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Inorganic nanomaterial-based biocatalysts

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Over the years, nanostructures have been developed to enable to support enzyme usability to obtain highly selective and efficient biocatalysts for catalyzing processes under various conditions. This review summarizes recent developments in the nanostructures for enzyme supporters, typically those formed with various inorganic materials. To improve enzyme attachment, the surface of nanomaterials is properly modified to express specific functional groups. Various materials and nanostructures can be applied to improve both enzyme activity and stability. The merits of the incorporation of enzymes in inorganic nanomaterials and unprecedented opportunities for enhanced enzyme properties are discussed. Finally, the limitations encountered with nanomaterial-based enzyme immobilization are discussed together with the future prospects of such systems. [BMB reports 2011; 44(2): 77-86]

INTRODUCTION

As the use of enzymes as biocatalysts is extended within various fields, including biotechnology, industry and environmental technology, enzyme stabilization has given rise to a great number of research works of both its academic and practical uses. Many approaches to improve the enzyme stability have been attempted, for example, enzyme immobilization, enzyme modification, protein engineering and medium engineering. Among them, enzyme immobilization is most widely used in the processing of variety of products and increasingly used in the fields of medicine, food and pharmaceutical industries (1-3). Immobilization of enzymes to solid support allows repetitive use, rapid cessation of the reaction, and easy recovery and separation of the enzymes as well as improvement of enzyme stability.

Inorganic materials are attractive as immobilizing supports due to stronger mechanical strength, higher resistance to organic solvents and thermal stability. With recent progress in nanoscience, many inorganic nanomaterials are available for enzyme immobilization. Nanostructures provide a large surface area and comfort compartment for the immobilization of enzyme. Most of the nanomaterials used today are silica having nonporous and porous formats. However, it is expanding to a broad variety of materials and structures. Manners of enzyme attachment should be considered to obtain higher activity and stability. Prior to enzyme attachment, the surface of particles was usually modified to express specific functional groups which promoted the attachment. After enzyme immobilization, there are changes in enzymatic functions, for example, activity, stability, selectivity, and utilization and those are depends on the combination of enzyme and carrier. Consequently, a variety of future applications are envisaged for such immobilized biocatalysts in the medical, industrial, pharmaceutical and environmental realms, including biosensor, bioremediation or food production. In this article, we overview the state-of-the-art technology in rapidly developing field that focus on inorganic nanomaterial-based biocatalyst systems.

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Inorganic Nanoparticles for Enzyme Immobilization

In enzyme immobilization, it should be considered that the physical and chemical nature of carriers such as their nanostructure, hydrophilic or hydrophobic properties, the charges on the carriers, and the binding chemistry, strongly dictated the biocatalytic characteristics of the enzyme. These natures of carriers are usually determined by material itself but, sometimes, surface modification is performed to modify the nature of materials. Following the improvement of nanoscience and nanotechnology, the challenge in application of nanomaterials is to control not only the particle size but also the particle shapes and morphologies, which would allow unique nature for enzyme immobilization. In this section, we summarize what materials have been employed and what nanostructures adopted in developing biocatalyst systems.

Gold nanoparticles

Gold nanoparticles (AuNPs) have high affinity for biomolecules either with or without linkers. Surface of gold is easily modified with sulfur-containing compounds such as alkane thiols and ditiocarbamates. These types of compounds deprotonate upon self-assembled monolayers (SAMs), a process that neutralizes the negatively charged sulfur-containing compound assuming the formation of gold (4, 5). SAMs of alkane thiols

on gold containing a functional terminal group have served to associate additional monolayers of enzymes by covalent conjugation, supramolecular association, physical adsorption and electrostatic interactions (6-11). Previous study found that immobilization of enzyme onto gold nanoparticle's surfaces, without any surface modification, provided colloidal stability, which result to significantly enhanced catalytic activity (11). In addition, gold nanoparticles facilitate electron transfer and can be easily modified using a wide range of biomolecules and chemical ligands.

It was reported that nanoporous gold electrodes with increased surface area improved enzyme immobilization and, ultimately, bioelectrocatalytic activity (12). This nanoporous electrode could be employed in biofuel cells with a potentially higher power output. In the other study, nanoporous gold with a pore size of 40-50 nm was prepared by dealloying Au/Ag alloy (50:50 wt%) for 17 h (13).

Nanosphere was the most widely used structure among gold nanoparticles. Horseradish peroxidase was modified with cysteamine-capped Au nanospheres and further immobilized on sodium alginate-coated Au electrode through polyelectrostatic interactions for developing mediatorless H_2O_2 quantifying biosensor (14). Moreover, the gold nanosphere-mesoporous silica composite represented excellent catalytic behavior to the oxidation of glucose, faster response time, lower detection limit, higher sensitivity and wider linear range with respect to solely use gold nanosphere. Zhang *et al*. reported that flower-like ZnO crystal was synthesized to be employed for anchoring hoseradish peroxidase (HRP)-labeled gold nanospheres (15). The introduction of flower-like ZnO crystals into chitosan matrix to be casted on the electrode could offer greatly increased loading surface area available for binding gold nanosphere and HRP in a larger scale.

Carbon nanotubes

Carbon nanotubes (CNTs) have been extensively used as the electrode modifying materials due to their high electrocatalysis (16), efficiency for immobilization of proteins (17) and excellent electron wiring to result direct electrochemistry of redox proteins (18). It also can serve as supporting materials for enzyme immobilization because carbon nanotubes can be broadly functionalized and have good dispersion in solution compared to other nanomaterials (19, 20). Highly efficient enzyme loading was reported by using single-wall carbon nanotubes without requiring enzyme purification for immobilization (21). After immobilization, 92% maximum activity of the native enzyme was maintained on single wall nanotubes (SWNTs). The electrical properties of SWNTs are suitable for use in biological electronics and electrochemical biosensors. Lee et al. improved electrical properties of an anode of enzyme-based biofuel cell via immobilization of glucose oxidase on single walled nanotubes (22).

Furthermore, CNTs can maintain direct electron transfer between redox enzymes and electrodes because of its good conductivity. Multi-walled carbon nanotubes (MWNTs) were also used to modify working electrode after immobilizing glucose oxidase (23). It exhibited fast response, high sensitivity and stability to glucose detection. The other study reported that sulfonated polyaniline network was formed on multi-walled carbon nanotubes (MWNTs) and, subsequently, glucose oxidase was immobilized onto polyaniline matrix on MWNTs to fabricate the biosensor (24). Immobilized enzyme on MWNTs was packed onto a ceramic bed to fabricate ceramic cylinder fed-bath reactor (25).

Magnetite nanoparticles

Enzyme immobilization on to magnetic supports such as magnetite nanoparticles allow selective and easy enzyme recovery from the medium under the magnetic force without need for expensive liquid chromatography systems, centrifuges, filters or other equipment (26). Even though functional groups could be introduced on the surface of magnetic beads through suspension polymerization in the presence of ferric ions (27), it is difficult to modify the surface of magnetite nanoparticles. In addition, magnetite nanoparticles are highly hydrophobic in aqueous solution, resulting to cause aggregation. Hence, the surface of magnetite nanoparticles was coated with silica before employing to enzyme immobilization. Silica is often employed as a coating material over the surface of nanoparticles. Silica is chemically inert, promotes the dispersion of the nanoparticles and has a high surface silanol concentration which facilitates a wide variety of surface reactions and the binding of biomolecules. Typically, silica coated magnetite is readily modifiable its surface to present a large number of functional groups, for example amino, aldehyde and alkyl chain groups. Consequently, dendritic silica coated magnetic nanoparticles were used to immobilize lipase and showed a higher enantioselectivity than that by free lipase (28).

Porous silica structures

Notwithstanding minimum diffusion limitation, enzyme loading per unit mass of nonporous nanoparticles is usually low. On the other hand, porous nanoparticles can afford high enzyme loading with a larger surface area. Especially, mesoporous silica particles have attracted an early attention as a host of enzyme immobilization due to high surface area, controllable pore diameter, and uniform pore size distributions (Fig. 1) (29-31).

Up to now, various type of mesoporous silicas have been synthesized and utilized for enzyme immobilization such as MCM-41, SBA-15 and mesocellular foam (MCM) (29, 32, 33). Typically, in mesoporous silica, many studies were reported to improve the stability of immobilized enzyme (34, 35). Functionalized porous silicas were synthesized to immobilized penicillin G acylase (36, 37). In addition, the immobilization behavior of enzyme in mesoporous silicas depending on morphologies has been investigated (38). Effect of textural and structural parameters was affected on the immobilization of

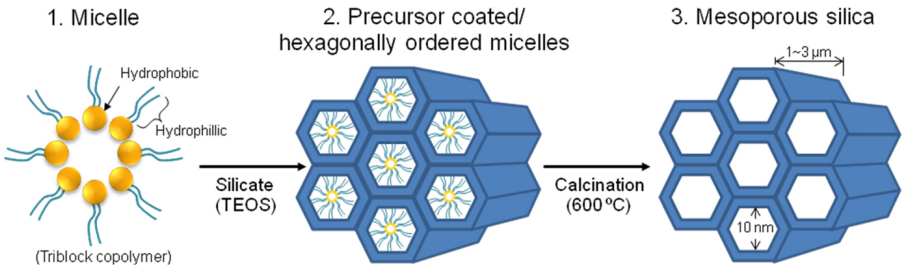

Fig. 1. Schematic fabrication of mesoporous silica nanoparticles.

enzyme in ordered mesoporous silicas (39). In this study, authors investigated effects of structure (cubic or hexagonal), the nature of the pores (channel-like or cage-like), the connectivity of the porous network and the pore size. The pH is also one of important parameters for the immobilization of enzyme (40). Now, magnetic mesoporous silica particles have been developed to easily separate immobilized enzyme from products (41, 42).

Other metallic materials

There are only few studies reported that used ZnO, copper and aluminum oxide (43-45). Patterned growth of ZnO nanorods using photoresist was successfully performed on epitaxial single crystalline ZnO using a hydrothermal method and glucose oxidase was immobilized (45). Because the large surface area of ZnO nanorods is expected to be advantageous for the detection of biomolecules, the immobilization of enzyme on their surface is one of the key issues for the fabrication of high sensitive biosensors. Furthermore, the integration of ZnO nanorods with field effect transistors is of importance for reducing the detection limit of target molecules and in a multifunctional biosensing system.

Nanoporous copper with a pore size of 100-200 nm was synthesized by simply dealloying $Al_{60}Cu_{40}$ alloy and horseradish peroxidase was immobilized on it (43). Since the nanoporous copper electrode has good electric conductivity and electrocatalytic activity, the enzyme immobilized nanoporous copper can be applied to an electrochemical biosensor.

After surface chemical polymerization, anodic aluminum oxide could be supports for enzyme immobilization (44). Since artificial membranes have high surface area per unit volume and possibility to combine separation with chemical reaction, it has interested biotechnologist in their attempt to find carriers for the immobilization of biocatalysts (46). Aluminum oxide membrane is a self-ordered nano-porous array suitable for preserving enzyme with high stability.

Manners of Enzyme Immobilization

Although, the basic manners of enzyme immobilization can be categorized into a few different methods only including adsorption, covalent bonding, entrapment, encapsulation, and cross-linking, various methods based on combinations of these original methods have been developed.

Physical adsorption

The approach of physical adsorption is very simple to perform to immobilize enzyme on the surface of nanoparticles since most of nanomaterials are hydrophobic in aqueous solution. Immobilized enzyme can be prepared by only immersing enzyme in buffer solution for certain time period to establish an adsorption equilibrium (34). However, aggregation of the particles should be avoided after immobilization. Direct contact of an enzyme to the surface of solid particles may hinder the flexible enzyme structure. In this case, the ligand on the surface of the particle is presumed to act as a spacer between the particle and the immobilized enzyme, thus mitigating this problem. Lee et al used sodium dodecyl sulfate to generate a hydrophobic surface and to create a spacer between the lipase and magnetic particles (26). To modify the surface of silica particles, hydrophobic alkyl chanin could be introduced on porous silica particles, which ultimately increase amount of immobilized enzyme (47). Chouyyok *et al*. compared significant roles of electrostatic interactions and meso-porous silica's pore characters on enzyme loading and stability (40).

Covalent bonding

However, the weak interactions between the enzyme and the nanoparticles may result in a rapid leaching of the immobilized enzyme through adsorption. Hence, covalent bond was used to immobilize enzyme on nanoparticles. Surface of nanoparticles can be modified to express covalent binding site. Especially, amino groups are introduced on the surface of particles, followed by modified with glutaraldehyde bearing a free aldehyde group. Those modified particles were reacted, via Schiff's base formation reaction, with the amino groups on the enzyme surface (14).

The other method for modification of surface is co-condensation (48, 49). This method allows modification of the surface of the particles in a single step by copolymerization of an organosilane with a silica or organosilica precursor in the presence of a surfactant template (36, 37). Introduction of func-

tional groups on the surface of porous silica particles can be easily achieved by using organosilanes, such as 3-aninopropyltriethoxysilane (APTES), 3-mercaptopropyltrimethoxysilane (MPTMS), phenyltrimethoxysilane (PTMS), vinyltriethoxysilane (VTES), and 4-(triethoxysilyl)butyronitrile (TSBN) (36, 37). Beside of that, glycidoxypropyl groups were introduced to the surface of mesoporous channels by the co-condensation of 1, 2-bis(triethoxysilyl)ethane (BTESE) and 3-glycidoxypropyltrimethoxysilane (GPTMS) under acidic conditions to immobilize enzyme (50).

Crosslinked enzyme aggregates

For mesoporous silica particles, the approach of crosslinked enzyme aggregates was reported as a simple and effective method for enzyme stabilization (51, 52). Crosslinked enzyme aggregates were prepared by crosslinking enzyme molecules after adsorption into the channel of mesoporous silica particles (53). It showed negligible activity decrease under harsh shaking condition while the conventional approaches including adsorption and covalent attachment resulted in more than 50-90% enzyme inactivation under the same condition (54). In addition, the covalent immobilization approach cannot take a full use of channels for enzyme immobilization, leading to lowered enzyme loading. This bottleneck can be prevented by physical adsorption of enzyme into mesoporous channels but the immobilized enzyme can be easily leached out from the channels. Therefore, crosslinking enzyme aggregates is simple and powerful approach in mesoporous nanostructures for enzyme immobilization.

However, the approaches described above do not consider active site of enzyme during immobilization. If active site of enzymes is blocked by enzyme attachment, intrinsic enzyme activity might be reduced after immobilization. To avoid this, enzyme can be designed to have amino acid tags to exposure the active site to surrounding and these amino acid tags are attempted to immobilize enzyme. Recently, Lee *et al*. reported that six histidine-tagged catechol 1, 2-dioxygenase was immobilized on Ni^{2+} functionalized silica coated magnetic nanoparticles (42). This immobilized enzyme using histidine residue may propose a potential for retained high catalytic and stability than its free type.

Enhancement of Enzyme Functions Via Immobilization

Enzymes might improve its catalytic properties when supported with solid materials because substrate specificity might be enhanced and the effect of inhibitors might be reduced. In this section, we describe apparent examples for enhanced enzyme functions by immobilization with various inorganic nanomaterials.

Improved enzyme activity

The immobilized enzymes to solid inorganic nanomaterials can effectively improve the activity of the biocatalysts (55). Lee *et al*. prepared hydrophobic nano-sized magnetite particles (diameter 8-12 nm) functionalized with sodium dodecyl sulfate (26). The particles were used for the supporting materials of lipase. The specific activity of immobilized enzyme was observed to be comparative approximately 2-fold higher than that of free enzyme (8.12 U/mg and 4.75 U/mg, respectively). The greater surface area of the immobilization system in the nano-dimension structure may be viewed as the reason for better substrate-enzyme interaction, through exposure active site (56). Indeed, enzyme activity is strongly dependent to three-dimensional structure of enzyme molecules because active site (or catalytic domain) of enzyme should be conserved in order to occur catalytic reaction between enzyme and substrate (57, 58) Vertegel *et al*. studied whether particle size contributes to conserve protein 3-D structure (59). In this study, the greater loss of α-helicity and enzyme activity was observed for the lysozyme immobilized onto 100 nm SiO_2 nanoparticles comparing with 4 and 20 nm nanoparticles. Considering dimensions of lysozyme (4.5 × 3.5 × 3.5 nm), smaller nanoparticles (*i.e.*, 4 nm SiO_2 nanoparticles) perhaps promote the retention of more nativelike structure and function when compared to their larger (and hence less curved) particle counterparts (59, 60). Thus, the surface curvature of nanomaterials needs to be carefully selected to be able to improve enzyme activity.

Stabilization of enzymes

Generally, soluble enzymes are exposed to some possibilities such as aggregation, autolysis or proteolysis by proteases during catalysis (61). Enzyme immobilization to inorganic nanomaterials may reduce these risks due to endow physical stability to intrinsic enzyme structure (55, 61). In many studies, immobilized enzymes were observed more enhanced stability against high temperature (55, 62, 63), broad pH range (63) or harsh shaking (64) than their free counterparts. Moreover, proper surface modification can help supporting materials to increase enzyme stability. For instance, Jiang *et al*. reported that the activity of lipase immobilized onto magnetic nanoparticles supported ionic liquid (1-methyl-3-(tri-ethoxysilylpropyl)-imidazolium chloride) was remained 60% of its initial activity at 80°C when free enzyme was not observed any activity (62). Ionic liquids are suitable media for enzyme catalysis because they have very low vapor pressure and thermally stable property (65). Therefore, it suggest that ionic liquids supported to surface of magnetic nanoparticles could preserve the compact conformation of immobilized lipase at a tough conditions such as high temperature (66). Inorganic nanomaterials also permit stability of enzymes placed in nonaqueous environments that is the limiting factor for industrial application (55). The nanoporous-glass immobilized chymotrypsin exhibited greatly enhanced stability both in aqueous solution and organic solvents. The half-life of immobilized enzyme was > 1000-fold higher than that of the free enzyme, as measure either in aqueous buffer of anhydrous methanol. The phenomenon was implemented by surface modification of nanoporous-glass using a bifunctional ligand and trimethoxysilylpropanal.

Positive effects for promotion of enzyme selectivity

The improvement of the substrate selectivity of enzyme may be a critical requirement to get an industrially relevant process. However, enzymes can be distorted catalytic properties resulting in their conformational changes during catalysis (61). Considering this difficulty, immobilization of enzymes inside or outside the solid structure may permit to have a potential application for enzyme immobilization, which increases the enzymatic property (67). Shimomura *et al*. immobilized formaldehyde dehydrogenase which is an enzyme that catalyzes formaldehyde on mesoporous silica materials (FSM8.0) of 8.0 nm in pore diameter (34). It was found that the immobilized enzyme exhibited higher substrate selectivity (over 93%) for formaldehyde than other aqueous volatile organic compounds (VOCs) such as acetaldehyde, methanol, ethanol, benzene and acetone. The most noteworthy observation was that the high selectivity can provide potential for developing high-performance amperometric biosensor for specific VOCs detection. In addition, Zeng *et al*. prepared novel dendritic silica magnetic Fe_3O_4 composite carriers (MS-type) and assessed its application in immobilizing lipase (28). As a result, they described that the hydrolysis of 2-phenyl-1-propyl acetate catalyzed by lipase immobilized on the MS-type carriers showed a higher enantioselectivity (18 to 30% up) than that by free enzyme.

Application

Enzyme conducted inorganic nanomaterials have a potential for application of versatile nanoscale biocatalysts and attractive for a range of applications in many areas, including biosensor technology, food industry, pharmaceutics, bioenergy area and environmental applications (Table 1).

Biosensors

Nano-sized structure of inorganic nanomaterials can provide many advantages such as a lower reagent consumption, minimized sample volumes, lover energy consumption, less space requirement (sensor portability), a faster reaction kinetics (reduced diffusion paths), and a reduced packaging (68). Inorganic nanomaterials also possess some outstanding properties of

Table 1. Application areas of enzyme immobilized inorganic nanomaterials

Application	Enzyme	Reactions	Carrier		Attachment method	Ref.
			Material	Structure		
Biosensor	Glucose oxidase	Glucose oxidation	Silica	Nanoporous matrix	Covalent bonding	(68)
		Glucose oxidation	Zinc oxide	Nanorod	Electrostatic adsorption	(45)
		Glucose oxidation	Indium tin oxide	Layer-by-layer dendrimer-nanoparticle	Covalent bonding	(69)
		Glucose oxidation	Gold/polycarbonate composite	Porous nanoparticle track-etched membrane	Covalent bonding	(70)
		Glucose oxidation	Gold/cellulose acetate composite	Film of nanorod composite	Covalent bonding	(72)
	Horseradish peroxidase	H_2O_2 oxidation	Gold	Nanoparticle	Electrostatic adsorption	(15)
		H_2O_2 oxidation	Cysteamine-capped gold	Nanoparticle	Polyeletrostatic interaction	(14)
		H_2O_2 oxidation	Copper	Nanoporous particle	Electrostatic adsorption	(43)
Food industry	α-Amylase	Starch hydrolysis	Halloysite	Nanotube	Physical adsorption	(77)
	Diastase	Starch hydrolysis	Octadecanoic acid/silica composite	Porous nanoparticle	Physical adsorption	(47)
	Lactase	Lactose hydrolysis	Gold	Nanoparticle	Covalent bonding	(75)
	Lipase	Olive oil hydrolysis	Silica	Nanosphere	Covalent bonding	(76)
Pharmaceutical approach	L-lactate dehydrogenase	Conversion of 2-oxo acids into (S)-2-hydroxy acids	Silica coated magnetic cluster	Nanoparticle	Covalent bonding	(79)
	Penicillin G acylase	Penicillin G hydrolysis	Silica	Nanoporous material	Physical adsorption	(80)
		Penicillin G hydrolysis	Silica	Porous hollow nanotube	Physical adsorption and entrapment inner hollow cores	(81)
		Penicillin G hydrolysis	Silica	Mesoporous foam	Covalent bonding	(37)
Bioenergy (Bio fuel cell)	Glutamate dehydrogenase	Glucose oxidation	Gold	Porous electrode	Covalent bonding	(12)
Bioenergy (Biorefinery)	Laccase	Lignin oxidation	Zinc tetraaminophthalocyanine-iron oxide (Fe_3O_4)	Nanoparticle	Covalent bonding	(83, 84)
Environmental technology	Lignin peroxidase	Dye decolorization	Gold	Nanoporous particle	Physical adsorption	(13)

large surface-to-volume ratio and high electrical conductivity that provides electron communication of electrical signals (15, 69, 70). Thus recent years witness the vigorous applications of inorganic nanomaterials in the development of biosensors (71). To develop biosensor systems, oxidizing enzymes (*i.e.*, glucose oxidase and horseradish peroxidase) are conducted to sensing elements because they do not consume itself during the recognition event (catalyzing reaction of O_2 or H_2O_2), hence can be reused thus providing a long lifetime for the device (43, 45, 72). Delvaux and Demoustier-Champagne reported that arrays of nanoscopic gold tubes were prepared by electroless deposition of the metal within the pores of polycarbonate particle track-etched membranes (70). Glucose oxidase was immobilized onto self-assembled monolayers on gold nanotubes via cross-linking with glutaraldehyde or covalent attachment by carbodiimide coupling. The resulting gold nanotubes-based biosensor showed remarkable sensitivity to glucose, up to 400 nA mM^{-1} cm^{-2}, and a large dynamic range, up to 20 mM when measured amperometrically with a detection potential as low as 0.35 V versus Ag/AgCl via the *p*-benzoquinone mediator. The improvement of the enzyme immobilization procedure with higher porosity is under way to increase the enzyme content.

The association properties of ZnO and glold nanoparticles were also studied to conduct biosensors by horseradish peroxidase (HRP) immobilization (14, 15). The nanoparticles provide a large area for the conjugation of enzyme therefore it enhances the chance of the oxidation reaction of H_2O_2.

Food industry

Thermal stability, storage stability and reusability of enzymes should be ensured to conduct enzymes for harsh fermentation process (73). In this context, compare with organic materials, inorganic materials possess advantages of high stability, resistibility against organic solvents and microbial attacks and ease of disposal or reusability (74). To date, some food related enzymes (*i.e.*, α-amylase, diastase, lactase, and lipase) were immobilized onto various types of inorganic nanomaterials including silica porous nanoparticles, gold nanoparticles, and silica nanospheres (47, 75, 76).

Recently, halloysite nanotubes (HNTs) were introduced to immobilization of α-amylase, which is a hydrolyzing enzyme of starch, as novel support materials (77). Naturally occurring HNTs are easily obtainable and much cheaper than other nanoparticles such as carbon nanotubes. More importantly, the inner surface of HNTs retains positive charged when pH is below 8.5, which promotes loading of HNTs with negative charged enzymes. From the experiments, the immobilized α-amylase exhibited thermal stability (at 80°C), good storage stability (over 15 days), and reusability (over 7 cycles), which indicate that halloysite is a promising support materials for enzyme immobilization.

Pharmaceutical approach

Conventional chemical synthesis of pharmaceutical compounds containing a chiral centre generally yields equal mixtures of enantiomers (78). Thus, it is necessary to maintain high enantioselectivity and regioselectivity of enzymes in order to use enzymes for production of the chiral compounds (58). Yusdy *et al*. immobilized L-lactate dehydrogenase (LDH) on silica-coated magnetic nanoclusters for chiral conversion of L-lactate to pyruvate (79). The immobilized LDH showed a better enzyme selectivity regarding its substrate than the soluble LDH does. This was due to the possibility of hydrophobic interaction in controlling a better LDH orientation on particle surface, and thus avoiding any severe deactivation.

Moreover, penicillin G acylase (PGA), which is involved in the production of a wide range of semi-synthetic penicillin, is commonly conducted to immobilize on inorganic nanomaterials having various types of silica materials for pharmaceutical application (37, 80, 81). The immobilized PGA exhibited higher resistance to the change of temperature and stronger tolerability to the pH variance in surroundings (37). Therefore, it is expected that the biocatalyst system may facilitate large scale production of pharmaceutical chemicals including penicillin.

Bioenergy and environmental technology

Currently, bioenergy and environmental technology has been focused on the replacement of petroleum-based fuels and the eco-friendly remediation of polluted environments (82). There are some attempts to improve enzyme activity via conducting inorganic nanomaterials to conduct bioenergy and environmental technology.

Recently, Szamocki *et al*. prepared gold porous electrodes and immobilized glutamate dehydrogenase (GDH) to internal surface of electrode (12). The GDH immobilized porous electrodes showed an increased overall signal and therefore a potentially higher power output when employed in biofuel cells. In addition, zinc tetraaminophthalocyanine (ZnTAPc)-Fe_3O_4 nanoparticle composites were prepared by organic-inorganic complex technology (83). The particles were used for immobilization of laccase enzyme that has catalytic activity of lignin breakdown (83, 84). This kind of immobilized laccase had good thermal, storage and operational stability, and could be used as biocatalyst for lignin contained to biomass-based feedstocks. Meanwhile, lignin peroxidase (LiP) can oxidize a wide range of environmentally persistent aromatic compounds and, therefore, many attempts have been made to degrade aromatic pollutants with this enzyme (84). Qia and co-workers demonstrated that Lip immobilized nanoporous gold revealed a potential of very effective decolourization of aromatic dye molecules such as fushcine, rhodamine B and pyrogallol red (13).

Concluding Remarks and Future Outlook

The versatility of size, shape and morphology of various inorganic nanomaterials can introduce unique properties to high-performance enzymatic systems, making it possible to develop a revolutionary class of biocatalyst that differs from traditional immobilized enzymes in terms of preparation, catalytic efficiency and application potential. As shown in this review, the surface of materials was modified in many ways by express specific functional groups which promoted the enzyme attachment. Especially, silica based nanomaterials can provide a high surface silanol concentration which facilitates a wide variety of surface reactions. Consequently, introduction of functional groups on the silica surface can be easily achieved by using organosilanes for effective enzyme immobilization. To increase enzyme catalytic activity of enzymes, however, it is necessary to consider about preserving intrinsic active site of enzymes for optimal applications of the biocatalysts. Therefore, more examples can be expected in the near future. Further elaboration of this field will lead to the creation of powerful strategies to synthesize novel immobilization technology by combining methods to incorporate nanomaterials with proper surface modification methods.

We anticipate that the development of inorganic nanomaterials-based biocatalyst requires multidisciplinary inputs from the diverse fields such as chemistry, biochemistry, molecular biology and material science, and so on.

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