Hybrid chemo-enzymatic heterogeneous catalysts

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Abstract

Through selected recent examples, we show that a new class of catalysts – hybrid chemo-enzymatic heterogeneous catalysts (HCEHC) – is currently emerging. These multi-functional solid materials have a bright forecast for the development of green and sustainable chemistry. By combining the actions of a solid chemical catalyst and that of an enzyme, these formulations can catalyze multiple reactions in one pot thereby merging the best of two worlds: robustness and recyclability of heterogeneous catalysts on the one hand and outstanding selectivity and specificity of biocatalysts on the second hand. The preparation of such hybrid materials, however, represents a multidisciplinary challenge for catalysis scientists and materials chemists.

Keywords

Enzyme ; heterogeneous catalysis ; biocatalysis ; hybrid materials ; chemoenzymatic; green chemistry ; cascade reactions; metal nanoparticles

1. Introduction

Enzymes are nature's catalysts, developed along the evolution to catalyze chemical reactions with outstanding efficiency, and mostly under mild conditions. With the tools of biotechnology, the power of enzymes can be harnessed to suit the needs of the chemists [1], and this opened up avenues of development for green and sustainable chemistry [2,3]. In parallel, the field of heterogeneous catalysis is continuously being propelled by advances in materials science, through which a plethora of efficient catalytic materials emerge at a fast pace [4].

The integration of heterogeneous catalysts and enzymes is increasingly being seen as one of the most promising lines of attack for the development of innovative catalytic routes which involve multi-step reactions that can be carried out in intensified processes [5]. In such chemo-enzymatic approaches, the idea is to leverage on the assets of each type of catalyst, while tuning down their respective downsides. For example, a heterogeneous catalyst may be selected – despite a modest intrinsic activity – for its robustness and ease of recovery, while an enzyme would be selected – despite its relative instability – for its excellent (enantio-)selectivity or specificity.

2. The concept of "hybrid" in materials science and in catalysis

Processes utilizing simultaneously two types of catalysts are denoted as "hybrid catalysis" and include all possible combinations of homogeneous, heterogeneous and enzymatic catalysts [6-8]; these have been reviewed recently [9-11]. Arguably, the most exciting developments in the field of hybrid catalysis concern the development of catalytic materials, in which an enzyme and a chemical catalyst are both integrated in the same solid material (Figure 1). The preparation of such bi- or even multi-functional catalysts, represents a true synthetic challenge for catalysis scientists; the tools of materials chemistry and biochemistry have to be mastered and exploited.

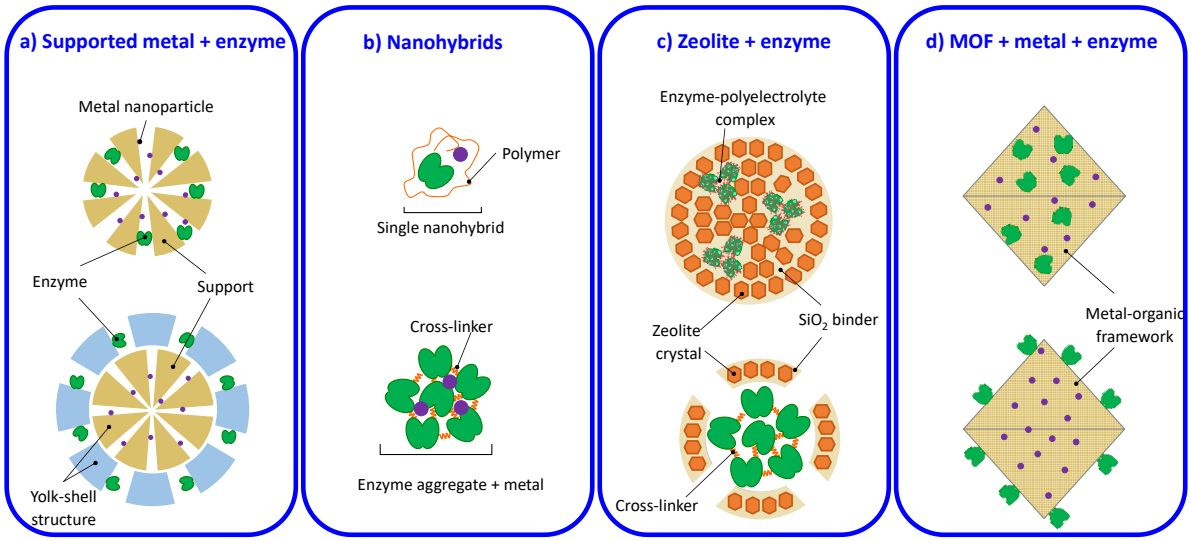

Figure 1. Examples of hybrid chemo-enzymatic heterogeneous catalysts: (a) metal nanoparticles and enzyme deposited (or grafted) onto a support, with or without spatial compartmentalization, (b) nanohybrids either based on a single enzyme molecule and a single metal nanoparticle embed in a polymer molecule, or based on crosslinked enzyme aggregates (CLEA) serving as a host for metal nanoparticles, (c) combination of catalytically active zeolite nanocrystals and an enzyme, with or without spatial compartmentalization, and (d) MOF-based catalysts featuring metal nanoparticles and enzymes in the porosity or at the surface (the MOF itself can be catalytically active too).

In the field of materials science, the term “hybrid” has been used for decades to refer to materials that entail both organic and inorganic moieties [12]. Based on this definition, many types of catalytic materials should be considered as “hybrid catalysts” [13], including for example oxide-supported organocatalysts [14], surface-functionalized oxide catalysts [15], mesoporous organosilica and their metallosilicate counterparts [16,17], MOF-based catalysts [18], and enzyme supported on inorganic carriers [19,20]. To avoid any confusion, in the field of hybrid catalysis, we propose to use an unambiguous denotation for solid catalytic materials that feature both chemical and enzymatic catalytic functionalities: “hybrid chemo-enzymatic heterogeneous catalysts” (HCEHC). In the following, we cover some of the most recent and representative results reported in this field in the primary literature, for a list of representative chemo-enzymatic reactions (Figure 2).

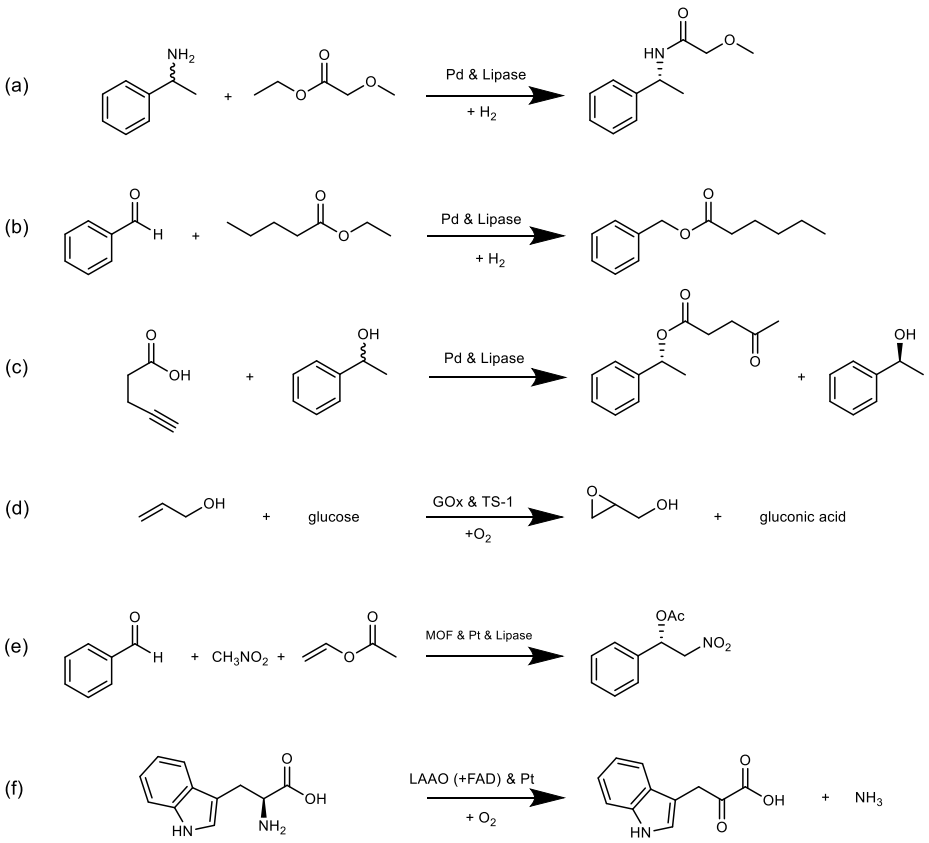

Figure 2. Examples of chemo-enzymatic reactions. (a) Dynamic kinetic resolution (DKR) of a secondary amine, (b) synthesis of benzyl hexanoate from benzaldehyde and ethyl hexanoate, with the intermediate formation of benzyl alcohol, (c) kinetic resolution of a secondary alcohol, via the intermediate formation of 5-methylene-dihydrofuran by cyclization of 4-pentynoic acid, (d) epoxidation of allyl alcohol with H_2O_2 formed in situ as a co-product of the oxidation of glucose to gluconic acid, (e) synthesis of an optically pure acetylated nitroalcohol via a nitroaldol reaction, racemization of the formed nitroalcohol, and kinetic resolution, (f) synthesis of indole-3-pyruvic acid from L-tryptophan via the intermediate formation of the L-imino acid, assisted by the catalytic decomposition of H_2O_2 formed as a by-product of the reaction.

3. Supported enzyme and metal nanoparticles

A simple way to envisage the preparation of a HCEHC is to modify the surface of an inert carrier so that it can host both a chemo and a bio-catalyst (Figure 1(a)). For example, a silic support can simply be impregnated by a metal salt (which is further reduced to form catalytically active metal nanoparticles) and functionalized by silanization to generate anchoring points for the enzyme (e.g. amino groups which couple via a dialdehyde with the lysine amino acids of the enzyme). Such strategy has been reported to prepare catalysts based on Pd nanoparticles and lipase, exploited a.o. in the dynamic kinetic resolution (DKR) of secondary amines: a chiral amine is racemized by the metal, and the enzyme acts as a resolving agent that selectively converts only one enantiomer of the starting amine into the

corresponding amide [21]. In a recent study, Pd-Lipase@silica catalysts were also supplemented with magnetic nanoparticles to further facilitate recovery [22]. In an advanced example, Zhang et al. had previously shown that Pd and lipase could be physically separated respectively in the "yolk" and in the "shell" of structured silica nanoparticles [23]. Interestingly, they clearly demonstrated a synergy in the HCEHC, which performed much better than the physical mixture of two catalysts loaded respectively with Pd nanoparticles and with the lipase. Apart from silica supports, the amine functions present at the surface of a Pd-loaded C_3N_4 material have also been used as anchoring points to immobilize an enzyme, using glutaraldehyde as a coupling agent [24]. This catalyst allowed performing the one-pot chemoenzymatic conversion of benzaldehyde to benzyl hexanoate.

4. Enzyme-metal nanohybrids

A similar strategy can be implemented in the absence of a support, with so called "enzyme-metal nanohybrids" (Figure 1(b)). For example, protein-polymer conjugates were obtained, in which a single enzyme molecule (lipase) was coupled to an aldehyde-functionalized block copolymer and used as a nanoreactor to form a Pd nanoparticle with a controlled size (down to 0.8 nm) [25]. These support-less hybrid chemo-enzymatic nanoparticles were exploited in the DKR of chiral amines of pharmaceutical interest. These nanocatalysts (or "colloidal catalysts" [26]) were shown to be recoverable by centrifugation and reusable. Advantageously, it can be envisaged to prepare a network of aggregated enzyme, functioning as support for the metal nanoparticles [27]. Thus, cross-linked enzyme aggregates (CLEAs [28]) of a lipase were obtained by cross-linking with glutaraldehyde, and the so-formed CLEAs were treated with palladium acetate, subsequently reduced with $Na(CN)BH_3$. These particles catalyzed the cycloisomerization of 4-pentynoic acid to form a lactone that acted as an acyl donor for the lipase-catalyzed kinetic resolution of a set of *sec*-alcohols. The same catalyst was recently exploited for the dynamic kinetic resolution of primary benzylic amines [29]. One advantage of such unsupported HCEHC based on cross-linked aggregates, is to avoid a reduction of the catalytic activity per mass unit of the catalytic material, which is otherwise the case for biocatalysts supported on inert carriers. Often, however, the average enzymatic specific activity in CLEAs is somewhat lower than for free enzymes.

5. Combining a microporous zeolite and an enzyme

The direct immobilization of an enzyme onto and active heterogeneous catalyst is not necessarily straightforward; the case of zeolite catalysts is a telling example. Indeed, (i) the zeolite micropores are too small to accommodate an enzyme, and (ii) a grafting on the

external surface is not satisfactory (in particular when the zeolite features a low concentration of defects such as silanols which serve as anchoring points). To overcome this issue, a method was developed to encapsulate an enzyme inside hollow microspheres built from zeolite nanocrystals (Figure 1(c)) [30]. The microspheres were obtained by a spray drying technique [31] and featured a central cavity which was used to entrap the enzyme in the form of CLEAs. The strategy was validated by performing a chemo-enzymatic reaction: entrapped glucose oxidase (GOx) catalyzed the *in situ* production of H_2O_2 subsequently utilized by the TS-1 zeolite to catalyze the epoxidation of allylic alcohol toward glycidol (Figure 3). The encapsulation strategy was also applied to multi-enzyme CLEAs [30].

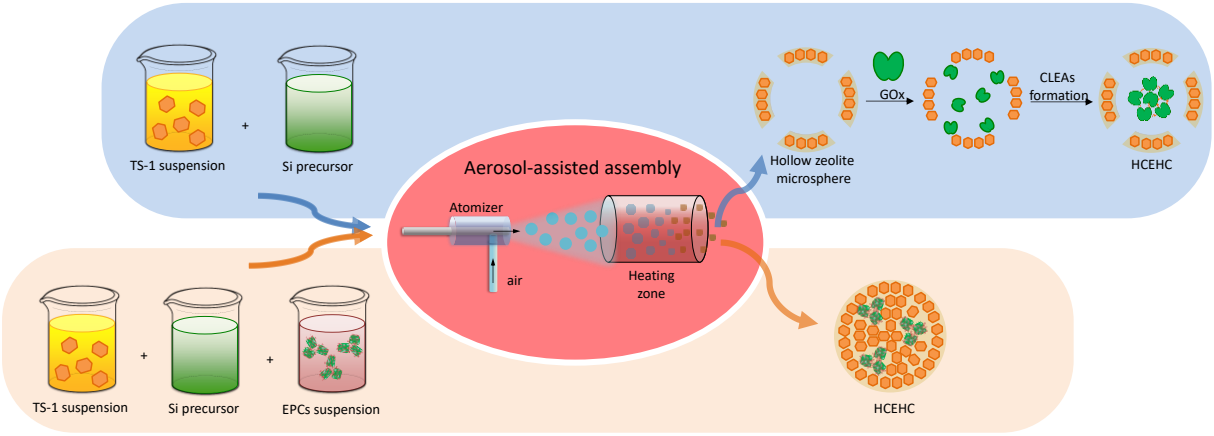

Figure 3. Preparation of HCEHC based on TS-1 nanocrystals and GOx, leveraging on an aerosol-assisted assembly process, via two possible approaches. (top, blue) Inorganic hollow zeolite microspheres are first prepared by spray drying starting from a suspension of TS-1 nanocrystals and tetraethyl orthosilicate, and then GOx is impregnated and trapped inside the microspheres in the form of CLEAs upon precipitation (ethyl acetate) and cross-linking (glutaraldehyde). (bottom, orange) Enzyme polyelectrolyte complexes (EPCs) are formed to stabilize GOx and these are directly added to the mixture before spray drying, to yield the final hybrid chemo-enzymatic heterogeneous catalyst in one step.

Starting from the same catalytic partners, a more direct approach has recently been proposed to simultaneously spray dry the enzyme and the zeolite nanocrystals to obtain the HCEHC in one step (Figure 3) [32]. The direct spray drying of the TS-1 suspension together with free GOx provoked the complete deactivation of the enzyme. Thus, to obtain an active HCEHC, the enzyme was first stabilized via the formation of enzyme-polyelectrolyte complexes (EPCs [33]) before spray drying. In this case, the glucose oxidase activity was partly preserved and the catalyst was shown to reach higher glycidol yields in the chemo-enzymatic reaction. Interestingly, the utilization of EPCs was also shown to stabilize the enzyme against leaching

and against pH variations. Moreover, the hybrid material was significantly more active than the combination of TS-1 and GOx (or EPCs) added as separated catalysts in the reaction medium. It was surmised (i) that the close proximity between the two catalytic partners favored the rapid utilization of H_2O_2 thereby preventing enzyme deactivation or (ii) that the polyelectrolyte imposed a beneficial pH buffer effect in the close proximity of the enzyme.

6. Combining a MOF-based catalyst and an enzyme

Metal organic frameworks (MOFs) offer crystalline and porous structures that can be tailored for a multitude of applications, including heterogeneous catalysts [34]. Recently, MOFs have successfully been used for the preparation of HCEHC (Figure 1(d)). Dutta et al. carried out the crystallization of a cobalt-based zeolitic imidazolate framework (ZIF67) in the presence of Pd nanoparticles and of polyvinylpyrrolidone, to create large mesopores (~30 nm) and favor the formation of defects (unsaturated metal sites) [35]. The so-obtained material was incubated with *Candida antarctica* lipase A to form the final multifunctional catalyst featuring three catalytic counterparts that were put at work simultaneously: (i) unsaturated metallic nodes in the MOF acted as Lewis acid sites for a nitroaldol reaction, (ii) Pd nanoparticles played the role of racemization catalyst and (iii) CalA realized a kinetic resolution. This three-step cascade reaction allowed to synthesize several chiral acetylated nitroalcohols in high yield and high *ee*, under ambient conditions.

Wu et al. have confined Pt nanoparticles inside the crystals of the zirconium-based UiO-66 MOF, and then adsorbed L-amino acid oxidase (LAAO) on the external surface [36]. This HCEHC was exploited in the biocatalytic synthesis of indole-3-pyruvic acid from L-tryptophan. The co-product, H_2O_2, was rapidly decomposed by Pt nanoparticles, which allowed suppressing the undesired decarboxylation of the product to indole-3-acetic acid. Interestingly, the authors showed that compartmentalization was key in this success; the enzyme deactivated rapidly if it was put in close contact with the Pt nanoparticles.

The aminated version of the same UiO-66 MOF was also used as a host for Pd nanoparticles and then used to physically adsorb a lipase (*Candida antarctica* lipase B, CalB) on the external surface of the crystals [37]. Utilizing a ligand exchange reaction with lauric acid, the MOF was made more hydrophobic. This allowed enhancing the amount of immobilized enzyme and dispersing the HCEHC in organic solvents of different polarity. The lipase-Pd-MOF was exploited to convert benzaldehyde into benzyl alcohol and then benzyl hexanoate. The yield in the final product was higher with the HCEHC than in a control experiment with

separate Pd-MOF and lipase-MOF. In its immobilized form, the lipase can be recovered and reused; yet, a relatively drastic deactivation was observed in the consecutives runs.

7. Perspectives for green and sustainable chemistry

The forecasts for hybrid chemo-enzymatic heterogeneous catalysts (HCEHC) in the field of green and sustainable chemistry are bright. While some of the early developments discussed herein should be seen as proofs of concept based on model reactions with limited industrial interest, some relevant examples have been highlighted as well (e.g. synthesis of optically pure pharmaceutical intermediates). The ability of enzymes to catalyze reactions with an excellent enantioselectivity and enantiospecificity is arguably the most crucial feature of biocatalysis; developments of efficient HCEHC will undoubtedly allow us to exploit this property more effectively, in economically relevant synthesis processes. One important task of the community will be to improve the robustness of HCEHC, which often suffer from the relative instability of the enzymatic part of the catalyst.

Beyond the exploitation of enzymes and their heterogenization (which facilitates handling, recovery and reuse), the concept of HCEHC paves the way to process intensification. By combining two (or more!) catalytic entities in the same material, orthogonal reactions can be carried out in a single reactor, with a single solid. The key idea, here, is to avoid transitional separation and purification of intermediate products. Since the economic and environmental cost of purification in multi-step synthesis processes typically represents a large fraction of the total production cost [38], the design of synthesis routes that involve a lower number of separate steps can have a significant impact on process sustainability. Furthermore, HCEHC could advantageously be implemented in continuous flow mode, which would further favorably impact the environmental performance of the newly designed processes [39,40].

A key factor in the synthesis of efficient HCEHC is the spatial distribution of the chemo- and bio-catalytic species. In some cases, a close proximity is desirable, for example when the intermediate of the reaction is a poison to the enzyme (e.g. H_2O_2) and should be eliminated as fast as possible by the chemical catalyst. The close proximity between the two entities can also boost the kinetics of sluggish equilibrated reaction (the product of the first reaction is readily converted, thereby favorably shifting the thermodynamic equilibrium). On the other hand, in many cases, compartmentalization (site isolation) is required, for example if the chemo-catalyst (e.g. Pt nanoparticles) provokes the denaturation of the enzyme. Addressing

the challenge of the relative location of the different catalytic species in such chemo-enzymatic catalysts is a stimulating undertaking for materials chemists.

Conflict of interest statement

Authors have no competing interests to declare

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