33)GGQ showed that the structure of α-helices in the N-terminal fragment is aborted and mobility of the N-terminal fragment of the protein increased. Spatial structure of the mutant PPQ(31-33)AAA is stabilized - helix is lengthened. The activity of the mutant PPQ (31-33) GGG protein is significantly impaired. In the case of mutants PPQ(31-33)AAA and PPQ (31-33)AAQ activity of the protein reduced slightly.

The results of this work lead to enhance knowledge of the structure and molecular basis of the mechanism for inhibiting the immune response by herpesviruses. In addition, they can help develop new tools to fight the virus.