ABSTRACT

Glycosidases belong to a large group of enzymes, which catalyze hydrolysis of the glycosidic bond causing breakdown of different glycosylated molecules. The proper action of glycosidases is vital for living organisms functioning and for the numerous applications in various fields of science and industry.

The aim of this project was to design, prepare and investigate fluorescent indicators of β-glycosidases' activity, evaluate the mechanism of action of these indicators to find sensitive, selective, reproductive and applicative method for monitoring of these enzymes' activity. This aim was assessed by the investigations of interaction of β-glycosidases with novel indicators consisting of a chiral glycoside-part, which due to its unique structure and suitability to the enzyme active site enabled high selectivity of the designed method. Described probes use the excited-state intramolecular proton-transfer (ESIPT) phenomenon, which due to the abnormally high Stokes shift enables insensitivity of fluorescence to some quenching effects, including reabsorption. Due to the latter features, use of the ESIPT fluorophores allows to avoid some problems connected with the concentration restrictions and scattering of light in solutions of biological samples with increased turbidity.

2-Substituted derivatives of 3-hydroxychromen-4-one, including 4'-substituted derivatives of flavonol were chosen as the ESIPT fluorophores. Their glycosylation was carried out using respective bromide (less often chloride) in a two-phase system CH_2Cl_2/H_2O in the presence of K_2CO_3 and TBAB as a phase-transfer catalyst. Next, the obtained glycosides were *O*-deacetylated.

To optimize the conditions of determination of β-glycosidases' activity, absorption and fluorescence spectra of aglicones were investigated. At this stage as well as at the stage of enzymatic hydrolysis of glicosides various methods of the fluorescence enhancement were applied: extraction to aprotic solvent, complexation with albumines, and metal enhanced fluorescence (MEF) phenomenon. The key idea realized in this work was introduction of the synthesized glycosides to interaction with glycosidase and carring out enzymatic hydrolysis, which releases corresponding fluorophore. The wide spectrum of fluorophores used enabled the comparative studies on the kinetics of their enzymatic hydrolysis at various pH, including the physiological one. Influence of a flavonol 4'-substituent on the rate and mechanism of an enzymatic hydrolysis was also explored. Action of the selected probes was verified for various glycosidases: β-D-glucosidase, β-D-glucuronidase and *N*-acetyl-β-D-glucosaminidase.