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THE EFFECT OF AMINO ACID ENANTIOMERS ON ACTIVITY OF SELECTED ENZYMES IN SOIL

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Abstract

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This work was aimed to test the effect of selected amino acid enantiomers on activity of casein-protease and acid phosphomonoesterase in soil. Casein-protease was selected due to its key role in nitrogen mineralization and acid phosphomonoesterase due to its importance in soil organic P mineralization. The results showed that 5 mg of L- and D-glutamic acid added to fresh soil from Ah horizon of a moderately mown mountain meadow significantly ($P < 0.05$) decreased casein-protease activity, whereas alanine enantiomers slightly increased activity of this enzyme. Testing the effect of cystine on activity of acid phosphomonoesterase in soil showed slight increase of this activity after application of 3.2 mg L- or D-cystine to fresh soil (equivalent to 8 mg to dry soil).

glutamic acid, alanine, cystine, enantiomers, soil, protease, phosphomonoesterase

D-amino acids occur in soil either free or incorporated in organic matter (Brückner and Wasthauser, 2003; Amelung *et al*., 2006). The utilization of D-amino acids by soil microorganisms (when artificially supplied to soil) was studied and published in different works; generally, D-enantiomers of amino acids are less mineralized compared to their L-counterparts (Martens and Frankenberger, 1993; Hopkins and Ferguson, 1994; Hopkins *et al*., 1994, 1997; O'Dowd *et al*., 1997, 1999; O'Dowd and Hopkins, 1998; Landi *et al*., 2000). Several works have been published on the subject of D-amino acids occurrence in soil hydrolysates (Amelung and Zhang, 2001; Glaser and Amelung, 2002; Amelung and Brodowski, 2002; Amelung *et al*., 2006). Knowledge on occurrence of L-enantiomers of amino acids and their metabolism in soil as well as on the use of D-amino acids for purposes of dating (soil organic matter, archeological finds) or determination of origin of soil organic matter is summarized in work of Rejšek *et al*. (2010).

Occurrence of amino acids in soil is related to activity of proteases. Casein-protease assay has been widely used to determine proteolytic activity of soils. Proteases (EC 3.4.4) play an important role in mineralization of soil nitrogen. They are involved in cleavage of proteins to polypeptides, and oligopeptides to amino acids. The main sources of proteases in soil are microorganisms; proteolytic activity of soil is a key step of nitrogen mineralization (Rejšek *et al*., 2008). D-amino acids naturally occurring in bacterial cell walls protect bacterial peptidoglycan from proteases and have been used as indicator of bacterial origin of soil organic matter (Voet and Voet, 1995). Therefore, we have attempted to determine the effect of enantiomers of glutamic acid and alanine of which D-enantiomers occur in bacterial cell walls, on activity of soil casein protease. Knowledge on the regulation of proteolysis in soil is valuable to better understand nitrogen cycling in ecosystems.

Acid phosphomonoesterases (E.C. 3.1.3.2) catalyze hydrolysis of a variety of organic phosphomonoesters and are therefore important in soil organic P mineralization and plant nutrition. Assay of this enzymatic activity is based on release of *p*-nitrophenol after cleavage of a synthetic substrate *p*-nitrophenyl phosphate. Phosphomonoesterase activity indicates changes in the quantity and quality of phosphorylated substrates in soil and is a good indicator of its biological state as well as of the presence of pollutants (Trasar-Cepeda *et al*., 2000; Car-

reira *et al*., 2000; Nowak *et al*., 2006). As there is no information in the literature related to the effect of amino acids on acid phosphomonoesterase activity in soil, or on how is acid phosphomonoesterase activity in soil related to metabolism of amino acids, we have attempted to test if enantiomers of cystine may affect soil acid phosphomonoesterase. Cystine was selected due to relatively high abundance of its L-enantiomer in soil of mountain meadows and forests (Formánek *et al*., 2008b).

MATERIAL AND METHODS

The soil was taken from Ah horizon of a moderately mown meadow at the experimental site "Bílý Kříž" located in the Moravian-Silesian Beskids Mountains in the northeast part of the Czech Republic (N 49°30'17", E 18°32'28"), on a slope with the altitude of 825–860 m. a. s. l. and southeast orientation. The local subcontinental climate in this region is characterized by mean annual air temperature of 4.9 °C, mean relative air humidity of 80% and by mean annual precipitation of 1100 mm. The number of days with snow cover is 160 per year. The moderately mown mountain meadow plant community belongs to the *Nardo-Callunetea* class. Soil type is classified as a Gleyic Luvisol (ISSS-ISRIC-FAO 1998). Soil sampling was performed in October 13, 2009, and after sieving through 5 mm sieve the samples were stored for more than 5 months at 5 °C. Activity of casein-protease was determined according to Rejšek *et al*. (2008) when 5 mg of L- and D- enantiomers of alanine or glutamic acid per g fresh weight were added into the reaction mixture. Glutamic acid and alanine were selected due to occurrence of their D-enantiomers in bacterial cell walls. Casein-protease activity was measured by incubation of 1 g wet soil with 0.05 M Tris–HCl buffer (2 ml, pH 8.52) containing individual amino acids and 2 ml 1% casein (sodium salt, C-8654, Sigma) solution (pH 6.7) at 49 °C for a period of 2 h. Control samples were prepared in the same way as described above, except that incubations were performed without 1% casein which was applied into the reaction mixture at the end of incubation. After stopping the reaction by addition of 1 ml of 17.5% trichloroacetic acid (TCA) and subsequent centrifugation, 1ml of supernatant was mixed with 3.7% aqueous Na_2CO_3 solution, and 1ml 0.06% aqueous $CuSO_4$ solution. After mixing and 30min incubation at room temperature, 1ml of Folin-Ciocalteau reagent (diluted 1:3 by demineralized water) was applied. Following further incubation at 37 °C and 15 min at room temperature, the concentration of aromatic amino acids released by proteolytic cleavage of casein and expressed in L-tyrosine equivalents was determined colorimetrically at 578 nm. The calibration line of dependence of absorbance on L-tyrosine concentration was prepared from stock solution of L-tyrosine in demineralized H_2O and a solution of 0.05M Tris–HCl = 17.5% TCA (ratio 3:1).

The effect of cystine enantiomers on activity of acid phosphomonoesterase was tested on soil taken from cultivation of *Miscanthus* x *Giganteus* in Botanic Garden and Arboretum of Mendel University in Brno, Czech Republic (N 49°12'54.240", E 16°36'41.989", 235.19 m. a.s.l). Soil was air-dried and consequently re-moistened to 60% (w/w) and incubated for 18 h at 22 °C prior to the experiment. Acid phosphomonoesterase activity was determined using a modified method of Rejšek (1991). Fresh soil (1 g) was incubated with *p*-nitrophenyl phosphate (p-NPP) as substrate in 12 mL of succinate-borate buffer (pH 4.8) at 37 °C for 1 h when cystine enantiomers were added into the reaction mixture at the concentration of 8 mg per g dry weight. Diamino amino acid cystine was chosen due to its abundant occurrence in mountain forest and meadows soils (Formánek *et al*., 2008b). As in case of testing the effect of alanine or glutamic acid enantiomers on casein-protease activity, also enantiomers of cystine were applied to soil in randomly selected concentrations, which were in all cases much higher compared to concentrations of these amino acids in nature.

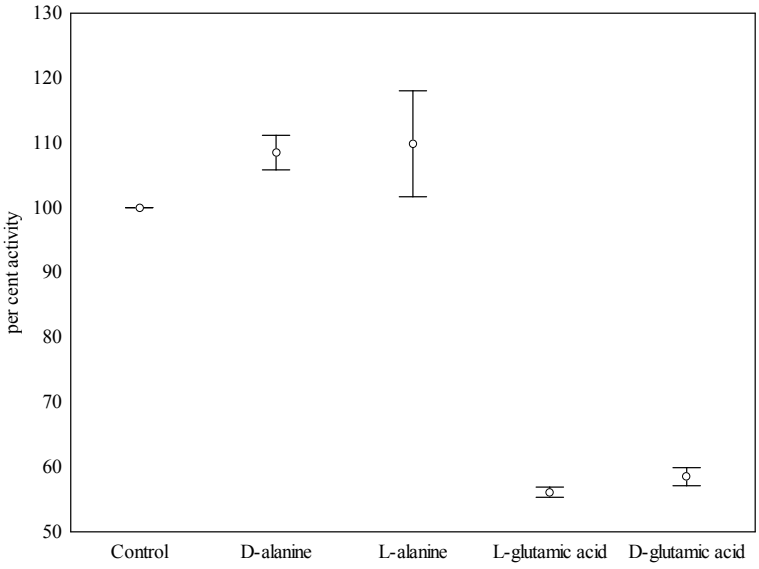
Statistical analysis of the data was performed through one-way ANOVA (analysis of variance) and Fisher's LSD test (least significant difference). All statistical analyses were undertaken using the Statistica 9.0 program.

RESULTS AND DISCUSSION

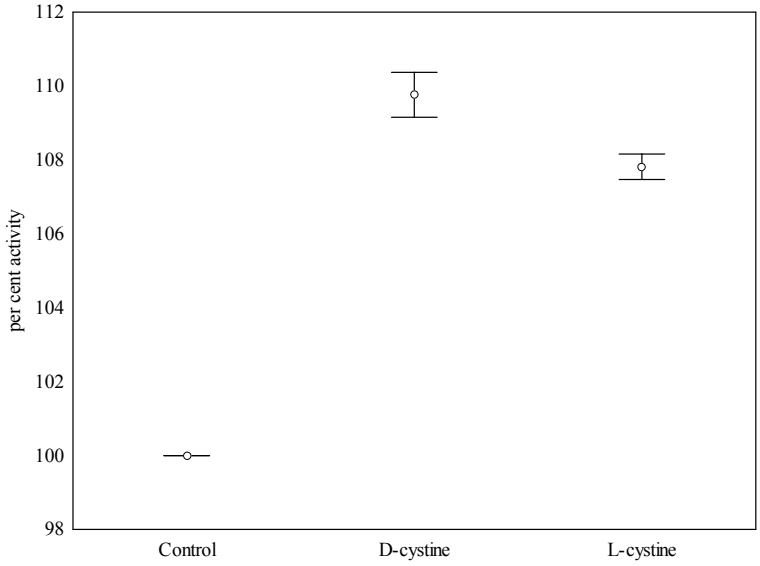
Application of alanine enantiomers in concentration of 5 mg per g fresh soil slightly increased casein-protease activity in soil (Fig. 1). Both enantiomers of glutamic acid significantly ($P < 0.05$) decreased casein-protease by ca. 40%. Application of L- or D- cystine enantiomers in the concentration of 3.2 mg per g fresh soil (= 8 mg per g dry soil) increased acid phosphomonoesterase activity by 9–10% compared to control.

It has been known that protease activity in soil may be inhibited or stimulated by easily-degradable C sources and amino acids; on the other hand, this activity may be stimulated by peptides, proteins and mineral nitrogen (Glenn 1976; Asmar *et al*. 1994; Vágnerová and Macura 1974).

D-amino acids are naturally occurring in bacterial cell walls protect bacterial peptidoglycan from proteolytic cleavage (Voet and Voet, 1995). Results presented in this work are in agreement with this finding; further research is necessary to clarify the influence of amino acid enantiomers on enzymatic processes in soil. Casein-protease activity in the same soil as used in this work was studied throughout whole vegetation season 2006. Activity of casein-protease may be affected by many factors including temperature, concentration of casein, pH, moisture etc. (Vranová *et al*., 2009). Glutamic acid and alanine were added to soil to determine their effect on casein-protease in artificially high concentrations. More research is necessary to evaluate mainly



1: *The effect of addition of L- and D- alanine and glutamic acid (5 mg per g fresh soil) on activity of casein-protease (mean ±SE, n = 3)*



2: *The effect of addition of L- and D- cystine (8 mg per g dry soil) on activity of acid phosphomonoesterase (mean ± SE, n = 2-3)*

the effect of both amino acids when supplied in naturally occurring concentrations (Formánek *et al.*, 2008 a, b). Especially glutamic acid, which belongs to the most abundant "free" soil amino acids in many ecosystems (Rejšek *et al.*, 2010), should be further studied; if this amino acid significantly inhibits soil casein-protease, it may have a significant effect on nitrogen mineralization in soil and plant nutrition of different ecosystems.

Sulphur-containing free amino acids like cystine and methionine were found to compose 0–18.4% of the pool of total free amino acids in different soils of differently managed ecosystems (Rejšek *et al.*, 2010 b). Diamino amino acid cystine has been found between dominant free amino acids in organomineral soils of mountain meadows or in forest humus floor of mountain forests (Formánek *et al.*, 2005, 2008 b) when extracted by water and 0.5 M ammonium acetate (Formánek *et al.*, 2005). Determination of the effect of cystine enantiomers on acid phosphomonoesterase is the first work in this research direction as no comparable studies are available so far. Cystine was applied into soil in artificially high concentrations and we may hypothesize that natural concentrations of cystine in soil may have negligible effect on acid phosphomonoesterase activity and phosphorus cycling.

CONCLUSION

Amino acids in soil affect the enzymatic activities in soil. This work has proved that enantiomers of alanine at artificially high concentrations increase and enantiomers of glutamic acid decrease cleavage of casein in soil. Application of enantiomers of cystine in artificially high concentration increases acid phosphomonoesterase activity in soil by 9–10%. More research on the effect of amino acids on enzymatic activities in soil is necessary.

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REFERENCES

AMELUNG, W., BRODOWSKI, S., 2002: In vitro quantification of hydrolysis-induced racemization of amino acid enantiomers in environmental samples using deuterium labeling and electron-impact ionization mass spectrometry. *Analytical Chemistry* 74, 13, 3239–3246. ISSN 0003-2700.

AMELUNG, W., ZHANG, X., 2001: Determination of amino acid enantiomers in soils. *Soil Biology and Biochemistry* 33, 4–5: 553–562. ISSN 0038-0717.

AMELUNG, W., ZHANG, X., FLACH, K. W., 2006: Amino acids in grassland soils: Climatic effect on concentrations and chirality. *Geoderma* 130, 3–4: 207–217. ISSN 0016-7061.

ASMAR, F., EILAND, F., NIELSEN, N. E., 1994: Effect of extracellular-enzyme activities on solubilization rate of soil organic nitrogen. *Biology and Fertility of Soil* 17, 1: 32–38. ISSN 0178-2762.

BRÜCKNER, H., WASTHAUSER, T., 2003: Chromatographic determination of L- and D-amino acids in plants. *Amino Acids* 24, 2: 43–55. ISSN 1948-5948.

CARREIRA, J. A., GARCA-RUIZ, R., LIÉTOR, J., HARRISON, A. F., 2000: Changes in soil phosphatase activity and P transformation rates induced by application of N- and S-containing acid-mist to a forest canopy. *Soil Biology and Biochemistry* 32, 13: 1857–1865. ISSN 0038-0717.

FORMÁNEK, P., KLEJDUS, B., VRANOVÁ, V., 2005: Bio-available amino acids extraction from soil by demineralized water and 0.5M ammonium acetate. *Amino Acids* 28, 4: 427–429. ISSN 0939-4451.

FORMÁNEK, P., REJŠEK, K., VRANOVÁ, V., MAREK, M. V., 2008a: Bio-available amino acids and mineral nitrogen forms in soil of moderately mown and abandoned mountain meadows. *Amino Acids* 34, 2: 301–306. ISSN 0939-4451.

FORMÁNEK, P., REJŠEK, K., VRANOVÁ, V., KLEJDUS, B., 2008b: Selected Diamino Acids in Soils of Differently Managed Mountain Meadow and Forest Ecosystems: Assessment of their Role in the Ecosystem Nutrition. In: SCHÄFER, H. A., WOHLBIER, L. M.: *Diamino Amino Acids*. 1. vyd. New York: Nova Science Publishers, 182–218. ISBN 978-1-60456-892-9.

GLASER, B., AMELUNG, W., 2002: Determination of ^{13}C natural abundance of amino acid enantiomers in soil: methodological considerations and first results. *Rappid Communications in Mass Spectrometry* 16, 9: 891–898. ISSN 0951-4198.

GLENN, A. R., 1976: Production of extracellular proteins by bacteria. *Annual Review of Microbiology* 30: 41–62. ISSN 0066-4227.

HOPKINS, D. W., FERGUSON, K. E., 1994: Substrate induced respiration in soil amended with different amino acid isomers. *Applied Soil Ecology* 1, 1: 75–81. ISSN 0929-1393.

HOPKINS, D. W., ISABELLA, B. L., SCOTT, S. E., 1994: Relationship between microbial biomass and substrate induced respiration in soils amended with D- and L-isomers of amino acids. *Soil Biology and Biochemistry* 26, 12: 1623–1627. ISSN 0038-0717.

HOPKINS, D. W., O'DOWD, R. W., SHIELD, R. S., 1997: Comparison of D- and L-amino acid metabolism in soils with differing microbial biomass and activity. *Soil Biology and Biochemistry* 29, 1: 23–29. ISSN 0038-0717.

LANDI, L., RENELLA, G., MORENO, J. L., FALCHINI, F. L., NANNIPIERI, P., 2000: Influence of cadmium on the metabolic quotient, L-D-glutamic acid respiration ratio and enzyme activity: microbial biomass ratio under laboratory conditions. *Biology and Fertility of Soils* 32, 1: 8–16. ISSN 0178-2762.

MARTENS, D. A., FRANKENBERGER W. T. JR., 1993: Metabolism of tryptophan in soil. *Soil Biology and Biochemistry* 25, 12: 1679–1687. ISSN 0038-0717.

NOWAK, J., KAKLEWSKI, K., KLÓDKA, D., 2006: Influence of various concentrations of selenic acid (IV) on the activity of soil enzymes. *Science of the Total Environment* 291, 1–3: 105–110. ISSN 0048-9697.

O'DOWD, R. W., HOPKINS, D. W., 1998: Mineralization of carbon from D- and L-amino acids and D-glucose in two contrasting soils. *Soil Biology and Biochemistry* 30, 14: 2009–2016. ISSN 0038-0717.

O'DOWD, R. W., PARSONS, R., HOPKINS, D. W., 1997: Soil respiration induced by the D- and

L-isomers of a range of amino acids. *Soil Biology and Biochemistry* 29, 11: 1665–1671. ISSN 0038-0717.

O'DOWD, R. W., BARRACLOUGH, D., HOPKINS, D. W., 1999: Nitrogen and carbon mineralization in soil amended with D- and L- leucine. *Soil Biology and Biochemistry* 31, 11: 1573–1578. ISSN 0038-0717.

REJŠEK, K., 1991: Acid phosphomonoesters activity of ectomycorrhizal roots in Norway spruce pure stands exposed to pollution. *Soil Biology and Biochemistry* 23, 7: 667–671. ISSN 0038-0717.

REJŠEK, K., FORMÁNEK, P., PAVELKA, M., 2008: Estimation of protease activity in soils at low temperatures by casein amendment and with substitution of buffer by demineralized water. *Amino Acids* 35, 2: 411–417. ISSN 0939-4451.

REJŠEK, K., FORMÁNEK, P., VRANOVÁ, V., 2010: *The Soil Amino Acids: Quality, Distribution and Site Ecology*. 1. vyd. New York: Nova Science Publishers, Inc. 222 s. ISBN 978-1-61668-335-1.

TRASAR-CEPEDA, C., LEIRÓS, M. C., GIL-SOTRES, F., 2000: Biochemical properties of acid soils under climax vegetation (Atlantic oakwood) in an area of the European temperate-humid zone (Galicia, NW Spain): Specific parameters. *Soil Biology and Biochemistry* 32, 6: 747–755. ISSN 0038-0717.

VÁGNEROVÁ, K., MACURA, J., 1974: Relationship between plant roots, proteolytic organisms and activity of protease. *Folia Microbiologica* 19, 6: 525–535. ISSN 0015-5632.

VOET, D., VOET, J. G., 1995: *Biochemistry*. John Wiley & Sons Ltd, New York. ISBN 0-471-19350-X.

VRANOVÁ, V., FORMÁNEK, P., REJŠEK, K., PAVELKA, M., 2009: Impact of land-use change on proteolytic activity of mountain meadows. *Soil and Water Research* 3, 4: 122–125. ISSN 1801-5395.

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