Inhibition, Repression and Induction of Phosphatase Activity in a Salt Lake Sediment

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The inhibitory/repressive effect of the final reaction product (inorganic o-phosphate) and the inducing effect of a substrate (β-glycerophosphate) on the phosphatase activity and synthesis in a salt lake sediment were studied. The determinations have been differentially carried out, for acid, neutral and alkaline phosphatase, and in reaction mixtures without buffering. The acid phosphatase activity was the most intense, and the alkaline phosphatase activity was the lowest. The inhibitory effect was obvious at 0.2 and especially at 2 mg PO_4^{3-}–P/ml concentration, without significant differences between the four types of activities. A significantly negative correlation has been established between the concentration of added inorganic o-phosphate and the intensity of phosphatase activity. Both the repressive effect of inorganic o-phosphate and the inducing effect of β-glycerophospate were stronger in the case of acid phosphatase synthesis. In the case of acid phosphatase, the inducing effect of β-glycerophosphate prevailed over the repressive effect of inorganic o-phosphate. In the other three cases, the repressive effect of the final reaction product was dominant.

Keywords: phosphatase, sediment, inhibition, repression, induction

The main P form used by autotrophic organisms is the PO_4^{3-} ion, so that mechanisms by which it can be released from organic phosphorus compounds have a great importance for the equilibrium of the ecosystems where the enzymatic hydrolysis is practically the only way to release the biologically active orthophosphate from these organic compounds. The process is accomplished by phosphatases. These enzymes are useful key compounds for the study and interpretation of the complex mechanisms which are the background of the organic phosphorus transformation, particularly of its mineralization in ecosystems [10, 11, 14].

According to their optimum activity related to pH, the phosphatases are alkaline, neutral and acid. The literature data, concerning the constitutive or adaptive nature of the phosphatases in aquatic habitats, especially in sediments, are contradictory. It has been stated that the alkaline phosphatases, produced particularly by the phytoplankton, are adaptive and dominant in the euphotic zone, and the acid phosphatases, produced mainly by the bacteria, are constitutive and dominant in the deep zone of aquatic basins, including sediments [1, 11, 12].

In the present paper, we attempted to study the inhibitory/repressive effect of PO_4^{3-} (final reaction product) and the inducing effect of calcium ß-glycerophosphate (phosphatase substrate), respectively, on the synthesis and activity of the the acid, neutral, and alkaline phosphatase, as well as on the phosphatase activity measured in reaction mixtures without buffering, at the natural *p*H (8) in the sediment of the salt lake Ursu (Sovata, Romania). Parts of our previous researches in the field have been already published [8, 9].

Experimental part

Material and methods

The experiment was performed on a mean sample resulted by mixing three samples taken from different sites of the Ursu lake. The sediment (*p*H 8) is black, greasy, and characterized by a high enzymatic potential. As inhibitor of phosphatase activity, potassium orthophosphate (K_2HPO_4) was used in concentrations specified in table 1.

Table 1
EXPERIMENTAL VARIANTS FOR DETERMINING THE INHIBITORY EFFECT OF ORTHOPHOSPHATE ON THE PHOSPHATASE ACTIVITY

Experimental variant	$K_2HPO_4 \cdot 3H_2O$ (mg/ml)	P (mg/ml)	Molar P concentration (mM)
Control – V1	-	-	-
V2	0.0015	0.0002	0.006
V3	0.015	0.002	0.064
V4	0.15	0.02	0.645
V5	1.5	0.2	6.451
V6	15	2	64.516

The constitutive or adaptive nature of phosphatases in the studied salt lake sediment was tested using the orthophosphate as a repressive agent, and calcium β-glycerophosphate ($C_3H_7O_6PCa$) as an inducing agent. One kg of sediment in 1-L graded cylinders was completed to 1 L with filtered lake water. The substances were added according to the scheme presented in table 2 and the mixtures were incubated in the dark, at 28°C. The activity of the four types of phosphatases was determined after 3, 10, 20, 40, 100 and 200 days of incubation.

For each sample, acid (*p*H 5.5), neutral (*p*H 7), and alkaline (*p*H 10) phosphatase activity of the sediment was measured, as well as the phosphatase activity in reaction mixtures with distilled water, without buffering. Reaction mixtures were obtained using Tris universal buffer.

Phosphatase activity was measured using the Krámer and Erdei method [7]. Reaction mixtures consisted of 2.5 g sediment + 2 mL toluene + 5 mL buffer solution + 5 mL 1% disodium phenylphosphate solution ($C_6H_5PO_4Na_2$) as substrate. Comparatively, phosphatase activity was measured in reaction mixtures without buffer solution, this being replaced by the same quantity of distilled water, so that we obtained in all the reaction mixtures a 10 mL water phase volume and an 0.5% disodium phenylphosphate concentration. As controls, we used reaction mixtures with: sediment + buffer + toluene,

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Table 2

EXPERIMENTAL VARIANTS FOR THE REPRESSIVE EFFECT OF PO_4, AND THE INDUCING EFFECT OF *B*-GLYCEROPHOSPHATE ON THE SYNTHESIS AND ACTIVITY OF PHOSPHATASE (FOR EACH VARIANT 1 KG OF SEDIMENT AND LAKE WATER TO 1 L WERE USED)

Variant	Glucose (g)	$C_3H_7O_6PCa$ (g)	$K_2HPO_4 \cdot 3H_2O$ (g)	$PO_4 - P$ (mg/ml)	NH_4Cl (g)	Urea (g)	C (g)	N (g)	P (g)
V1	-	-	-	-	-	-	-	-	-
V2	-	-	1.5	0.2	-	-	-	-	0.203
V3	-	-	15	2	-	-	-	-	2.039
V4	10	-	-	-	0.8	0.5	4	0.442	-
V5	10	2	-	-	0.8	0.5	4.292	0.442	0.252
V6	10	4	-	-	0.8	0.5	4.585	0.442	0.504
V7	10	-	1.5	0.2	0.8	0.5	4	0.442	0.203
V8	10	-	15	2	0.8	0.5	4	0.442	2.039
V9	10	4	1.5	0.2	0.8	0.5	4.585	0.442	0.707
V10	10	4	15	2	0.8	0.5	4.585	0.442	2.543

without substrate; substrate + toluene and distilled water + toluene, without sediment, respectively, at the same final volume. Incubation was carried out at 37°C, for 24 h. Phosphatase activity is expressed in mg phenol/2.5 g sediment (dry matter).

Results and discussions

The results of research which surveyed the inhibitory effect of PO_4^{3-} on the phosphatase activity are presented in figure 1. The most intense activity is that of the acid phosphatase. Alkaline phosphatase activity represents 60%, neutral phosphatase activity 84%, and phosphatase activity in reaction mixtures without buffer 72% of the acid phosphatase activity.

The inhibitory effect of inorganic phosphate on the phosphatase activity is obvious in all the 4 cases. Negative correlation coefficients have been established between the concentration of inorganic phosphate and the phosphatase activity. Their values are higher than –0.900 ($p < 0.05$). The inhibitory effect became obivous especially at the high concentrations of 0.2 and 2 mg PO_4^{3-} – P/mL. Phosphatase activity decreased by approximately 50% in the variant with 0.2 mg PO_4^{3-} – P/ml. As an exception, acid phosphatase activity decreased only by 27.26%. In the variant with 2 mg PO_4^{3-} – P/mL, phosphatase activity decreased by over 80% (maximum 95.21% in reaction mixture without buffering).

The significant inhibition of the phosphatase activity by the added inorganic phosphate appeared in our experiment at higher phosphate concentrations compared to the data reported by other researchers [1, 3, 5].

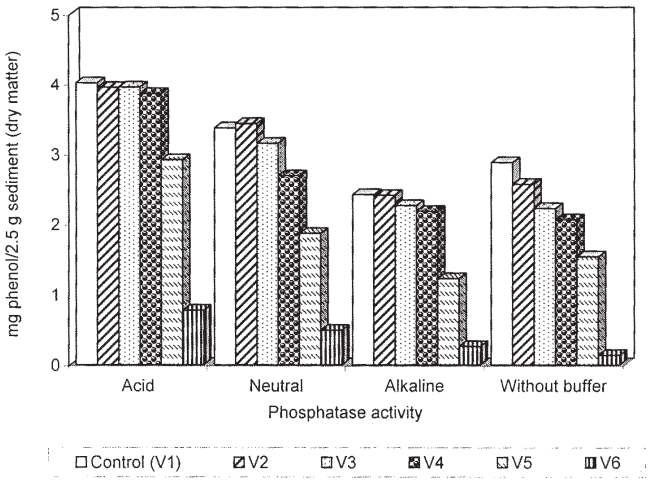

Fig. 1. Inhibition of phosphatase activity by added inorganic phosphate. Explanations (table 1)

We now present the results of the experiment pursuing the constitutive or adaptative nature of the four phosphatase types studied. The influence of ortho-phosphate and β-glycerophosphate on synthesis and activity of phosphatase is presented in figure 2. In spite of its marked decrease, PO_4^{3-} concentration in the variants to which orthophosphate was initially added remained higher than in the other variants, maintaining a repressive effect on phosphatase synthesis.

Initial phosphatase activities, before the substances presented in table 2 were added to the experimental variants, had the following mean values, expressed in mg phenol/2.5 g sediment (dry matter): acid phosphatase: 4.017; neutral phosphatase: 3.374; alkaline phosphatase: 2.426; in reaction mixture without buffering: 2.880.

After the first three days of incubation, a sudden decrease in all four activities occurred. This can be explained by the inhibitory effect of inorganic phosphate initially added to the experimental variants. After an oscillatory evolution during the first 40 days of incubation, there is a relative uniform decrease in phosphatase activity in all four cases. With no exception, phosphatase activity in the control variant (V1) is higher than that of the variants supplemented with 0.2 and 2 mg/mL PO_4^{3-}-P. This confirms the repressive effect of the PO_4^{3-} ion on *de novo* synthesis of the enzyme, and is explained by the fact that at the end of the incubation period (after 200 days), PO_4-P concentration is much too low (by order of μg/mL) to provide an inhibitory effect. Naturally, the repressive effect is stronger in variant V3, to which 2 mg/mL PO_4-P were added.

Even if after 200 days of incubation, other indicators of biological activity (the number of heterotrophic aerobic bacteria, catalase activity, dehydrogenase activity) in the studied sediments had a decreasing evolution too, comparison of variants V2 and V3 with the control (V1) after the whole incubation period is essential for the studied phenomenon – the repression of phosphatase synthesis – and results confirm the existence of this phenomenon, which is more prominent in the case of acid phosphatase.

The nutrient substance supplementation led to a spectacular increase in phosphatase activity. After 200 days of incubation, phosphatase activity in the case of control variant (V1) is only 58.59% (acid), 32.53% (neutral), 28.94% (alkaline), 41.15% (without buffering) of that of the variant V4, to which supplementary C and N sources were added. This is in good agreement with the observations of Huang and Morris (2005), who found that fertilization with only P had no effect on alkaline phosphatase activity in marsh sediments along a

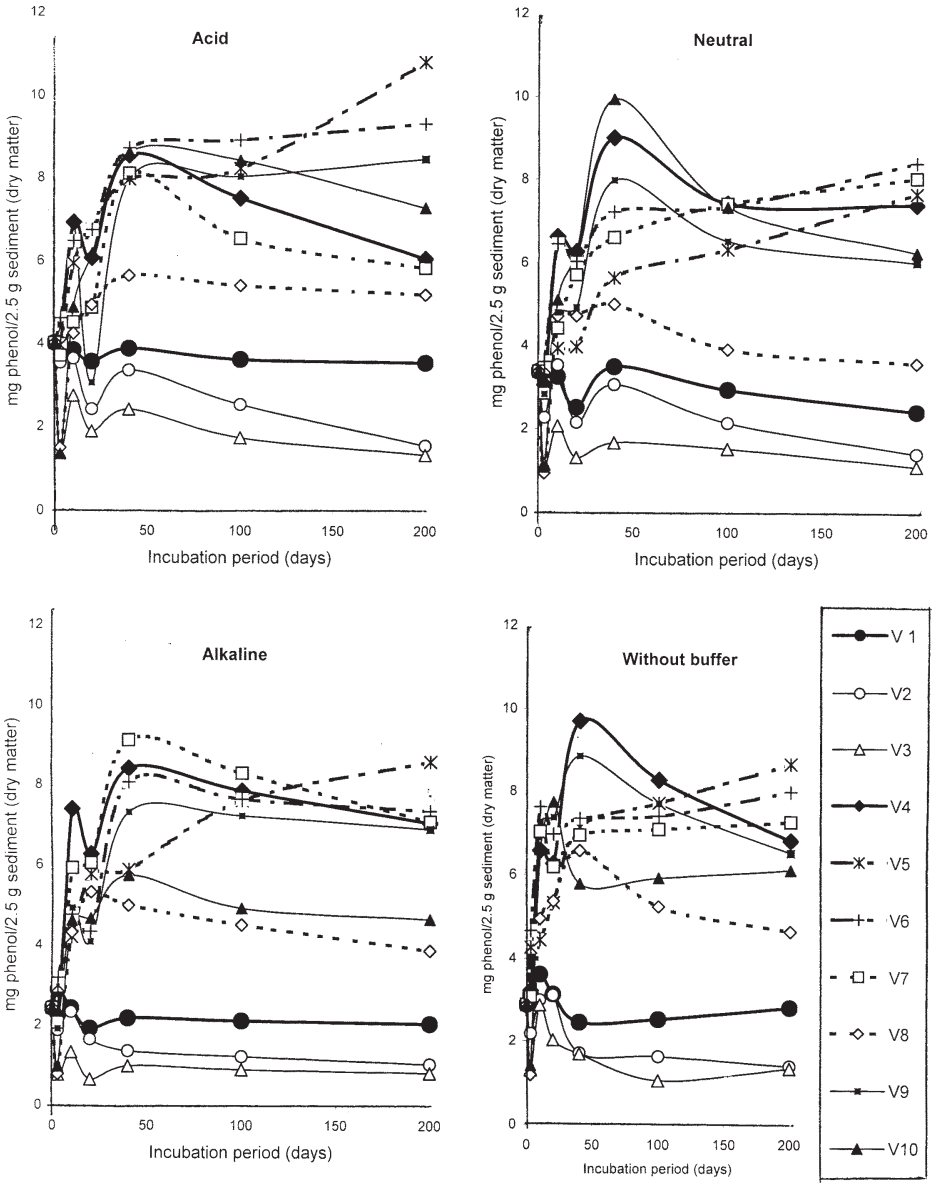

Fig. 2. Influence of orthophosphate and ß-glycerophosphate on synthesis and activity of phosphatase. Explanations(table 2)

salinity gradient, while N-only or N + P fertilization significantly increased the enzymatic activity [2]. On the other hand, there are reports which suggests that proteins or lipids induce an increase in the phosphatase activity of the sediments, but carbohydrate addition has no effect [6].

The repressive effect of inorganic orthophosphate can be found in the case of experimental variants V7 and V8 as well, to which C and N sources were added. At a concentration of 0.2 mg/mL added PO_4-P, the repressive effect of orthophosphate is insignificant, enzymatic activity in variant V7, with the exception of acid phosphatase, slightly exceeding that of the control (V4). In the case of variant V8, with 2 mg/mL PO_4-P initially added, the repressive effect is obvious in all cases. The intensity of neutral phosphatase activity decreases to less than half, while the intensity of acid phosphatase activity decreases by only 15% compared to the control (V4). Even if PO_4-P concentration decreases at the end of the incubation period to only 32 µg/mL, this concentration seems to be sufficient to maintain the synthesis rate of all phosphatase types at a low level.

The increase of phosphatase activity as a result of C and N addition to the experimental variants, is explained by the massive development of microbiota, as a consequence of C and N supplementation. If at the beginning of the experiment the number of heterotrophic aerobic bacteria was of the 10^6/g sediment (dry matter) order, after 200 days of incubation this number was 20-35 times higher. At the same time, the other enzymatic activities runs (catalase and dehydrogenase) intensified as well.

The addition of β-glycerophosphate, the enzymatic phosphatase substrate, to initial mixtures of experimental variants led to an increase of the enzymatic activity, compared with the control variant V4. The induction is stronger in the case of acid phosphatase, but it is not correlated with the β-glycerophosphate concentration. The intensity of acid phosphatase activity increases by 53.54% in V6, and by 78.13% in V5, respectively, compared to the control (V4). In the case of the other phosphatase types, the difference from the control is smaller and it is positively correlated with the substrate (β-glycerophosphate) concentration only in the case of neutral phosphatase.

Only in the second part of the incubation period, after 100 days, the phosphatase activity level in the experimental variants V5 and V6 exceeds that of the control (V4). Thus, the manifestation of the inducing effect of β-glycerophosphate requires a time lag, during which microorganisms adapt their enzymatic system of phosphatase synthesis. This interval is shorter in the case of acid phosphatase, in which induction is the strongest. Positive correlations between the alkaline phosphatase especially, and the total or the organically-bound phosphorus in sediments are frequently encountered in the literature [4, 13, 15].

In the case of simultaneous supplementation of the experimental variants with both orthophosphate and β-glycero-

phosphate, the acid phosphatase reacts differently compared to the other phosphatase types, confirming the results already presented in connection with the two phenomena, induction and repression. In the case of acid phosphatase, induction is stronger than repression: after 200 days of incubation, in V9 the intensity of acid phosphatase activity is by 39.49%, and in V10 by 20.15% higher as compared to the control (V4). In the other cases, the repressive effect is stronger than the inducing effect. The strongest repression occurs in the case of alkaline phosphatase in variant V10, in which the activity is reduced after 200 days of incubation by 33.93% compared to that of the control V4.

Conclusions

The existence of a differentiated phosphatase activity according to the *p*H in a salt lake sediment has been ascertained. The acid phosphatase activity was the most intense, and the alkaline phosphatase activity was the lowest.

The inorganic phosphate, final enzymatic reaction product, had an inhibitory effect on the 4 types of phosphatase activities in the salt lake sediment. The inhibition was very obvious starting from an added inorganic phosphate concentration of 0.2 mg PO_4^{3-} – P/mL. No notable difference between the 4 types of studied phosphatase activity related to the inhibitory effect of the final reaction product added to the initial reaction mixtures has been observed.

The synthesis activity of the phosphatase is repressed by orthophosphate released ion concentration. After 200 days of incubation the level of enzymatic activity in all four cases was lower than 50% in the variant to which 2 mg/mL PO_4 – P were initially added, and around the same value in the variant initially supplemented with 0.2 mg/mL PO_4 – P.

When supplementary C and N sources are added to the experimental variants, the repressive effect of orthophosphate is substantially diminished and manifests itself only in the variant initially supplemented with 2 mg PO_4 – P. The less affected is the acid phosphatase. The manifestation of the repressive effect of orthophosphate may be diminished by the massive development of microbiota, which provides a sufficiently high level of phosphatase synthesis, so that this effect seems to be lacking. However, at the initial 2 mg/mL PO_4 – P concentration the repressive effect is obvious, being more evident in the case of the neutral phosphatase and lower in that of the acid phosphatase.

The β-glycerophosphate has an inducing effect on the synthesis and activity of phosphatase, especially of the acid phosphatase. The effect manifests itself after 100 days of incubation, even earlier in the case of the acid phosphatase.

Simultaneous supplementation of the experimental variants with orthophosphate and β-glycerophosphate demonstrates that in the case of the acid phosphatase the inducing effect prevails. In the case of the other phosphatase types, the repressive effect of the final reaction product prevails. Differences when compared to the control are small and they are not related to the concentration of the supplemented substance.

References

1. CHRÓST, R.J., OVERBECK, J., Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in Lake Plußsee (North German eutrophic lake), Microb. Ecol., **13**, 1987, p. 229
2. HUANG, X., MORRIS, J.T., Distribution of phosphatase activity in marsh sediments along an estuarine salinity gradient, Mar. Ecol. Progr. Ser., **292**, 2005 p. 75
3. JANSSON, M., OLSSON, H., BROBERG, O., Characterization of acid phosphatases in the acidified Lake G rdsjön, Sweden, Arch. Hydrobiol., **92**, 1981, p. 377
4. JIN, X.C., WANG, S.R., ZHAO, H.C., BU, Q.Y., CHU, J.Z, CUI, Z., ZHOU, X.N., WU, F.C., Effect of lake sediments of different trophic states on alkaline phosphatase activity, Lakes & Reservoirs: Research & Management, **11**, nr. 3, 2006, p. 169
5. KARL, D.M., CRAVEN, D.B., Effects of alkaline phosphatase activity on nucleotide measurements in aquatic microbial communities, Appl. Environ. Microbiol., **40**, nr. 3, 1980, p. 549
6. KEN'ICHI, N., CHIEKO, T., Changes in phosphatase activities in tideland sediments by loading of chemical substances, J. Environ. Chem., **14**, nr. 3, 2004, **p.** 495
7. KRÁMER, M., ERDEI, G., Primenenie metoda opredeliniya aktivnosti fosfatazy v agrokhimicheskikh issledovaniyakh, Pochvovedenie, **9**, 1959, p. 99
8. MUNTEAN, V., Inhibition of phosphatase activity in a salt lake sediment, a leached chernozem and a brown luvic soil, Stud. Univ. Babeş-Bolyai, Biol., **39**, nr. 2, 1994, p. 105
9. MUNTEAN, V., PASCA, D., CRISAN, R., Influence of substrate and final reaction product on synthesis and activity of phosphatase in a salt lake sediment. Stud. Univ. Babeş-Bolyai, Biol., **42**, nr. 1-2, 1997, p. 173
10. REJMÁNKOVÁ, E., MACEK, P., Response of root and sediment phosphatase activity to increased nutrients and salinity, Biogeochemistry, **90**, nr. 2, 2008, p. 159
11.SIUDA, W., Phosphatases and their role in organic phosphorus transformation in natural waters. A review, Pol. Arch. Hydrobiol., **31**, 1984, p. 207
12. SIUDA, W., GÜDE, H., A comparative study on 5'-nucleotidase (5'-nase) and alkaline phosphatase (APA) activities in two lakes, Pol. Arch. Hydrobiol., **131**, 1994, p. 211
13. ZHANG, T., WANG, X., JIN, X., Variations of alkaline phosphatase activity and P fractions in sediments of a shallow Chinese eutrophic lake (Lake Taihu), Environ. Pollut., **150**, nr. 2, p. 288
14. ZHOU, Y., SONG, C., CAO, X., CHUNLEI, S., LI, J., CHEN, G., LIANG, P., Variations in kinetics of alkaline phosphatase in sediments of eutrophic, shallow, Chinese lakes, Hydrobiologia, **581**, nr. 1, 200 , p. 109
15. ZHOU, Y., SONG, C., CAO, X., LI, J., CHEN, G., XIA, Z., JIANG, P., Phosphorus fractions and alkaline phosphatase activity in sediments of a large eutrophic Chinese lake (Lake Taihu), Hydrobiologia, **599**, 2008, p. 119

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