Abstract

In plants the shikimate pathway followed by the phenylpropanoid pathway leads to the formation of not only aromatic amino acids but also a plenty of secondary metabolites. The crucial enzyme shikimate dehydrogenase is there a part of bi-functional protein 3-dehydroquinate dehydratase/shikimate dehydrogenase (DHD/SDH; EC 4.2.1.10 and EC 1.1.1.25). Whereas the regulation of the shikimic pathway is complex, little is known about the feedback regulation of plant SDH. The aim of this project was to find plant source with high SDH activity and to find out, if and in which way this SDH is regulated by compounds of phenylpropanoid metabolism, specifically by simple polyphenols.

SDH from parsley root (*Petroselinum crispum*) has been prepared by 3-step purification to final specific activity 470 $\mu mol.min^{-1}.mg^{-1}$. The enzyme exhibited one band after both isoelectric focusing and red native electrophoresis using detection of the activity. Relative molecular mass of native enzyme was determined by gel chromatography (M_r 60 000) and red native electrophoresis (M_r 63 000). Isoelectric point pI 4.5 was established by isoelectric focusing. Optimal pH for the reaction catalyzed by SDH was determined in rage pH 9.5 – 10.0.

The kinetic parameters (K_m, V_{max}) of SDH for substrate shikimate, and coenzyme $NADP^+$ were determined by the initial velocity studies. K_m SDH for shikimate was 161 ± 9 μM and for $NADP^+$ 54 ± 11 μM. The mechanism of two-substrate reaction catalyzed by SDH was determined as sequential. A number of compounds of secondary metabolism affected parsley SDH, from which *p*-coumaric acid and *t*-ferulic acid were subjected to detailed inhibition study. A mixed-type of inhibition and respective inhibition constants were determined for both inhibitors. A significant decrease of SDH activity was observed in the presence of $ZnSO_4$ a $CuSO_4$.

Key words

shikimate dehydrogenase, phenylpropanoids, coumaric acid, ferulic acid, inhibition, shikimate pathway