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Nature-inspired design and synthesis of heterogeneous and macromolecular catalysts

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ABSTRACT

Advances in understanding enzyme catalysis have made possible development of synthetic schemes and strategies to incorporate the essential functions of enzymes into artificial structures. Two of these functions, environmental effect inherent to the active site cavity defined by the protein conformation and cooperative effect of functional groups in the cavity, have been successfully introduced into artificial structures and examined. Illustrative examples of these studies are described, as well as remaining challenges and illustrative application potentials.

1. Introduction

Enzymes are among the most active and selective catalysts known. Unlike artificial catalysts, enzymes catalyze reactions effectively under typical physiological conditions at near room temperature and near neutral pH. These are conditions when artificial catalysts would be inactive. What is amazing is the fact that the active sites of enzymes are composed of only a few simple organic functional groups, which are hydroxyl (–OH), carboxylic acid (–COOH), amines (–NH_2 and histidine), and thiol (–SH). Sometimes metal ions are also involved, but they are quite different from the late transition metals commonly used in artificial systems. Evidently, enzymes possess properties that are not found in typical artificial systems.

In spite of their high activities and selectivities, enzymes have disadvantages. Their superior properties depend critically on the protein conformation, such that the activity can be lost when the conformation is significantly distorted when the protein denatures. Since the conformation is dictated by a large number of relatively weak hydrogen bonds between amino acids, it is sensitively dependent on the processing conditions such as temperature, pH, and hydrophobicity of the solvent.

It would be ideal, then, to be able to design and synthesize artificial catalysts based on robust organic/inorganic structures that possess functional properties of enzymes. Then, one would have highly active and selective catalysts that perform under mild conditions that are environmentally friendly, but tolerant to process condition variations for optimization. This has been the goal of catalysis researchers for many years. Recently advances in understanding enzyme catalysis, particularly the availability of detailed structural information coupled with mechanistic information, have enabled design and synthesis of sophisticated target structures as enzyme analogs. Whereas such advances are more prominent in homogeneous catalysis, they are also noticeable in heterogeneous catalysis. In this brief review, a few examples taken from the literature and from our laboratory will be presented to highlight such advances.

2. Important properties of enzymes

The reasons behind the superior properties of enzymes are quite well established [1]. The enzyme protein is folded into a configuration that creates a cavity for the active site, and the local environment of the cavity can significantly change the properties of the catalytic functional groups. Broadly speaking, there are two principal factors at play. One factor is the hydrophobicity of the cavity environment, which affects the energetics of charge separation by changing the charge-screening ability of the environment and consequently the electrostatic interaction among charged groups. This changes the energetics of reactions that involve charged groups. One manifestation of this phenomenon is the *pK shift effect*, where the protonation/deprotonation tendency of a functional groups inside the cavity is significantly different from that when the groups is in an aqueous solution freely. For example, the protonation constant of the second lysine

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in acetoacetate decarboxylase is shifted from a pK_a of 10 to about 6 due to electrostatic repulsion between two charged ammonium ions and the low dielectric constant of the hydrophobic environment of a protein [2], where solvation stabilization and charge screening are much weaker than in an aqueous medium.

The second factor is the *cooperative effect*. It is due to interaction among functional groups in the cavity, often through hydrogen bonding, that results in significant enhancement of nucleophilicity or nucleophilicity of these groups. This effect has been attributed to be the main reason for the extremely high hydrolysis activity of the protease enzyme to break the amide linkage between protein amino acids [3].

3. pK shift in designed structures

pK shift has been observed in artificial structures. For example, the protonation constant of amines in the interior of a G4-PAMAM dendrimer is shifted by 1–2 pH unit to lower pHs because of the high density of hydrophobic hydrocarbon segments in the region [4]. For the same reason, the protonation constant of amines inside the aggregate of long hydrocarbon tails of a surfactant in a micelle is shifted by 1–2 pH unit [5,6]. The protonation constants of propylamine and octylamine in an aqueous solution differ by ~2 pH unit due to agglomeration of octylamine consequent to its hydrophobic octyl group, thus affecting solvation stabilization of the ammonium ion [7]. These shifts are all results of a combination of effects of electrostatic repulsion and hydrophobic medium.

These examples, while demonstrating the importance of the nature of the medium by embedding the functional group inside a hydrocarbon medium, differ in detail from the effect of positioning the function group inside the enzyme cavity. In the latter case, the functional groups may not have hydrocarbon segments as nearest neighbors. Instead, they are surrounded by solvent molecules entrapped inside a cavity defined by a hydrophobic wall. Thus, in order to mimic the effect of a protein cavity more closely, we need cage-like structures.

We have been investigating methods to synthesize nanometer-size cages (nanocages) for this purpose. We chose siloxane and carbosilane cross-linked oligomeric structures to serve as the cavity wall. Siloxane and carbosilane are hydrophobic material. The fact that siloxane oligomers contain heteroatoms probably make them more similar to a protein than a hydrocarbon molecule. Silicon-based materials are also generally rather flexible, offering the structure an ability to respond to the changing interactive forces over the course of a chemical reaction, which is deemed essential in enzymes.

We have explored two different methods to synthesize cage-like structures [8,9]. The "unit-by-unit" method, which simulates peptide synthesis, builds a structure by adding individual monomeric silicon complexes one at a time. Similar to peptide synthesis, this method offers a high degree of control of the composition and structure of the material, and is limited only by the availability of monomers and their stability/reactivity under the synthesis conditions. A bicyclic siloxane structure that resembles a cage has been synthesized (Fig. 1A) [10].

Templating or imprinting is another method. In this method, a sacrificial template, which can be a molecule or other structures, is used to generate the desired cavity after the defined shell or wall is formed. Ideally, the shell should be thin and porous to enable rapid diffusion of reaction molecules of interest, stable under reaction as well as synthesis conditions, and capable of attaching functional groups either directly as part of the synthetic scheme or by post-synthesis modification. The template, on the other hand, should be readily degradable into small fragments to generate the cavity at the end of synthesis, but stable throughout the synthesis.

We explored two different materials for template: micelle and dendrimer. Micelles are made of surfactant molecules that possess a hydrophilic head section and a hydrophobic tail section (Fig. 1B). In a hydrolytic medium, the surfactant molecules form aggregates with the hydrophobic tails in the interior and the polar head section at the exterior. The size of a micelle is typically a few nm in diameter, and is determined by the size of the head and tail sections. For the surfactant (triethoxysilyl)propylcetylcarbamate, 2 nm micelles were obtained [11]. In addition to being polar, the head group of this surfactant consists of ethoxysilyl groups that can be hydrolyzed into silanol, which in turn condenses with each other to form a network. The tail section is connected to the head section by a carbamate bond, which is labile to hydrolysis or cleavage with silyl iodide, such that the tail section can be removed from the cross-linked shell when desired. Using this method, we synthesized a ~2 nm diameter nanocage that is defined by a siloxane shell with 7–9 amine groups tethered to the interior wall of the shell (Fig. 1C) [11].

Dendrimers are well-defined macromolecules formed with repeating, branching units radiating from a core. Each shell of repeating units constitutes a generation. For use as a template to generate a cavity, there should be a labile linkage in every repeating unit that can be cleaved on demand, and the dendrimer would be decomposed into small fragments for removal from the final structure to generate the cavity. Fig. 1D shows the dendrimer we used for this purpose [12,13]. It is a generation-4 dendrimer, implying that it has 4 layers of repeating units. There is a carbamate linkage in each repeating unit. There are chemically active groups at the periphery that can be used for shell cross-linking. For the dendrimer in the figure, these are C=C bonds that can be used for hydrosilylation with cross-linking molecules. After forming the shell, the carbamate bonds are cleaved to generate the cavity. The cavity size is determined by the dendrimer size, which

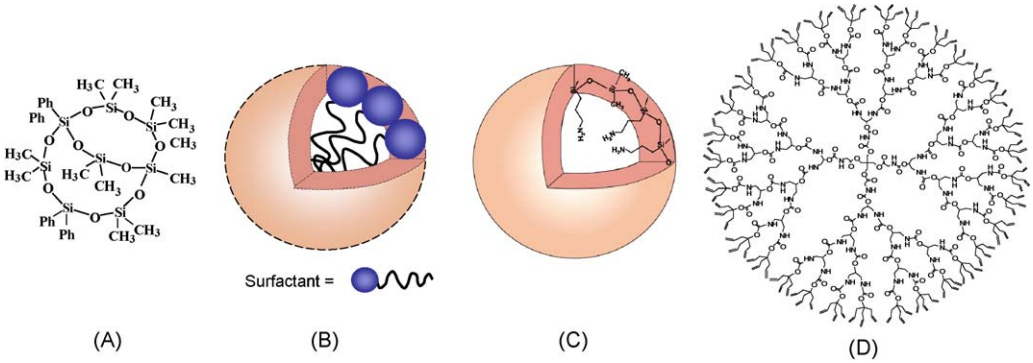

Fig. 1. Examples of cage-like structures. (A) A bicyclosiloxane synthesized with unit-by-unit method. (B) Schematic drawing of a micelle. (C) Siloxane nanocage synthesized using a micelle template. (D) Example of a dendrimer template.

can be tuned by using different generation dendrimers and with different size repeating units as well as degree of branching in each generation. Thus, the dendrimer template offers a high degree of control and a large range of possible variations. They are also well-defined molecules, such that there can be a high degree of uniformity in the cavity formed. These are distinct advantages compared to micellar templates. A disadvantage, however, is the much more involved synthesis process.

The protonation affinity of the amine groups in structure 1C was determined. It is expected that, at the minimum, packing a high density of amine groups in such a small volume would have an effect. A simple calculation, neglecting the ionic strength or other nonideal solution effects, shows that the pH of a 2 nm diameter water droplet containing one protonated amine group is ~13.5, and is ~11.5 for a 10 nm droplet (Fig. 2) [14]. Thus, inside a water-filled, 2 nm cavity of a nanocage, no more than one amine group would be protonated because it would increase the pH of the water droplet to be higher than the pK of the amine.

The result of this simple calculation can be tested experimentally. Neutral amine ($-NH_2$) contains an electron lone pair, making it a much stronger base and nucleophile than a protonated amine ($-NH_3^+$). Thus, a neutral amine is a good ligand for noble metal complexes (e.g. Au complexes) but not a protonated amine. Using this, it is possible to quantify the extent of protonation of amine groups inside the nanocage by monitoring the formation of Au–NH_2 bonds by reaction of $AuCl_4^-$ with amine (Eq. (1)). If the amine is protonated, binding of the anionic complex would be by electrostatic interaction without ligand exchange (Eq. (2)). The difference in the coordination sphere of Au can be monitored by both X-ray absorption (XAS) and UV–vis spectroscopy. In XAS, the different scattering properties of N versus Cl results in different X-ray absorption fine structures near the absorption edge of Au, and in UV–vis spectroscopy, the ligand-to-metal transfer band at 227 nm is sensitive to the coordinating atom.

$$-RNH_2 + AuCl_4^- = -RN(H)_2AuCl_3 + Cl^- \quad (1)$$

$$-RNH_3^+ + AuCl_4^- = -RNH_3^+[AuCl_4]^- \quad (2)$$

The results of such an experiment are shown in Fig. 3 [14], which shows the decrease in the Au–Cl coordination as a function of the proton concentration in solution. When the solution contains amine as free molecules, there is little change in the Au–Cl coordination at high H^+ concentrations, suggesting no detectable ligand exchange, consistent with the fact that when the amine is protonated, Au complex binds by Eq. (2). Changes in the coordination begin to appear when the H concentration is lower than 10^{-7} M. In contrast, in the presence of nanocages in the solution, a unit decrease of Au–Cl coordination is observed even when the H^+ concentration is as high as 10^{-4} M, equivalent to pH 4. The pK_a for a protonated amine is typically between 9 and 10. Thus, the result then suggests a pK shift of about 5 pH units. Clearly, the

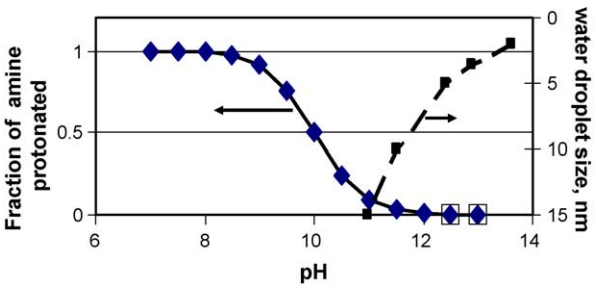

Fig. 2. Fraction of amine (pK 10) protonated as a function of pH, and pH of a water droplet containing one hydroxyl group as a function of droplet size.

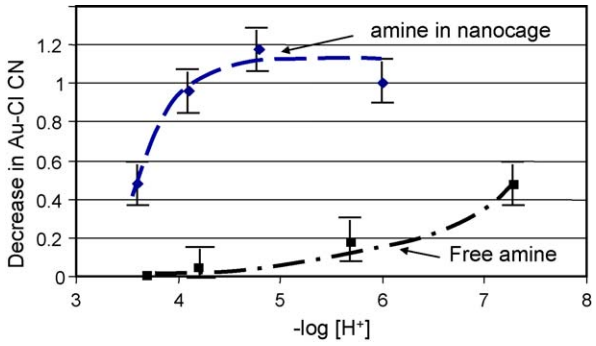

Fig. 3. Change in Au–Cl coordination in $AuCl_4^-$ as a function of proton concentration in solution containing either nanocages with interior amines or free amines [14].

nanocage environment exerts significant effects on the properties of interior functional groups.

Other expected effects of the cage environment are also observed. Interior functional groups are shown to be accessible to smaller molecules that can readily penetrate the porous shell but not larger molecules [11,13]. The close proximity of the shell wall enhances attractive nonbonding interaction with molecules, which results in concentrating the molecules inside the cavity. This concentration effect has two consequences in a catalytic reaction. It concentrates the reactant and increases the reaction rate. It also concentrates the primary product and increases the rate of any consecutive reactions, changing the observed product distribution (i.e. the apparent reaction selectivity) [11].

4. Cooperative effects

There are literature examples of successful demonstration of cooperative effects. The most common ones involve acid–base cooperation for catalytic carbon–carbon coupling, which is achieved by anchoring both an acid (e.g. sulfonic) and a base (e.g. amine) function in close proximity onto a support [15]. Cooperative effect utilizing a designed structure has been demonstrated using dendrimers. An example is shown in Fig. 4 for the Co–salen complex catalyzed hydrolytic ring-opening of epoxide.

The Co–salen catalyzed hydrolysis of epoxide is believed to be facilitated by a pair of Co complexes, one to bind the epoxide and the other to bind the nucleophile (water). Therefore, a scaffold structure that positions Co–salen complexes in close proximity would enhance the catalytic efficiency, and amine-terminated PAMAM dendrimers has been exploited for this purpose [16]. The peripheral amine groups in these dendrimers can be used to anchor Co complexes by covalent linking to the salen ligand. Thus, Co complexes can be brought to close proximity of each other at the surface of these dendrimers. Since the density of peripheral amine groups differs for different dendrimer generations, it becomes possible to investigate the effect of Co complex density using dendrimers of different generations. In all cases, Co complexes bound to the dendrimer periphery are more active than if they are freely in solution. The effectiveness also depends on the dendrimer generation, with the first generation dendrimer-supported sample more active than the second generation, which is more active than the third generation.

A similar observation was made with the Zn-catalyzed hydrolysis of 2-hydroxypropyl-*p*-nitrophenyl phosphate [17]. In this case, instead of changing the density of Zn at the periphery by using different dendrimer generations, it is changed by binding different ratios of acetamide to Zn complexes. It was observed that the catalytic activity increased as second order in Zn density at the periphery, suggesting cooperation between two adjacent Zn complexes. It was hypothesized that one Zn complex binds the

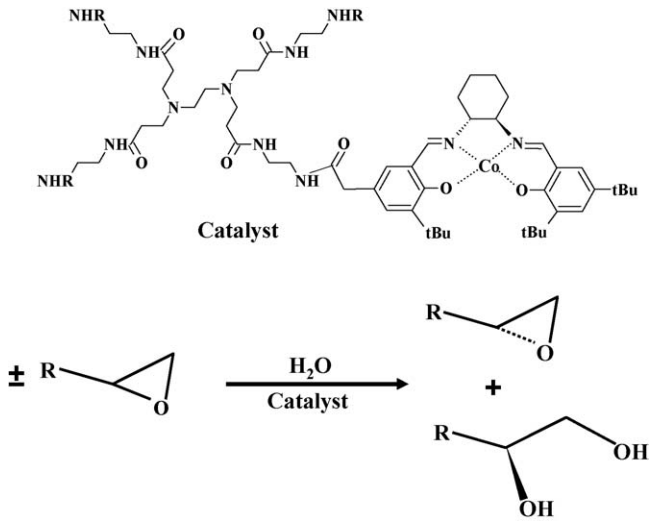

Fig. 4. Catalyst consisting of Co–salen complexes anchored on a first generation PAMAM dendrimer periphery (top), used to catalyze hydrolysis of epoxide. One of Co–salen complex is shown in detail. Based on Ref. [16].

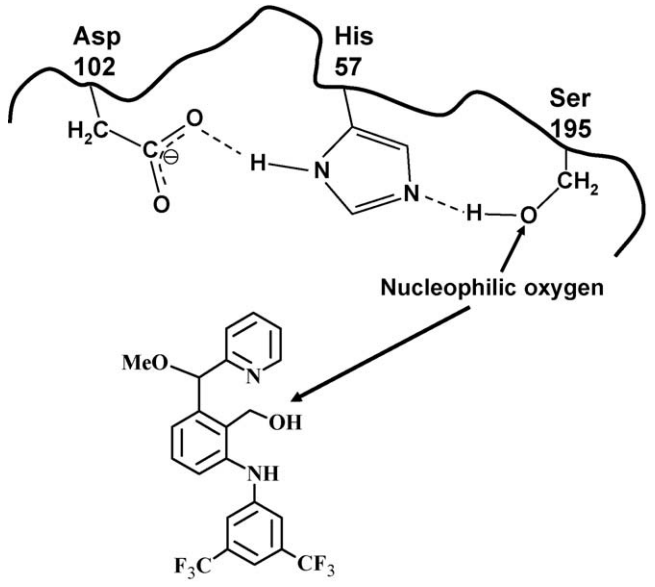

Fig. 5. Schematic drawing depicting the active site of a protease enzyme (top), showing cooperative interaction involving three amino acid residues (asp102, his57, and ser195), resulting in much enhanced nucleophilicity of the serine oxygen, and (bottom) an artificial structure in an attempt to mimic the cooperative interaction of the enzyme. Adapted from Refs. [3,18].

phosphate leaving group of the reactant, and a second Zn complex activates the electrophile to attack the phosphate.

There are also examples involving more complicated cooperative effects. In one example, the molecule (A) shown in Fig. 5 was synthesized to mimic the interaction known as the catalytic triad in the enzyme protease [18]. In this structure, the nucleophilicity of the oxygen of the hydroxyl group is significantly enhanced by a series of hydrogen bonding involving an adjacent imidazole group and a more distant carboxylate group. Through this multiple interaction, the hydroxyl group catalyzes hydrolysis of an amide bond orders of magnitude faster.

5. Conclusion

The selected examples discussed in this brief review demonstrate that synthetic advances have made possible the construction of designed structures that exhibit properties of enzymes critical to catalysis. In particular, two of the most important functions of an enzyme protein can be incorporated into designed structures, namely the environmental effect of the cavity and the cooperative effect of functional groups. However, this field is only at the beginning. There is yet to be a general synthetic scheme or strategy to incorporate both environmental and cooperative effects in a structure. More research is needed to develop methods to introduce multiple, cooperating functional groups into a cavity. Whereas it is necessary to synthesize structures that can be readily characterized fully and relatively easily at this developmental stage, which necessitates synthesizing soluble species, improving the methods to generate structures suitable for heterogeneous catalysis will be the next challenging step. However, the potential impact of this direction of research to technology can be huge. For example, conversion of biomass, especially cellulose, is readily degraded by living organism using the enzyme cellulase. If an artificial structure can be synthesized to possess the functions of a cellulase, we may be able to convert cellulose to much more easily processable compounds under much milder, energy efficient, and environmentally friendly conditions. We hope that the few highlights of examples of recent advances in this area would arouse the interest and imagination of more researchers in the field to tackle the remaining challenges, such that eventually, it becomes common practice that artificial catalysts possess enzyme functions but are superior in their tolerance to processing conditions.

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