

Activation energy and kinetic parameters of phosphomonoesterase activity in different soil types

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Abstract

In the present research we aimed to determine kinetic and thermodynamic constants of the acid and alkaline phosphomonoesterases activities such as Michaelis-Menten constant, maximum enzymatic reaction velocity (V_{max}), as well as activation energy (E_a). We did it by examining different types of soil. Soil samples were collected from the humus horizon (0-15 cm) of the following soil types: 1. planosol – Ub; 2. solonetz – Kumane; 3. chernozem - Zemun Polje; 4. vertisol – Umka; 5. humogley -Makis. The Michaelis-Menten constant of acid phosphomonoesterase activity varied between 5.7 mm in planosol and 72.8 mm in solonetz. The highest acid phosphomonoesterase V_{max} was found in solonetz (809.8) and the lowest in chernozem (81.2 $\mu\text{g p-npg}^{-1} \text{ h}^{-1}$). The E_a of acid phosphomonoesterase varied between 28.8 kJ mol^{-1} (solonetz) and 77.54 kJ mol^{-1} (chernozem). The Michaelis-Menten constant of alkaline phosphomonoesterase activity was between 7.59 mm in solonetz and 21.4 mm in chernozem. Alkaline phosphomonoesterase V_{max} varied between 217.2 $\mu\text{g p-npg}^{-1} \text{ h}^{-1}$ in planosol and 789.4 $\text{p-npg}^{-1} \text{ h}^{-1}$ in chernozem, and E_a between 28.8 kJ mol^{-1} in solonetz and 59.3 kJ mol^{-1} in planosol. This research confirms the importance of kinetic and thermodynamic indicators of the active enzyme phosphomonoesterase in mineralization of organic phosphorous in soils and its uptake by plants.

Key words: activation energy, acid phosphomonoesterase activity, alkaline phosphomonoesterase activity, kinetic parameters

Introduction

Enzymes as biological catalysts play a crucial role in important biochemical processes: synthesis and decomposition of humus, hydrolysis of organic compounds, as well as in the biogeochemical cycling of nutrients (22). Activity of soil enzymes has been related to structure of microbial populations (8), vegetation (19), physical and chemical properties of soil (1) etc. Soil enzyme data provide information about coherency between microorganisms and nutrient dynamics (16).

Alkaline phosphomonoesterase (EC 3.1.3.1) and acid phosphomonoesterase (EC 3.1.3.2) are enzymes that play a major role in biological activity of soil (2), soil quality (24) and in the circulation of phosphorus as they catalyze the hydrolysis of monoesters of orthophosphoric acid

and transform them into available forms for plants. The morphology and physiology of root system is directly affected by microbial activity in rhizosphere (18), especially by microbial enzymes.

Determination of kinetic and thermodynamic indicators of the active enzyme complex is important parameter for understanding of enzymatic reactions in soil. The Michaelis-Menten constant is one of fundamental constants in enzymology that enables determining the effect of substrate concentration on the velocity of enzymatic reaction. Studies of enzyme kinetics in soil are useful for understanding the activity of enzymes depending on the physical and chemical properties of soil. The Michaelis-Menten constant is often described by parameters as V_{max} and K_m , who shows the enzyme activity (10).

The aim of this paper was to determine the Michaelis-Menten constant, maximum velocity of enzymatic reaction and activation energy of acid and alkaline phosphomonoesterase in different soil types.

Materials and methods

Samples for determining the kinetic and thermodynamic constants of the activity of acid and alkaline phosphomonoesterase were collected from the surface (0-10 cm) of soils differing in types, localities, and consequently chemical properties (Table 1).

Table 1. Chemical properties of the tested soil types

Soil type/ locality	pH (H ₂ O)	Humus (%)	N (%)	P ₂ O ₅ (mg/100g)	K ₂ O (mg/100g)
Planosol / Ub	5.72	1.99	0.110	14.40	16.80
Solonetz / Kumane	6.40	6.05	0.400	7.30	18.80
Chernozem / Zemun Polje	8.02	2.64	0.182	11.00	18.40
Vertisol / Umka	7.64	3.85	0.180	6.80	27.60
Humogley / Makis	7.62	4.55	0.24	11.70	21.60

The activity of acid and alkaline phosphomonoesterase was determined by the Tabatabai & Bremner (21) method. Briefly, 1 g of soil was incubated with 0.2 ml toluene, 4 ml MUB (pH 6.5 for assay of acid phosphatase or pH 11 for assay of alkaline phosphatase), and 1.0 ml of p-nitrophenyl phosphate for 60 minutes at 37⁰C.

The effect of substrate concentration (K_m) was determined by supplementing the reaction mixture with p-nitrophenyl phosphate in the amount required to make the concentration of the reaction mixture reach 1, 2, 5, 10, 15, 20 mM. Each concentration of supplemented substrate had a control. All experiments were conducted in triplicate. K_m and V_{max} kinetic parameters were calculated from a linear regression analysis of the initial velocity of reaction against $1/S$ using the Lineweaver-Burk transformation.

Using by temperature and activation energy, the rate of chemical reaction was determined according to the Arrhenius equation:

$$k = Ae^{-E_a/RT}$$

In the formula given above, 'k' stands for the constant rate of chemical reaction, 'Ae' for pre-exponential factor, 'E_a' for the activation energy, 'R' for the universal gas constant, and 'T' stands for the absolute temperature.

Ea was determined by linear regression analysis. Written in logarithmic form, the Arrhenius equation reads as follows:

$$\log V = \log A - E_a/2.303 RT$$

Results and Discussion

The activity of acid and alkaline phosphomonoesterase varied in different soil types. The Michaelis constant (Km) of the acid phosphomonoesterase activity varied between 5.7 mM in planosol and 72.8 mM in solonetz (Table 2), while the values of Km of alkaline phosphomonoesterase activity varies between 7.59 mM in solonetz and 21.4 mM in chernozem (Table 2). Value of Km of acid phosphomonoesterase in planosol was lower compared to Km of alkaline phosphomonoesterase. The soil types with higher pH value have higher Km of acid phosphomonoesterase, while Km values of alkaline phosphomonoesterase were particularly low.

Maximum velocity of the enzymatic reaction of acid and alkaline phosphomonoesterase varies in different soil types. The highest Vmax of acid phosphomonoesterase was found in acid soil samples (planosol and solonetz), and the lowest was observed in chernozem. However, the highest Vmax of alkaline phosphomonoesterase was noticed in chernozem, while the lowest in planosol.

Table 2. The Michaelis constant (Km), maximum velocity (Vmax) and activation energy (Ea) of acid phosphomonoesterase in different soil types

Soil type/ locality	Acid phosphomonoesterase		
	Km (mM)	Vmax ($\mu\text{g p-NP g}^{-1} \text{ h}^{-1}$)	Ea (kJ mol ⁻¹)
Planosol / Ub	5.7	570.1	43.77
Solonetz / Kumane	72.8	809.8	25.03
Chernozem / Zemun Polje	33.3	81.2	77.54
Vertisol / Umka	26.8	290.7	40.19
Humogley / Makis	26.0	329.9	70.99

The activation energy for acid and alkaline phosphomonoesterase varies in the different soil types (Table 3). The lowest Ea of acid and alkaline phosphomonoesterase was noticed in solonetz (25.03 and 28.8 kJ mol⁻¹, respectively). The highest Ea of acid phosphomonoesterase was found in chernozem (77.54 kJ mol⁻¹), while in planosol was observed a highest value of alkaline phosphomonoesterase (59.3 kJ mol⁻¹).

Soil types with acid pH values (planosol and solonetz) have higher values of activation energy for alkaline phosphomonoesterase, compared to acid phosphomonoesterase. On the other hand, in alkaline soils we noticed the lower energy of activation for alkaline phosphomonoesterase, particularly when compared to acid phosphomonoesterase.

The enzymes in soil are linked with different biotic and abiotic factors and originate from animals, plants and microorganisms (14). Microorganisms provide most of the soil enzyme activity, thanks to their vigorous metabolism, diversity and biomass content (17). Several reports showed that characteristics of soil and water can influence the activity of enzymes, e.g. saccharase, protease, phosphatase etc (5, 23).

Table 3. The Michaelis constant (Km), maximum velocity (Vmax) and activation energy (Ea) of alkaline phosphomonoesterase in different soil types

Soil type/ locality	Alkaline phosphomonoesterase		
	Km (mM)	Vmax ($\mu\text{g p-NP g}^{-1} \text{ h}^{-1}$)	Ea (kJ mol ⁻¹)
Planosol / Ub	11.0	217.2	59.33
Solonetz / Kumane	7.59	759.2	28.80
Chernozem / Zemun Polje	21.4	789.4	40.50
Vertisol / Umka	8.05	444.6	34.70
Humogley / Makis	9.24	308.3	40.23

The activity of acid and alkaline phosphomonoesterase correlated with humus and nitrogen contents, pH, soil texture, microbiological and biochemical properties (4). High activity of phosphomonoesterases was obtained in solonetz and probably resulted from high content of nitrogen and humus, which is in coordinance with previous researches (3, 15).

Phosphatase activity is also correlated with pH value of soils. In our investigation we noticed the high acid phosphomonoesterase activity in acid soils. The conclusion of our research corresponds with the results of Sarapatka (13). The same author noticed negative correlation between activity of alkaline phosphomonoesterase and pH, and those particular findings significantly differ from ours, primarily because we discovered highest activity of alkaline phosphomonoesterase in most alkaline soil.

Our results are similar with observation by Pang & Kolenko (12), who reported that Km of acid phosphomonoesterase varies between 25 and 91 mM, but differs to the investigations by Tabatabai (20), who found the Km to vary between 1.3 and 4.5.

The lower Km values for alkaline phosphomonoesterase indicate a greater enzyme affinity for the substrate. In this research Km values of alkaline phosphomonoesterase in vertisol and humogley were lower compared with planosol. Similar data was obtained by Juma & Tabatabai (7), who found the Km values for alkaline phosphomonoesterase to be lower in alkaline than in acid soil. Also, in alkaline soils, Ea values for alkaline phosphomonoesterase were lower than for acid phosphomonoesterase. Tabatabai (20) reported Ea of alkaline phosphatase in alkaline soil to be significantly lower than in acid soils. The lowest Ea of acid and alkaline phosphomonoesterase was found in solonetz, resulting from the presence of water soluble cations Mg²⁺ and Ca²⁺ in the humus horizon (11). Mg ions stimulate phosphomonoesterase activity (6) by allosteric changes in the substrate (9).

This research confirms the importance of kinetic parameters of phosphomonoesterases in mineralization of organic phosphorous in soils and its utilization by plants, especially under low concentration of phosphorus.

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