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REVIEW

A Review of the Reactivity of Phosphatase Controlled by Clays and Clay Minerals: Implications for Understanding Phosphorus Mineralization in Soils

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Abstract Mineralizable macronutrients (e.g. C, N, P, and S) are sorbed readily (i.e. adsorption and precipitation) in clays and clay minerals. Phosphorus (P) is one of the limiting macronutrients in soils because both phosphate and organic P undergo chemisorption in soil minerals. Furthermore, phosphatases that mineralize the organic P species tend to partition into soil minerals, suppressing the interactions between organic P and phosphatase. Adsorbed phosphatase on the mineral surfaces can regulate the enzyme activity and influence the biochemical properties of the enzyme (e.g. kinetics, conformation, and stability), affecting the P cycle in the terrestrial environment. Phosphatase–mineral interactions are widely reported to decrease the enzyme activity while enhancing the enzyme stability (e.g. thermal and proteolysis stability). Contradictory findings have also been reported. Specific enzymes, mineral characteristics, and reaction conditions are probably responsible for various reactivity (e.g. mineralization). The purpose of the present review was to summarize current and past investigations of acid and alkaline phosphatase sorption in clays and clay minerals and to examine phosphatase chemical properties (e.g. kinetic activity, thermal and proteolysis stability) and factors (e.g. pH, saturating cations of the mineral, enzyme structure, and mineral surface polarity) influencing the phosphatase-mineral interaction. Lastly, also reviewed is the application of phosphatase–mineral interactions with some expansion to other enzymes as an indication of potential future application for phosphatase and future research needs.

Keywords Adsorption · Mineralization · Minerals · Phosphatase · Phosphorus

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Introduction

Globally, around half of the topsoils contain 15–30% clay and the clay content in ~ 20% of the topsoils is > 30% (Wei et al., 2014). Depending on factors such as parent material and weathering, the dominant clays and clay minerals vary in different soils. For example, in weathered soils, Al and Fe (oxyhydr)oxides are the dominant clay minerals (Zimmerman & Ahn, 2011). In young soils developed from volcanic parent materials, allophanes and ferrihydrite are found commonly (Allison, 2006). Clays from the illite and mica groups are commonly found in arid and high-latitude regions, while humid tropical soils contain predominantly 1:1 kaolinite groups (Ito & Wagai, 2017). Fibrous silicates such as palygorskite and sepiolite are found in calcareous and gypsiferous soils in arid regions

(Shirvani et al., 2020). Smectite-group minerals are ubiquitous in many soils, especially in the semi-arid and arid regions, in Vertisols, and in continental and oceanic sediments (Ito & Wagai, 2017; Odom, 1984).

The extracellular enzymes are common in soils. They are excreted by soil microbes such as fungi and actinomycetes, and by plants. The extracellular enzymes carry out many critical functions including mineralizing soil nutrients. They are also involved in the formation and decomposition of soil organic matter, the bioremediation of polluted soils, and the nutrient cycling processes such as mineralization and immobilization (Bollag et al., 1994; Burns et al., 2013; Naidja et al., 2000; Zimmerman & Ahn, 2011). Furthermore, the activity of some enzymes can be used as a soil quality indicator to assess soil health, soil productivity, and ecological functionality (Alkorta et al., 2003; Gil-Sotres et al., 2005).

The secreted, extracellular enzymes in soils are known to be susceptible to adsorption and immobilization by soil minerals. The interaction between soil enzymes and minerals has been discussed for decades, covering a wide range of studies from the investigation of pure layered silicate–enzyme reactions to models mimicking the realistic soil mineral colloid–enzyme interaction (Naidja et al., 2000). Datta et al. (2017) also summarized some key results on the adsorption process (e.g. driving forces, adsorption isotherm) of enzymes on minerals and on adsorption kinetics. The general effects of some soil parameters on enzyme adsorption and some key factors that affect enzyme activity were also reviewed in the same work.

Phosphatases, as a group of important enzymes in soils, along with their properties and activities, have been studied extensively but only brief discussion about the interaction between phosphatases and clay minerals has been included in reviews with a general topic of enzyme–clay mineral interaction (Nannipieri et al., 2011). More comprehensive reviews focusing on phosphatase behavior and properties during adsorption are still lacking. The objective of the present review, therefore, was to focus specifically on the adsorption of phosphatases on clay minerals and how it alters several important properties of phosphatases.

In recognition of the potential application of immobilized (e.g. adsorbed) enzymes, many reviews discuss potential industrial applications. For instance, Jesionowski et al. (2014) focused on selecting an appropriate carrier as the adsorbent for an enzyme and compared the advantages of different kinds of commonly used carriers (e.g. silicas, metals, clay minerals, and natural and synthetic polymers). Many recent reviews discussed different industrial applications of commonly used clay mineral-immobilized enzymes (e.g. An et al., 2015; Basso & Serban, 2019; Maghraby et al., 2023). Application of the interaction between phosphatase and minerals is also discussed in the present review, therefore. As current application of such interactions is still limited, this review also expands the discussion into enzymes that can mineralize other macronutrients as indications of future potential application of phosphatases.

Mineralizable Macronutrient Retention in Soils by Clays and Clay Minerals

Soil minerals play an important role in numerous soil reactions and one of the most important roles is the retention of mineralizable macronutrients (C, N, P, and S). With small particle size and, therefore, large specific surface area, the minerals carry abundant positive and negative charges on their internal and external surfaces, making them effective in interacting with different forms of mineralizable macronutrients, including inorganic anions (e.g. phosphate, nitrate, and sulfate), cations (e.g. NH_4^+), and organic compounds (e.g. enzymes, organic acids).

The distribution of charges on the variable-charge mineral surface is not homogeneous, and the two sources of charges are permanent charge, which is an inherent characteristic of the mineral, and pH-dependent charge. For some clay minerals, such as allophane, kaolinite, and Al/Fe (oxyhydr)oxides, the charges are predominantly pH-dependent while a large percentage of permanent charge is often observed in chlorite and 2:1 clays such as smectite and vermiculite (McBride, 1989; Sumner, 2000).

Dissolved ions attracted and retained by clays can exchange with free ions in the surrounding soil solution and the cation- and anion-exchange capacities of a specific clay mineral are pH-dependent. pH-dependent charges arise through the adsorption of ions and protonation and deprotonation of the exposed hydroxyl groups on the edges of the mineral surfaces where the mineral unit layer is disrupted (Cross & Yariv, 1979).

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The complexation between ions and the charged mineral surfaces can be divided into two mechanisms: inner-sphere and outer-sphere complexation. In the case of outer-sphere complexation, water molecules are involved and form a bridge between the adsorbed ion and the clay-mineral surface. Outer-sphere complexation is, therefore, rather weak, rapid, and reversible, as it mainly involves electrostatic interactions and occurs only between surfaces and ions that bear the opposite charges (Sparks, 2003).

In contrast, the adsorbed ion is bonded directly to the mineral surface in inner-sphere complexation, which provides a relatively strong bonding environment and is often irreversible compared to outer-sphere complexation (Sparks, 2003). Depending on the characteristics of the adsorbate and adsorbent, inner-sphere adsorbed ions are less susceptible to being exchanged easily by ions other than the outer-sphere adsorbed ones (Sposito, 1984).

Through ion-exchange reactions, the minerals can store and supply the adsorbed ions, largely affecting the fate and bioavailability of nutrients in soils. For example, a large amount of nitrate has been found to be retained in Oxisols and Ultisols (Kome et al., 2019; Lehmann et al., 2004). Ammonium is commonly fixed in the interlayers of 2:1 phyllosilicates (e.g. vermiculite, montmorillonite) (Mamo et al., 1993). Clays such as Fe and Al (oxyhydr)oxides (e.g. ferrihydrite, gibbsite), and clay minerals such as allophane and imogolite are effective at retaining phosphate and organic phosphorus compounds through adsorption and precipitation (Redel et al., 2016; Sims & Sharpley, 2005).

The retention of mineralizable macronutrients may also be a result of the interaction between the minerals and enzymes involved in the mineralization. The enzyme–clay mineral interaction can regulate enzyme activities and alter their kinetic properties (Datta et al., 2017). As the free enzymes are probably prone to rapid denaturation, the bound forms may serve as a reservoir of potential enzyme activity (Burns, 1982; Rejsek et al., 2012; Shindo et al., 2002). In some cases, the association between enzymes and minerals stabilized the structure of the enzymes, and the lifetime of the enzyme was prolonged while high activity was maintained (Bollag et al., 1994; Burns et al., 2013). The stabilization effect can also protect the enzymes against proteolysis and abiotic processes such as thermal denaturation and dehydration (Zimmerman & Ahn, 2011). Decreased enzyme activity upon enzyme adsorption, however, has also been documented widely when the adsorption made the active site of the enzyme less accessible or when enzyme deformation was induced (Dick & Tabatabai, 1987; Hughes & Simpson, 1978; Makboul & Ottow, 1979; Tietjen & Wetzel, 2003; Zimmerman & Ahn, 2011). Other properties related to the enzyme activity (e.g. optimal temperature, pH, or substrate affinity) may be largely influenced by adsorption to minerals, which would indirectly affect the mineralizable macronutrient cycles, such as enzymatic degradation of organic C, N, P, and S (Rao et al., 2000).

Importance of Organic P Mineralization

Of all the macronutrients, P is acknowledged to be rather immobile and unavailable due to precipitation with various metals and the partitioning reaction (e.g. adsorption) with minerals and organic matter (Abel et al., 2002; Quiquampiox & Mousain, 2005; Vance et al., 2003). Adsorbed phosphates are difficult to exchange due to transformation into more recalcitrant precipitates. Furthermore, the clay-sized colloidal phosphate has also been reported to have enhanced mobility and is prone to loss via surface runoff and subsurface routes such as preferential flow, leading to nutrient loss (Chen & Arai, 2020). Phosphorus deficiency, therefore, has been observed in many soils, such as tropical forests and weathered soils, and biomass accumulation and productivity can be severely constrained in P-deficient soils (Oliverio et al., 2020). Furthermore, due to the strong fixation of phosphate in soils, the inefficiency of phosphate fertilizer has been a problem, so an excessive amount is usually applied (Vance et al., 2003; Zhang et al., 2019). A consequence of excessive P fertilization is the loss of P to waters, resulting in eutrophication. By estimation, the inexpensive yet finite and non-renewable source of the widely used rock phosphate fertilizer is likely to be depleted within decades, especially given the increasing requirement for P fertilization and the political instability of some areas where rock phosphate is found (Abelson, 1999; Garske & Ekardt, 2021; Richardson et al., 2011). As a result, the price of phosphate fertilizer has increased to >$700/ton (with an increase of $100–400/ton since 2009) recently in 2021 following the sharp

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price increase in 2008 (Beghin & Nogueira, 2021; Cordell & White, 2014).

Soils can provide phosphate through weathering of mineral phosphate and mineralization of organic P compounds (Quiquampiox & Mousain, 2005). Organic P accounts for 20–80% of total P in soils and it can represent a particularly important potential pool to provide phosphate, especially in soils with no fertilizer input and/or poor phosphate solubility (Tiessen et al., 1984). Other strategies such as promoted mineralization of the organic P, therefore, could be another option to increase the P availability in soils. Phosphohydrolases are a group of enzymes that can catalyze the hydrolysis of organic P and some inorganic P such as linear polyphosphate and pyrophosphate (Quiquampiox & Mousain, 2005). Another group of enzymes that can release P from organic P compounds in soils is lyases involved in the P cycle (Rodríguez et al., 2006).

Classification of Soil Phosphohydrolases and Phosphate-Releasing Lyases

Phosphohydrolases can catalyze the hydrolysis of different bonds and they can be classified depending on the bond type according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) (Fig. 1). The Enzyme Commission (EC) number is used as a numerical classification to categorize enzymes based on the reactions catalyzed. For example, many phosphohydrolases target the ester bonds. Phosphoric monoester hydrolases (EC 3.1.3) are enzymes that can cleave the ester bonds on a phosphomonoester. Of all phosphoric monoester hydrolases, phosphatases have been studied widely, and depending on their optimal pH (below or above pH 7.0), phosphatases can be categorized into acid phosphatases (EC 3.1.3.2) and alkaline phosphatases (EC 3.1.3.1) (Vincent et al., 1992). Depending on substrate specificity, phosphatases can also be grouped into, for example, protein serine/threonine phosphatase (PSTP or PSP), protein tyrosine phosphatase (PTP), histidine phosphatase, lipid phosphatase, sugar phosphatases, and glycerophosphatases (Alef & Nannipieri, 1995; Fahs et al., 2016). Other examples of phosphoric monoester hydrolases include phytases and nucleotidases.

Other ester bond-targeting phosphohydrolases consist of phosphoric diester hydrolases (EC 3.1.4) such as phospholipases, triphosphoric monoester hydrolases (EC 3.1.5), diphosphoric monoester hydrolases (EC 3.1.7), and phosphoric triester hydrolases (EC 3.1.8).

Phosphohydrolases also include enzymes acting on guanosine triphosphate (GTP) (EC 3.6.5), enzymes targeting phosphoryl-containing anhydrides (EC 3.6.1) such as pyrophosphotases (or diphosphate phosphohydrolase) (EC 3.6.1.1) which catalyze the hydrolysis of pyrophosphate, phosphoamidases which act on the P–N bonds (EC 3.9.1), and phosphonatases (or phosphonate hydrolases) (EC 3.11) (e.g. phosphonoacetaldehyde hydrolase, phosphonopyruvate hydrolase, and phosphonoacetate hydrolase) which target the C–P bond and hydrolyze C-phosphono groups (Alef & Nannipieri, 1995; Chin et al., 2016; Nannipieri et al., 2011; White & Metcalf, 2007).

Lyases are enzymes that can catalyze the cleavage of bonds by means other than hydrolysis or oxidation and they can also be classified according to the bonds they attack. For example, a group of P-O lyases (EC 4.6), the ribonucleases, are usually secreted under phosphate starvation conditions, and they can catalyze the cleavage of P–O bonds in RNAs (Abel et al., 2002; Bariola et al., 1994). C-P lyases (EC 4.7) have also been found in phosphate-limited environments and they target the C–P bonds in organophosphonates (Oliverio et al., 2020; Tapia-Torres et al., 2016).

Reaction Mechanisms of Phosphohydrolases and Phosphate-Releasing Lyases

The majority of P-containing organic compounds contain the ester bond, and the activity of the phosphomonoesterases is usually greater than that of phosphodi- or phosphotri-esterases in soils (Alef & Nannipieri, 1995). Among all phosphomonoesterases in soils, alkaline and acid phosphatases are those studied most widely (Nannipieri et al., 2011).

Alkaline phosphatases have a common conserved core structure containing a serine residue that binds with phosphate on the phosphomonoester during the catalysis, resulting in the formation of a phosphoserine intermediate. The fully active site for each alkaline phosphatase monomer also contains two Zn(II) which are bridged by the phosphate group, and one Mg(II) coordinated with a water molecule that is hydrogen-bonded to the phosphate (Coleman, 1992; Kim & Wyckoff, 1991; Stec et al., 2000). Unlike alkaline phosphatases, a

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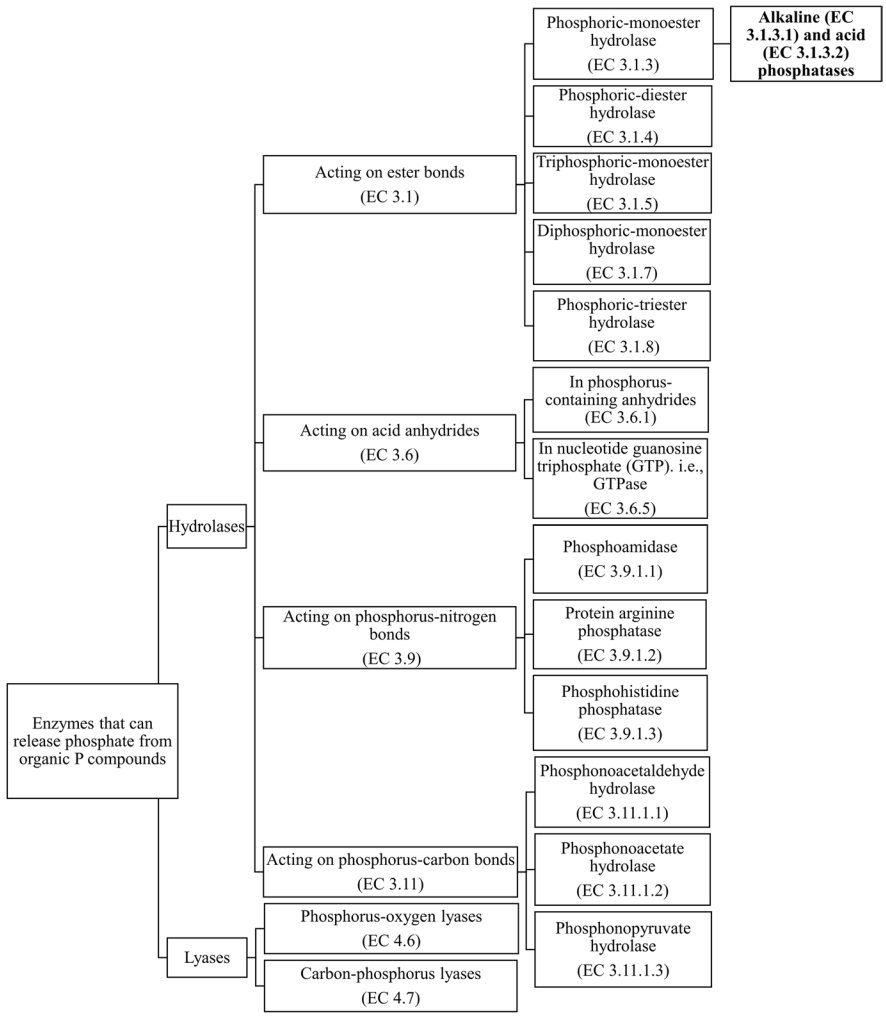

Fig. 1 Classification of enzymes that can release phosphate from organic P compounds, based on the enzyme database from the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). Alkaline (EC 3.1.3.1) and acid (EC 3.1.3.2) phosphatases are the main focus of the subsequent sections

histidine residue acts as the nucleophilic acceptor in the catalytic process of acid phosphatases, and phosphohistidine intermediates are formed. Acid phosphatases also have conserved arginine residues and many of them carry a characteristic RHGXRXP (R: arginine; H: histidine; G:glycine; P: proline; X: any amino acid) motif (some acid phosphatases such as *Aspergillus niger* acid phosphatase only have the RHG motif) (Ostanin et al., 1992; Ullah et al., 1991). For both alkaline and acid phosphatase, the final hydrolysis products are phosphate and corresponding hydrocarbons such as alcohol or phenol (Fig. 2). Phosphodiesters are converted to corresponding phosphomonoesters through phosphodiesterases, which then go through subsequent hydrolysis via phosphatases and release phosphate (Blake et al., 2005). Phosphotriesters are hydrolyzed to phosphodiesters which then experience subsequent hydrolysis while releasing phosphate. *myo*-Inositol hexakisphosphate, or phytic acid, is the predominant source organic P in many soils, accounting for more than half of the organic P pool (Anderson, 1980; Liu et al., 2018). The enzymes that hydrolyze phytic acid are referred to as phytases and, depending on catalytic mechanisms, they are generally classified into four categories. The histidine acid phytases (HAPhys) share a similar catalysis mechanism, such as the conserved

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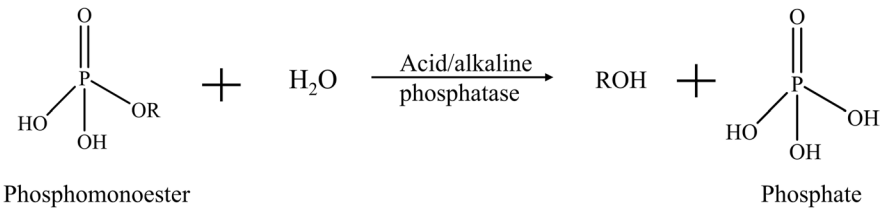

Fig. 2 Phosphomonoester hydrolysis reaction catalyzed by alkaline and acid phosphatases

RHGXRXP motif and the formation of phosphohistidine intermediate (Oh et al., 2004; Ullah et al., 1991). β-Propeller phytases (BPPhys) have an optimum alkaline pH, and metal ions, especially Ca(II), are found in the active site and contribute to the catalytic activity of BPPhy (Kim et al., 1998). Shin et al. (2001) classified the active sites on the enzyme and categorized them into "cleavage site" and "affinity site." Two adjacent phosphate groups on a phytic acid molecule bind simultaneously with Ca(II) in the cleavage site and affinity site, respectively. The phosphate bound to the cleavage site would later be removed, while the other one increases the affinity of enzymes for the remaining *myo*-inositol polyphosphate intermediate. Cysteine phytases (Cphys) are members of the cysteine phosphatases (CP). The Cphys share some substantial properties with the protein tyrosine phosphatases, another group of enzymes from the CP family. For example, they have a conserved nucleophilic cysteine residue and the catalytic hydrolysis involves the formation of a cysteinyl phosphate intermediate. Furthermore, a characteristic active-site motif of these enzymes is HCXXGXXR(T/S) (C: cysteine; G: glycine; S: serine; T: threonine). The formation of a "cysteinyl-phosphate trigonal–bipyramidal" pentavalent intermediate has been postulated and sequential hydrolysis is facilitated with the reorientation of the intermediate (Chu et al., 2004; Turner et al., 2007; Yanke et al., 1999). Purple acid phytases (PAPhys) are named for to their distinct purple color and they have binuclear metal bridging at the active sites, and most PAPhys are coordinated with five conserved consensus motifs (Dionisio et al., 2011; Rodríguez, 2018). The metal bridge is usually in the form of Fe(III)-divalent metal where Fe(III) is on the chromophoric site and the divalent metal can be Fe(II), Zn(II), or Mn(II) (Faba-Rodriguez et al., 2022; Hegeman & Grabau, 2001; Nasrabadi et al., 2018). The scissile phosphate group binds with the metal ions on the active site through hydrogen bonds. The substrate is then rearranged so that the phosphate group is coordinated directly with metal ions, forming a catalytic complex that facilitates the nucleophilic attack, initiating the hydrolysis (Rodríguez, 2018). Depending on the species of phytases, hydrolysis products are phosphate and different *myo*-inositol polyphosphates.

As well as the compounds containing P in the highest oxidation state (+5) such as phosphates and phosphate esters, reduced P forms are also found in soils, such as the organophosphonate, glyphosate, which involves a directly connected C–P bond (Kehler et al., 2021; Ternan et al., 1998).

Organophosphonates can be catabolized by phosphonatases or C-P lyases. The characteristic property of the substrate of the phosphonatases is the presence of an electron-withdrawing β-carbonyl group which facilitates the heterolytic cleavage of the C–P bond (Kamat & Raushel, 2013). The C-P lyases have a redox-active [4Fe-4S]-cluster and the catalysis process involves a radical-based homolytic cleavage of the C–P bond. The products of the C-P lyase degradation are inorganic phosphate and corresponding hydrocarbon, e.g. alkanes, alkenes, and benzenes from alkyl-, vinyl-, or phenyl-phosphonate degradation (Daughton et al., 1979; Stosiek et al., 2020; Wackett et al., 1987). More detailed mechanisms of phosphonatases and C-P lyases have been reviewed in the literature (Kamat & Raushel, 2013; Stosiek et al., 2020).

Enzyme Activity and other Properties Affected by the Formation of the Enzyme–Clay Mineral Complex

Enzyme Adsorption Mechanisms in Clays and Clay Minerals

Protein enzymes bear a variety of functional groups which can interact with the clay mineral surface and

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the adsorption of enzymes onto the mineral surface can be the result of various forces and interactions including salt linkage, ligand exchange, hydrogen bonding, hydrophobic interaction, conformational entropy, Coulombic force (or electrostatic interaction), and van der Waals forces (Boyd & Mortland, 1990; Datta et al., 2017; Roth & Lenhoff, 1995; Theng, 2012).

Among all of the forces mentioned, electrostatic attraction, as a long-range force, has been proposed by some authors to be the one factor responsible for the first contact between the enzyme and clay (Datta et al., 2017). Positively charged amino acids (e.g. arginine, histidine, and lysine above pH 4) can be attracted electrostatically to a negatively charged mineral surface. Furthermore, ligand exchange between the carboxylic groups of the enzyme and hydroxyl groups of the mineral has been proposed as the mechanism for enzyme adsorption on metal oxide surfaces (Sepelyak et al., 1984).

Hydrophobic interaction also contributes significantly to the enzyme–mineral reaction. Phyllosilicates such as montmorillonite have hydrophobic siloxane layers and hydrophilic exchangeable ions on the mineral surfaces (Staunton & Quiquampoix, 1994). Some amino acids have a hydrophobic side chain (e.g. tryptophan, tyrosine), which would probably engage in hydrophobic interaction with the siloxane layer after the enzyme exchanges with the surface exchangeable ions, and the dehydration from such hydrophobic interaction facilitates the adsorption of most enzymes (Norde, 2008). For a hydrophobic mineral surface, even when both the enzyme and surface have charges of the same sign, hydrophobic force may, therefore, overcome the electrostatic repulsion and bring the surface and the enzyme together (Norde, 2008; Quiquampoix et al., 1995). The adsorption process may be further augmented by enzyme conformational change (Fig. 3), van der Waals forces, and hydrogen bonding.

The adsorption of the enzyme on the mineral surface can induce a conformational change in the enzyme, leading to changes in the enzyme's conformational entropy and Gibbs free energy of the system. For example, a decrease in the ordered secondary structure of the enzyme due to adsorption would lead to increased conformational entropy, thus reducing the Gibbs free energy of the system. Subsequently, the modification of enzyme structure and adsorption becomes more irreversible (Quiquampiox & Mousain, 2005).

Van der Waals interaction consists of three components, permanent dipole-permanent dipole (Keesom force), permanent dipole-induced dipole (Debye force), and induced dipole-induced dipole (London force). Van der Waals forces increase as the distance between the enzyme and mineral surface decreases and as the molecular size increases (Roth & Lenhoff, 1995; Stotzky, 1986). Although the attractive force is rather weak, it can work on all molecules despite charges and can be strengthened through the formation of a salt bridge between enzyme-bound salt ions and the mineral surface or between clay-complexed cations and the enzyme, providing a polarizable layer, or through adjacent van der Waals forces acting together, creating a stronger interaction

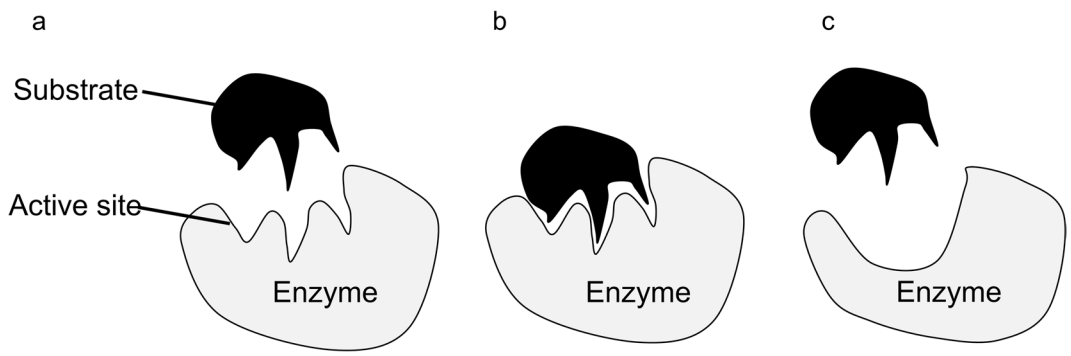

Fig. 3 **a** The enzyme is in its active conformation and the substrate enters the active site. **b** Substrate binds to the active site, forming an enzyme–substrate complex. **c** Conformational change of the enzyme alters the shape of the active site, preventing substrate binding

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(Quiquampiox & Mousain, 2005; Roth & Lenhoff, 1995; Stotzky, 1986).

Hydrogen bonds can be formed between OH groups or O on the mineral surface, or more importantly, clay-associated water, and amino acid functional groups (e.g. carbonyl, amine) (Stotzky, 1986). Like the van der Waals forces, hydrogen bonding becomes a strong binding force when numerous hydrogen bonds are functioning together (Stotzky, 1986). Hydrogen bonds are generally formed between the enzyme and a polar surface while a nonpolar surface doesn't interact with the enzyme via hydrogen bonds (Norde, 2008).

Factors Affecting the Adsorption of Phosphatase on the Mineral Surface

The adsorption of enzymes on a mineral surface is affected by many factors in various aspects. The general effects of temperature, pH, soil moisture, and dissolved ion concentration on enzyme adsorption have been reviewed by Datta et al. (2017). The interaction between clay minerals and phosphatases is the most widely studied and the question of how the adsorption onto the clay mineral affects other P-related enzymes (e.g. phytase) has been investigated much less commonly, or not at all. This review, therefore, focused on the influences of several widely studied factors on alkaline and acid phosphatase adsorption and, subsequently, the effects of adsorption on phosphatase activity and other properties.

The electrostatic interaction between an enzyme and the mineral surface depends on the pH, ionic strength of the bathing solution, and background cation charge (Roth & Lenhoff, 1995). At a pH above the point of zero charge of the mineral and below the isoelectric point of the enzyme, i.e. when the enzymes are positively charged, greater adsorption generally occurs with a negatively charged mineral surface (Carrasco et al., 1995; Datta et al., 2017). pH not only affects the charges on the mineral surface and the enzyme but enzyme conformation is also altered upon pH changes, influencing the adsorption by minerals. For example, a decrease in pH, i.e. an increase in the positive charge on the enzyme, could induce the enzyme to unfold on an electronegative surface (Quiquampoix et al., 1995). Furthermore, strongly acidic or alkaline pH could inhibit adsorption by denaturing the enzymes and a near-neutral pH is likely more appropriate for enzyme adsorption (Sarkar & Leonowicz, 1989). The effects of ionic strength are less commonly studied, and Leprince and Quiquampoix (1996) found decreased adsorption of acid phosphatases on montmorillonite with increasing ionic strength and they attributed it to the competition between phosphatase and Na^+ for negative sites.

The adsorption also depends on the structure of enzymes and the polarity of the mineral surface. Norde (2008) adopted two notions, "hard" and "soft", to describe proteins and, therefore, enzymes, depending on their behavior upon adsorption. "Hard" enzymes (e.g. rigid or tightly coiled) undergo limited conformational change when adsorbed on the polar mineral surface and the adsorption is only favored in the case of electrostatic attraction. On the other hand, "soft" enzymes undergo structural change upon adsorption and can gain conformational entropy, as discussed in the section *Enzyme adsorption mechanisms in clays and clay minerals*, via decreased ordered structure, thus facilitating adsorption even on an electrostatically unfavorable surface.

The types of clay minerals may influence their ability to adsorb enzymes and many factors such as swelling, interlayer space, and charge density contribute to their adsorption capacity. Montmorillonite has been found to have a larger adsorption capacity for acid phosphatase than kaolinite, possibly due to large surface area, high charge density, and interlattice fixation of the enzyme in montmorillonite (Gianfreda & Bollag, 1994; Makboul & Ottow, 1979). Greater adsorption of alkaline phosphatase by goethite than by montmorillonite was discovered by Tan et al. (2018), while the opposite trend was reported by Zhu et al. (2016). Such discrepancy was attributed to different pH used in the adsorption experiment and, therefore, different charge properties of the phosphatases and minerals (Tan et al., 2018).

According to Shindo et al. (2002), the adsorption of an acid phosphatase followed the order: montmorillonite > > kaolinite > Mn oxide > Fe oxide > Al oxide > > allophane. In their study, a positive correlation between the amount of adsorption and mineral-specific surface area could not be established. The charges of the enzyme and mineral surfaces, and thus electrostatic interaction and charge density, mineral microstructure, and ligand exchange capacity of the oxides likely played a larger role in affecting the extent of adsorption. More adsorption of acid

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phosphatase was observed (Huang et al., 2005) on goethite than on fine clays; this, in turn, was greater than the adsorption on coarse clays from an Ultisol; and, finally, this, in turn, was also greater than the adsorption on kaolinite. Those authors concluded that large surface area and ion exchange capacity were responsible for greater adsorption.

Even with the same mineral, different saturating cations may influence the extent of adsorption, which was probably due to the difference in how easily the cations can be displaced by the enzyme (Boyd & Mortland, 1990). For example, Carrasco et al. (1995) found that the amount of alkaline phosphatase adsorption followed the order: Ca-sepiolite > Na-sepiolite > H-sepiolite and that H-sepiolite-adsorbed enzyme was desorbed easily under high salinity and pH.

On the contrary, little effect from varying clay minerals has been reported. For instance, Sedaghat et al. (2009) found a similar amount of adsorption of an alkaline phosphatase on sepiolite and bentonite despite a much larger specific surface area of sepiolite. They concluded that the enzyme was preferentially immobilized on the external surface of the mineral, instead of penetrating the interlayer. Discrepancies among different literature might be due to different reaction conditions such as pH, the specific microstructure of the mineral, and properties of different enzymes and, therefore, varied electrostatic attraction or repulsion between the mineral surface and the enzyme.

The interaction between clays and organic matter could also affect the adsorption of enzymes because the organic matter may serve as a protective layer on the clay surface, decreasing enzyme adsorption (Quiquampoix et al., 1995). Kelleher et al. (2004) found less acid phosphatase immobilized on organic molecule-intercalated montmorillonite (i.e. the organic molecules were immobilized between layers of the montmorillonite) than on pure montmorillonite. The opposite trend was also observed. For example, Tang et al. (1993) reported more adsorption of alkaline phosphatase by protamine-intercalated bentonite at pH 10.4. Both the phosphatase and mineral surface were negatively charged while the protamine was positively charged, acting as a bridge to connect the enzyme and mineral. Excess protamine decreased phosphatase adsorption due to steric blockage of active sites, however. Huang et al. (2005) found that within the same size fraction of clays, natural clays that contained organic matter expressed more enzyme adsorption than inorganic clays (natural clays with organic matter removed by H_2O_2). According to those authors, the enhanced adsorption was attributed to the additional adsorption of phosphatase by humic substances as well as their ability to trap the phosphatase within the "macromolecular net" of humic acids.

Enzyme Activity and Kinetics Parameters Affected by its Adsorption

Upon adsorption by clay minerals, phosphatase activity was mostly inhibited, while enhanced catalytic activity has seldom been observed (Table 1). Interestingly, enhanced phosphatase activity was more commonly observed when adsorbed on poorly crystalline minerals such as allophane and ferrihydrite. The mesoporosity of allophanes was considered to be responsible for making the minerals more suitable supports in biocatalytic processes (Calabi-Floody et al., 2012; Wang, 2006). From the literature, possible contributors of enhanced enzyme activity upon adsorption could be: greater concentrations of the enzyme and the substrate on the clay mineral surface, conformational change, or stabilization of the enzyme structure induced by adsorption (Allison, 2006; An et al., 2015; Tietjen & Wetzel, 2003). Inhibited enzyme activity is mostly attributed to conformational change or even deformation of the enzyme, the incorporation of enzymes into the internal structure of the clay, steric hindrance, the interaction between amino acids on the active site and the mineral surface, or diffusional limitation, leading to restricted access of the substrate to the enzyme (Datta et al., 2017; Dick & Tabatabai, 1987; Olagoke et al., 2019; Tietjen & Wetzel, 2003; Zimmerman & Ahn, 2011).

The most commonly used model to study enzyme kinetics is the Michaelis–Menten equation:

$$v = \frac{[S]}{\mathbf{K_m} + [S]} V_{max} \quad (1)$$

where v is the velocity of the reaction, [*S*] the substrate concentration, K_m the Michaelis–Menten constant, and V_{max} the maximum velocity. The mechanism of how a clay mineral, i.e. the inhibitor, inhibits the enzyme activity can be represented by changes in K_m and V_{max} (Johnson & Goody, 2011). Four types

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Table 1 Experimental conditions and changes in phosphatase properties in phosphatase-clay mineral sorption studies

References	Enzymes	Sources of the enzyme	Clays and clay minerals	pH and temperature[a]	Changes after the sorption[b]			
					Catalytic activity	Ordered secondary structure	Proteolytic stability	Thermal stability
Paul et al. (2022)	Acid phosphatase	Potato	Clays from Inceptisol (mica and kaolinite), Vertisol (smectite and kaolinite), and Alfisol (kaolinite and mica)	5, 30°C	Decreased	n.a	n.a	n.a
Calabi-Floody et al. (2012)	Acid phosphatase	Sweet potato	Montmorillonite clay and allophanic clay extracted from an Andisol	5.0, 30°C	Increased	n.a	n.a	n.a
Zhu et al. (2010)	Acid phosphatase (Type II)	Potato	Uncalcined and calcined Mg/Al-CO_3 layered double hydroxides	4.5–7.0, 15–70°C	Decreased	n.a	Increased	Increased
Huang et al. (2009)	Acid phosphatase (Type II)	Potato	Montmorillonite, kaolinite, and colloids (mainly hydromica, vermiculite and kaolinite) from an Alfisol	7.0, 37°C	Decreased	Increased	Increased	n.a
Rosas et al. (2008)	Acid phosphatase	Sweet potato	Allophanic clay extracted from an Andisol	5.0, 30°C	Increased	n.a	n.a	n.a
Allison (2006)	Acid phosphatase	Sigma-Aldrich	Ferrihydrite, allophane	5.0°C, room temperature	Increased	n.a	n.a	n.a
Huang et al. (2005)	Acid phosphatase (Type II)	Potato	Kaolinite, goethite, clays (mainly illite and kaolinite) from an Ultisol	4.0–7.0, 15–85°C	Decreased	n.a	n.a	Increased
Kelleher et al. (2004)	Acid phosphatase (Type I)	Wheat germ	Montmorillonite	1–13, 20°C	Decreased	Increased	n.a	n.a
Shindo et al. (2002)	Acid phosphatase (Type I)	Wheat germ	Kaolin, montmorillonite, allophane, Fe, Al, Mn oxides	5.5, 20°C	Decreased	n.a	n.a	n.a
Huang and Shindo (2000)	Acid phosphatase (Type I)	Wheat germ	Kaolin, goethite, δ-MnO_2	5.0–6.0, 20°C	Decreased	n.a	n.a	n.a
Rao et al. (2000)	Acid phosphatase (Type I)	Potato	Montmorillonite, $Al(OH)_{18}$-montmorillonite, aluminum hydroxide, OH-Al-tannic acid-montmorillonite	4.0–8.0, 10–60°C	Decreased	n.a	Increased	Increased

Table 1 (continued)

References	Enzymes	Sources of the enzyme	Clays and clay minerals	pH and temperature[a]	Changes after the sorption[b]			
					Catalytic activity	Ordered secondary structure	Proteolytic stability	Thermal stability
Leprince and Quiquampoix (1996)	Acid phosphatase	*Hebeloma cylindrosporum* Romagnesi (HC82111)	Montmorillonite	2–8.5, 28°C	Decreased	n.a	n.a	n.a
Gianfreda and Bollag (1994)	Acid phosphatase (Type I)	Wheat germ	Montmorillonite, kaolinite	5.0, 25°C	Decreased	n.a	n.a	n.a
Sarkar and Leonowicz (1989)	Acid phosphatase	Sigma	Bentonite, kaolinite	6, 30°C	Decreased	n.a	Increased	n.a
Dick and Tabatabai (1987)	Acid phosphatase	Corn roots grown under sterile condition	Na kaolinite, Na montmorillonite, Na illite	4.0, 37 °C	Decreased	n.a	n.a	n.a
Makboul and Ottow (1979)	Acid phosphatase	Wheat plant seed lipase	Montmorillonite, illite, kaolinite	9.6, 37°C	Decreased	n.a	n.a	n.a
Paul et al. (2022)	Alkaline phosphatase	Bovine intestinal mucosa	Clays from Inceptisol (mica and kaolinite), Vertisol (smectite and kaolinite), and Alfisol (kaolinite and mica)	8, 30°C	Increased	n.a	n.a	n.a
Shirvani et al. (2020)	Alkaline phosphatase	Sigma-Aldrich	Palygorskite, sepiolite	8.0, 37°C	Decreased	n.a	n.a	n.a
Tan et al. (2018)	Alkaline phosphatase	Bovine intestine mucosa	Montmorillonite, goethite	9.4, 37°C	Decreased	n.a	n.a	n.a
Wang et al. (2017)	Alkaline phosphatase	Bovine intestinal mucosa	Montmorillonite, goethite	pH of the substrate solution was 9.4 and pH of the enzyme solution was 7.4, 37 °C	Decreased	n.a	n.a	n.a
Zhu et al. (2016)	Alkaline phosphatase	Sigma-Aldrich	Montmorillonite, goethite, sediments from lakes	9.0, 37°C	Decreased	n.a	n.a	n.a

Table 1 (continued)

References	Enzymes	Sources of the enzyme	Clays and clay minerals	pH and temperature[a]	Changes after the sorption[b]			
					Catalytic activity	Ordered secondary structure	Proteolytic stability	Thermal stability
Ghiaci et al. (2009)	Alkaline phosphatase	Calf intestinal mucous membrane	Surfactant-modified and unmodified Na bentonite	5–12, 30–80°C	Decreased	n.a	Increased and decreased with different incubation time	No significant change and decreased for Na-, surfactant-modified-bentonite, respectively
Sedaghat et al. (2009)	Alkaline phosphatase	Calf intestinal mucous membrane	Surfactant-modified and unmodified Na sepiolite	5–12, 30–80°C	Decreased	n.a	n.a	Increased
Tietjen and Wetzel (2003)	Alkaline phosphatase	*Escherichia coli*	Montmorillonite, clay from a lake basin (mostly kaolinite and smectite)	7, 21°C	Decreased	n.a	n.a	n.a
Carrasco et al. (1995)	Alkaline phosphatase (Type I-S)	Bovine intestinal mucous membrane	Na sepiolite	5–12, 0–80°C	Decreased	n.a	Decreased	Decreased
Tang et al. (1993)	Alkaline phosphatase	Bovine intestinal mucosa	Protamine-intercalated bentonite	8.0–11.5, 37°C	Decreased	n.a	n.a	n.a

[a] The pH and temperature are the conditions used for enzyme activity measurement. RT: room temperature

[b] Compared to respective properties of the free enzyme

of reversible inhibition mechanisms are generally acknowledged: competitive inhibition, non-competitive inhibition, uncompetitive inhibition, and mixed inhibition. Competitive inhibition is observed with unchanged V_{max} and increased K_m, and the inhibitor competes with the substrate generally in a mutually exclusive form, and often for the same active site on the enzyme. The noncompetitive inhibitor binds with the enzyme or the enzyme–substrate complex with the same affinity, thus leading to unchanged K_m and decreased V_{max}. The uncompetitive inhibitor binds only to the enzyme–substrate complex to prevent product formation and, therefore, decreases both the V_{max} and K_m. Mixed inhibition is often observed with increased or decreased K_m and decreased V_{max}, and the inhibitor can bind to the enzyme or the enzyme–substrate complex with different affinity (Copeland, 2000; Cornish-Bowden, 1979; Fange et al., 2011).

Mixed inhibition is the most widely observed mechanism for clay minerals with regard to their influence on phosphatase activity (Quiquampiox & Mousain, 2005; Shirvani et al., 2020). For instance, kaolinite, kaolin, mica, montmorillonite, tannic acid-montmorillonite complex, goethite, allophane, Fe/Al/Mn oxides, uncalcined and calcined Mg/Al-CO_3 layered double hydroxide, and δ-MnO_2 have been reported to be inhibitors for many acid phosphatases (Gianfreda & Bollag, 1994; Huang & Shindo, 2000; Makboul & Ottow, 1979; Paul et al., 2022; Rao et al., 2000; Shindo et al., 2002; Zhu et al., 2010), and palygorskite, sepiolite, goethite, and montmorillonite for some alkaline phosphatases (Ghiaci et al., 2009; Sedaghat et al., 2009; Shirvani et al., 2020; Wang et al., 2017; Zhu et al., 2016). Discrepancies and other inhibition mechanisms have also been reported. For example, illite and montmorillonite can be uncompetitive inhibitors for some acid phosphatases (Kelleher et al., 2004; Makboul & Ottow, 1979), but noncompetitive inhibitors for other acid phosphatases (Dick & Tabatabai, 1987). Montmorillonite has also been found to be an uncompetitive inhibitor for alkaline phosphatase (Wang et al., 2017).

Other Properties Affected by the Adsorption

After being adsorbed, the enzymes experience a different microenvironment (e.g. altered diffusion, charge, or steric hindrance) from that experienced by free enzymes (Zimmerman & Ahn, 2011). Therefore, in addition to catalytic activity, other properties including optimum pH, enzyme conformation, and thermal and proteolytic stability can be influenced. pH-dependent phosphatase adsorption has been observed by several researchers. For instance, the optimum pH of acid phosphatase was shifted to more alkaline values when they were adsorbed on negatively charged mineral surfaces, which was also attributed to the pH-dependent modification of the enzyme conformation (Leprince & Quiquampoix, 1996). Instead of exhibiting an optimum pH, the activity of a montmorillonite-adsorbed alkaline phosphatase continued to decrease with pH increasing from 4.0 to 8.0 (Rao et al., 2000). No change in the optimum pH was reported when an alkaline phosphatase was immobilized on sepiolite, bentonite, and protamine-intercalated bentonite in some studies. This was possibly due to high ionic strength and the compression of the diffuse double layer of the mineral, leading to less local pH variation on the clay mineral surface from the solution pH (Carrasco et al., 1995; Ghiaci et al., 2009; Sedaghat et al., 2009; Tang et al., 1993). Adsorption of acid phosphatase on goethite, kaolinite, and clay minerals from an Ultisol failed to affect significantly the enzyme's optimum pH, and the adsorbed phosphatases expressed less sensitivity toward pH changes (Huang et al., 2005). Unlike the more widely investigated reasons for shifted optimum pH, the factors contributing to unchanged phosphatase optimum pH are poorly understood and have not been studied extensively; studies in the past decade did not probe the optimum pH of adsorbed phosphatase.

Conformational changes of adsorbed enzymes have been recognized (Table 1, Fig. 3). The attractive electrostatic force between minerals and the enzyme could induce the unfolding of the enzyme, exposing the hydrophobic chain, and leading to a hydrophobic interaction with the siloxane layers of phyllosilicates (Staunton & Quiquampoix, 1994). Hydrophobic interaction between the mineral and enzyme can also induce a conformational change, as discussed in the section *Enzyme adsorption mechanisms in clays and clay minerals*. The extent of conformational change depends on the balance between several factors including the intramolecular forces of the enzyme, the effects from the mineral surface, and the solvent molecule (Boyd & Mortland, 1990; Datta et al., 2017). Some enzyme structures, e.g. fibrous proteins,

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are more likely to unfold due to more interaction with the mineral surface upon adsorption (Boyd & Mortland, 1990).

An increased ordered secondary structure of phosphatase upon mineral adsorption has been reported. The conformational change of an acid phosphatase when adsorbed on montmorillonite, kaolinite, and inorganic and organic colloids from an Alfisol were studied by Huang et al. (2009). This change increased the ordered secondary structure of the phosphatase and the 2:1 clay led to a more significant change than the 1:1 clay. Such a difference was attributed to the difference in the hydrophobicity between kaolinite and montmorillonite. All of the external planar surfaces of montmorillonite are siloxane surfaces which are typically hydrophobic. As for kaolinite, half of the external planar surface is siloxane and, therefore, the kaolinite surface is considered to be less hydrophobic. The more hydrophobic surface of montmorillonite promoted the formation of intramolecular hydrogen bonds.

Adsorption of enzymes on clay minerals can serve as a means to protect the enzymes against, for example, proteolysis, heat, light, and other inhibitors (Huang et al., 2009; Rao et al., 2000; Sarkar & Leonowicz, 1989). Different minerals vary in their ability to affect the sensitivity of phosphatases toward proteolysis (Table 1). For example, adsorption by montmorillonite was found to provide more proteolytic protection than kaolinite (Huang et al., 2009). Increased proteolytic resistance was attributed to the conformational change of the enzyme when immobilized, and to the alteration of the cleavage site, disturbing the recognition from the proteinase. A more significant extent of the conformational change induced by montmorillonite than kaolinite led to greater resistance of montmorillonite-adsorbed phosphatase. In another study, increased hydrolytic stability of an acid phosphatase was induced in the order: adsorption on calcined Mg/Al-CO_3 layered double hydroxide (LDH) > adsorption on uncalcined Mg/Al-CO_3 LDH > enzyme-free (Zhu et al., 2010). The latter authors suggested that the enzymes penetrated into the pores on the porous surface of LDH, rendering them less accessible for protease and, therefore, the greater porosity of calcined minerals accounted for more elevated proteolytic resistance of the enzyme. The difference in the proteolytic stability may be a result of the specific mineral site where the enzyme is adsorbed. If the enzyme is fixed in the interlayer space, it would be inaccessible to the protease; hence there is an increased proteolytic resistance. On the other hand, if the enzyme is immobilized on the surface or edges of the mineral which can act as a support for concentrated enzymes and proteases, the proteolysis would be enhanced, resulting in decreased proteolytic stability of immobilized enzyme (Boyd & Mortland, 1985).

As for the effects on thermal stability, less temperature sensitivity from montmorillonite-adsorbed acid phosphatase was reported by Rao et al. (2000). The acid phosphatase adsorbed on kaolinite and goethite had greater thermal stability than the free enzyme (Huang et al., 2005). The greater thermal stability of kaolinite-adsorbed than goethite-adsorbed acid phosphatase was probably a result of tighter binding on kaolinite as less desorption was detected from kaolinite. They also reported greater thermal stability of an organic clay-adsorbed acid phosphatase, which was attributed to the protective effect of organic matter. Zhu et al. (2010) observed enhanced thermal stability of uncalcined and calcined Mg/Al-CO_3 LDH-adsorbed acid phosphatase than the free enzyme, which was attributed to the loss of enzyme conformational flexibility. Increased thermal stability was also observed for the alkaline phosphatase adsorbed on a bilayer-surfactant-covered sepiolite (Sedaghat et al., 2009). Decreased thermal stability of Na-sepiolite-adsorbed alkaline phosphatase was attributed to a rapid loss of moisture from the clay during the incubation, which affected the immobilized enzyme (Carrasco et al., 1995). No significant changes in the thermal stability of an alkaline phosphatase immobilized on bentonite were observed (Ghiaci et al., 2009). Light sensitivity has been studied less, and Tietjen and Wetzel (2003) found less light sensitivity and photodegradation of montmorillonite- and lake basin clay-immobilized alkaline phosphatase.

Adsorption on clay minerals may protect the enzyme against other inhibitors, e.g. metal cations (aq) or oxyanions. For instance, a decreased inhibitory effect of Cd^{2+} on the activity of palygorskite-, sepiolite-, goethite-, and montmorillonite-adsorbed alkaline phosphatase than free phosphatase has been observed (Shirvani et al., 2020; Tan et al., 2018). Wang et al. (2017) reported less sensitivity of goethite- and montmorillonite-adsorbed alkaline phosphatase toward the inhibitory effects of arsenate.

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Rosas et al. (2008) reported the protective effect of an allophanic clay on an acid phosphatase against Mn^{2+} and $Mo_7O_4^-$ inhibition. Huang and Shindo (2000) observed different effects from kaolin, goethite, and δ-MnO_2 in protecting an acid phosphatase against Cu^{2+} inhibition. At the same $CuCl_2$ concentration, the goethite-adsorbed enzyme showed greater activity than the δ-MnO_2-adsorbed enzyme and this, in turn, was greater than the activity of the kaolin-adsorbed enzyme; the activity of the latter was similar to that of the free enzyme. When copper citrate was used, however, its inhibitory effect was more significant on the clay-adsorbed enzymes than on the free ones. The observed difference induced by varied copper forms was postulated to be a result of the interaction between the carboxylic group of the citrate and the hydroxyl group on the mineral surface, modifying the enzyme conformation.

The protection was attributed to the adsorption of the inhibitors by clay minerals, resulting in lowered inhibitor concentration in the solution and decreased interaction between inhibitors and enzyme active sites. Another factor contributing to the diminished inhibition effect from the inhibitors may be the conformational change of the enzyme after adsorption, burying the active site, which would limit access by the inhibitors (Shirvani et al., 2020; Wang et al., 2017). The extent of protection depends on many factors, including the concentration and identity of the inhibitor, exposure time, order of addition of the inhibitor, and clay mineral type. For example, adsorption on montmorillonite and goethite diminished the sensitivity of an alkaline phosphatase toward Cd^{2+} inhibition with an exposure time of >1 h. The opposite effect was observed when the exposure to Cd^{2+} was <1 h (Tan et al., 2018). When Mn^{2+} was added together with allophane to an acid phosphatase, its activity decreased with increasing Mn^{2+} concentration. When added to allophane-adsorbed acid phosphatase, Mn^{2+} did not affect significantly the enzyme activity. On the contrary, the opposite trend was observed with $Mo_7O_4^-$ (Rosas et al., 2008).

The adsorption of phosphatases on clay minerals is, therefore, usually a tradeoff between decreased activity and protection against environmental stress (Fig. 4). From the literature, it is not surprising to find that even with the same mineral, the inhibition mechanism and the way in which the enzyme properties are affected can be different. Effects of different forces in the adsorption and the variety of enzyme sources and, thus, enzyme species and behavior (e.g. conformation, orientation on the mineral surface) with respect to different reaction conditions make enzyme adsorption a complex process and increase the difficulty of predicting the results. Specific enzyme structure and characteristics and reaction conditions including pH and temperature could all lead to different properties and behavior of the mineral surface and enzyme. If one were to investigate or compare the effects of a certain mineral on an enzyme, careful attention should, therefore, be paid to clay and enzyme species and individual reaction conditions.

The Phosphatase–Mineral Interaction affects the Fate of P in Soils

The fate of P in soils is dependent on the biological and geochemical activities in the soil and, from the aspect of biology, the release of bioavailable P is achieved through mineralization (e.g. by phosphatase) and solubilization (e.g. by phosphorus-solubilizing microorganisms) (Alori et al., 2017; Hussain et al., 2021). Up to 85% of the total P in soils may exist as organic P and a meta-analysis study that summarized results from 149 soils showed that ~30–60% of the organic P in the studied soils can be mineralized by phosphatases and/or phytases (Bünemann, 2008; Dalal, 1977; Tarafdar et al., 2001).

An enhanced phosphatase activity promotes the mineralization of organic P and the release of phosphate (Fig. 2), which is a more bioavailable fraction. For instance, Tarafdar et al. (2001) observed a positive linear relationship between the activity of acid phosphatase and the amount of phosphate released from different organic P compounds. Zou et al. (1995) found a positive correlation between phosphatase activity and gross P mineralization rate. Accordingly, depletion of organic P was usually observed with increasing phosphatase activity in the bulk soil (McConnell et al., 2020; Schaap et al., 2021). An enhanced depletion of different organic P forms (e.g. sodium hydroxide-extractable, bicarbonate-extractable) was also found to be related to increased activity of phosphatases in the rhizosphere of rape, onion, wheat, clover, barley, Norway spruce, and radiata pine, indicating the important role of phosphatases in the organic P mineralization and in providing

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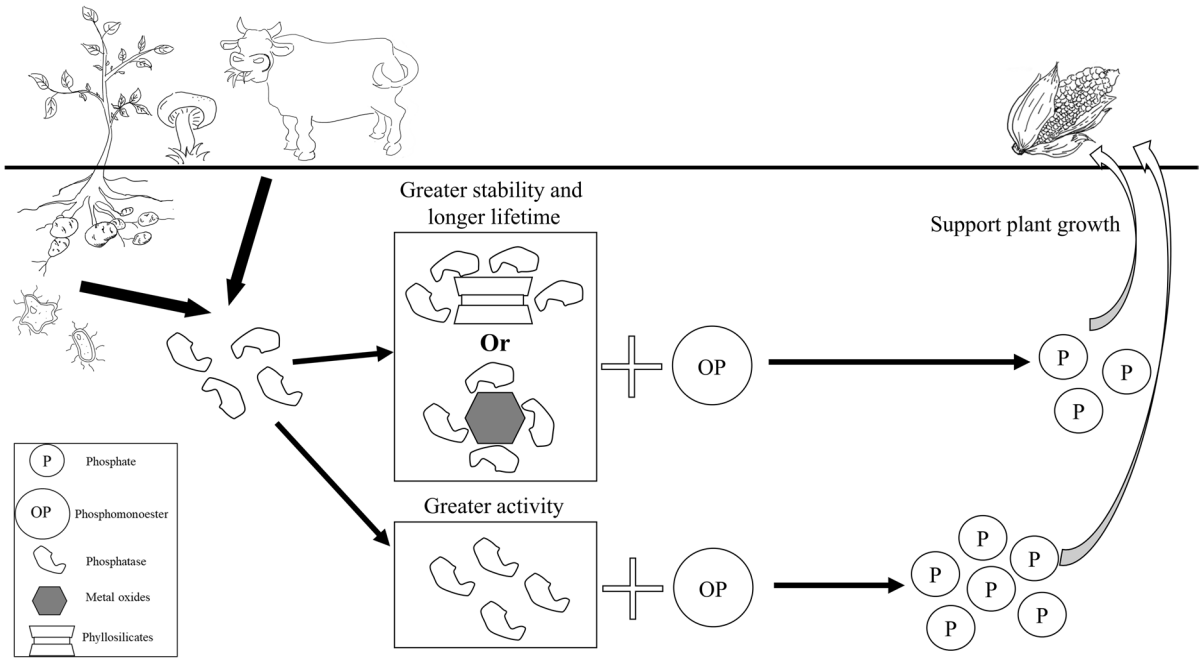

Fig. 4 Phosphatase activities in the terrestrial environment. The role of clays and clay minerals as phosphatase sinks is highlighted. The interaction could suppress P mineralization and enhance enzyme stability

bioavailable P for plants (Asmar et al., 1995; Chen et al., 2002; Häussling & Marschner, 1989; Liu et al., 2004; Tarafdar & Jungk, 1987).

As well as for phosphate, other P forms are also affected by phosphatase activities. Margalef et al. (2017) collated data from 183 studies from 378 sites around the world and found that some P forms may be correlated to the activity of phosphatases in the soil. They found a positive correlation between alkaline phosphatase activity and Olsen-P (the "Olsen-P test" provides estimation on the available P content in the soil), and between organic P and acid phosphatase activity. Yu et al. (2006) observed significant correlations between acid and/or alkaline phosphatase activity and different P fractions, including hydrochloric acid-extractable P, Olsen P, and total P, and significant correlations between neutral, as well as natural phosphatase activity (i.e. measured at soil pH) and P fractions such as Olsen P, water-extractable inorganic P, and sodium bicarbonate-extractable organic P. Compton and Cole (2001) observed a positive relationship between phosphatase activity in the bulk soil and some organic P forms (e.g. the organic P after sodium bicarbonate extraction and labile organic P) at pH 5, which might be attributed to the stimulation of phosphatase excretion induced by high organic P content.

The promoted release of phosphate and other labile P by phosphatases, while more bioavailable, are also prone to leaching. Yu et al. (2006) found natural phosphatase activity to be related to the concentration of total P, total dissolved P, and phosphate in surface runoff. A negative correlation was observed between alkaline phosphatase activity and total P and total dissolved P. No correlations were found between acid phosphatase activity and any P fractions, which was probably due to the neutral to slightly alkaline nature of the runoff water. Yu et al. (2006) proposed, therefore, that soil natural phosphatase activity may be an index for the P-loss potential by surface runoff. A positive correlation was observed between neutral phosphatase activity and total P in the surface water of a rice paddy field, indicating a possible enhanced P runoff loss (Wang et al., 2012).

The interaction between soil minerals and phosphatases clearly affects phosphatase activity, which in turn influences both soil P availability and loss. Depending on which results are desired, either promoting or suppressing the adsorption of phosphatases on the mineral surfaces could be the goal of soil

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management. For instance, in strongly weathered soils, the organic P and secondary minerals (e.g. sasaite, wavellite, crandallite, cacoxenite) are usually the predominant P forms and these soils are often observed with limited P availability (Kovács et al., 2020; Margalef et al., 2017; Rocha et al., 2019; Wang et al., 2023). Furthermore, with the long-term development of ecosystems (e.g. forest), available P is gradually lost through surface runoff, for example (Turner et al., 2013). Phosphorus depletion and limitation in agricultural soils not only affect yield and decrease biomass but also influence plant community composition and diversity (Turner & Condron, 2013). For such soils, mineralization by extracellular enzymes and maintaining their stability (e.g. through clay adsorption) and activity are particularly crucial in providing bioavailable P (Fig. 4). Some studies observed enhanced phosphatase activity when adsorbed on the mineral surface, which can lead to useful application. Many heavy metals (e.g. Cd, Pb, Cu, Hg, As, W) inhibit phosphatase activity and in some heavy metal-polluted soils, the inhibitory effect, therefore, becomes a severe problem (Hong et al., 2020; Huang & Shindo, 2000; Mao et al., 2015; Yang et al., 2006). Phosphatase–clay interaction may be used to enhance organic P mineralization or to protect phosphatase against inhibitors or environmental stress, but the real-world application is still limited. For example, an analysis of the inhibition constants and ecological dose of arsenate (Tian et al., 2018) showed that the adsorbents (montmorillonite or soil) protected alkaline phosphatase from arsenate deactivation. Li and Xu (2018) found an increase in phosphatase activity and available P level in a Cd-polluted rice field when sepiolite was added. Although this could also be a result of Cd immobilization by clay minerals and, thus, decreased Cd, the addition of adsorbed or immobilized enzymes to soils still has the potential as one of the applications of enzyme–clay mineral association.

Industrial Application of Enzyme-Mineral Complexes

Many natural and anthropogenic activities contribute to the contamination of soils and waters, and the application of free enzymes or adsorbed enzymes has been developed as a means of bioremediation. One of the main requirements for effective bioremediation by enzymes is the stability of the enzyme under the environmental conditions of the contaminated site (Gianfreda & Rao, 2004). The indigenous extracellular enzymes or free enzymes added to soils are prone to rapid denaturation or degradation due to, for example, microbial and enzymatic degradation or chemical-mediated denaturation, thus generally having a short lifetime (Gianfreda et al., 2002; Sarkar & Leonowicz, 1989). The association of minerals and enzymes, however, can largely contribute to the stabilization and persistence of the extracellular enzymes in soils (Boyd & Mortland, 1990). As mentioned above, greater thermal stability, proteolysis stability, storage stability, and protection against other inhibitors may result from the adsorption to minerals. Upon adsorption and with increased stability, although usually at a cost of reduced activity, the enzymes can survive longer with long-term retention of activity and contribute to promoting the degradation of xenobiotic substances in aqueous and terrestrial environments to produce less toxic compounds (Gianfreda & Rao, 2004; Gianfreda et al., 2002; Hoehamer et al., 2005; Meng et al., 2019). Compared to the use of free enzymes, other advantages of applying adsorbed enzymes include improved enzyme reusability, enhanced enantioselectivity, and they can usually be recovered at the end of the process (Burns & Dick, 2002; Chen et al., 2023; Gianfreda & Rao, 2004).

Organophosphorus pesticides (e.g. glyphosate), accounting for ~40% of the global pesticide market, have been used commonly in agriculture and forestry (Chen et al., 2023). They are highly toxic and disposal methods might be inefficient and produce toxic pollution (Chen et al., 2023). As an alternative, using enzymes as a bioremediation method could be a more sustainable, renewable, and green approach that is more environmentally friendly and less invasive (Dzionek et al., 2016; Somu et al., 2022). For instance, organophosphorus hydrolases (e.g. phosphotriesterase, parathion hydrolase), organophosphate acid anhydrase, and methyl parathion hydrolase can mineralize organophosphorus compounds such as (methyl) paraoxon, (methyl) parathion, and soman. Nitrilases can be used for degrading herbicides, polymers, plastics, and cyanide (Mousavi et al., 2021). Enzymes such as tyrosinase, phenoloxidases, and peroxidases have been used for the removal of phenols,

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aromatic amines, and related compounds (Gianfreda et al., 2002; Mousavi et al., 2021; Somu et al., 2022).

The application of free and immobilized enzymes has, therefore, become more common. Daumann et al. (2014) found that glycerophosphodiesterases immobilized on functionalized magnetite nanoparticles had prolonged storage time and can be used for bioremediation of organophosphate pollution. Laccases have wide substrate specificity and can be used for degrading a variety of contaminants such as dyes, pesticides, benzenediol, and polycyclic aromatic hydrocarbons (Mousavi et al., 2021). Montmorillonite- and kaolinite-adsorbed laccase and peroxidase have been used to transform and detoxify 2,4-dichlorophenol, a degradation product from a herbicide (Gianfreda & Bollag, 1994; Ruggiero et al., 1989). The removal efficiency has been confirmed both with and without the presence of soils. The authors also reported that the immobilized enzyme can be separated from the reaction mixture and used repeatedly. Masaphy et al. (1996) used a crude parathion-degrading enzyme extract from *Xanthomonas* sp. to degrade parathion, an organophosphate pesticide. To simulate the soil environment, the enzyme was added to a Na-montmorillonite suspension for parathion degradation. Their study suggested the potential of using the enzyme as an approach in soil decontamination.

Many enzymes have also been used for the bioremediation of haloorganics and heavy metal (e.g. Se, As, Cr, Hg) pollution, which can be an indication of the future application of phosphatases (Burns & Dick, 2002). With the enzyme stabilization brought about via enzyme–clay mineral interaction, the beneficial influences such as eco-friendly remediation and, in some cases, enhanced mineralization can be prolonged with reusable enzymes. To further induce the excretion of enzymes and, therefore, to improve the remediation effect and mineralization efficiency, methods such as adjusting soil properties (e.g. moisture, pH, and fertilizer N amendment) can be applied (Arenberg & Arai, 2019).

Enzymes adsorbed on the mineral surfaces are also applied in other fields such as the biomedical, biosensor, biocatalyst, food, pharmaceutical, textile, and detergent industries, and in wastewater treatment (An et al., 2015; Basso & Serban, 2019; Jesionowski et al., 2014; Maghraby et al., 2023). Adsorption can increase thermal stability and protect enzymes against denaturation during the purification process in the food industry (Hassan et al., 2019). In the pharmaceutical industry, a loss of activity during shelf life can be a problem, and enzymes adsorbed to, e.g. silica surfaces, with greater stability may help alleviate the problem (Norde, 2008). Amorphous clay minerals such as allophane can adsorb water from the surrounding environment, providing an aqueous environment for adsorbed enzymes, which can be applied to the biotechnological field (Calabi-Floody et al., 2012).

Future Perspectives

Previous studies of the phosphatase–mineral interaction investigated the macroscopic behavior of mineralization, such as phosphatase activity and its thermal and/or proteolysis stability. The results showed that the interaction between phosphatases and minerals was a trade-off between mostly inhibited enzyme activity and protection against the environmental stress. Various and complex phosphatase behavior in the literature was attributed to the variety of specific properties of the enzymes and minerals involved. The wide application of adsorbed enzymes, the complexity of the phosphatase–mineral reactions, and the significant role of phosphatase in affecting P fate in soils have, however, made it important to further investigate the microscopic characteristics of the interaction and reaction mechanisms (e.g. the three-dimensional conformational change of the enzyme, effects on detailed biochemical pathway) between phosphatases and clay minerals. Spectroscopic techniques such as nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR), and in situ attenuated total reflectance Fourier-transform infrared (ATR-FTIR) are robust in probing such characteristics, and their recent applications have provided more insights into the microscopic aspects of the protein–clay mineral interactions (Reardon et al., 2016; Schmidt & Martínez, 2016; Ustunol et al., 2021). The future microscopic study of phosphatase–clay mineral interaction could, therefore, benefit from these techniques. Furthermore, description of the specific reaction conditions and well-characterized macro- and microscopic properties of the specific phosphatase and minerals could be very helpful when investigating their interaction and comparing results from different studies.

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Declarations

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