ABSTRACT

β-N-acetylhexosaminidase from tobacco leaves (*Nicotiana tabacum* L.) was partially purified to final specific activity 1,72 μmol · min^{-1} · mg^{-1} using p-nitrofenyl-β-N-acetyl-D-glucosaminide as substrate. The enzyme exhibited one band after both isoelectric focusing and native electrophoresis. Molecular mass of native enzyme was determined by gel chromatography (M_R 275000) and native electrophoresis (M_R 285000). Isoelectric point pI 5.4 was determined by isoelectric focusing. Activity of β-N-acetylhexosaminidase was measured using substrates p-nitrofenyl-β-N-acetyl-D-galactosaminide, p-nitrofenyl-β-N-acetyl-D-glucosaminide, N,N'-diacetylchitobiose, p-nitrofenyl-N,N'-diacetylchitobioside and N,N',N''-triacetylchitotriose. For substrates N,N'-diacetylchitobiose, p-nitrofenyl-N,N'-diacetylchitobioside and N,N',N''-triacetylchitotriose an enzyme assay of β-N-acetylhexosaminidase using capillary zone electrophoresis was developed. Optimal pH and temperature of β-N-acetylhexosaminidase were determined with individual substrates, as well as products of hydrolysis. Activity of β-N-acetylhexosaminidase was highest using p-nitrofenyl-β-N-acetyl-D-glucosaminide as substrate and lowest using N,N',N''-triacetylchitotriose (35% in relative comparison). Maximum velocity and Michaelis constant of β-N-acetylhexosaminidase were determined with substrates N-acetyl-D-galactosaminide, p-nitrofenyl-β-N-acetyl-D-glucosaminide and N,N'-diacetylchitobiose. Substrate inhibition of β-N-acetylhexosaminidase by p-nitrofenyl-β-N-acetyl-D-glucosaminide was observed, for the first time in plant β-N-acetylhexosaminidase studies. The inhibition effects of D-galactosamine, D-glucosamine, N-acetyl-D-galactosamine and N-acetyl-D-glucosamine on the activity of β-N-acetylhexosaminidase were determined.

(In Czech)

Keywords:

β-N-acetylhexosaminidase, capillary zone electrophoresis, substrate inhibition, N,N'-diacetylchitobiose, p-NP-N,N'-diacetylchitobioside, N,N',N''-triacetylchitotriose