ABSTRACT

Fluorescent labeling is a method used for visualization of various types of biomolecules including proteins and protein complexes. However, the effect of protein labeling on protein structure and functions has not been investigated so far.

The goal of the diploma thesis was to examine an influence of NHS-fluorescein binding on structure and function of human carbonic anhydrase I (hCA-I). The particular aims of this work were to prepare recombinant ^{15}N-hCA-I which was used for NMR structure analysis of carbonic anhydrase upon fluorescent labeling. Furthermore, enzyme activity was measured in order to find out a correlation between the concentration of NHS-fluorescein and protein function. In addition, the reaction mixtures were systematically analyzed by ESI FT-ICR mass spectrometry. The analysis revealed experimental conditions for fluorescent labeling of human carbonic anhydrase I with minimal effect on protein structure and function.

The results of this study show that the calculation of molar excess of NHS-fluorescein cannot rely on a simple procedure provided by manufacturer. However, due to decrease of enzyme activity upon fluorescent labeling, it is better to take into count the influence of NHS-fluorescein concentration on the relative enzymatic activity. Moreover, the calculation of molar excess of fluorescent reagent is not in accordance with the degree of labeling at all. In order to establish the general conditions for fluorescent labeling, it is necessary to perform further analysis of other proteins using other fluorescent labels in future research.

Keywords: carbonate dehydratase (carbonic anhydrase), NHS-fluorescein, NMR, ESI FT-ICR MS, the relative enzyme activity, fluorescent labeling