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Introduction

Artificial enzymes may be defined as the synthetic, organic molecule prepared to recreate/mimic the active site of an enzyme. The binding of a substrate close to functional groups in the enzyme causes catalysis by so called proximity effects. It is therefore possible to create similar catalysts from small molecule which will mimics the enzyme active sites. Since the artificial enzymes need to bind molecules, they are made based on the host molecule such as cyclodextrins, crown ethers or calixarene etc[1]. Living cells often synthesize complex molecules via multistep sequential reactions, each catalyzed by an enzyme. To allow all of the reactions to work well, nature uses “Compartmentalization” or “site isolation” through which the individual steps are spatially separated to optimize their action. The key to achieving site isolation in solution lay in the use of “star polymers”, macromolecules that are capable of being functionalized so that they can bind and encapsulate small catalytic molecules in their core, adjacent to the binding site for the “reactant.”[2-4] A number of artificial enzymes have been reported catalyzing various reactions with rate increases up to 10^3; this is nevertheless substantially lower than natural enzymes that typically causes rate increases above 10^6[1].

Acetylcholinesterase (acetylcholine ‘acetylhydrolase, EC 3.1.1.7) is a widely distributed en- zyme in excitable membranes of nerve and muscle. Its molecular properties are of particular interest because of its involvement in nerve impulse transmission . The enzyme catalyses the hydrolysis of acetylcholine to choline and acetate by means of a two-step process involving the formation of an acyl-enzyme intermediate . With few exceptions, the extraction of acetylcholinesterase from any tissue source releases -various molecular forms of the enzyme. The basis for such heterogeneity is not fully understood, but in part may relate to cellular location. It is widely suggested that the three main molecular forms correspond to three locations: intracellular, membrane bound and immobilized on basal lamina. Artificial enzyme concept can help us to identify the interaction between acetylcholinesterase activity and membrane potential by using artificial acetylcholine esterase membrane[5].

Ideal Requirement for Artificial Enzymes Environment

While constructing new and complex nano-environments which completely and closely surrounding the enzyme molecule, should imply a strong chemical modification of the enzyme and possibly a very significant loss of catalytic activity. So keeping view in mind, a careful design of immobilization and post-immobilization techniques could provide a suitable procedure to create complex nano-environments associated to minimal enzyme modifications:

a. The covalent immobilization of one enzyme on solid supports having large surfaces with given properties (e.g., hydrophilic or hydrophobic surfaces) should provide a nano-environment surrounding the area of the enzyme directly in contact with the support;

b. Further covalent immobilization of macromolecular polymers (hydrophilic, hydrophobic) on the same large internal surfaces of the solid support should provide an additional nano-environment surrounding the areas of the immobilized enzyme molecules next to the support;

c. Chemical modification of immobilized enzymes with polyfuncional macromolecules could also be an interesting way to greatly modify the enzyme nano-environment with minimal chemical modification of the enzyme (e.g., a single covalent modification per molecule of polymeric modifier introduced in the enzyme).

These new environments surrounding the enzyme molecule should be mainly constituted by fairly open structures that permit the substrates and products to diffuse toward the enzyme active center[6]. Agarose gels are composed by a 3D network of hydrophilic thick fibers 20 nm in diameter and much larger than protein sizes (around 2–5 nm in diameter); thus, proteins and other macromolecules become immobilized on large internal agarose surfaces. Such support geometry becomes a key feature in order to get very intense enzyme-support multipoint covalent immobilizations. On the other hand, such support geometry may be suitable to permit the adjacent immobilization of the polymer surrounding the previously immobilized enzyme. In this way, the co-immobilized hydrophilic polymer may fully protect a great portion of the surface of immobilized enzyme

molecules without introducing any direct chemical modification on the enzyme structure.

Dextrans are very hydrophilic commercial polymers that are available with well-defined and very different molecular weights. In fact, dextrans constitute the polar phase of many aqueous two-phase systems, partitioning the organic solvent toward the other phase. Dextrans or their derivatives have been used to modify proteins and they may be useful for chemical hydrophilization of both support and enzyme surfaces.

Design Approach for Artificial Enzyme[10]

The traditional approach to enzyme mimics has focused on the de novo design of macromolecular receptors with appropriately placed functional groups. These catalytic groups are usually chosen to mimic the amino acid residues known to be involved in the enzyme catalysed reaction. The realisation of ideas in such a process can be an arduous affair and although there are some impressive successes (vide infra), ef?cient catalysis rivalling enzyme rate accelerations still seems a long way down the line.

1. Cyclodextrins as enzyme mimics

One of the most prodigious aspects of enzyme mechanism is the functional group cooperation often displayed in the active site. The electrostatic environment in the binding site maintains the delicate balance of pK_as required for the various groups to participate in a catalytic fashion. In particular, histidine is often able to function as both an acid and as a base in simultaneous bi or multifunctional catalysis. Intrigued by the challenge of imitating this phenomenon Breslow and co-workers chose to mimic the enzyme ribonuclease A. This enzyme uses His and His as its principle catalytic groups in the hydrolysis of RNA.

To mimic this enzyme, two imidazole rings were attached to the primary face of b-cyclodextrin as depicted below in **figure 1**. This mimic **7** catalyses the hydrolysis of the cyclic phosphate **8** and shows greater than 99:1 selectivity for **9**. This is in comparison to the simple solution reaction with NaOH which gives a 1:1 mixture of both products. Isotope effects showed that the two catalytic groups were operating simultaneously and the pH rate pro?le, which was almost identical to the enzyme itself, shows that one imidazole functions in its protonated form whilst the other is unprotonated.

The relative positioning of the imidazole groups on the ring of the b-cyclodextrin was found to be crucial. Only when the imidazole groups were attached to adjacent sugars was a single product **9** detected. This regioisomer of b-cyclodextrin was not only more selective but also provided the fastest rate of hydrolysis and displayed the strongest binding to the substrate **8**. Importantly, this result gave information about the mechanism involved since the imidazolium ion in this isomer would be better placed to protonate the phosphate anionic oxygen, which it can access more easily than the other catalyst isomers(**Figure 2)**.

This observation, when coupled with the evidence that the imidazoles function in a simultaneous co-operative way, allowed the group to postulate that the mechanism was as depicted below(**Figure 2)** and hence was the same as used by the enzyme, ribonuclease itself.

See Illusration 1&2

2. Cyclophane enzyme mimics

Another impressive application of the design approach is Diederich's pyruvate oxidase mimic. Pyruvate oxidase employs two co-factors ThDP and Flavin to water or alcohols to carboxylic acids or esters respectively by simple thiazolium ions in the presence of lavin.

Diederich's pyruvate oxidase mimic combines a well defined binding site with both the ?avin and thiazolium groups attached in covalent fashion (**Figure 3**). The proximity of the groups to the binding site and the intramolecularity of the oxidation step was therefore expected to improve catalysis relative to previous two component systems. It should also mimic the situation in the enzyme where the cofactors are bound in the enzyme active site thus increasing the effective molarity of the reagents.

See Illustration 3

Reversibly self assembled dimers as enzyme mimics

In a different approach, Rebek et al. have investigated the in?uence of a designed binding cavity on the rate of the Diels–Alder reaction. In this case, no catalytic groups are required. Furthermore, although a Diels–Alderase is known, no natural enzyme catalyst has been isolated or is available for synthetic applications as yet. This is important, since the desirability of designing arti?cial enzymes for reactions which have no convenient natural enzyme equivalent is self evident.

Rebek and co-workers have carried out much research into the synthesis of reversibly self-assembled dimers. The extended polycyclic system in **26** (**Figure 4**) exists as a hydrogen-bonded

dimer in organic solvents and adopts a pseudo spherical structure (described as a 'hydroxy-softball') which is able to form and dissipate on a timescale of milliseconds. This dynamic behaviour, coupled with the microenvironment provided by the 'softball' led Rebek et al. to investigate the catalytic potential of **26** towards the Diels–Alder reaction of thiophene dioxide **28** and benzoquinone **27** (**Figure 4**).

See Illustration 4

Reactions supported by artificial enzymes

Palladium-Artificial Enzyme

See Illustration :5

Palladium catalysed reaction can be operated using these artificial enzymes[7].

Multiple step Chemical Reaction

See Illustration 6

In a demonstration experiment, they made a star polymer with a core functionalized with a "sulfonate" group, which could immobilize the catalyst imidazolidinone. The two starting materials in the reactant path bind to that site and are converted by the imidazolidinone to an intermediate product. This intermediate product then diffuses to the second star polymer designed to immobilize the second catalyst, a pyrrolidine derivative. There, it is converted to the final product through simultaneous catalysis by the pyrrolidine and a third catalyst, this one in solution. When all three catalysts were present, 90% of the starting material was converted to product, showing that a "cascade" of all reaction steps had occurred. No or very little product was observed with the polymers without the catalysts. Further, the catalysts used in this work were designed to be "chiral," in an attempt to control which mirror image of the product was to be produced. In this experiment, more than 99% of the product had the preferred "chirality." The group further demonstrated that all four possible mirror images of the cascade reaction could easily be made simply by changing the catalyst chirality. (There are two optically active centers in the molecules of this reaction, thus four possible mirror images.)[2-4]

Hemin Based Organic Phase Artificial Enzyme

Based on the newly discovered arti?cial enzyme formed by mixing hemin with supramolecular hydrogels via the self-assembly of amphiphilic oligopeptides, a novel organic phase arti?cial enzyme electrode by coating the arti?cial enzyme on an electrode which was then covered with sodium alginate for protection. Scanning electron micrograph showed that the supramolecular hydrogel kept its nano?bers structure on the electrode surface. Hemin dispersed in the supermolecular hydrogel as monomer greatly promotes its direct electrochemistry behavior in organic solvents. At the same time, this electrode exhibited higher electrocatalytic ability to tert-butyl hydroperoxide (TBHP) than free hemin modi?ed electrode (free hemin mainly present as dimer). The biosensor can reach 95% of the steady-state current in about 10±2s. More importantly, it can be applied in both hydrophilic and hydrophobic solvents without adding extra buffer or mediators to them that cannot be received by most traditional organic phase enzyme electrodes. This unique property greatly promotes the development of the organic phase enzyme electrodes by facilitating the detection of different kinds of substrates of the hemin-based arti?cial enzyme soluble in hydrophilic and hydrophobic solvents. The arti?cial enzyme electrode was successfully used to determine organic peroxides in body lotion samples[8].

Vitamin B12 Artificial Enzyme

A novel amphiphile ($N^+C_5Arg2C_{16}$) having an arginine residue and a dihexadecyl moiety for the double-chain segment was synthesized, and it's aggregate behavior was examined by electron microscopy, differential scanning calorimetry, and ?uorescence polarization measurements. The hybrid vesicles formed with an alanine lipid, N^+-C_5Ala2C_{16}, and an arginine lipid, N^+C_5 $Arg2C_{16}$, are morphologically very stable. Hydrophobic vitamin B_{12} derivatives, which have carboxylic ester groups in place of the peripheral amide moieties of the naturally occurring vitamin B_{12}, were incorporated into the single-walled vesicles of $N^+C_5Ala2C_{16}$ and N^+C_5 $Arg2C_{16}$ to construct an arti?cial holoenzyme with vitamin B_{12}, activity. In these hybrid bilayer vesicles, the enzyme mimicking reaction of methylmalonyl-CoA mutase, which is 1,2-migration of carboxylic ester group on an axial ligand of the cobalt, has been enhanced by microenvironmental effects[9].

Significance

The field of artifcial enzymes is a rapidly evolving subject. As the barriers between chemistry and biology become less distinct a range of new methods, which combine expertise from both areas, are developing. In recognition of both the fact that the de novo design approach can be time consuming, and that a tiny miscalculation will be catastrophic, a trend in all these recent techniques is the use of 'selection approaches'. The natural process of selection and ampli?cation is after all, the way in which enzymes have evolved their sophisticated function.

The basic problem in arti?cial enzyme synthesis is choosing the selection event. Experimentally, selection based on binding af?nity is the easiest method to use. However, many methods which have employed binding criteria, using TSAs as the ligands, have stopped short of producing the rate accelerations required to rival natural enzymes. This is perhaps understandable, since the electrostatic and geometric ?delity of a TSA to the real transition state cannot be entirely complete. To a certain extent, the effect of this problem can be alleviated by introducing an element of design into the system. Using information available on the natural enzyme mechanism and catalytic groups, several researchers have improved the rate accelerations available with TSA methods by incorporating catalytic groups into TSA binding as we have seen above with catalytic antibodies and MIPs.

Within the apparently differing areas of enantioselective transformations induced by enzyme-like mimics or by transition metal catalysts, many of the most fundamental principles of design should still apply to both. The vocabulary and emphasis used by each specialist group is such however, that effective interaction and transmission of useful ideas and concepts is almost discouraged, even though techniques such as combinatorial searches are now used by both. At present, when the transition state or intermediate in a catalysed reaction may involve partial formation and cleavage of several bonds, and such factors as shape, polarity match, electrostatic potential and acidity and basicity as a function of molecular environment all have to be factored in, it would seem timely to devise some new pictorial way of representing and calculating the relative incremental value of these factors for various alternatives. In reality however, although an enormous data set is available, and spectacular advances have been made, catalysis still remains a delightful area of discovery[10].

References

1. http://en.wikipedia.org/wiki/Artificial_enzyme
2. Jean M. J. Fréchet, (510) 643-3077,
4. Materials Sciences Division (510) 486-4755, Berkeley Lab.
Yonggui Chi, Steven T. Scroggins, and Jean M. J. Fréchet, “One-Pot Multi-Component Asymmetric Cascade Reactions Catalyzed by Soluble Star Polymers with Highly Branched Non-Interpenetrating Catalytic Cores,” J. Amer. Chem. Soc. 130, 6322–6323 (2008).
5. A Friboulet & D Thomas. Electrical excitability of artificial enzyme membranes II. Electrochemical and enzyme properties of immobilized acetylcholinesterase membranes. Biophysical Chemistry, 1982;16:145-151.
6. R Fernandez-Lafuente et al. Facile synthesis of artificial enzyme nano-environments via solid phase chemistry of immobilized derivatives: Dramatic stabilization of penicillin acylase versus organic solvents. Enzyme and microbial technology. 1999;24:96-103.
7. Nam Sook Cho et al. Synthesis of heteromacromolecules as ligands of a Palladium-Artificial Enzyme and crystal structure. Bull. Korean Chemical Society. 2009;30(7):1647-1650.
8. Pei-Yu ge et al. A novel hemin based organic phase artificial enzyme electrode and its application in different hydrophobicity organic solvents. Biosensors and Bioelectronics. 2009; 24:2002-2007.
9. Yoshio Hisaeda, Eiji Hoshima, Makiko Arimura. Aggregation behavior of synthetic peptide lipids with arginine in aqueous solution and construction of a vitamin B12 artificial enzyme. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2000; 169:143-153.
10. W B Motherwell, M J Bingham and Y Six. Recent Progress in the design and synthesis of artificial enzymes. Tetrahedron. 2001; 57:4663-4686.

Illustrations

Illustration 1

Cyclodextrin As Artificial Enzyme

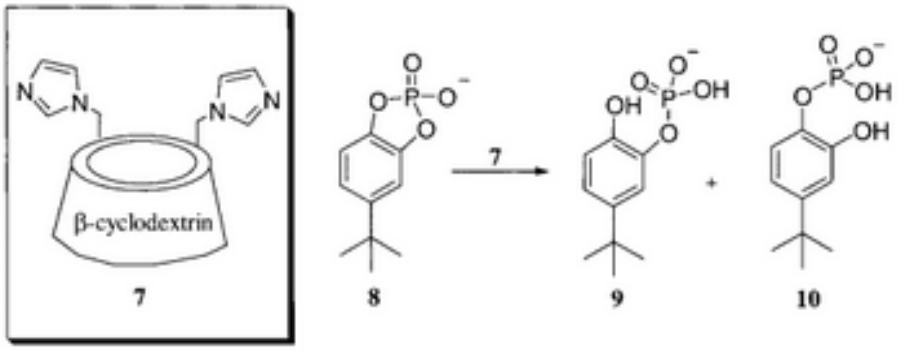


Figure 1 Cyclodextrin As Artificial Enzyme

Illustration 2

Artificial Enzyme

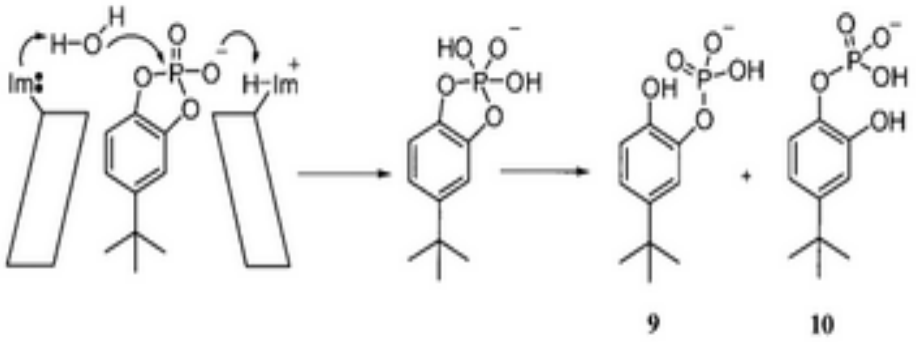


Figure 2 Artificial Enzyme

Illustration 3

Cyclophane Enzyme Mimics

Illustration 4

Reversibly self-assembled dimers as enzyme mimics

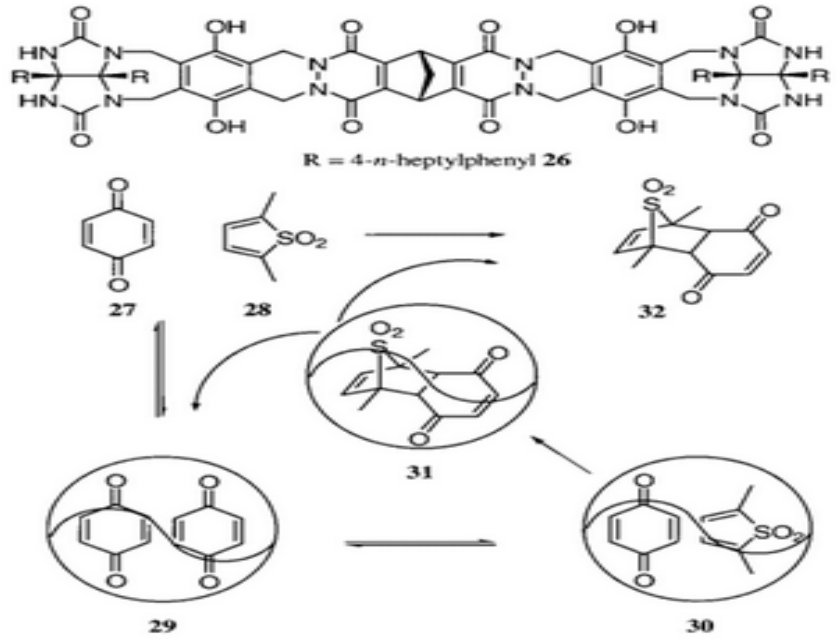
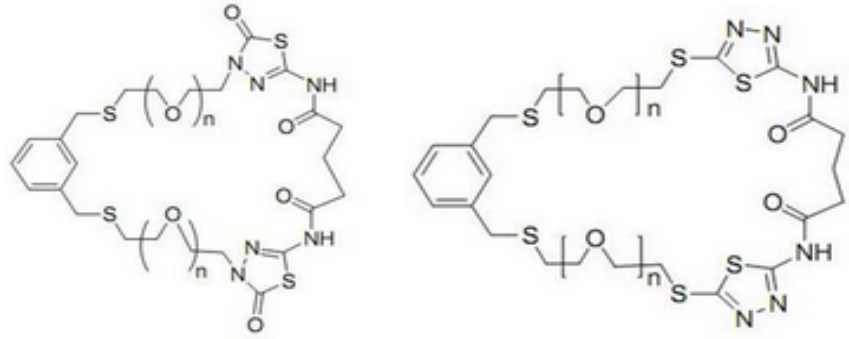


Fig 4: Reversibly self-assembled dimers as enyzme mimics

Illustration 5

Palladium-Artificial Enzyme

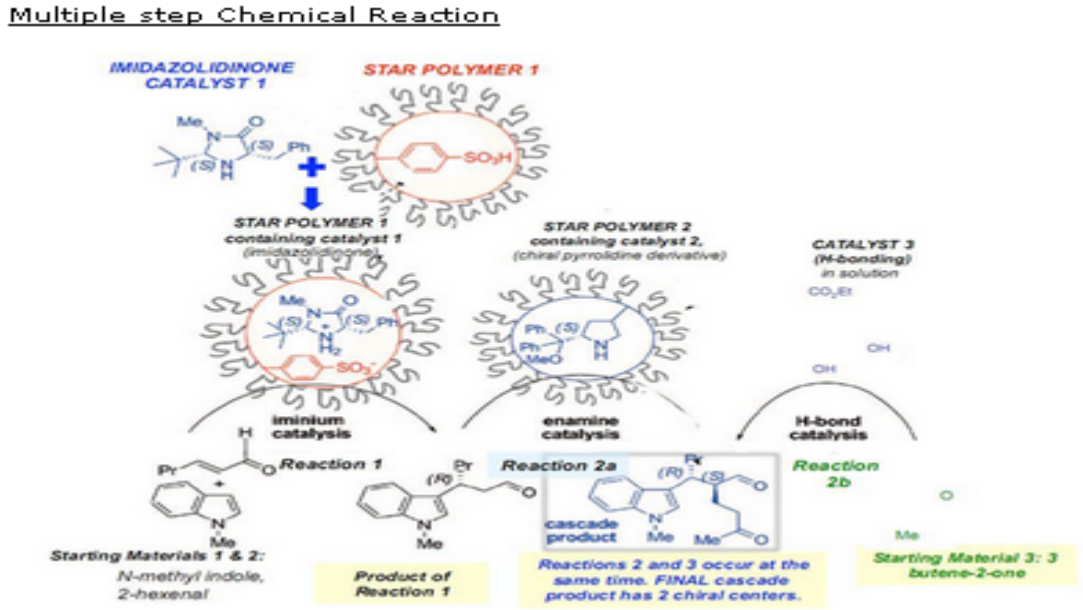
Palladium-Artificial Enzyme



Palladium catalysed reaction can be operated using these artificial enzymes[7].

Illustration 6

Multiple step Chemical Reaction



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