Chapter 15
Organo-Mineral–Enzyme Interaction and Soil Enzyme Activity

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15.1 Introduction

Both microbially produced enzymes and mineral surfaces can be considered catalysts of chemical transformation in soils and other geological environments such as sediments and subsurface aquifers. While both are important direct agents of organic matter (OM) remineralization and transformation, mineral surfaces can also act as "heterogeneous co-catalysts," influencing the kinetic properties of biological enzymes by providing surfaces upon which reactions can take place. Enzymes may be categorized, according to their location as "intracellular," those present in living and proliferating cells, or "abiontic," i.e. all others (Skujins 1976). Within the latter group, extracellular enzymes may be leaked or lysed from dead cells or actively secreted by living bacteria and fungi cells (Burns 1982). They are of particular importance to the biogeochemistry of soils and sediments, in that they hydrolyze large polymeric organic compounds into small monomers, which then can be passed through cell walls and fuel microbial respiration and growth. Thus, their activity may be the rate-limiting step in governing the degradation of OM and the remineralization of carbon and other nutrients.

While classical enzyme experiments have typically been carried out in buffered solutions and under easily controllable conditions, the soil environment represents a wholly different set of conditions and extracellular enzyme activity cannot be expected to conform to the "ideal." In addition to the variables often cited as influencing enzyme activity such as temperature, pH, and substrate availability, the activity and stability of a particular enzyme may be enormously influenced by its interaction

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with surfaces. Whether in soil, groundwater, or sediment systems, the interactions of mineral or indigenous OM with both enzymes and enzyme-targeted organic substrates may largely determine the effectiveness of enzymatic degradation, and thus, microbial accessibility to organic carbon and nutrient sources. These geological environments are extremely heterogeneous in their biotic (microorganisms, enzymes, micro- and macrofauna) and abiotic components (labile organic compounds, humic substances, mineral types, organo-mineral aggregates). Thus, the actual activity and stability that an extracellular enzyme would be expected to exhibit in a particular system might be considered a sum of the enzyme and enzyme-modulating component pairs in the system. However, this does not account for the interactive and synergistic effects between multiple components that we are now beginning to realize exist. We have begun to examine, in isolation or combination, many of the factors that influence extracellular enzyme activity and, thus, may become better able to estimate the behavior of these enzymes in the natural environment.

A number of papers have reviewed specific aspects of enzyme–organo-mineral interaction such as that of protein (enzyme) adsorption to minerals (Quiquampoix et al. 2002; Quiquampoix and Burns 2007) and enzyme–clay interaction (Boyd and Mortland 1990; Naidja et al. 2000). This chapter outlines the broader range of organo-mineral interactions in soils and their varied effects on enzyme activity, with special focus on providing historical prospective and recent developments in our understanding of this research area. For the purposes of this discussion, OM will be used here to refer to generic environmental OM that is neither enzyme, nor enzyme-targeted substrate.

15.2 History of Enzyme–Organo-Mineral Interaction Studies

Our understanding of the mechanisms and importance of enzyme–organo-mineral interaction has proceeded from a number of fields including biochemical and environmental engineering, material science, microbiology, and soil and sediment organic geochemistry. Techniques and concepts developed in each of these fields have provided insight into the effects of these interactions on soil enzyme activity and microbial ecology. Among the earliest reference to the measurement of surface-associated enzyme activity is the work of Griffin and Nelson (1916) and Nelson and Griffin (1916) who found no change in yeast-derived invertase activity whether sorbed to charcoal, aluminum hydroxide, or even colloidal proteins such as egg albumin, as long as pH was held constant using an appropriate buffer. Since then, these so-called "immobilized enzymes" have seen wide commercial application in processes that require a biocatalyst such as in the food and pharmaceutical industries. The benefits of immobilized enzymes are that, on a solid support, an enzyme can be easily removed from the reaction solution following its use and then reused and can, in some cases, be stabilized (i.e., made to have increased longevity relative to the unbound enzyme).

By one count, more than one thousand reports on the immobilization of about two hundred different enzymes had been published by the mid-1970s (Mosbach 1976). The methods of immobilization can be categorized as involving adsorption, entrapment, covalent attachment, or co-polymerization or cross-linking (Srere and Ueda 1976), and myriad solid supports have been used ranging from natural minerals and ceramics to organic polymer beads and gels. The goal of the immobilization method chosen for any particular application is to prevent the loss of enzyme activity while strongly binding the enzyme. In general, any attachment that changes the nature (structure or chemistry) of reactive groups in the binding site of the enzyme will lead to activity loss. While physical adsorption is the simplest and most economical of these methods (and the most analogous to "bound" extracellular soil enzymes), it is the weakest and least controllable binding method, and offers the greatest possibility of enzyme release and loss of activity. In addition, the understanding derived from work in this field that the chemical nature of the support material determines the amount, stability, and activity of bound enzymes (Goldstein and Katchalski-Katzir 1976), is directly applicable to our understanding of extracellular soils enzymes.

Early work on enzyme immobilization often focused on enzyme–clay, or more generally, protein–clay interaction (e.g., Ensminger and Gieseking 1939; Zittle 1953). Clay-adsorbed proteins were found to degrade more slowly than their "free" counterparts, both by microbes (Estermann and McLaren 1959; Pinck and Allison 1951; Pinck et al. 1954; Skujins et al. 1959) and by proteinases (Ensminger and Gieseking 1941; Ensminger and Gieseking 1942; McLaren 1954; McLaren and Estermann 1956; Sorensen 1969). In other words, the stability of enzymes, i.e., resistance to denaturization *via* proteinases or other physical factors including high and low temperatures, dehydration, and radiation, was found to be increased in the adsorbed state.

From the earliest detection and study of soil enzymes at the beginning of the twentieth century (reviewed in Skujins 1978), workers noted, in addition to microbial factors, the importance of both organic and inorganic soil fractions in controlling catalytic and, specifically, enzyme activity. However, perhaps due to methodological obstacles and a lack of cross-disciplinary expertise, these relationships did not become a widespread focus of research until the 1960–1970s. Though early work on soil enzymology failed to produce its highly sought after "fertility index," it found, instead, strong correlations between enzyme activities and physical and chemical soil characteristics. For example, a number of workers noted a predominance of enzymatic activity in the clay and silt soil size fractions relative to sand, and surmised preferential adsorption to be at play (McLaren and Packer 1970). Durand (1963) reported that clay sorption of either uricase or the substrate urate resulted in lower activity than that of the free enzyme.

At the same time, a line of research developed investigating the association between enzymes and humic substances and other organic components of soils. For example, Ladd and Butler (1969) discovered that protease activity was, in many cases, dramatically inhibited by the presence of soil humic acids (HA). At solution concentrations of 1 and 10 μg/ml, respectively, 80 and 10% of pronase activity, 32 and 14% of trypsin activity, and 74 and 48% of carboxypeptidase A activity remained. An effect on these enzymes similar in magnitude was also observed with other polymeric anions

such as a benzoquinone and a catechol polycondensate. However, phaseolain, tyrosinase, and chemotrypsin showed little effect from the presence of HA, and the activity of papain, ficin, and thermolysin actually increased with increasing HA concentrations (by 30, 120, and 115% at 10 μg/ml HA, respectively, depending upon the substrate used). In addition, working with these same proteases, these workers found that HA inhibited enzyme activity to a greater extent than fulvic acids and ascribed this difference to the relatively greater molecular weight and rigidity of HA, which may have led to more structural deformation of certain enzymes (Butler and Ladd 1971). Additional hypotheses that could explain the differential effects of HA on various enzymes, such as metal ion requirements (Ladd and Butler 1969) and the presence of carboxyl groups in the inhibitory organic component (Butler and Ladd 1969), proved not to be universally true. For example, although Kunze (1970, 1971) showed that although a number of acidic organic compounds including syringic and gallic acids inhibited catalase activity, tannins also had a similar effect. Mayaudon and Sarkar (1974, 1975) found that diphenol oxidase enzymes were associated with HA and protein complexes extracted from soils. Further, these enzymes, when complexed with OM, retained significant activity.

In the field of aquatic science, examinations of extracellular enzymatic initially drew from concepts and techniques developed in soil science. During the 1980s, the first measurements of enzymatic activity in mineral-based marine snow (Amy et al. 1987), and marine (Meyer-Reil 1986), freshwater (King and Klug 1980), and intertidal sediments (King 1986; Rego et al. 1985) were made. Though with the primary focus of understanding the degradation of aquatic OM, these studies have led to important contributions by marine scientists, to the understanding of enzyme–organo-mineral interactions, particularly with regard to OM–mineral sorption and surface protection (discussed below).

As research in this field progresses through the development of analytical tools and the cross-fertilization from a variety of disciplines, we are beginning to understand the modes of mineral and OM interaction with enzymes and the chemical mechanisms by which this interaction may affect enzyme activity. However, we are also beginning to see that there are many ways in which soil OM, mineral, and enzyme may interact (Fig. 15.1), and these interactions are both complex and synergistic, and not simply additive. In the following, the processes and mechanisms of enzyme–mineral, enzyme–OM, and ternary interactions between all three are outlined.

15.3 Enzyme–Mineral Interactions

Enzymes, as with most proteins, exhibit a strong affinity for mineral surfaces of many types. Because they are made up of amino acids of a range of properties including hydrophilic, hydrophobic, and negatively, positively, and neutrally charged, and because they may change their shape in response to environmental changes, the mechanisms of protein–mineral adsorption are varied and complex.

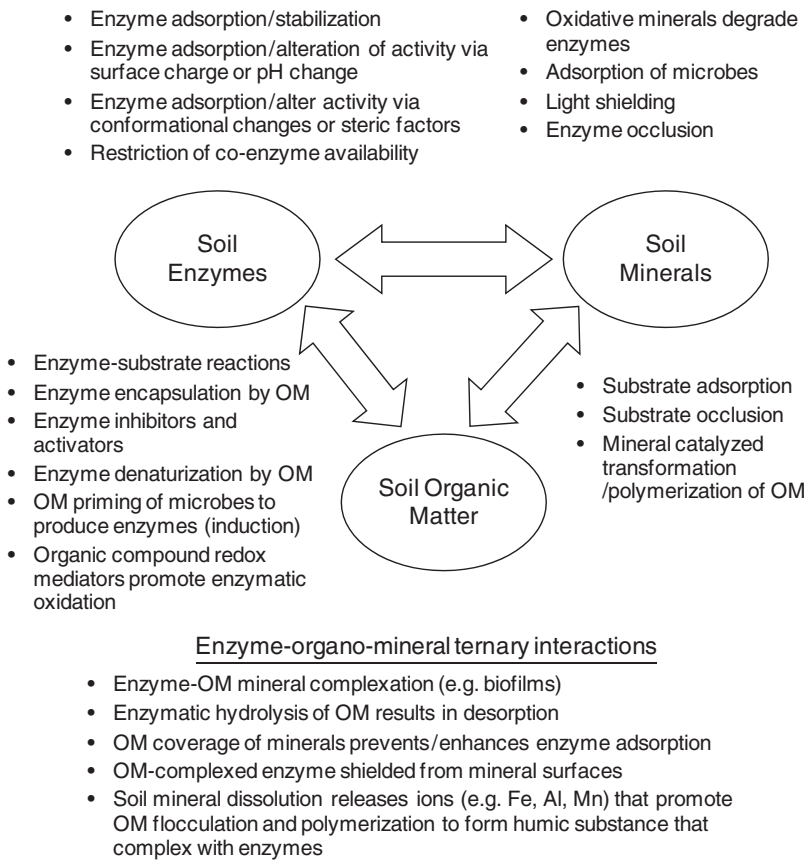

Fig. 15.1 Summary of soil enzyme-mineral-organic matter interactions

Early work examining the adsorption of proteins to clays put forward ionic exchange and electrostatic attraction as the primary mode of bonding (Boyd and Mortland 1990; Naidja et al. 2000). Evidence for this was cited as the pH change that occurs when protein and clay are mixed. However, though the surface of many minerals is negatively charged, and proteins, though amphiphilic, usually carry some net charge, this may not be the dominating factor in controlling protein adsorption. There is no significant relationship between the net charge of adsorbed protein and the charge density of a mineral sorbent's surface, though one does exist for amino acid monomers and dimers (Zimmerman et al. 2004b). Clay interlayer adsorption of protein was thought to occur for expandable clays such as smectites, and especially montmorillonite, but this mechanism has been shown to occur only partially and only in certain cases such as with albumin (De Cristofaro and Violante 2001), glucose oxidase (Garwood et al. 1983), and aspartase (Naidja and Huang 1996).

While some studies have found electrostatic interactions to dominate protein–mineral interaction (e.g., Ding and Henrichs 2002; Servagent-Noinville et al. 2000), entropic effects such as hydrophobic interactions and lowering of free energy due to conformational changes are, increasingly, thought to be a predominant adsorption mechanism for protein onto mineral surfaces. This mechanism explains a number of common observations such as the adsorption of protein onto hydrophobic surfaces such as that of silica oxides even under non-favorable electrostatic conditions (Arai and Norde 1990; Fusi et al. 1989; Giacomelli and Norde 2001), as well as the maximal adsorption of protein occurring at or near the isoelectric point of a mineral surface (e.g., Barral et al. 2008; McLaren 1954; Staunton and Quiquampoix 1994). Though the relative importance of enthalpic and entropic effects may vary with the protein–sorbent pair, and these are not completely independent (Quiquampoix et al. 2002), adsorbed proteins have been clearly shown to exhibit conformation changes such as internal and external unfolding upon mineral adsorption (Baron et al. 1999; Naidja et al. 2002; Servagent-Noinville et al. 2000).

Changes in the kinetic properties of enzymes (activity) are usually observed upon adsorption to mineral surfaces. Table 15.1 is a non-exhaustive list of the changes in enzyme activity that have been observed in enzyme potential activity upon adsorption to various common soil sorbents. Most typically, a decrease in enzyme activity is recorded though the amount of activity inhibition varies from non-existent to complete. The hypotheses that have been proposed to explain this activity decrease are (1) a difference in the pH or ionic strength that an enzyme experiences close to a mineral's surface *versus* the bulk solution (Claus and Filip 1988; Skujins 1976; Skujins et al. 1959), (2) a conformational change in the enzyme when adsorbed to a mineral surface (Leprince and Quiquampoix 1996; Quiquampoix 1987a), or (3) steric hindrance (Baron et al. 1999; Naidja et al. 2000; Quiquampoix and Burns 2007). However, the first hypothesis cannot explain the shift toward a higher pH of maximal activity that is commonly observed when an enzyme is adsorbed (Baron et al. 1999; Pflug 1982; Quiquampoix 1987b; Skujins et al. 1974). Evidence for the second hypothesis is found in more recent studies that have shown pH-dependent structural changes in enzyme conformation upon mineral adsorption that may cause enzyme deactivation (Servagent-Noinville et al. 2000), and the nonreversibility of enzyme activity loss upon desorption (Leprince and Quiquampoix 1996; Quiquampoix 1987a). In further support of this theory, cases in which there is no loss of enzyme activity also show no sign of enzyme secondary structure alteration (e.g., laccase on Al hydroxide; Ahn et al. 2007).

Substrate accessibility to an enzyme's active site, or vice versa (steric restriction), may also play a role in reduced sorbed enzyme activity. The orientation of enzyme attachment has also been shown to be pH dependent (Baron et al. 1999) and this would explain why some minerals exhibit enhanced activity in the bound state and why mineral edge-bound versus face-bound enzymes may display varying activity. Variations in bound enzyme orientation, and differing mineral surface morphology may also explain why enzymes adsorbed to minerals similar in chemistry may widely differ in adsorbed enzyme activity (Table 15.1). For example, the

Table 15.1 Changes in enzyme activity following mineral interaction

Study	Enzyme	Sorbent	Activity change (%)
Morgan and Corke (1976)	Glucose oxidase	Ca-montmorillonite	−57 to −96
Ross and McNeilly (1972)	Glucose oxidase	Kaolinite	−17
Ross and McNeilly (1972)	Glucose oxidase	Illite	−21
Ross and McNeilly (1972)	Glucose oxidase	Ca-montmorillonite	−77
Ross and McNeilly (1972)	Glucose oxidase	Allophane	−52
Hughes and Simpson (1978)	Arylsulphatase	Ca-montmorillonite	−52
Hughes and Simpson (1978)	Arylsulphatase	Kaolinite	−18
Haska (1981)	Mixed endopeptidases	Montmorillonite	−66
Claus and Filip (1988)	Laccase	Bentonite	−11 to −100
Claus and Filip (1988)	Tyrosinase	Bentonite	−84 to 100
Claus and Filip (1988)	Laccase	Kaolinite	−30 to −83
Claus and Filip (1988)	Tyrosinase	Kaolinite	0 to −75
Quiquampoix (1987a)	Glucosidase	Na-montmorillonite	−35 to −100
Quiquampoix (1987a)	Glucosidase	Kaolinite	−13 to −100
Quiquampoix (1987a)	Glucosidase	Goethite	0 to 40
Skujins et al. (1974)	Chitinase	Kaolinite	−95
Naidja et al. (1997)	Tyrosinase	$Al(OH)_x$-coated-montmorillonite	−24 to −62
Naidja and Huang (1996)	Aspartate	Ca-montmorillonite	−20
Rao et al. (2000)	Acid phosphatase	Montmorillonite	−80
Rao et al. (2000)	Acid phosphatase	Al hydroxide	−55
Calamai et al. (2000)	Catalase	Ca-montmorillonite	−81 to −99
Gianfreda et al. (1991)	Invertase	Na-montmorillonite	−88 to −96
Gianfreda et al. (1991)	Invertase	$Al(OH)_x$-montmorillonite	−89 to −95
Gianfreda et al. (1991)	Invertase	$Al(OH)_x$	−94 to −99
Gianfreda et al. (1992)	Urease	Na-montmorillonite	−41
Gianfreda et al. (1992)	Urease	$Al(OH)_x$-montmorillonite	−49 to −67
Gianfreda and Bollag (1994)	Laccase	Montmorillonite	0
Gianfreda and Bollag (1994)	Laccase	Kaolinte	−14
Gianfreda and Bollag (1994)	Peroxidase	Montmorillonite complex	0
Gianfreda and Bollag (1994)	Kaolinite	Montmorillonite complex	−21
Gianfreda and Bollag (1994)	Acid phosphatase	Montmorillonite	−68
Gianfreda and Bollag (1994)	Acid phosphatase	Kaolinite	−64
Huang et al. (2005)	Acid phosphatase	Goethite	−32
Huang et al. (2005)	Acid phosphatase	Kaolinite	−43
(Ahn et al. 2007)	Tyrosinase	Al hydroxide	−11[a]
(Lozzi et al. 2001)	Peroxidase	Ca-montmorillonite	0 to −69
(Lozzi et al. 2001)	Peroxidase	Na-montmorillonite	−88 to −99
Tietjen and Wetzel (2003)	Alkaline phosphatase	Elledge clay	−75
Tietjen and Wetzel (2003)	Alkaline phosphatase	Montmorillonite	−67

(*continued*)

Table 15.1 (continued)

Study	Enzyme	Sorbent	Activity change (%)
Tietjen and Wetzel (2003)	Glucosidase	Elledge clay	+55
Tietjen and Wetzel (2003)	Glucosidase	Montmorillonite	+50
Tietjen and Wetzel (2003)	Protease	Elledge clay	−82 to 82
Tietjen and Wetzel (2003)	Protease	Montmorillonite	−100
Tietjen and Wetzel (2003)	Xylosidase	Elledge clay	+50
Tietjen and Wetzel (2003)	Xylosidase	Montmorillonite	+200
Quiquampoix (1987b)	Glucosidase	Montmorillonite	0 to −82
Quiquampoix (1987b)	Glucosidase	Natural clay fraction	0 to −99
Quiquampoix (1987a)	Glucosidase	Kaolinite	0 to −88
Rao et al. (2000)	Phosphatase	Na-montmorillonite	−80
Rao et al. (2000)	Phosphatase	Al hydroxide	−55
Rao et al. (2000)	Phosphatase	$Al(OH)_x$-montmorillonite	−42

[a]Calculated from V_{max}

enhanced catalytic activity of nanoparticle-bound enzymes relative to their "free" counterpart (Serefoglou et al. 2008) may be due to favorable active site orientation on these surfaces or to alleviation of reactant or product diffusion limitation that may occur on particles of larger sizes. Of course, it is recognized that the nature of the specific sorbent–enzyme pair (e.g., "hard" or "soft" enzyme, hydrophobicity of mineral surface) may also determine which of these possible mechanisms play a dominating role in altering enzyme activity.

As pointed out by Quiquampoix and Burns (2007), the study of enzyme interaction with mineral surfaces is difficult because changes in conformation both result from and drive enzyme adsorption to mineral surfaces and because the structure of enzymes in the adsorbed state cannot be fully examined by the methods currently available. Spectroscopic methods have only permitted elucidation of protein secondary structure, though advances in this regard are being made (Smith et al. 2009). Further, neither the chemical environment at the surface of a mineral nor the orientation of a sorbed enzyme can be completely known. An additional impediment to the advancement of our understanding is that enzyme activity experiments are not often conducted with associated enzyme adsorption and substrate adsorption experiments. Without these, it cannot be known what portion of the activity measured is produced by adsorbed enzymes versus "free" enzymes. Variation in the enzyme activity with substrate concentration, as described, for example, by the Michaelis–Menten equation is not commonly determined for mineral-adsorbed enzymes (discussed below). Even less often is a range of enzyme-mineral surface area loadings tested. Lastly, soils are commonly composed of a heterogeneous assemblage of minerals. But pure clays have been, by far, the main focus of attention with regard to enzyme– mineral interaction experiments. In many clay-poor soils, amorphous or poorly crystalline minerals such as Al and Fe oxyhydroxides may play a dominant role in regulating enzyme stability and activity and require greater study.

15.4 Enzyme–Organic Matter Interactions

It is likely that, particularly in soils with higher OM or sand contents, enzyme–OM interaction plays a more important role in regulating soil enzyme activity and stability than enzyme–mineral interaction. Research in this area has utilized two general methods. The first is to examine the association of enzymes with different natural soil OM fractions through various extraction schemes and, perhaps, to measure the residual potential activity present in each fraction. The other approach is to compare the kinetic properties of free enzyme forms with those of OM–enzyme complexes prepared in the laboratory. Each approach has yielded important insights.

Most soil extracellular enzymes are thought to be intimately associated with soil OM as they cannot be efficiently extracted without also extracting a great deal of humic substances (Ladd 1972). Further, many purification techniques fail to separate enzymes from their associated OM, and enzymatic activity and organic-C contents of the extracts are generally correlated (Ceccanti et al. 1978). It can be assumed that enzymes will form complexes with the OM components of soil much as do proteins, which are associated and react chemically with phenols, quinones, tannins, lignin components, and HA (Ladd and Butler 1975 and references therein). Both hydrogen and other electrostatic bonds and covalent bonds are known to occur, and the natures of both are strongly dependent upon pH.

Studies of extracted HA–enzyme complexes and attempts at purification have not revealed any single mode of association, but rather, a combination of mechanisms have been suggested including ion exchange, physical entrapment within three-dimensional structures, hydrogen and covalent bonding. For example, the involvement of inorganic cations such as Ca^{+2} in encouraging protein complexation and complexed-enzyme inactivation would suggest the influence of electrostatic forces (Ladd and Butler 1969; Mayaudon and Sarkar 1975; Pflug 1981). However, the effectiveness of sodium pyrophosphate in extracting active soil OM-bound extracellular enzymes (Ceccanti et al. 1978; Nannipieri et al. 1988) suggests that a large fraction of enzymes are covalently bound or, at least, strongly associated with humic substances rather than ionically bound. Another prospective is provided by the observation of a direct relationship between molecular weight of the fractionated soil humic extracts and its resistance to thermal denaturation and proteolysis (Butler and Ladd 1971; Nannipieri et al. 1988). This would suggest a HA porous structure that has to permit the diffusion of substrates toward and products away from the enzyme, but restricts the movement of larger molecules such as proteases. Some amount of diffusion limitation would be expected to occur, however, no matter the size of the substrate, and this may play a role in the usually observed reduced activity of OM-bound enzymes (Table 15.2). This type of encapsulation may be maintained mainly by hydrophobic interactions, which is supported by recent calorimetric measurements of protein–HA complexation reactions (Tan et al. 2008) and ^{15}N and ^{13}C NMR spectrographic monitoring of OM degradation (Zang et al. 2000, 2001).

Table 15.2 Changes in enzyme activity following organic matter interaction

Author	Enzyme	Sorbent/Complexant	Activity change (%)
Grego et al. (1990)	Pronase	Resorcinol	−80
Grego et al. (1990)	Pronase	Pyrogallol	−100
Grego et al. (1990)	Pronase	Catechol	−100
Rowell et al. (1973)	Trypsin	Benzoquinone	−56 to −98
Rowell et al. (1973)	Pronase	Benzoquinone	−32 to −96
Serban and Nissenbaum (1986)	Peroxidase	Humic acid	+18 to +125
Serban and Nissenbaum (1986)	Catalase	Humic acid	+100
Ruggiero and Radogna (1988)	Tyrosinase	Humic acid	−5 to +19
Pflug (1981)	Malate dehydrogenase	Humic acid	−16 to −57
Butler and Ladd (1969)	Pronase	Humic acid	−40 to −73
Butler and Ladd (1969)	Trypsin	Humic acid	−44 to −64
Butler and Ladd (1969)	Papain	Humic acid	+88 to +160
Butler and Ladd (1969)	Carboxypeptidase	Humic acid	−35 to −76
Gianfreda et al. (1995b)	Urease	Tannic acid	−29 to −72
Rao et al. (2000)	Acid phosphatase	Tannic acid	−49 to −93
Criquet et al. (2000)	Laccase	Humic acid	−95 to +60
Allison (2006)	Glucosidase	Humic acid	−70 to −100
Allison (2006)	Acid phosphatase	Humic acid	−20 to −65
Allison (2006)	Urease	Humic acid	−10 to −35
Allison (2006)	Glucosaminidase	Humic acid	−75 to −90
Allison (2006)	Polyphenol oxidase	Humic acid	−35 to −70
Boavida and Wetzel (1998)	Phosphatase	Humic acid	+10 to +100
Canas et al. (2007)	Laccase	Acetosyringone	−10 to −50
Canas et al. (2007)	Laccase	Syringaldehyde	0 to −50
Canas et al. (2007)	Laccase	Vanillin	−50 to −60
Canas et al. (2007)	Laccase	Acetovanillone	−70 or +50
Canas et al. (2007)	Laccase	2,4,6-Trimethylphenol	0 to −30
Canas et al. (2007)	Laccase	*p*-coumaric acid	−85 or +250
Canas et al. (2007)	Laccase	Ferulic acid	−30 or +80
Canas et al. (2007)	Laccase	Sinapic acid	−50 or +100
Freeman et al. (2004)	Sulphatase	Phenolic compound	−47[a]
Freeman et al. (2004)	Phosphatase	Phenolic compound	−18[a]
Freeman et al. (2004)	Glucosidase	Phenolic compound	−26[a]
Freeman et al. (2004)	Xylosidase	Phenolic compound	−16 [a]
Freeman et al. (2004)	Chitinase	Phenolic compound	−22[a]
Ladd and Butler (1969)	Pronase	Humic acid	−25 to −35
Freeman et al. (2004)	Trypsin	Humic acid	−18 to −52
Freeman et al. (2004)	Carboxypeptidase A	Humic acid	−33 to −49
Freeman et al. (2004)	Papain	Humic acid	+49 to +226

[a]Not pH buffered

HA–enzyme complexes have been prepared in vitro through simple adsorption techniques, such as with trypsin and pronase adsorbed to benzoquinone (Rowell et al. 1973) and pronase adsorbed to polyphenolic HA precursors (Grego et al. 1990). However, these complexes retained little of their pre-interaction activity. In contrast, better activity retention was achieved when phenoloxidase enzymes, such as peroxidases and laccases, were combined with HA precursors and lignin subunits (Canas et al. 2007; Sarkar and Burns 1983, 1984), which carry out oxidative coupling reactions much like those thought to occur during the formation of natural soil humic substances. Recently, ^{15}N-^{1}H HSQC NMR spectrometry has provided evidence for protein–quinone group covalent bonding of HAs using a tetrapeptide

(Hsu and Hatcher 2005). But while these synthetic model studies, along with those utilizing preformed humic materials, generally show greater stability and decreased activity of OM-associated enzymes (Table 15.2), a wide range of magnitude of changes have been demonstrated with no clear pattern emerging. It seems that, much as with enzyme–mineral interactions, bound enzyme activity is highly specific to the enzyme–sorbent pair and deactivation may occur due to the orientation of an enzyme's active site relative to each specific substrate's accessibility or to distortion of an enzyme's structure due to intermolecular forces in its bound chemical environment.

One way that the effects and mechanisms of enzyme inactivation due to mineral versus OM interaction can be directly compared is through the use of Michaelis–Menten parameters, which model enzyme kinetic properties. Though most of the enzyme interaction experiments (Tables 15.1 and 15.2) have recorded enzyme activity at a given substrate and enzyme concentration, a range of substrate concentrations must be tested to derive K_m, the Michaelis–Menten constant, and V_{max}, the maximum enzyme reaction velocity. The former is a measure of the affinity of an enzyme for a substrate (a higher K_m value indicates lower affinity), and the latter, describes the rate of substrate conversion activity that occurs when enzyme active sites are saturated with substrate (Fig. 15.2). These are useful because they are independent of the enzyme concentration used and also provide insight into the operative inhibition mechanism.

While they can only strictly be applied to simple enzyme–substrate systems with homogeneous reactions, the "apparent" kinetic values obtained for bound enzymes, i.e., heterogeneous catalysts with diffusion limitations, may have mechanistic implications. For example, reversible competitive inhibition, which occurs when a substance competes for the same active site as the substrate, will be characterized by an increase in K_m without a change in V_{max} (Fig. 15.2). This type of inhibition may be analogous to the temporary binding of OM to an enzyme's active site.

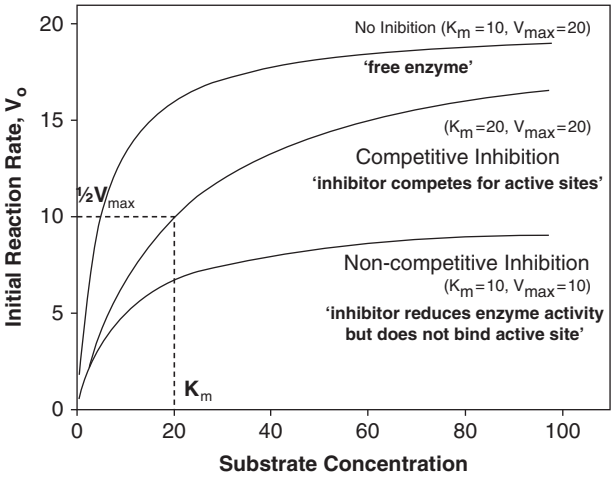

Fig. 15.2 Model Michaelis-Menton plots showing predicted kinetic changes with enzyme inhibition

Noncompetitive inhibition, indicated by a decrease in V_{max} without a change in K_m, occurs when an inhibitor binds to either the enzyme–substrate complex or the enzyme itself, resulting in lowered enzymatic efficiency. This might be likened to an enzyme conformational change, which occurs upon mineral or OM interaction. With irreversible inhibition, one would expect to see time-dependent changes in both K_m and V_{max}. Among those soil mineral and OM interaction experiments that have calculated Michaelis–Menten parameters (Table 15.3), mixed inhibition was most common, indicating that both competitive and noncompetitive inhibition may be operative. That is, substrate binding to both active and nonactive sites of the enzyme, conformation alteration, and diffusion, charge, and steric effects may decrease enzyme affinity for substrate. Decreased K_m values, indicating increased enzyme–substrate affinity, have been observed, mainly for montomorillonite. This may be attributable to the formation of an adsorption bond orientation, which causes enhanced active site accessibility to substrate. And increased enzyme activity has been observed in the presence of urea that has been attributed to loosening of the enzyme's structure, which confers increased conformational flexibility at the active site (Fan et al. 1996; Zhang et al. 1997). Other kinetic constants such as turnover frequency and enzyme efficiency may provide additional information with which to compare the effects of complexents. However, the extent to which these parameters are applicable to the extremely heterogeneous nature of soil systems is debatable.

15.5 Mineral-Organic Matter–Enzyme Synergistic Interactions

While the enzyme complexation experiments of the type reviewed, above, provide some insight into the fundamental processes affecting enzyme activity in soils, an understanding of the actual effect of minerals and OM on soil enzyme activity cannot be achieved without consideration of the possible synergistic interactions between enzymes and these complexants. Soil OM and soil minerals are intimately associated, so it is likely to be only rarely that an enzyme is associated with one or the other, exclusively. That being so, the relatively low number of experiments that have been carried out in ternary systems or examining alternative enzyme–substrate-sorbent relationships is rather surprising. Furthermore, of the wide variety of ways in which minerals, OM and enzymes may interact (Fig. 15.1), only a few have been studied to any great extent.

One method used to study the ternary interaction between enzyme, OM, and mineral is to measure the activity of OM-complexed enzymes fixed to mineral surfaces. In one case, β-D-glucosidase, which had been made somewhat resistant to protease attack over 24 h due to copolymerization with phenolic compounds, was completely resistant when these copolymers were fixed to bentonite (Sarkar and Burns 1984). Interestingly, while the copolymer immobilization was accompanied by increases in K_m and decreases in V_{max}, changes in these parameters were not as large as those that would be expected had enzyme–phenol and enzyme–bentonite changes been additive. These data imply that association with OM can, to a degree, shield an enzyme from the conformational or steric limitations that might otherwise

Table 15.3 Changes in Michaelis–Menten kinetic parameters following enzyme complexation with mineral or organic matter

Study	Enzyme	Sorbent/Complexant	K_m[a] (% change from free to complexed)	V_{max}[a]
Minerals				
Makboul and Ottow (1979a)	Acid phosphatase	Montorillonite	+5779	−46
Makboul and Ottow (1979a)	Acid phosphatase	Illite	+500	−56
Makboul and Ottow (1979a)	Acid phosphatase	Kaolinite	+443	−69
Makboul and Ottow (1979b)	Urease	Montorillonite	+191	−50
Makboul and Ottow (1979b)	Urease	Illite	+88	−50
Makboul and Ottow (1979b)	Urease	Kaolinite	+161	−48
Dick and Tabatabai (1987)	Acid phosphatase	Montorillonite	0	−38 to −67
Dick and Tabatabai (1987)	Acid phosphatase	Illite	0	−21 to −46
Dick and Tabatabai (1987)	Acid phosphatase	Kaolinite	+68 to +307	0
(Dick and Tabatabai 1987)	Pyrophosphatase	Montorillonite	0	−39 to −48
(Dick and Tabatabai 1987)	Pyrophosphatase	Illite	0	−18 to −41
(Dick and Tabatabai 1987)	Pyrophosphatase	Kaolinite	+71 to +138	0
Serefoglou et al. (2008)	β-glucosidase	Montmorillonite	−60	−3
Gianfreda et al. (1992)	Urease	Montorillonite	−73	−30
Sarkar and Burns (1984)	β-glucosidase	Bentonite	+45	−98
Sarkar and Burns (1984)	β-glucosidase	$Al(OH)_x$	−9.4	−81
Sarkar and Burns (1984)	β-glucosidase	Montorillonite-Al $(OH)_x$	−48	−36
Marzadori et al. (1998b)	Urease	Hydroxyapatite	−6.7	−34
Ahn et al. (2007)	Laccase	$Al(OH)_x$	−16	−11
Rosas et al. (2008)	Acid phosphatase	Allophonic clay	−14	+42
Shindo et al. (2002)	Acid phosphatase	Fe oxide	+67	−77
Shindo et al. (2002)	Acid phosphatase	Al oxide	−20	−87
Shindo et al. (2002)	Acid phosphatase	Mn oxide	+120	−87
Organic matter				
Rosas et al. (2008)	Acid phosphatase	Tannic acid	+135	−35
Gianfreda et al. (1995b)	Urease	Tannic acid (0.02 mM)	−11	−25
Gianfreda et al. (1995b)	Urease	Tannic acid (0.1 mM)	−63	−71
Vuorinen and Saharinen (1996)	Phosphomonoesterase	Soil OM extract (0.05 mg/L)	+124	+2.6
Vuorinen and Saharinen (1996)	Phosphomonoesterase	Soil OM extract (0.5 mg/L)	+22	−0.7
Marzadori et al. (1998a)	Acid phosphatase	Ca-polygalacturonate	+102	−59
Rao et al. (2000)	Acid phosphatase	Tannic acid	+100	−75
Rao et al. (2000)	Acid phosphatase	Tannic acid + Fe	+233	−75
Rao et al. (2000)	Acid phosphatase	Tannic acid + Mn	+167	−63
Sarkar and Burns (1984)	β-glucosidase	Resorcinol	+13	−45
Sarkar and Burns (1984)	β-glucosidase	Tyrosine	+52	−75

[a]Michaelis–Menton constant and maximum conversions rate, K_m and V_m, respectively, calculated using Linweaver–Burk equation

accompany mineral boundedness. In another study (Ahn et al. 2006), laccase activity in the ternary system of laccase with catechol and humic-like polymers, produced through birnessite catalysis of catechol, was less than the sum of enzyme activity in binary systems of laccase and birnessite or laccase and catechol. Enzyme inactivation both by birnessite-generated humic-like polymers and by Mn^{+2} ions released from the mineral were considered possible causes of the inhibition. An effect of metal ions, which are commonly released from minerals especially when redox changes occur, on enzyme activity has been observed in other cases. For example, Fe^{+2} has been shown to stimulate phenol oxidase activity (Van Bodegom et al. 2005) and soluble Fe^{+3}, Mn^{+2}, and Al^{+3} species stimulated the activity of tannate–urease complexes (Gianfreda et al. 1995a; Gianfreda et al. 1995b). Interestingly, these species had little effect on the activity of phosphatase–tannic acid complexes unless montomorillite was also present (Rao et al. 2000).

Perhaps due to the possibility of industrial and contaminant remediation applications, while many studies have examined the activity of "free" and mineral-adsorbed enzymes, only a few studies have examined enzyme activity when provided mineral- or OM-adsorbed substrates. This may be carried out by performing substrate adsorption prior to enzyme addition; however, the possibility of substrate desorption and enzyme adsorption must be considered. In one study (McLaren and Estermann 1957), the activity of chymotrypsin, when supplied kaolinite-sorbed lysozyme as a substrate, exhibited no loss of activity relative to "free" lysozyme. However, an increase in the pH of maximum activity was observed, much as occurs with most mineral-adsorbed enzymes. In another study (Skujins et al. 1974), an 84% decrease in chitinase activity occurred when provided with kaolinite-sorbed chitin as a substrate, but this was less than the 94% decrease observed when the chitinase was first adsorbed to kaolinite. Zimmerman et al. (2004a) found that oxidation of an amorphous alumina-adsorbed phenol by laccase was somewhat less (by a mean of 7%) than that of the free compound, while that of amorphous silica-adsorbed phenol was enhanced (by a mean of 20%). In this same study, almost complete loss of laccase activity occurred when the substrate was occluded within alumina and silica nanopores, showing that mineral surface morphology can play a major role in substrate accessibility to enzymes. Thus, bound substrates may or may not be completely protected from enzymatic digestion, depending upon the situation, but sorbed-enzyme degradation of sorbed substrate is likely to be extremely limited.

Native OM can also control the adsorption of enzymes to mineral surfaces. For example, HAs added before urease resulted in less urease adsorption to hydroxyapatite and less urease activity than when urease was added first (Borghetti et al. 2003). Interestingly, the order of addition, in this case, had no effect on long-term enzyme stability. Conversely, minerals, particularly short-range-ordered minerals such as Fe, Al, and Mn oxyhydroxides promote the immobilization of OM and enzyme-associated OM via polymerization and copolymerization reactions (Ahn et al. 2006; Gianfreda et al. 1995a; Gianfreda et al. 1995b; Rao et al. 1996).

Soil OM may play an additional role in enzyme activity regulation by providing microbes with nutrients needed to produce enzymes (induction), providing co-enzymes, activator or inhibitor molecules, or substrate competitors. Measurements

of enzyme activity following experimental additions of various substrates have been carried out, but often produce complex results that vary with enzyme and substrate type, soil mineralogy, and response period. For example, acid phosphatase activity was only stimulated by citrate in a clayey soil, versus oxalate, glutamate, and citrate in a sandy soil (Renella et al. 2007). In the same study, urease activity was only stimulated by glucose and citrate in the clayey soil, and by no substrates in the sandy soil. In another study (Nannipieri et al. 1983), additions of glucose or rye grass to a clay-loam increased phosphatase and urease activities in proportion to increases in bacterial biomass, whereas casein hydrolysis was delayed. Often, enzymatic remineralization out of proportion to the amount of substrate added is observed, i.e. "OM priming" (Kuzyakov et al. 2000). Although some of the results of these types of addition experiments conform to prediction, i.e., increased enzyme activity when with labile substrates are abundant and decreased enzyme activity when only complex substrates are present (Allison and Vitousek 2005), many times they do not, suggesting that factors other than substrate supply influence soil enzyme activity. Among them, surely, is enzyme–organo-mineral interaction. In addition, the common observation that most enzyme activities returned to their pre-OM addition levels (e.g., Dilly and Nannipieri 2001; Kuzyakov et al. 2000; Nannipieri et al. 1983) is support for the hypothesized homeostatic level of microbial/enzymatic activity for each particular soil, first proposed by Burns (1982), controlled perhaps by the enzyme–organo-mineral interaction present in each soil type. Future work should combine substrate adsorption experiments with controlled incubations of mixed microbial populations in binary and ternary OM-mineral–enzyme systems to identify the mechanisms responsible for these interactions.

15.6 Consequences of Organo-Mineral–Enzyme Interactions

Aside from the obvious importance of soil enzymes in supporting all microbial life and, thus, making nutrients available to the plants upon which we all depend, organo-mineral–enzyme interactions, in particular, are of fundamental importance in explaining diverse phenomena such as the bioavailability and migration of organic contaminants (Luthy et al. 1997), the sequestration of organic carbon in soils and coastal and marine sediments (Mayer 1994; Mayer 1999; Torn et al. 1997), and the global carbon balance regulating atmospheric CO_2 concentrations. Although most biomolecular OM is inherently labile, some portion of it is preserved in soils and sediments over long timescales and remains apparently unavailable to microbial decomposers (Hedges and Keil 1995; Luthy et al. 1997). Direct correlations between soil and sediment surface area and organic carbon (Baldock and Skjemstad 2000; Kennedy et al. 2002) suggest that organo-mineral complexation stabilizes labile forms of OM against enzymatic attack, and this has been shown experimentally (Jastrow and Miller 1997; Kaiser and Guggenberger 2000; Zimmerman et al. 2004a). The mechanisms responsible for this OM protection are the same ones outlined, above, including enzyme activity limitation via adsorption

or complexation within OM or on mineral surfaces. Enzymes or substrates may be occluded within mineral micro- and nanofabric structures such as aggregates and clay domains, sorbed to mineral surfaces (particularly clays, poorly crystalline minerals or micro- or nanopores), or complexed within organic polymer structures.

15.7 Conclusion

Advances in enzyme technology will allow the examination of enzyme–organo-mineral interactions in greater detail than previously allowed. For example, advances in spectroscopic techniques will allow better detection of changes in enzyme tertiary structure that occur with OM and mineral interaction (Smith et al. 2009). Experimental and analytical techniques developed by biochemists and chemical engineers have recently been creatively employed to probe the enzyme–organo-mineral relationship. For example, Ziervogel et al. (2007) tethered a fluorescently labeled polysaccharide (covalently bonded pullulan) to agarose beads to examine the accessibility of mobility-restricted substrates to a "free" and clay-adsorbed enzyme (pullanase). In this study, apportionment of the products of enzymatic hydrolysis of the substrate into different molecular weight classes was monitored by gel permeation chromatography. In another study, decreases in the molecular size of "free" and montmorillonite-sorbed spin-labeled polysaccharides were monitored in real time using electron paramagnetic resonance spectroscopy (Steen et al. 2006), allowing high-resolution determinations of enzyme sorption and degradation kinetics. Finally, in a novel application of histochemical staining, Curry et al. (2007) were able to visualize OM protected from enzymatic digestion in nanoscale clay structures using acid-thiosemicarbazide-silver proteinate-tagged polysaccharides and transmission electron microscopy. With analytical and visualization techniques such as these, we will be able to examine enzyme–organo-mineral relationships in ever greater detail. While past research has mainly examined binary interaction of enzymes with certain minerals (mostly clays) or specific OM fractions, future work should focus on ternary enzyme–organo-mineral synergistic interactions, including those of mineral catalysis, OM priming, and mineral and OM protective structures.

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