1-Amino-3-phenylpropylphosphonic acid, the inhibitor of L-phenylalanine ammonia-lyase activity of higher plants*

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Keywords: L-phenylalanine ammonia-lyase, 1-amino-3-phenylpropylphosphonic acid, inhibitors, plants

L-Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) is a key enzyme of phenylpropanoid metabolism in higher plants. PAL catalyzes the reaction of elimination of ammonia and the pro-35 hydroxyapatite of L-phenylalanine to form trans-cinnamic acid [1].

Specific inhibitors of the enzyme activity: L-2-aminoxy-3-phenylpropionic acid (AOPP) [2], L-1-amino-2-phenylethylphosphonic acid (PheP) [3, 4] and 2-aminoindan-2-phosphonic acid (AIP) [5] have been used for elucidation of the intimate details of the mechanism of enzyme action and the structure of the active sites.

These compounds have been proved to inhibit PAL activity competitively in vitro [3, 5–7] but in vivo caused an increase ("superinduction") in extractable PAL activity in gherkin [8] and buckwheat [7] hypocotyls, carrot cell suspension cultures [9] and Spiraled oligorrhiza (Kurz.) Hegelm. [4].

PheP caused inhibition of anthocyanin synthesis in buckwheat hypocotyls [7] and S. oligorrhiza [4] and decreased the content of total phenols and chlorogenic acid [4]. At the same time the level of free phenylalanine was specifically increased in the presence of PheP [7, 10, 11].

The aim of this study was to analyze the activity of another structural analogue of phenylalanine, 1-amino-3-phenylpropylphosphonic acid (PhPP), to investigate the effect of elongation of alkyl chain by one -CH2- group.

The plant material consisted of the sliced potato tubers [3] and the 1-cm segments of buckwheat hypocotyls, dissected from the 4–6-day old etiolated seedlings [7]. Purification of PAL from potato tubers and analytical techniques have been described by Janas et al. [3].

Km of PAL purified from potato tubers was 0.23 mM.

The substrate saturation curves of partially purified PAL [3] were evaluated according to Dixon-Webb's double reciprocal plot (Fig. 1): four concentrations of D,L-PhPP were tested for the inhibitory effect on the enzyme activity in vitro. The data demonstrate a competitive inhibition of PAL by D,L-PhPP with KI 3.0 μM, i.e. the inhibitory effectiveness of PhPP is a half of that of PheP.

In further experiments the effect of D,L-PhPP (0.1 mM) on PAL activity in buckwheat hypocotyls was studied in vivo. This activity was enhanced by D,L-PhPP, the effect depended on the age of the seedlings. The highest PAL activity was found in the 4-day old plants (Fig. 2). The effect of PheP was higher by about 20% than that of PhPP.

PhPP reduced the level of both total phenols and chlorogenic acid in the 4-day old buckwheat hypocotyls (Fig. 3). The content of anthocyanins was also decreased proportionally to the concentration of D,L-PhPP (Fig. 3). The le-

*This research was supported in part by the Polish Ministry of Education (MEN), grant PB 0173/TZ/92/03/92.
Fig. 1. Dixon-Webb plot for determining the nature of inhibition of D.L-PhPP for PAL from sliced potato tuber [31].

Fig. 2. The effect of D.L-PhPP (0.1 mM) on PAL activity in vivo in excised buckwheat hypocotyls. Control (empty bars), PhPP (hatched bars).
Experiments were performed in three repetition. Data in figure show results of one representative experiment.
levels of these compounds were decreased about 20% more as compared to PhnP.

In the presence of D,L-PhPP (1 mM) the content of free phenylalanine increased about 13-fold (Fig. 4) while the levels of the other amino acids were within the range of control levels. The level of free phenylalanine in buckwheat hypocotyls increased about 40-fold in the presence of PhnP [7].

**Fig. 3.** The content of total phenols, chlorogenic acid and anthocyanin after treatment buckwheat hypocotyls (4-day old) with different concentrations of D,L-PhPP. Standard deviations of three independent experiments were shown.

In conclusion it can be stated that insertion of -CH₂-group results in approx. 2-fold reduction of effectiveness of PhPP as the inhibitor of PAL.

**REFERENCES**


**Fig. 4.** Content of soluble phenylalanine in 4-day old excised buckwheat hypocotyl transferred on 24 h on water (0) or D,L-PhPP (1 mM) and incubated 24 h in light.

Values represent the mean of three independent experiments.