The 14-3-3 proteins are a family of important regulatory proteins, found in all eukaryotes, which are involved in many cellular processes. In this diploma thesis, we studied structure/function relationships of 14-3-3 proteins, in this case it was the influence of the structure of H8-H9 loop on the binding affinity in barley isoform hv 14-3-3A and human isoform 14-3-3ζ. According to former results, hv 14-3-3A binds to a ligand with lowest affinity, which could be caused by present of a glycin in H8-H9 loop, while in other isoforms there is a serin on the same position. We measured the binding affinity in protein hv 14-3-3A WT and its mutant, which contained the serin instead of the glycin in H8-H9 loop. For comparison, we also measured the binding affinity of human isoform 14-3-3ζ containing the serin in H8-H9 loop and its mutant, where the the serin was replaced by the glycin. Proteins were expressed in *E. coli* cells strain BL21(DE3) and then purified. The dissociation constant for the binding of peptide pRaf-259 labeled with fluorophores FITC and ATTO was measured using both the fluorescence correlated spectroscopy and the steady-state fluorescence intensity. Our results showed that in both isoforms the mutation of H8-H9 loop causes decrease in the binding affinity.