Regulation and Characterization of Acid and Alkaline Phosphatase in Yeast

By A. SCHURR* AND E. YAGIL

*Department of Biochemistry, Tel-Aviv University,
Tel-Aviv, Israel*

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SUMMARY

The activity of acid and alkaline phosphatase in baker's yeast is depressed when the organisms are starved of phosphate. Mutants lacking phosphatase activity and mutants with constitutive phosphatase synthesis were isolated. In all of them acid and alkaline phosphatases were affected simultaneously. Nevertheless, characterization of these two enzymes by sensitivity to inhibition by orthophosphate, temperature inactivation, proteolytic digestion and cation dependence showed that they were clearly distinct. It is suggested that these two enzymes share a common genetic and structural component.

INTRODUCTION

The regulation of non-specific phosphomonoesterase (phosphatase) activity by the concentration of orthophosphate in the culture medium has been reported in several organisms. These include *Escherichia coli* (Torriani, 1960), *Bacillus subtilis* (Fan, 1966; Moses, 1967), *Neurospora crassa* (Nyc, Kadner & Crocken, 1966), *Aspergillus* (Dorn, 1965; Dorn & Rivera, 1966; Shieh, Wodzinski, & Ware, 1969), *Euglena* (Blum, 1965; Bennun & Blum, 1966), *Torulopsis utilis* (Rautanen & Kärkäinen, 1951) and *Saccharomyces cerevisiae* (Suomalainen, Linko & Oura, 1960; Schmidt *et al.* 1963). The best understood of these regulatory systems is that of alkaline phosphatase in *E. coli*, in which it was clearly demonstrated that the derepression of enzyme activity caused by phosphate starvation is due to *de novo* enzyme synthesis (Garen & Levinthal, 1960); one structural and two regulatory loci were characterized and mapped (Echols, Garen, Garen & Torriani, 1961; Aono & Otsuji, 1968; Bracha & Yagil, 1969).

Two non-specific phosphomonoesterases – acid and alkaline phosphatase (EC 3.1.3.2 and EC 3.1.3.1, respectively) – are known to occur in baker's yeast (*Saccharomyces cerevisiae*). These two phosphatases differ both in their optimum pH activity and their cellular location, and their activity is derepressed upon phosphate starvation (Suomalainen *et al.* 1960; McLellan & Lampen, 1963; Schmidt *et al.* 1963; Tonino & Steyn-Parvé, 1963). No information is as yet available on the genetic aspects of this regulatory system. In this paper we describe the isolation of mutants impaired either in phosphatase activity or in the regulation of phosphatase synthesis. In all mutants isolated both acid and alkaline phosphatases were affected simultaneously and we therefore determined whether these two enzymes differed with respect to several other properties.

* Present address: Negev Institute for Arid Zone Research, Beer-Sheva, Israel.

METHODS

Organisms. (Abbreviations used: *leu* = leucine, *his* = histidine, *met* = methionine, *can-r* = resistance to canavanine.) The strains of *Saccharomyces cerevisiae* used in this study were s288c (mating type α), and three of its derivatives: x2180-1A (mating type a), s878c *leu*, *his*7 (mating type α) and EY9 *met*8*can-r* (mating type a). All were obtained from Professor R. K. Mortimer.

Media

YEPD medium. A rich medium used for plating. It contained per litre: 10 g. yeast extract, 20 g. Bacto peptone (Difco), 20 g. dextrose, 20 g. agar.

Minimal medium. A modification of the medium described by Kitazume, Yčas & Vincent (1962) was used for both liquid cultures and for plating. One litre 0.02 M-citrate buffer (pH 5.0) contained: 2 g. NH_4Cl , 0.5 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 g. CaCl_2 , 0.1 g. KCl , 30 g. glucose, 50 mg. DL-asparagine, 60 mg. inositol, 6 mg. thiamine-HCl, 1 mg. pyridoxine, 0.15 mg. biotin, 0.5 mg. Ca-pantothenate, 0.5 mg. citric acid. $1\text{H}_2\text{O}$, 0.5 mg. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 mg. $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, 25 μg . $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 5 μg . $\text{MnSO}_4 \cdot 1\text{H}_2\text{O}$, 5 μg . H_3BO_3 , 5 μg . $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$. Where necessary, 60 mg. L-leucine, 40 mg. DL-histidine or 20 mg. L-methionine/l. were added. For plating, agar at 2% was added. This minimal medium was supplemented with KH_2PO_4 as follows: High-Pi minimal medium, 0.4 g. KH_2PO_4 /l. (3 mM); Low-Pi minimal medium, 0.05 g. KH_2PO_4 /l. (0.37 mM) (used only for plating); No-Pi minimal medium, no added phosphate.

Growth conditions. The strains were maintained in YEPD slants. Organisms from a single colony were grown overnight in a reciprocating shaker water-bath (Tutenauer, Jerusalem, Israel) at 30°, in a minimal medium containing a limiting concentration of glucose (0.8 g./l.). At this concentration the culture reached an absorbancy (recorded in a Zeiss PMQ II spectrophotometer at 440 nm.) of approximately unity ($\sim 10^8$ organisms/ml.). The next morning the organisms were diluted into a fresh minimal medium to resume exponential growth (generation time of 140 to 150 min.). Growth was followed by the change in extinction at 440 nm. (E_{440}). Dilutions for viable counts were carried out in 0.02 M-citrate buffer (pH 5.0).

Preparation of organisms for enzymic assays. Unless otherwise indicated all assays were carried out with enzyme prepared from strain s288c grown for 24 h. in No-Pi minimal medium. The samples taken from the growth medium were centrifuged, washed once and resuspended in the appropriate buffer (0.1 M-acetate buffer, pH 3.65, for acid phosphatase and 0.1 M-tris-chloride buffer, pH 8.9, containing 10^{-3} M- MgSO_4 for alkaline phosphatase). For cell disruption 8 g. of glass beads (0.25 to 0.30 mm. diameter size) were added to 10 ml. of the suspension, which was then shaken for 3 min. in a Braun mechanical cell homogenizer (model MSK, type 2376). In preliminary experiments using various sizes of glass beads and different shaking times these conditions proved to be optimum for maximal enzyme recovery.

Mixed enzyme preparation. In some of the experiments the enzyme assay was performed in a mixture (v/v) of a supernatant of cells disrupted in 0.1 M-acetate buffer with a supernatant of cells disrupted in 0.1 M-tris-Mg buffer; the mixture had a final pH of 6. This procedure preserved most of the acid and alkaline phosphatase activity.

Assay of acid phosphatase. The activity of acid phosphatase is located on the outer

cell surface (McLellan & Lampen, 1963; Schmidt *et al.* 1963; Tonino & Steyn-Parvé, 1963). The enzyme assay was carried out either with a suspension of intact cells or with the supernatant of disrupted cells. The reaction mixture, in a final volume of 2 ml., contained 1.9 ml. *p*-nitrophenyl phosphate (NPP, 0.8 mg./ml.) dissolved in 0.1 M-acetate buffer and 0.1 ml. enzyme solution. After incubation for 5 to 25 min. at 37° the reaction was terminated with 0.5 ml. 1 M-NaOH, the mixture centrifuged when necessary and the extinction read at 410 nm. The yellow colour of the liberated *p*-nitrophenol is only apparent at alkaline pH. One enzyme unit (e.u.) is defined as the amount of enzyme catalysing the liberation of 1 μ mole nitrophenol/min. Fig. 1 shows the linearity of the reaction as a function either of time (Fig. 1*a*) or dilution (Fig. 1*b*) when a suspension of intact organisms was assayed.

Fig. 1. The activity of acid phosphatase (○) and alkaline phosphatase (●) as a function of time (*a*) or dilution (*b*). Acid phosphatase was determined in a suspension of whole organisms and alkaline phosphatase in a suspension of disrupted organisms. Enzyme concentration defined as unity (*b*) is the amount of enzyme extracted from a culture of approximately 5×10^7 organisms/ml.; serial dilutions of this enzyme were assayed.

Assay of alkaline phosphatase. The activity of this enzyme is internally located (Tonino & Steyn-Parvé, 1963) and the assay was carried out either with a suspension or with the supernatant of disrupted organisms. The reaction mixture was the same as for acid phosphatase except that the substrate (NPP) was dissolved in 1 M-tris-chloride buffer, pH 8.9, and the reaction stopped with 0.5 ml. 5 M-NaOH. Enzyme units are as for acid phosphatase. Fig. 1 shows the linearity of the reaction as a function either of time (Fig. 1*a*) or dilution (Fig. 1*b*) when a suspension of disrupted organisms was assayed.

Determination of phosphatase activity in colonies. A modification of the method used for colonies of *Aspergillus* and *Escherichia coli* (Dorn, 1965; Bracha & Yagil, 1969) was used. Plates were sprayed with a mixture of equal volumes of α -naphthyl phosphate (1 mg./ml., Calbiochem) and tetrazotized-*o*-dianizidine (5 mg./ml., Sigma). Both compounds were dissolved in 0.1 M-acetate buffer at pH 3.65. Dark-purple coloration of the colony indicated phosphatase activity.

Mutagenesis by ethylmethane sulphonate (EMS). The treatment was carried out as

described by Lindegren, Hwang, Oshima & Lindegren (1965). The treated organisms were centrifuged, resuspended in 0.02 M-citrate buffer (pH 5.0) and plated on High-Pi and on Low-Pi minimal medium.

Chemicals. *p*-Nitrophenyl phosphate, α -naphthyl phosphate and pronase were obtained from Calbiochem, tetrazotized-*o*-dianizidine from Sigma, ethylmethane sulphonate from Koch-Light, ethylene diaminetetraacetate (EDTA) from Merck, 1,10-phenanthroline from Eastman Organic Chemicals and 8-hydroxyquinoline from British Drug Houses.

Fig. 2. Effect of pH on phosphatase activity. Derepressed organisms were centrifuged and re-suspended either in 0.1 M-acetate buffer (pH 4) or in 0.1 M-tris-Mg buffer (pH 8). The two suspensions were disrupted and acid phosphatase (○) and alkaline phosphatase (●) determined at the appropriate pH; for pH 2.95 to 6.5 0.1 M-acetate buffer was used, and for pH 7.0 to 9.6 0.1 M-tris-HCl buffer was used.

RESULTS

Effect of pH on phosphatase activity

Organisms were grown in minimal medium under conditions of phosphate starvation which derepress the phosphatases (see below). The organisms were disrupted either in 0.1 M-acetate buffer (pH 5) or in tris-Mg buffer (pH 8) and the activity of each supernatant determined over a pH range of 2.95 to 9.6 (Fig. 2). The two optima, one at pH 3.65 and the other at pH 8.9, represent the activity of acid phosphatase and alkaline phosphatase respectively. Suomalainen *et al.* (1960) reported equivalent

pH optima of 4.4 and 7.9 to 8.9, and Tonino & Steyn-Parvé (1963) 3 to 4 and 9 to 10. The activity of acid phosphatase is approximately five times greater than that of alkaline phosphatase. In other experiments an even greater difference was obtained.

Fig. 3. Derepression of phosphatase by phosphate starvation. Organisms growing exponentially in minimal medium were washed and resuspended either in High-Pi medium (closed symbols) or in No-Pi medium (open symbols). Following resuspension, samples were removed at intervals of 2 h. for the determination of growth (insert) and of acid (○, ●) and of alkaline phosphatase (△, ▲). Enzyme activity is expressed as a function of growth.

Table 1. *Effect of phosphate in the culture medium on acid and alkaline phosphatase activity*

Organisms were grown for 24 h. in minimal medium containing KH_2PO_4 at the indicated concentration and the enzyme activity determined. Specific activity is expressed as e.u./ E_{440} .

Concentration of KH_2PO_4 (mM)	Acid phosphatase (sp. act. $\times 10^3$)	Alkaline phosphatase (sp. act. $\times 10^3$)
0	2.23	0.48
0.25	0.36	0.40
0.5	0.21	0.33
1.0	0.34	0.17
2.0	0.16	0.17
5.0	0.19	0.15

Regulation of phosphatase activity in the culture

Table 1 shows the enzyme activity of acid and alkaline phosphatases in cultures grown for 24 h. in minimal medium with various concentrations of phosphate. The response of acid phosphatase to regulation by phosphate is much more marked than that of alkaline phosphatase. Under phosphate starvation acid phosphatase becomes derepressed by a factor of approximately 12 and at 0.25 mM-KH₂PO₄ it is almost completely repressed. Alkaline phosphatase is only derepressed by a factor of 3 and approaches maximum repression at 1 mM-KH₂PO₄. Under maximum repression both enzymes show the same basal level activity.

Fig. 3 shows the kinetics of derepression of both phosphatases when a culture is resuspended either in No-Pi or High-Pi minimal medium. The data are presented as differential rate curves, i.e. enzyme activity as a function of growth (cf. Monod, Pappenheimer & Cohen-Bazire, 1952). In the phosphate-free culture acid phosphatase activity is markedly derepressed and reaches a steady state after approximately three generations of growth, at which time the growth of the culture slows down markedly due to lack of inorganic phosphate (Fig. 3, insert). The activity of alkaline phosphatase, which is derepressed to a lesser extent, seems to reach a steady state much earlier.

Structural and constitutive mutants for phosphatase synthesis

When wild-type yeast organisms are plated on Low-Pi (0.37 mM) minimal medium there is sufficient phosphate to permit growth of colonies, but due to exhaustion of phosphate the activity of phosphatase becomes derepressed. When sprayed for phosphatase activity wild-type repressible colonies are stained on Low-Pi plates and remain unstained on High-Pi plates. Mutants which have lost the capacity to synthesize active enzyme should not stain on either plate whereas constitutive mutants, in which enzyme synthesis is unaffected by the concentration of phosphate, should stain on both Low-Pi and High-Pi plates.

Wild-type organisms were treated with EMS and plated on either Low-Pi or High-Pi media. Surviving colonies were sprayed and those which did not stain on Low-Pi plates were suspected of being structural and those staining on High-Pi constitutive mutants. Eight out of 953 colonies growing on Low-Pi plates did not show enzyme activity and 13 out of 942 colonies growing on High-Pi were enzymically active. The enzyme assays of three structural mutants (PhoA⁻) shows that activity is either lost completely (PhoA⁻¹) or partially (PhoA⁻⁵⁷, PhoA⁻⁶⁷), but in all mutants isolated so far the activity of both acid and alkaline phosphatase is affected (Table 2). The constitutive mutants (PhoR⁻) show a high enzymic activity in the presence of phosphate. In some of them the activity is completely independent of phosphate (PhoR⁻⁵⁴) and in others it is partially repressed (PhoR⁻²⁹, PhoR⁻³¹). Also in the constitutive mutants both acid and alkaline phosphatase are affected. Furthermore, it may be noticed that the enzyme activity of the constitutive mutants is greater than in the wild-type derepressed cultures (Table 2). This is probably because phosphate starvation in the latter, which is required for derepression, is incomplete (Markham & Byrne, 1968). Full expression of the gene(s) involved is therefore prevented in the wild-type strains.

In all mutants tested, phosphatase-negative as well as constitutive, the activity of both enzymes was affected simultaneously. These results suggested that the two enzymes have structural as well as regulatory gene(s) in common. If this is so, in what ways do

these two enzymes differ? It has already been established that they are located differently in the cells and have different pH optima for maximum activity. The rest of the experiments to be described establish that these two enzymes also differ in other properties.

Table 2. *Phosphatase activity in structural and constitutive mutants and their wild-type parental strains*

The cultures were grown for 24 h. in either No-Pi or in High-Pi minimal medium before the enzyme activity was assayed. Specific activity is expressed as e.u./ E_{440} .

Strain	Mating type	Phenotype*	Medium	Acid phosphatase		Alkaline phosphatase	
				Sp. act. ($\times 10^3$)	Induction ratio	Sp. act. ($\times 10^3$)	Induction ratio
X-2180-1A	a	+	No-Pi	2.48	8.4	0.93	3.3
			High-Pi	0.29		0.28	
s878c	a	+	PhoA ⁻¹	No-Pi	—	0	—
			High-Pi	0			
			No-Pi	3.88	10.5	0.55	4.6
			High-Pi	0.37		0.12	
			PhoA ⁻⁵⁷	No-Pi	0.7	0.07	1.2
			High-Pi	0.06		0.06	
EY9	α	+	PhoR ⁻²⁹	No-Pi	1.3	0.80	1.2
			High-Pi	12.60		0.65	
			PhoR ⁻³¹	No-Pi	2.0	0.43	1.8
			High-Pi	6.70		0.24	
			No-Pi	2.20	9.5	0.44	4.9
			High-Pi	0.23		0.09	
			PhoA ⁻⁶⁷ (leaky)	No-Pi	3.6	0.15	1.9
			High-Pi	0.97		0.08	
			PhoR ⁻⁵⁴	No-Pi	1.0	1.73	1.1
			High-Pi	15.80		1.52	

* PhoA⁻¹ = phosphatase-negative, isolation number 1; PhoR⁻²⁹ = phosphatase-constitutive, isolation number 29.

Inhibition by orthophosphate and the reaction velocity

The activity of alkaline phosphatase of *Escherichia coli* and *Aspergillus* is inhibited competitively by orthophosphate (Torriani, 1960; Dorn, 1968; Shieh *et al.* 1969; Simpson & Vallee, 1969) as is the activity of acid phosphatase from *Euglena* (Bennun & Blum, 1966) and *Neurospora* (Nyc *et al.* 1966). The activity of acid phosphatase is strongly inhibited by K_2HPO_4 while that of alkaline phosphatase is almost unaffected; at 10^{-2} M only 15% of acid phosphatase remained active whereas alkaline phosphatase retained 75% of its activity (Fig. 4). Fig. 5(a, b) shows the reaction velocity of acid and alkaline phosphatase, as a function of substrate (*p*-nitrophenyl-phosphate) concentration at three concentrations of phosphate. The reaction kinetics of acid phosphatase (Fig. 5a) show inhibition by the product (phosphate) liberated during the reaction. Alkaline phosphatase (Fig. 5b) is much less inhibited by phosphate. Lineweaver-Burk plots of data from Fig. 5(a, b) are shown in Fig. 5(c); the K_m value of both enzymes is 4.5×10^{-4} M. A K_m value of 5.3×10^{-4} M was reported for acid phosphatase by Boer & Steyn-Parvé (1969). K_2HPO_4 proves to be a competitive inhibitor of acid phosphatase with a K_i value of 1.36×10^{-4} M. It should be pointed out that acid phosphatase extracted from a constitutive strain is likewise inhibited by K_2HPO_4 .

Temperature inactivation

An enzyme preparation was treated for 30 min. at the temperature indicated on the abscissa in Fig. 6, and both acid and alkaline phosphatase activities were measured. Acid phosphatase is completely inactivated at 45° at which temperature alkaline phosphatase is still completely active; inactivation of the latter is obtained only at 65°. According to Rothman & Coleman (1968), alkaline phosphatase in crude extracts of *Escherichia coli* is even more heat-resistant than acid phosphatase from the same organism.

Fig. 4. Effect of phosphate on phosphatase activity. Acid phosphatase (○) and alkaline phosphatase (●) were assayed at increasing concentrations of K_2HPO_4 .

Inactivation by pronase

A preparation of phosphatases was treated for various periods with pronase (20 μ g./ml.) at 37° before assaying enzyme activity (Fig. 7). It is evident that only acid phosphatase is sensitive to the proteolytic enzyme while alkaline phosphatase is practically unaffected. The alkaline phosphatase of *Escherichia coli* is similarly resistant to digestion by pronase whereas the acid phosphatase is sensitive (Rothman & Coleman, 1968).

Inactivation by chelating agents

Escherichia coli alkaline phosphatase is a zinc metalloenzyme (Plocke, Levinthal & Vallee, 1962; Simpson & Vallee, 1969), and alkaline phosphatase from various other sources requires divalent cations for its activity (Dorn, 1968; Kaneko, 1968). Chelating agents, being metal-binding compounds, can therefore abolish the activity of this enzyme and its activity can be restored by replacement of the required metal ions.

The effect of three chelating agents was tested on yeast phosphatase activity. An enzymically active supernatant was assayed with increasing concentrations of chelates (Fig. 8). All three chelating agents – EDTA (Fig. 8*a*), 1,10-phenanthroline (Fig. 8*b*) and 8-hydroxyquinoline (Fig. 8*c*) – specifically inhibit alkaline phosphatase activity and have either no effect (in the case of EDTA) or a relatively small effect on acid phosphatase. The inactivation kinetics of alkaline phosphatase by 10^{-2} M-EDTA is shown in Fig. 9; maximum inhibition of activity is obtained instantaneously after

Fig. 5. Phosphatase activity as a function of substrate (*p*-nitrophenyl phosphate) concentration at various phosphate concentrations. (a) Acid phosphatase; (b) alkaline phosphatase; (c) Lineweaver-Burk plot of data from (a) and (b) O, ● = no K_2HPO_4 ; Δ , $\blacktriangle$ = 10^{-3} M- K_2HPO_4 ; $\square$, $\blacksquare$ = 10^{-2} M- K_2HPO_4 .

the addition of the chelate. Restoration of enzyme activity after the addition of increasing concentrations of either zinc or magnesium ions is shown in Fig. 10. While Zn^{2+} has very little effect, Mg^{2+} completely restores enzyme activity. The activity obtained with 10^{-3} M- Mg^{2+} is even higher than that of the original enzyme preparation extracted in tris buffer without $MgSO_4$. These results clearly show that alkaline phosphatase of *Saccharomyces cerevisiae* requires magnesium ions for its activation whereas the activity of acid phosphatase is cation-independent.

DISCUSSION

Both acid and alkaline phosphatase of baker's yeast are regulated by the concentration of phosphate in the culture medium. After phosphate starvation these two enzymes are derepressed; the activity of acid phosphatase increases by a factor of about 10 and that of alkaline phosphatase by a factor of 4. It is expected that the acid

Fig. 6

Fig. 7

Fig. 6. Effect of temperature on phosphatase activity. A mixed enzyme preparation was incubated for 30 min. at the indicated temperatures, and acid phosphatase (○) and alkaline phosphatase (●) assayed.

Fig. 7. Effect of digestion by pronase on phosphatase activity. Pronase (20 μ g./ml.) was added at time 0 to a mixed enzyme preparation which was incubated at 37°. At intervals samples were removed for the determination of acid phosphatase (○) and alkaline phosphatase (●).

Fig. 8. Effect of chelating agents on phosphatase activity. Acid phosphatase (○) and alkaline phosphatase (●) were assayed in a mixed enzyme preparation in the presence of various concentrations of the chelate. (a) Ethylene diaminetetraacetate (EDTA); (b) 1, 10-phenanthroline; (c) 8-hydroxyquinoline.

phosphatase is the more important enzyme in the yeast cell since optimum growth is obtained at acid pH. Garen & Levinthal (1960) and Blum (1965) have shown that derepression of phosphatases in *Escherichia coli* and *Euglena gracilis* is the result of *de novo* enzyme synthesis. It is reasonable to assume that this is also the case in yeast.

Fig. 9

Fig. 9. Kinetics of alkaline phosphatase inactivation by EDTA. A mixed enzyme preparation was assayed at short time intervals for acid (○) and alkaline (●) phosphatase; at 30 sec. EDTA was added to final concentration of 10^{-2} M.

Fig. 10

Fig. 10. Effect of Mg^{2+} and Zn^{2+} on the activity of alkaline phosphatase inhibited by EDTA. The enzyme was prepared in tris buffer without $MgSO_4$ and the activity of the preparation is shown by the broken line. It was treated with EDTA at a final concentration of 10^{-2} M and assayed in the presence of increasing concentrations of either $MgSO_4$ (▲) or $ZnSO_4$ (●).

All structural or constitutive mutants isolated so far affect both acid and alkaline phosphatase simultaneously. It should be pointed out, however, that the mutants were isolated under the acidic conditions which are optimal for growth, so that enzyme activity observed during selection of the mutants was that of acid phosphatase. Dorn (1965) has similarly isolated mutants of *Aspergillus* lacking both enzymes, but he also found additional mutants which had lost the activity of each enzyme separately.

The two enzymes are non-specific monoesterases (Schmidt & Laskowsky, 1961); nevertheless they seem to be very distinct enzymes. In addition to their different pH optima they are located differently in the organisms, react differently to inhibition by phosphate, to temperature inactivation, to digestive attack by pronase and to cation depletion. Furthermore, acid and alkaline phosphatases from several sources differ in their specificity for various substrates (Neumann, 1968). It is therefore surprising that constitutive and, in particular, structural mutations affect both enzymes simultaneously. Due to the relatively high mutation rate obtained ($\sim 1\%$), it is unlikely that double mutants were selected. It is more plausible that both enzymes have a genetic regulatory mechanism and a genetic structure in common. They may either share a common polypeptide or one of the enzymes is a subunit of the other.

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