Acta Biochimica Polonica

Vol. 41 No. 2/1994

QUARTERLY

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

Krystyna M. Janas and Dorota Olechnowicz[a]

Department of Plant Growth Substances, University of Łódź, S. Banacha 12/16, 90–237 Łódź, Poland and [a]Department of Agrobiochemistry, Pedagogical College Opole, Wandy 4, 45–709 Opole, Poland

Key words: 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*-3S hydrogen from L-phenylalanine to form *trans*-cinnamic acid [1].

Specific inhibitors of the enzyme activity: L-2-aminooxy-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 *Spirodela 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 -CH_2- 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 tuber tissues and analytical techniques have been described by Janas *et al.* [3]. K_m 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 K_i 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), grand PB 0173/PZ/92/03/92.

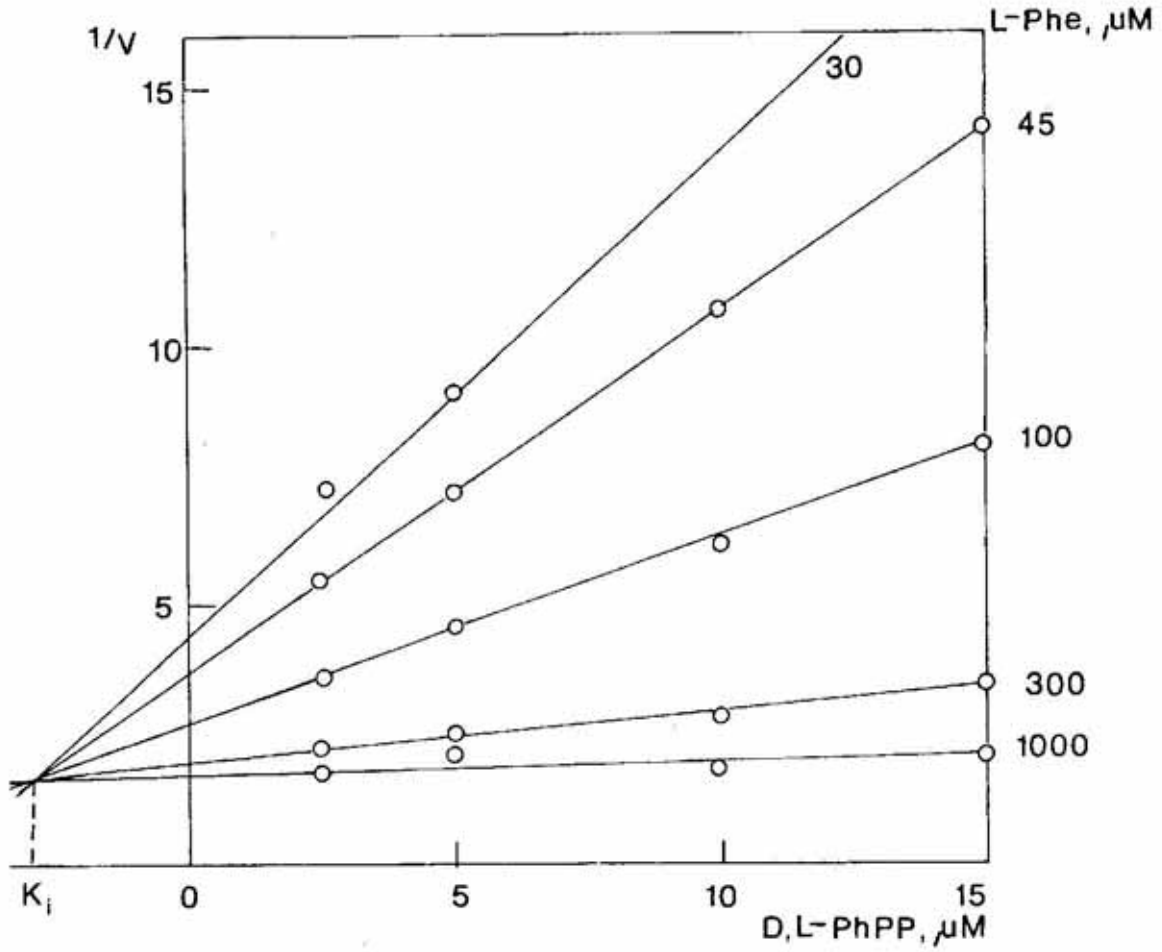

Fig. 1. Dixon-Webb plot for determining the nature of inhibition of D,L-*PhPP for PAL from sliced potato tuber [3].*

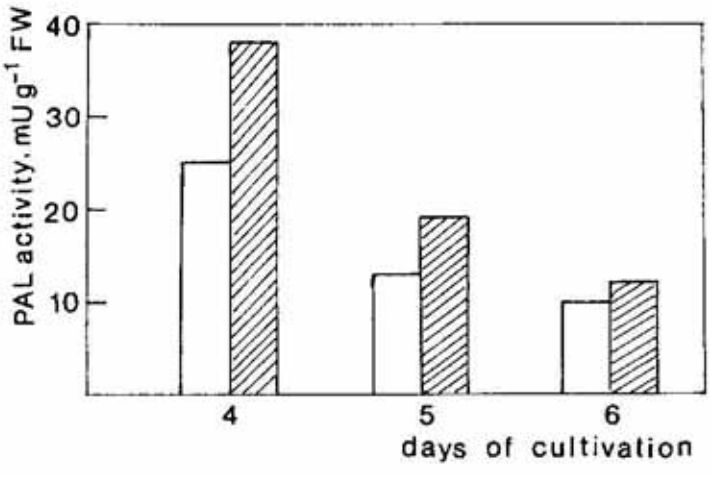

Fig. 2. The effect of D,L-*PhPP (0.1 mM) on PAL activity in vivo in excised buckwheat hypocotyls. Control (empty bars), PhPP (hatched bars).*

Experiments were performed in three repetition. Data in figure show results of one representative experiment.

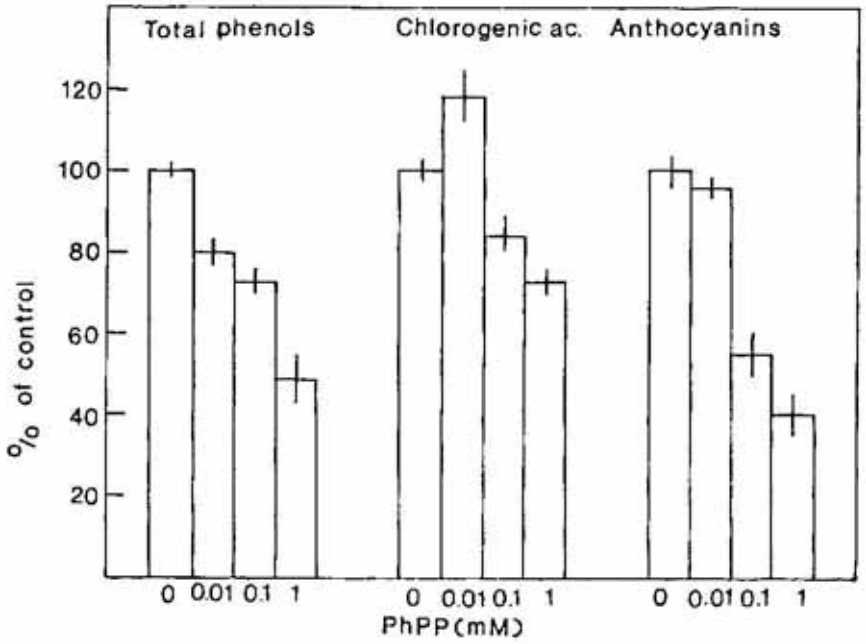

Fig. 3. The content of total phenols, chlorogenic acid and anthocyanin after treatment buckwheat hypocotyls (4-day old) with different concentrations of D,L-PhPP.
Standard deviations of three independent experiments were shown.

vels of these compounds were decreased about 20% more as compared to PheP.

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 PheP [7].

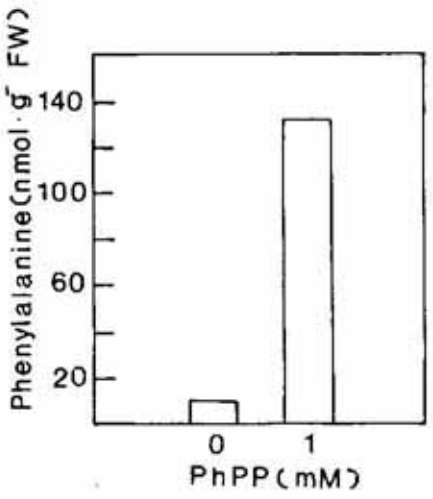

Fig. 4. Content of soluble phenylalanine in 4-day old excised buckwheat hypocotyl transferred on 24 h on water (0) or D,L-PhPP (1 mM) and incubated 24 h in light.
Values represent the mean of three independent experiments.

In conclusion it can be stated that insertion of $-CH_2-$ group results in approx. 2-fold reduction of effectiveness of PhPP as the inhibitor of PAL.

REFERENCES

1. Hanson, K.R. & Havir, E.A. (1981) *in The Biochemistry of Plants. A Comprehensive Treatise* (Stumpf, P.K. & Conn, E.E., eds.) vol. 7, pp. 577–625, Academic Press, New York.
2. Amrhein, N. & Gödecke, K.H. (1977) *Plant Sci. Lett.* **8**, 313–317.
3. Janas, K.M., Filipiak, A., Kowalik, J., Mastalerz, P. & Knypl, J.S. (1985) *Acta Biochim. Polon.* **32**, 131–143.
4. Janas, K.M., Knypl, J.S. & Wolska, M. (1986) *Physiol. Plant.* **66**, 543–549.
5. Zoń, J., Amrhein, N. (1992) *Justus Liebigs Ann. Chem.* 625–628.
6. Noé, W. & Seitz, U. (1983) Z. *Naturforsch.* **38c**, 408–412.
7. Laber, B., Kiltz, H.H. & Amrhein, N. (1986) Z. *Naturforsch.* **41c**, 49–55.
8. Amrhein, N. & Gerhard, J. (1979) *Biochim. Biophys. Acta* **583**, 434–442.
9. Noé, W., Langebartels, Ch. & Seitz, H.U. (1980) *Planta* **149**, 283–287.
10. Knypl, J.S. & Janas, K.M. (1990) *Acta Physiol. Plant.* **12**, 127–130.
11. Knypl, J.S. & Janas, K.M. (1990) *J. Plant Physiol.* **136**, 750–753.