

This thesis deals with production and properties of disulfide-stabilized single-chain variable fragments of the 5D3 antibody (dsscFv), which specifically recognizes and binds to glutamate carboxypeptidase II (GCPII), an antigen closely related to the prostate carcinoma processes and other tumor diseases. Small antibody fragments are in current focus of development of diagnostic and therapeutic reagents. However, compromised stability of antibody derivatives often results in low production yield or loss of function. Introduction of structural changes by protein engineering is often used to solve the issue.

The aim of the study was based on enhancement of protein stability by the introduction of interdomain disulfide bond into the structure of single-chain variable fragment. The effect of modification was evaluated by estimation of production yield and affinity of studied protein.

The aforementioned antibody derivative was produced using an *Escherichia coli* expression system, using specific signal sequences leading the production to the bacterial periplasm. The attempted stabilization was carried out by introducing mutations at L_v-G44 and H_v-G100 positions, replacing glycines with cysteines. The binding affinity of the derivative for human GCPII was determined using ELISA. This thesis also shows a solved 3D structure of the single-chain variable fragment protein, the parent molecule of dsscFv.

The primary result of the experimental part of this thesis was the discovery that the chosen strategy wasn't a viable method of stabilizing the fragment. Alternative means to potential stabilization are discussed, however. Also measured were dissociation constants of the 5D3 antibody and its two fragments. The obtained value for the intact antibody was in agreement with available literature, values for the single-chain variable fragments differed. This was most likely caused by lower purities of assayed proteins.

(in Czech)