

Abstract

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Title of diploma thesis: Introduction of the MST method for the study of interactions between biomolecules

MicroScale thermophoresis (MST) is currently a recognized method for studying interactions between biomolecules; the method is using directed movement of biomolecules in a temperature gradient, which allows to analyze practically all interactions where changes in analyte properties are possible. This thesis was focused on introduction of the MST method for research of protein interactions with low molecular weight substances focusing on verifying the possibility of using MST in monitoring the interaction of glutathione S-transferase (GST) with selected terpenes ((-)-fenchone, (-)-isopulegone, (+)-isomenthone, (-)-carvone, (+)-carvone, piperitone, and thujone). Another aim was to determinate the properties of terpenes by classical spectrofluorimetric methods and MST. Using spectrofluorimetric methods it was found that terpenes affect the fluorescence of human serum albumin, and they do not affect the fluorescence spectrum of the dye NHS, which is used as a dye for samples for MST. Next part of experimental work was to determine activity of GST, and to find out if monoterpenes affect the GST activity. GST shows lower activity after the addition of terpenes. The MST method determined that all used monoterpenes affected the fluorescence spectrum of GST. Using MST method was verified the binding of terpenes with GST, and then the complexes were subjected to affinity studies and dissociation constants were determined. Obtained values of dissociation constants differed from pM in (-)-carvone ($K_d=785$ pM) to μ M in piperitone ($K_d=4,1$ μ M). The results show that the use of the MST method is suitable for future GST inhibition studies.