ABSTRACT

β-N-acetylhexosaminidases are ubiquitous in all living organisms, but there is little information about these enzymes in plants, especially in leaves. Suggested functions of plant β-N-acetylhexosaminidases are participation in defence responses against fungal and other pathogens and also in degradation of storage glycoproteins. β-N-acetylhexosaminidase from tobacco leaves (*Nicotiana tabacum* L.) was purified to final specific activity 190 nmol min^{-1} mg^{-1} with p-NP-GlcNAc as substrate. Precipitation by ammonium sulphate, gel filtration chromatography on Sephacryl S-300 column and affinity chromatography on Con A-Sepharose column were used. Michaelis constant and maximal reaction rate of β-N-acetylhexosaminidases determined for p-NP-GlcNAc was 0.33 mM and 414 nmol min^{-1} mg^{-1}, respectively. The cleavage of diacetylchitobiose catalyzed by β-N-acetylhexosaminidase was studied using capillary electrophoresis. Determined activity of β-N-acetylhexosaminidase for diacetylchitobiose was more than 10-times lower compared to β-N-acetylhexosaminidase activity for p-NP-GlcNAc. These results indicate that chitobiose and probably other chitooligomers are not natural substrates of β-N-acetylhexosaminidase and thus this enzyme is most likely not involved in protection against fungal pathogens. Natural substrate would rather be saccharides chains of glycoproteins.

(In Czech)