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Phosphatase Hydrolysis of Organic Phosphorus Compounds

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Abstract

Phosphatases are diverse groups of enzymes that deserve special attention because of their significant roles in organic phosphorus (OP) mineralization to inorganic available forms (Pi). This work 1) compared the catalytic potentials of commercially acid phosphatase from wheat germ, sweet potato, and potato, and alkaline phosphatase from *E. coli*; 2) demonstrated that the rate of hydrolysis, catalytic efficiency, thermal stability, and optimal pH of these enzymes depended on enzyme sources and the stereochemical or stereoisomeric structures of the substrates; 3) revealed that both acid and alkaline phosphatases exhibited broad range of substrate hydrolysis with high affinity for *p*-nitrophenyl phosphate bis (cyclohexylammonium) than the widely used *p*-nitrophenyl phosphate disodium hexahydrate for phosphatase assay. Sweet potato had relatively higher reaction kinetics (V_{max}, K_m, K_{cat}, K_{cat}/K_m) values with most substrates tested. The order of catalytic activity was in the order: sweet potato > wheat germ > potato, while the order of substrate hydrolyzed was: PNPBC > PNP > PNP2A2E > DG6P2Na > DG6PNa > Bis-PNP > phytate. The optimum pH for the acid phosphatase was observed to be 5.0. Generally, the activity of alkaline phosphatase was similar to that of acid phosphatase with optimal pH between 10 and 13, depending on the substrates. Knowledge derived from this work would be helpful in enzyme catalysis in soils and water environments.

Keywords

Enzymes, Phosphatases, Organic Phosphorus Mineralization, Wheat Germ, Sweet Potato, *E. coli*

1. Introduction

Phosphatases are diverse groups of enzymes that deserve special attention because of their significant roles in

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organic phosphorus (OP) mineralization to inorganic available forms [1]-[4]. These enzymes have been classified into five major groups: (phosphoric monoester hydrolases (EC 3.1.3); phosphoric diester hydrolases (EC 3.1.4); triphosphoric monoester hydrolases (EC 3.1.5); polyphosphate hydrolase (EC 3.1.8); and phosphoamidase (EC 3.9.1.1), depending on the bonds they cleave [5].

While phosphatases generally hydrolyze phosphoric (H_3PO_4) esters and anhydrides to release phosphate, they differ in their pH optima, metal ion requirements, substrate specificities, and reaction mechanisms. Acid and alkaline phosphatases have been used to mineralize specific OP compounds in animal manure, soils, water, and sediments [6]-[12]. Both acid and alkaline phosphatases are known to hydrolyze a variety of phosphomono esters. The hydrolysis of organic P compounds using specific phosphatase enzymes both in soils and biological systems is vital for energy metabolisms, metabolic regulations, and cellular signal transductions pathways [13]-[16].

Low P availability in soils may trigger phosphatase secretions to the rhizosphere; however, soil phosphatases are sometimes derived from 1) intracellular enzymes or enzymes bound to cell components and 2) abiotic or extracellular enzymes leaking from intact cells or released from dead or lysed cells that originate from the cell membrane [17]. It has been shown that acid phosphatase in soils are of both plant and microbial origins, while alkaline phosphatase is mostly of microbial origin [4] [18]. Significant positive correlation between alkaline phosphatase activity and soil bacterial populations have been reported [19]. The dephosphorylation of OP compounds in soils largely depends on the stereochemical and stereoisomeric structures of the OP compounds in question, in addition to the type and enzyme sources involved in such catalytic hydrolysis. The high affinity of some enzymes for particular substrates has been used as the basis to identify and quantify specific OP compounds, or as part of post separation quantification step [20]-[23]. Strickland and Solorzano [24] used alkaline phosphatase to assess bioavailable P hydrolyzed by exocellular algal phosphatase released due to phosphate depletion.

A distinctive feature of alkaline phosphatase is the presence of two Zn^{2+} and one Mg^{2+} ions per sub unit [25], [26] which is lacking in acid phosphatase [13]. Our understanding of the precise role and function of phosphatase enzymes in mineralizing OP in soils is constrained to some extent by limitations of the methods used since there is no standard method to determine soil enzyme reactions or hydrolysis. Para-nitrophenyl phosphate and bis-para-nitrophenyl have been widely used to assay soil phosphomonoesterase and phosphodiesterase activities [27]. Although para-nitrophenyl has been commonly used to assay soil phosphatase hydrolysis, this may not accurately reflect the relative hydrolysis of various soil phosphohydrolases [28]. Thus, understanding the behavior of enzymes in pure systems with different substrates would improve our understanding of enzyme hydrolysis in soils and water environments. Such studies may enhance our understanding of the contributions of individual enzymes to specific reactions in soils and water environments, and enable predicting the status of key reactions participating in rate limiting steps during the decomposition or degradation of organic materials and transformation of elements [11] [20] [29]. Previously, we have investigated the hydrolysis of organic phosphates by commercially available phytase from wheat and fungi [30]. With this study, we have focused on commercially available acid phosphatase from wheat germ, sweet potato, and potato, and alkaline phosphatase from *E. coli* that catalyze simple monoester bond. These four enzymes are selected because some have been used in characterizing and quantifying OP in various environmental samples [22] [29] [31] [32]. The goals are to show that: 1) phosphatase enzyme catalytic efficiency and thermal stability are functions of the enzyme sources and the type of substrates involved, and 2) phosphatase enzyme exhibits broad range of substrate hydrolysis.

2. Materials and Methods

2.1. Organic Phosphate Compounds and Enzymes

P-nitrophenyl phosphate disodium hexahydrate (>97%) (PNP), *p*-nitrophenyl phosphate di (2-amino-2-ethyl-1, 3 propanediol) (PNP2A2E), *P*-nitrophenyl phosphate bis (cyclohexylammonium) (PNPBC), Bis *p*-nitrophenyl phosphate sodium (Bis-PNP), D-glucose 6-phosphate sodium salt (98%) (DG6PNa), D-glucose 6-phosphate disodium hydrate (98% - 100%) (DG6P2Na), and inositol hexakisphosphate (phytic acid sodium salt) substrates are shown in **Figure 1**, and acid phosphatase from wheat germ, sweet potato, and potato (EC 3.1.3.2; 0.4, 14, and 1 $U \cdot mg^{-1}$), and alkaline phosphatase from *E. coli* type III (EC 3.1.3.1, 24 $U \cdot mg^{-1}$) were used in this study. They were all purchased from Sigma-Aldrich, St. Louis, Missouri, USA; however, the enzymes were used without further purification.

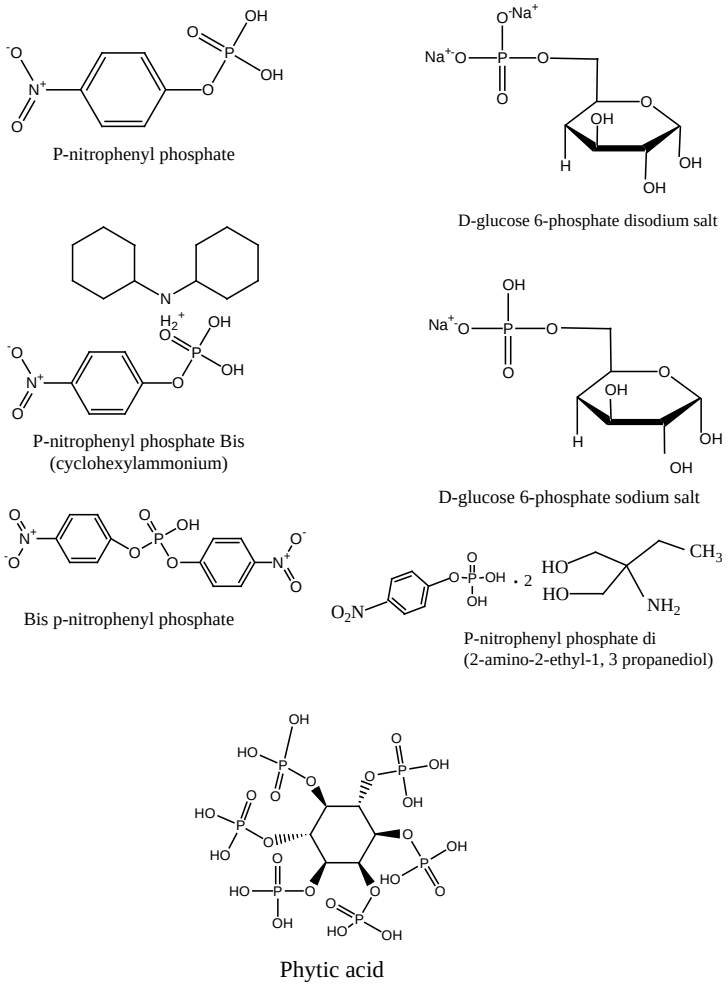

Figure 1. Stereochemical structures of substrates.

2.2. Assay Conditions

The optimal pH for the phosphatases as reported by the supplier is 4.8 for acid phosphatase, and 10.4 for alkaline phosphatase, while the optimal temperature is 37˚C for both acid and alkaline phosphatase. One unit (U) of the enzyme is reported to liberate 1.0 µmol orthophosphate with the appropriate substrate at the appropriate pH and temperature. With acid and alkaline phosphatases, concentrations of 0.013 - 0.166 $U \cdot mL^{-1}$ were used to hydrolyze the substrate. The effects of pH, temperature, and time on each enzyme was determined by exposure to a wide range of temperatures ranging from 10˚C to 80˚C; pH ranging from 2 to 9, and time ranging from 1 to 10 hours using a substrate concentration of 5 times the K_m [33].

2.3. Kinetic Determination

To determine the kinetic parameters of each enzyme, each substrate (phosphate compound) analog was hydrolyzed at increasing concentrations with each enzyme at the supplier's reported pH and temperature for 1 hour. The substrate was dissolved in 100 mM acetate buffer (pH 4.8) and a final enzyme concentration of 0.066, 0.013, and 0.166 $U \cdot mL^{-1}$ for wheat germ, sweet potato, and potato respectively used for acid phosphatase hydrolysis. For alkaline phosphatase hydrolysis, each substrate was dissolved in 87 mM glycine buffer, (pH 10.4) and a final enzyme concentration of 0.166 $U \cdot mL^{-1}$ used. All reaction mixtures were carried out in a total volume of 3 mL and the reaction stopped using 10% sodium dodecyl sulfate at the end of the incubation period. The controls were set up by incubating the substrate without enzyme to correct for the Pi released due to chemical hydrolysis. The amount of Pi released was determined colorimetrically [34] and results plotted against substrate concentra-

tions using the Michaelis-Mentenmodel (V = V_{max}[S]/[S] + K_m) which has been reported to account for the kinetic properties of several enzymes. The Michaelis-Menten constant (K_m), indicative of enzyme-substrate affinity; V_{max}, the maximum reaction velocity at enzyme saturation; K_{cat}, ratio of V_{max} to concentration of active sites; and K_{cat}/K_m, the turnover number of an enzyme, were obtained from the linear regression fit of the Michaelis-Menten equation (plots not shown). The K_{cat}/K_m is also the rate constant due to the interaction of substrate and enzyme and measures enzyme catalytic efficiency. Catalytic efficiency provides a better estimate of enzyme-substrate affinity than the K_m. The value may be used to compare an enyme's preference for various substrates or in characterizing enzymes in pure systems where the actual enzyme concentration is known.

Chemical reaction rates generally double with every 10˚C increase in temperature and is also known as temperature coefficient (Q_{10}). The calculations [phosphatase activity at T(˚C)/phosphatase activity at T(˚C) − 10˚C] to determine the temperature coefficients (Q_{10}) were at 10˚C intervals between 0 and 70˚C. When an enzyme reaction obeys the Arrhenius equation [k = A. exp ($-E_a/RT$)], the activation energy (E_a) or slope can be estimated from the logarithmic transformed equation [log k = ($-E_a$/2.303RT) + log A], where k is the rate constant, A is the Arrhenius constant, R is the gas constant (8.314 $mol^{-1}{\cdot}k^{-1}$), and T is the temperature on the Kelvin scale.

3. Results and Discussion

3.1. Activation Energies and Kinetic Parameters

The Arrhenius plots were linear between 10˚C and 50˚C for acid phosphatase from wheat germ (**Figure 2(a)**), between 10˚C and 70˚C for acid phosphatase from sweet potato (**Figure 2(b)**), between 10˚C and 60˚C acid phosphatase from potato (**Figure 2(c)**), and between 10˚C and 90˚C for alkaline phosphatase (**Figure 3**). The calculated E_a expressed in $kJ{\cdot}mole^{-1}$ ranged from 18.0 to 36.1 for acid phosphatase from wheat germ, 19.6 - 30.5 for acid phosphatase from sweet potato, 19.2 - 49.6 for acid phosphatase from potato, and 15.7 - 41.2 for alkaline phosphatase from *E. coli* (**Table 1**). The average temperature coefficient (Q_{10}) values, for acid phosphatase from wheat germ and potato between 20˚C and 70˚C ranged from 1.12 - 1.37 (**Table 2**) and 1.10 - 1.62 (**Table 3**) respectively, while the average Q_{10} values for acid phosphatase from sweet potato between 20˚C - 90˚C ranged from 1.03 - 1.19 (**Table 4**) and for alkaline phosphatase from *E. coli* ranged from 1.21 - 1.63 (**Table 5**).

The kinetic parameters calculated for the hydrolyzed substrates by acid phosphatase are shown in **Table 6** and **Table 7**. The maximal rate (V_{max}) reveals the turnover number of an enzyme, which is the number of substrate molecules converted into products by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate. The K_{cat} reveals the turnover number of an enzyme and is equal to the kinetic constant. The higher the K_{cat} value the greater the specificity of the enzyme in catalyzing the substrate. Berg *et al.* [35] reported that the K_{cat} of most enzymes using their physiological substrates fall in the range of 1 to 10^4 s^{-1}. This study indicates the substrates can be divided into three groups (specific; less specific; and non-specific), based on their kinetic parameters. The specific group includes PNP, PNP2A2E, and PNPBC, less specific group includes DG6PNa, and DG6P2Na, and the non specific group includes Bis-PNP and phytate. Siddiqua *et al.* [36] considers *p*-NPP, phenyl phosphate, *α*- and *β*-naphthyl phosphate and *β*-glycerol phosphate preferable to phosphoamino acids, nucleoside phosphates and sugar phosphates for acid phosphatase.

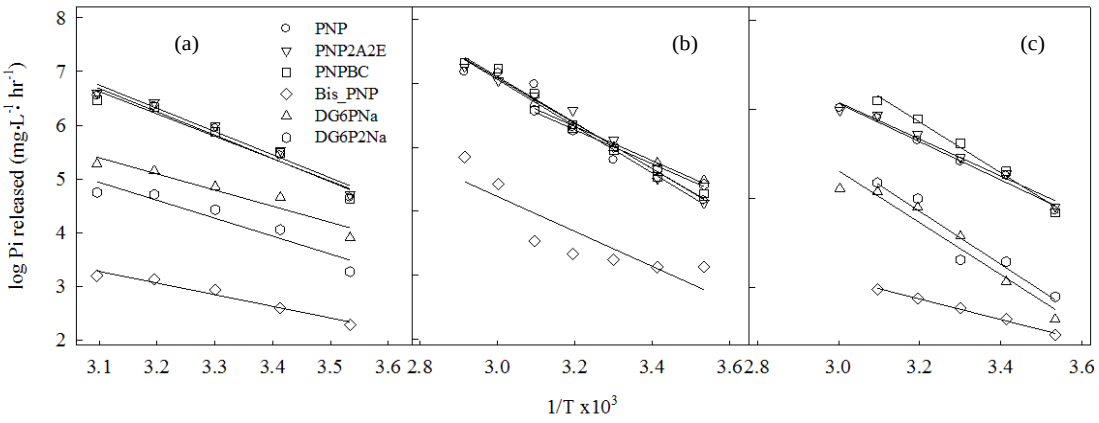

Figure 2. Arrhenius equation plot of acid phosphatase. (a) wheat germ; (b) sweet potato; (c) potato.

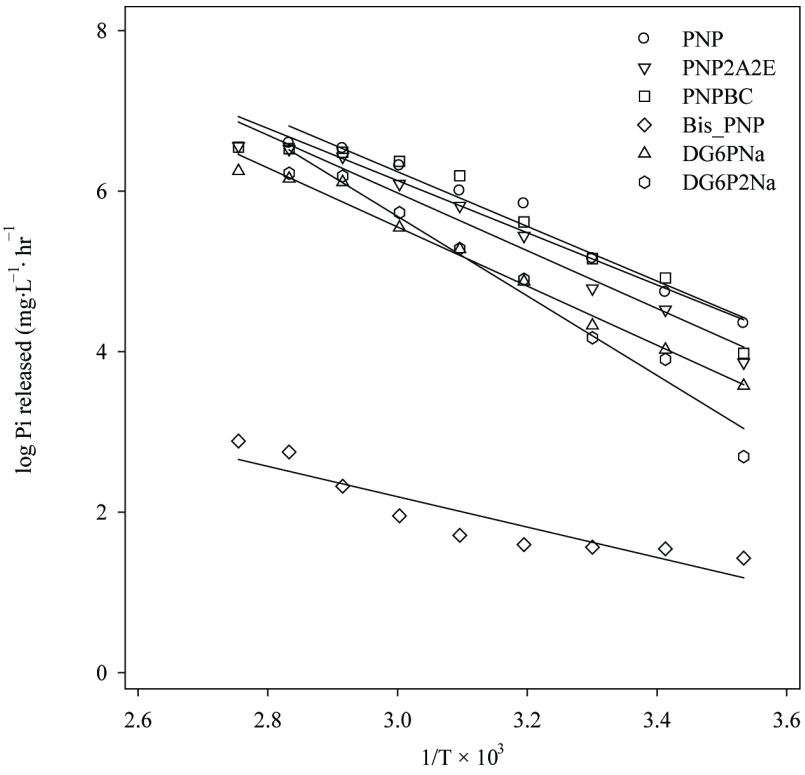

Figure 3. Arrhenius equation plot of alkaline phosphatase (*E. coli*) activity.

Table 1. Activation energy (E_a) values of acid and alkaline phosphatase activities.

Substrate	Acid phosphatase		Alkaline phosphatase	
	Wheat germ	Sweet potato	Potato	*E. coli*
	kJ·mol^{-1}			
PNP	36.1	30.5	36.8	28.3
PNP2A2E	35.9	29.5	34.5	29.3
PNPBC	34.7	30.1	47.4	27.2
Bis-PNP	18.0	22.8	19.2	15.7
DG6PNa	25.0	19.6	48.5	30.6
DG6P2Na	27.8	22.3	49.6	41.2

PNP (*p*-nitrophenyl phosphate disodium hexahydrate); PNP2A2E (*p*-nitrophenyl phosphate di [2-amino-2-ethyl-1, 3 propanediol]); PNPBC (*P*-nitrophenyl phosphate biscyclohexylammonium); Bis-PNP (Bis *p*-nitrophenyl phosphate sodium); DG6PNa (D-glucose 6-phosphate sodium salt); DG6P2Na (D-glucose 6-phosphate disodium hydrate).

Table 2. Temperature coefficients (Q_{10}) of the acid phosphatase activity from wheat germ.

Substrate	20	30	40	50	60	70	Mean
	˚C						
PNP	2.20	1.65	1.51	1.21	0.75	0.74	1.34
PNP2A2E	2.28	1.58	1.54	1.19	0.76	0.88	1.37
PNPBC	2.37	1.48	1.58	1.14	0.73	0.80	1.35
Bis-PNP	1.37	1.41	1.21	1.10	0.92	0.69	1.12
DG6PNa	2.13	1.22	1.35	1.13	0.49	0.42	1.12
DG6P2Na	2.19	1.45	1.33	1.04	0.53	0.33	1.14

Q_{10} = phosphatase activity at T(˚C)/phosphatase activity at T(˚C) − 10˚C, PNP (*p*-nitrophenyl phosphate disodium hexahydrate); PNP2A2E (*p*-nitrophenyl phosphate di [2-amino-2-ethyl-1,3propanediol]); PNPBC (*P*-nitrophenyl phosphate biscyclohexylammonium); Bis-PNP (Bis *p*-nitrophenyl phosphate sodium); DG6PNa (D-glucose 6-phosphate sodium salt); DG6P2Na (D-glucose 6-phosphate disodium hydrate).

Table 3. Temperature coefficients (Q_{10}) of acid phosphatase activity from potato.

Substrate	20 °C	30 °C	40 °C	50 °C	60 °C	70 °C	Mean
PNP	2.19	1.38	1.61	1.65	1.25	0.42	1.42
PNP2A2E	2.09	1.43	1.70	1.54	1.10	0.44	1.38
PNPBC	2.56	1.86	1.72	1.51	0.67	0.50	1.47
Bis-PNP	1.42	1.29	1.25	1.22	0.77	0.63	1.10
DG6PNa	2.32	2.78	1.92	1.41	1.07	0.20	1.62
DG6P2Na	2.2	1.05	3.95	1.43	0.43	0.57	1.61

Q_{10} = phosphatase activity at T(˚C)/phosphatase activity at T(˚C) − 10˚C; PNP (*p*-nitrophenyl phosphate disodium hexahydrate); PNP2A2E (*p*-nitrophenyl phosphate di [2-amino-2-ethyl-1,3propanediol]); PNPBC (*P*-nitrophenyl phosphate biscyclohexylammonium); Bis-PNP (Bis *p*-nitrophenyl phosphate sodium); DG6PNa (D-glucose 6-phosphate sodium salt); DG6P2Na (D-glucose 6-phosphate disodium hydrate).

Table 4. Temperature coefficients (Q_{10}) of acid phosphatase activity from sweet potato.

Substrate	20 °C	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C	90 °C	Mean
PNP	1.40	1.34	1.67	1.96	1.19	1.02	0.61	0.300	1.19
PNP2A2E	1.46	1.83	1.59	1.25	1.26	1.25	0.39	0.47	1.19
PNPBC	1.29	1.52	1.50	1.63	1.48	1.09	0.40	0.13	1.13
Bis-PNP	1.00	1.12	1.10	1.22	2.44	1.52	0.42	0.40	1.15
DG6PNa	1.31	2.27	1.35	1.50	0.91	0.27	0.85	0.77	1.03
DG6P2Na	1.28	1.41	1.30	1.36	0.96	0.93	0.04	1.00	1.03

Q_{10} = phosphatase activity at T(˚C)/phosphatase activity at T(˚C) − 10˚C.

Table 5. Temperature coefficients (Q_{10}) of alkaline phosphstaseactivity from *E. coli*.

Substrate	20 °C	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C	90 °C	Mean
PNP	1.50	1.53	1.98	1.17	1.37	1.24	1.10	0.92	1.35
PNP2A2E	1.93	1.30	1.92	1.46	1.32	1.40	1.10	1.04	1.43
PNPBC	2.55	1.25	1.58	1.78	1.20	1.12	1.04	1.02	1.44
Bis-PNP	2.20	1.02	1.03	1.12	1.27	1.45	1.53	1.15	1.21
DG6PNa	1.57	1.35	1.73	1.49	1.31	1.76	1.04	1.10	1.42
DG6P2Na	3.36	1.31	2.08	1.46	1.57	1.58	1.04	0.67	1.63

Q_{10} = phosphatase activity at T(˚C)/phosphatase activity at T(˚C) − 10˚C; PNP (*p*-nitrophenyl phosphate disodium hexahydrate); PNP2A2E (*p*-nitrophenyl phosphate di [2-amino-2-ethyl-1, 3 propanediol]); PNPBC (*P*-nitrophenyl phosphate biscyclohexylammonium); Bis-PNP (Bis *p*-nitrophenyl phosphate sodium); DG6PNa (D-glucose 6-phosphate sodium salt); DG6P2Na (D-glucose 6-phosphate disodium hydrate).

Table 6. V_{max}, K_m, K_{cat}, K_{cat}/K_m values of acid phosphatase activities.

	Wheat germ				Sweet potato				Potato			
	V_{max} (mg·L^{-1}·hr^{-1})	K_m (mM)	K_{cat} (hr^{-1})	K_{cat}/K_m (hr^{-1}·mM^{-1})	V_{max} (mg·L^{-1}·hr^{-1})	K_m (mM)	K_{cat} (hr^{-1})	K_{cat}/K_m (hr^{-1}·mM^{-1})	V_{max} (mg·L^{-1}·hr^{-1})	K_m (mM)	K_{cat} (hr^{-1})	K_{cat}/K_m (hr^{-1}·mM^{-1})
Phytate	65	11.8	984	83	72.1	10.4	1803	173	165	14.0	330	24
PNP	152	3.65	2303	631	135	2.69	10 385	3861	143	4.53	861	190
PNP2A2E	154	4.34	2333	538	130	3.32	10 000	3012	162	4.58	976	213
PNPBC	145	3.47	2197	633	126	2.25	9692	4308	166	5.00	1000	200
Bis-PNP	5.21	2.20	78.9	36	5.35	6.92	409	59.0	2.73	2.26	17	7.50
DG6PNa	38.8	5.16	588	114	223*	8.86	1343	152	44.61	3.20	269	84.0
DG6P2Na	31.6	1.70	479	282	251*	11.13	1512	136	43.00	1.90	259	136

PNP (*p*-nitrophenyl phosphate disodium hexahydrate); PNP2A2E (*p*-nitrophenyl phosphate di [-2-amino-2-ethyl^{-1}, 3 propanediol]); PNPBC (*P*-nitrophenyl phosphate bis (cyclohexylammonium); Bis-PNP (Bis *p*-nitrophenyl phosphate sodium); DG6PNa (D-glucose 6-phosphate sodium salt); DG6P2Na (D-glucose 6-phosphate disodium hydrate); * (0.166 U/ml of enzyme used).

Table 7. V_{max}, K_m, K_{cat}, K_{cat}/K_m values of alkaline phosphatase (*E. coli*) activity.

Substrate	V_{max} (mg·L^{-1}·hr^{-1})	K_m (mM)	K_{cat} (hr^{-1})	K_{cat}/K_m (hr^{-1}·mM^{-1})
PNP	216	6.55	1301	199
PNP2A2E	204	6.40	1229	192
PNPBC	198	5.79	1195	206
Bis-PNP	nd	nd	nd	nd
DG6PNa	88.5	4.87	533	110
DG6P2Na	82.8	5.50	499	91

PNP (*p*-nitrophenyl phosphate disodium hexahydrate); PNP2A2E (*p*-nitrophenyl phosphate di [-2-amino-2-ethyl^{-1}, 3 propanediol]); PNPBC (*p*-nitrophenyl phosphate bis (cyclohexylammonium); Bis-PNP (Bis *p*-nitrophenyl phosphate sodium); DG6PNa (D-glucose 6-phosphate sodium salt); DG6P2Na (D-glucose 6-phosphate disodium hydrate); nd: not determined.

The K_m value for acid phosphatase from sweet potato was the lowest (2.25 - 3.32 mM) for the specific group while that of potato was the highest (4.53 - 5.00 mM). For the specific group, a relatively low K_m value of 2.25 mM and 3.47 mM were obtained for sweet potato and wheat germ respectively using PNPBC. For the specific group, the K_{cat}/K_m value for acid phosphatase from sweet potato was the highest (3012 - 4308 h^{-1}·mM^{-1}) while that of potato was the lowest (190 - 213 h^{-1}·mM^{-1}). The K_{cat}/K_m value of acid phosphatase from all species using PNPBC was greater than when PNP and PNP2A2E were used respectively. Acid phosphatase from sweet potato had the highest K_{cat} values (9692 - 10,385 h^{-1}) while acid phosphatase from potato had the lowest values (861 - 1000 h^{-1}) for the specific group (**Table 6**).

For the less specific group, acid phosphatase from sweet potato had the highest K_m values ranging from 8.86 - 11.13 mM, and K_{cat} values ranging from 1343 - 1512 h^{-1}, than acid phosphatase from wheat germ and potato. Acid phosphatase from wheat germ and potato had low K_m values (1.7 and 1.9 mM, and high values of K_{cat}/K_m of 282 and 84 h^{-1}·mM^{-1} respectively) with DG6P2Na than DG6PNa as substrate. The non-specific group showed little activity with acid phosphatase from all three species. Wyss *et al*. [38] reported a broad range of substrate hydrolysis with acid phosphatase from *A. niger*, but with little activity using phytic acid. The order of catalytic activity for the acid phosphatase was in the order: sweet potato > wheat germ > potato, while the order of substrate hydrolyzed was: PNPBC > PNP > PNP2A2E > DG6P2Na > DG6PNa > Bis-PNP > phytate.

Results showing the enzyme kinetics for alkaline phosphatase from *E. coli* are presented in **Table 7**. The K_m values using PNPBC was lower than when PNP2A2E and PNP were used while the use of DG6PNa was lower than using DG6P2Na as substrate. The K_{cat} values with PNP was greater than with PNP2A2E and PNPBC. The K_{cat}/K_m of *E. coli* with PNPBC was four units higher than with PNP, and fourteen units higher than with PNP2A2E. The K_{cat} and K_{cat}/K_m value for alkaline phosphatase from *E. coli* on DG6PNa was higher than that of DG6P2Na. The order of substrate hydrolyzedwas: PNPBC > PNP > PNP2A2E > DG6PNa > DG6P2Na > Bis-PNP.

While other investigators [37] [39]-[43] [28] have reported K_m values for various substrates hydrolyzed by acid and alkaline phosphatase from various organisms, some of the values obtained in our study were higher than those reported which might be due to differences in substrate concentrations and the types and sources of the enzymes. The ratio (K_{cat}/K_m) is used as a measure of catalytic efficiency and to compare an enzyme's preference for various substrates. Higher values indicate higher substrate preference by the enzyme. This study suggests that acid phosphatase has a preference for PNPBC than the traditionally used PNP.

3.2. Effects of Time, Temperature, and pH on Activity

Figure 4 shows the dephosphorylation of PNP, PNP2A2E, PNPBC, Bis-PNP, DG6PNa, and DG6P2Naby acid phosphatase with time. The activity of wheat germ acid phosphatase with time was linear for up to 3 hours with PNP, PNP2A2E, and PNPBC, DG6PNa, and DG6P2Na, up to 4 hours, while Bis-PNP was linear up to 10 hours (**Figure 4(a)**). The rate of hydrolysis of these substrates with sweet potato acid phosphatase was slightly different (**Figure 4(b)**). The rate of hydrolysis with PNP, PNP2A2E, and PNPBC was linear for up to 4 hours, while with DG6PNa, and DG6P2Na, it was linear for up to 6 hours, and with Bis-PNP up to 10 hours. Similar curves were observed when these substrates analogs were hydrolyzed with potato acid phosphatase (**Figure 4(c)**), except that Bis-PNP, DG6PNa, and DG6P2Na were linear up to 10 hours.

Hydrolysis of these substrates by *E. coli* alkaline phosphatase (**Figure 5**), shows that PNP, PNP2A2E, DG6-

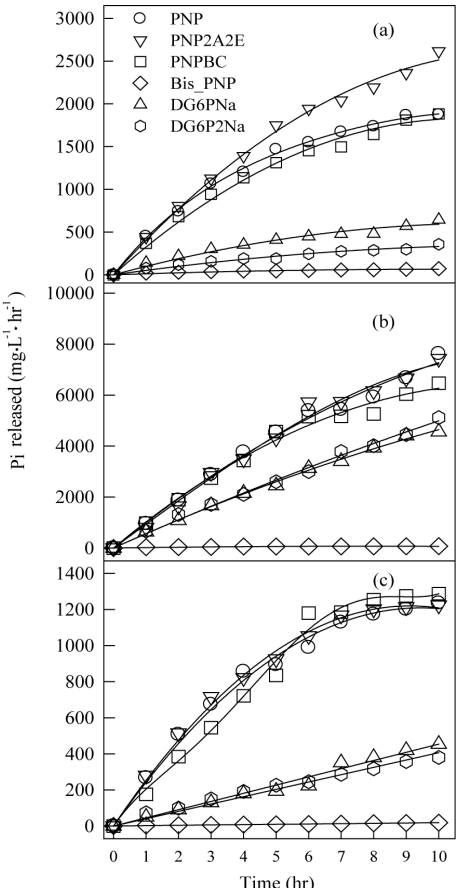

Figure 4. Effect of incubation time on acid phosphatase activity (a) wheat germ; (b) sweet potato; (c) potato.

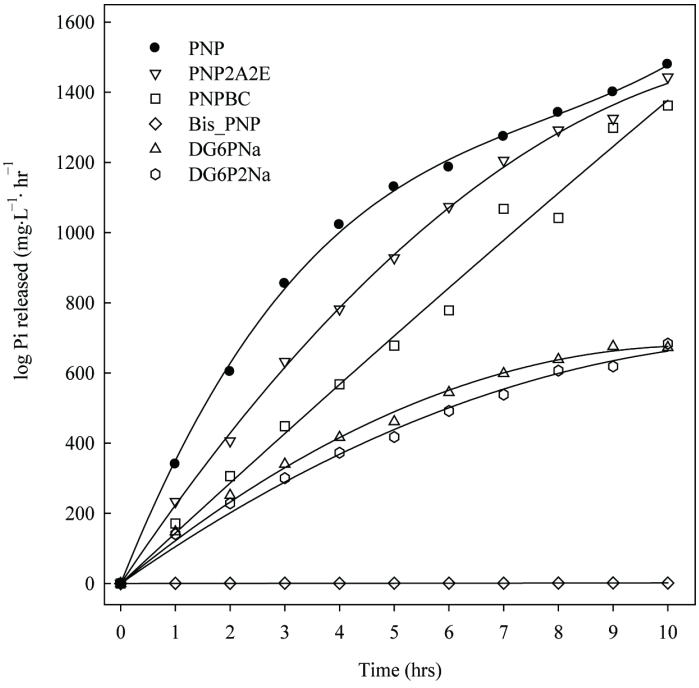

Figure 5. Effect of incubation time on alkaline phosphatase (*E. coli*) activity.

PNa, and DG6P2Na were linear up to 3 hours while PNPBC and Bis-PNP were linear up to 10 hours. Nigam *et al*. [43] reported that *β*-glycerophosphate was linear for a much shorter time than phenyl-phosphate and *p*-nitrophenyl phosphate using human prostatic acid phosphatase. The time at which the curve begins to be non-linear indicates enzyme saturation or product inhibition. Our findings show that both acid and alkaline phosphatase have similar time course curve with PNP, PNP2A2E, PNPBC, and Bis-PNP. Similar findings was reported by Martin *et al*. [41] in which potato acid phosphatase and *E. coli* alkaline phosphatase showed similar time course with both L-tyrosine O-phosphate and 3-fluoro-DL-tyrosine O-phosphate. According to these investigators, potato acid phosphatase and *E. coli* alkaline phosphatase are known to hydrolyze phosphate esters of dissimilar structures with equivalent maximum velocities.

The optimum temperature for the acid phosphatase is shown in **Figure 6**. Wheat germ acid phosphatase optimum temperature was at 50˚C (**Figure 6(a)**). Sweet potato acid phosphatase responded differently to temperature based on the substrate used. This enzyme was active for up to 70˚C with PNP, PNP2A2E, PNPBC, and Bis-PNP, and 50˚C with DG6PNa and DG6P2Na (**Figure 6(b)**). Potato acid phosphatase also responded differently to temperature depending on the substrate used. This enzyme was inactivated at temperatures above 50˚C

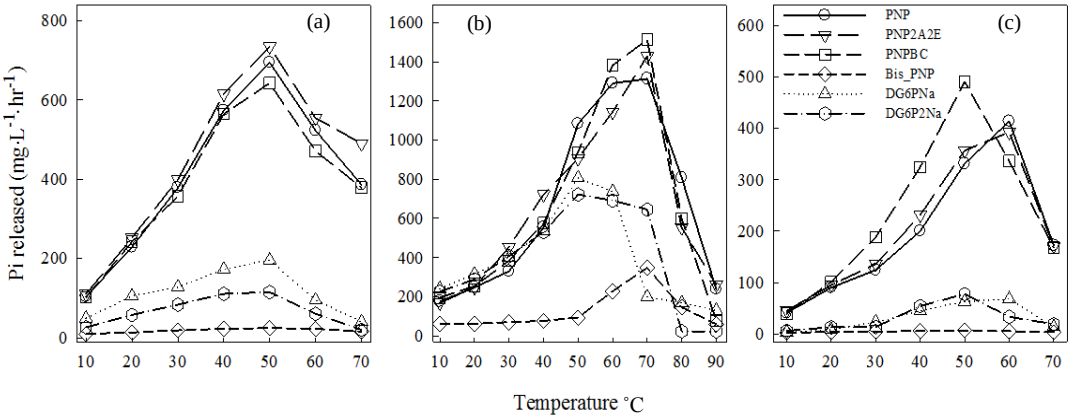

Figure 6. Effect of temperature on acid phosphatase. (a) wheat germ; (b) sweet potato; (c) potato.

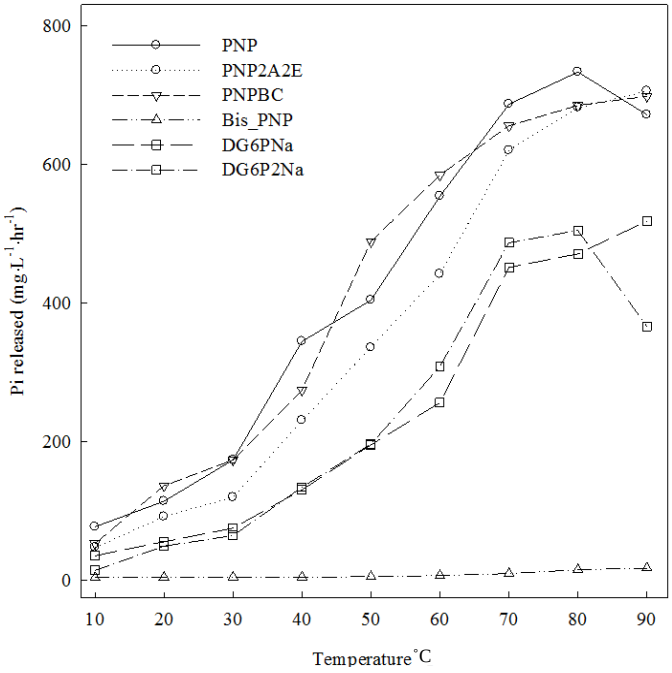

Figure 7. Effect of temperature on alkaline phosphatase. (*E. coli*) activity.

with PNPBC and DG6P2Na, 60˚C with PNP, PNP2A2E, and DG6PNa (**Figure 6(c)**). Asaduzzaman *et al*. [39] reported an optimum temperature of 55˚C for a germinating Black Gram (*Vignamungo L.)* seedling whereas Siddiqua *et al*. [36] reported an optimum temperature of 40˚C for *Rohu fish* liver acid phosphatase. The optimum temperature of activity for *E. coli* alkaline phosphatase was 80˚C with PNP and DG6P2Na, 90˚C with PNP2A2E, PNPBC, DG6PNa, and Bis-PNP (**Figure 7**).

The activity of each enzyme was also compared at various pH values (range, 2 - 9) using a concentration 5 times the K_m [33]. The pH for maximal hydrolysis of PNP, PNP2A2E, PNPBC, DG6PNa, and Bis-PNP by wheat germ acid phosphatase was 5.0 and 4.0 with DG6P2Na (**Figure 8(a)**). Sweet potato acid phosphatase showed a stable optimum pH between 5.0 and 6.0 for the hydrolysis of PNP, PNP2A2E, PNPBC, and Bis-PNP (**Figure 8(b)**). The optimum pH for the hydrolysis of DG6PNa was 6.0 and a pH of 5.0 using DG6P2Na (**Figure 8(b)**). These results are consistent with the findings by [39] [40] [43]-[45]. The pH optimum for potato acid phosphatase was 5.0 (**Figure 8(c)**).*Escherichia coli* alkaline phosphatase showed an optimal pH of 13.0 with PNP2A2E, PNPBC, and Bis-PNP, 10.0 with PNP, DG6PNa, and DG6P2Na (**Figure 9**), similar to the pH values reported by Cathala and Brunel [27] and Wilson *et al*. [46] but one unit higher than that reported by Mushak and Coleman [47]. The pH of alkaline phosphatase with PNP was two units higher than that reported by the supplier.

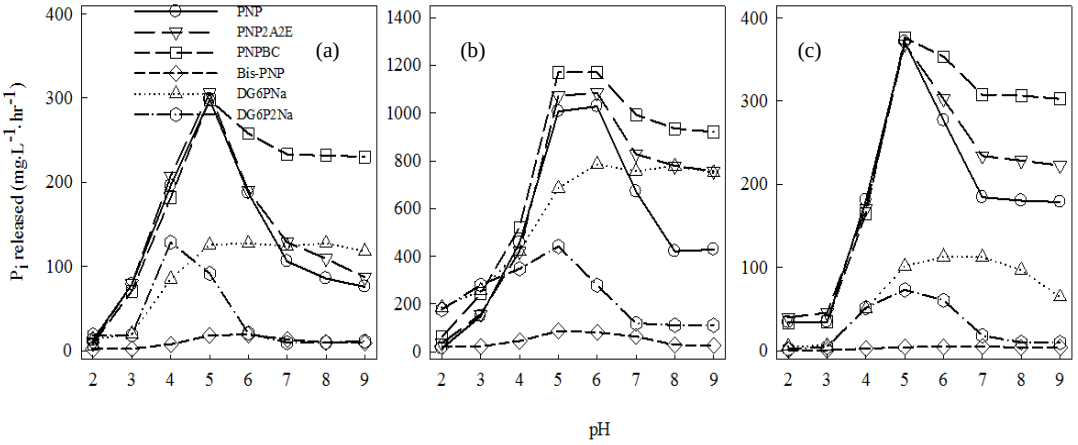

Figure 8. Effect of PH on acid phosphatase. (a) wheat germ; (b) sweet potato; (c) potato.

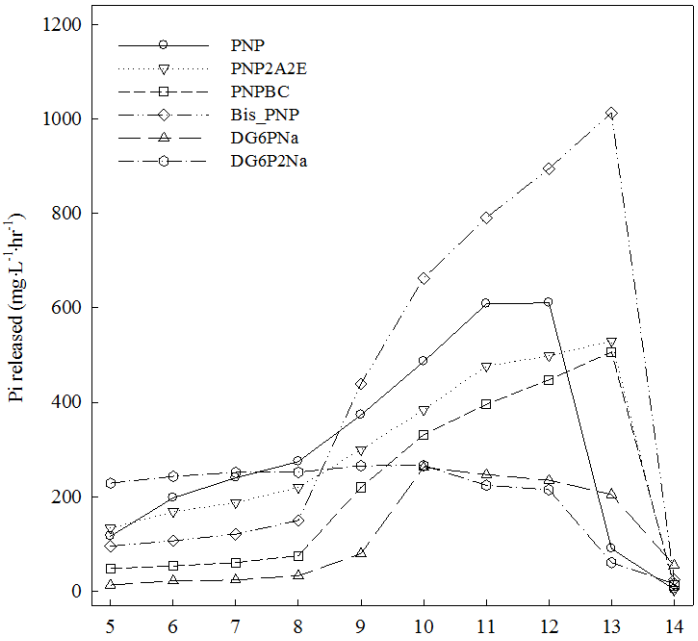

Figure 9. Effect of pH on alkaline phosphatase (*E. coli*) activity.

4. Conclusion

Our study indicates that the rate of hydrolysis, catalytic efficiency, thermal stability, and optimal pH of these enzymes may depend on enzyme sources and the stereochemical or stereoisomeric structures of the substrates. This study also reveals that acid and alkaline phosphatases do exhibit a broad range of substrate hydrolysis with high affinity for PNPBC than the commonly used PNP. Sweet potato had relatively higher reaction kinetics (V_{max}, K_m, K_{cat}, K_{cat}/K_m) values with most substrates tested. The order of catalytic activity for the acid phosphatase was in the order: sweet potato > wheat germ > potato, while the order of substrate hydrolyzed was: PNPBC > PNP > PNP2A2E > DG6P2Na > DG6PNa > Bis-PNP > phytate. The optimum pH for the acid phosphatase was observed to be 5.0. Alkaline phosphatase activity was similar to that of the acid phosphatase except that there was more alkaline phosphatase activity with DG6PNa than DG6P2Na.

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