Synzymes

These are also called ‘Artificial enzymes’. These are substances with catalytic capabilities. The name synzyme is derived from synthetic enzymes. Currently synzymes include organic molecules tailored in such a way that they catalyze specific reactions. These molecules bind a transition state of a substrate in an active site. Similar to enzymes, these synzymes also obey Michaelis-Menten kinetics.

Requirements for Synzyme Activity

Now a days, number of possibilities are there for the construction of synzymes. These are generally synthetic polymers or oligomers having enzyme like activities. A synzyme should have two different structural moieties, a substrate binding site and a catalytically effective site. It has been observed that producing the facility for substrate binding is relatively simple but catalytic sites are comparatively more difficult. Both sites may be designed separately but there are evidences that an enzyme having a binding site for the reaction transition state, exhibits both the functions. Normally, there is enzyme-substrate formation that is converted into the product and free enzyme gets released

Synzyme + S = synzyme-S complex = synzyme + P
Synzymes and Abzymes

Dr. Anil Kumar
Professor & Head

School of Biotechnology
Devi Ahilya University, Khandwa Road
Indore 452 017
Protein Derived Synzymes

Some synzymes are simply derivatised proteins. It is pertinent to mention that immobilized enzymes are not considered as synzymes. An example of synzyme is derivatisation of myoglobin that is the oxygen carrier in muscle, by attaching (Ru(NH3)5)3+ to three surface histidine residues. This derivatisation changes it from oxygen carrier to an oxidase. This derivatised myoglobin oxidizes ascorbic acid with reduction of molecular oxygen. The derivatised myoglobin as ascorbate oxidase is almost equally effective to ascorbate oxidases.

It is not preferable to design protein synzymes from scratch since their conformations are not predicted properly from their primary structure. Protein synzymes are also sensitive to denaturation, oxidation and hydrolysis. However, polyglutamic acid shows activity as an esterase similar to acid proteases. Similar to natural enzymes, polyglutamic acid synzyme showed a bell shaped pH-activity relationship with optimum activity at pH 5.3. It showed Michaelis Menten kinetics for the hydrolysis of 4-nitrophenyl acetate (artificial substrate for esterases).
Cyclodextrin Synzymes

Synzymic cyclodextrins are usually derivatised to introduce catalytically relevant groups. Cyclodextrins (Schardinger dextrins) are naturally occurring cyclic molecules consisted of 6 to 10 $\alpha$-1,4 linked glucosyl moieties linked head to tail in a ring form. These cyclodextrins can also be prepared from starch by the action of cyclomaltodextrin glucanotransferase (EC. 2.4.1.19). The enzyme has been isolated from Bacillus macerans. One example of cyclodextrin synzyme is $\beta$-cyclodextrin with C-6 hydroxyl group covalently derivatised by an activated pyridoxal coenzyme that showed transaminase activity with stereospecificity for L-amino acids. However, unlike derivatised myoglobin, it was not equally effective to natural transaminases.
Organic Synzymes

Polyethyleneimine is formed by polymerizing ethyleneimine resulting in a highly branched hydrophilic three dimensional matrix and about 25% resultant amines have been found to be primary amines.

The primary amines have been alkylated with 1-iodododecane and alkylated amine has hydrophobic binding sites. If primary amine is alkylated with 4(5)-chloromethylimidazole, it creates general acid base catalytic sites and resulting product acts as synzyme.

This synzyme shows the activity of $\alpha$-chymotrypsin against 4-nitrophenyl esters. However, due to its random structure, it shows very poor esterase specificity. Similarly, a synthetic polymer of polyethyleneimine has been prepared that contains about 15% of its nitrogen residues alkylated with methyleneimidazole side chains and 10% with dodecyl groups. The alkylation of polyethyleneimine (PEI-600) with methyleneimidazole side chains provided nucleophilic catalytic sites whereas dodecyl groups provided the binding sites to bind small substrate molecules. The imidazole function has been introduced by alkylation of water free PEI-600 with chloromethyl imidazole in the presence of potassium iodide and potassium tertiary-butoxide as catalysts.
The primary amines remaining after introduction of methyleneimidazole groups are alkylated with stoichiometric amounts of dodecyl iodide. The reaction of dodecyl iodide has been observed using ninhydrin reaction. The absence of any ninhydrin color indicates preferential reaction of alkyl iodide with primary amines of the polymers. This macromolecule has been shown to act as synzyme and catalyzes the hydrolysis of uncharged nitrophenyl esters in water at pH 7.0 (α-chymotrypsin activity). This molecule has been shown to have more catalytic rate compared to other synzymes under similar conditions.

Synzymes have also been constructed by combination of alkylated polyethylenimine and polyoxometalates. These synzymes have hydrophobic region that allowed the aqueous biphasic selective oxidation of very hydrophobic, water insoluble substrates with hydrogen peroxide. The presence of hydrophobic regions has been shown from the measurement of contact angles and a hypsochromic shift of a fluorescent probe. With the alkylated PEI/ {PO4[WO(O2)2]4}3- highly effective C-C bond cleavage of alkenes has been observed. These synzymes have been shown to have both tertiary and quarternary amine centers.
Superoxide Dismutase Synzyme

Superoxide dismutase is an enzyme present universally in all living organisms. Manganese superoxide dismutase has been found in the mammalian mitochondria that acts by scavenging superoxide free radicals. If free superoxide radicals are present, these may attack DNA and proteins. It is known that many diseases such as cancer, Parkinson’s, Alzheimer’s are associated with deficiencies in the natural enzyme. The possibility of using mimics of the natural enzymes (synzymes) active site for therapeutic use has been considered. The synzyme offers the possibility of treating such diseases with manganese complexes mimicking the function of natural enzyme. X-ray crystallographic studies have shown that the active site of manganese superoxide dismutase is a trigonal bipyramidal manganese complex. The Mn$^{+2}$ is present in either the $+2$ or $+3$ oxidation state. Binding of the superoxide free radical leads to the formation of a six coordinate intermediate. In one reaction cycle, the electron is transferred from the superoxide to the Mn complex and the resulting oxygen molecule is released to restore the five coordinate complex.
It has been found that various supramolecular complexes of various cytochrome c proteins with 18 crown-6 derivatives behave as cold active synzymes in the hydrogen peroxide oxidation of racemic sulfoxides. With the help of Electron Spray Ionization- Mass Spectrometry (ESI-MS), Ultraviolet (UV), circular dichroism (CD), Raman spectroscopy, it has been shown that four or five 18 crown-6-molecules strongly bind to the surface of the cytochrome c and also that non-natural low-spin hexacoordinate heme structures are induced in methanol. Crown ether complexation can convert catalytically inactive biological forms to catalytically active artificial forms. Synzyme’s behavior differ from native enzyme behavior where the employed proteins act as electron transfer carriers.
With natural system, oxidative decomposition of the heme moieties occurs at room temperature whereas, pigeon breast, yeast cytochromes c stereoselectively oxidize (S)-isomers of methyl tolyl sulfoxide and related sulfoxides upon crown ether complexion and these synzymes show the highest efficiency and enantiomer selectivity at –40°C in the hydrogen peroxide dependent sulfoxide oxidation. Among the cytochrome c complexes, yeast cytochrome c shows lowest activity and degradation reactivity. It has much different protein sequence, suggesting that crown ether complexation effectively activates heme coordination but may additionally alter the native backbone structure. Efforts are being made that proper combination of cytochrome c proteins, 18-crown-6-receptors and external circumstances may be used successfully to generate synzymes exhibiting non-biological reactivities.
Fullerene based Synzymes

Fullerene based synzymes have also been constructed and these synzymes have been shown to cleave DNA oligonucleotides efficiently. The reaction of cleavage has been monitored by UV-VIS spectroscopy and separating the cleaved fractions by membrane filtration. A small quantity of the fullerene derivatives is capable of cleaving a large excess of the oligonucleotide under ambient light conditions, leaving cleaved DNA in quantitative yields.

Medium of the Enzyme Reaction

Medium of the enzyme reaction is important in altering the rate of an enzyme reaction. Therefore, medium effects are studied by comparing the rate of reaction in different solvents. It is considered that catalytic efficiency of enzymes is due to combination of effects including medium effect. Max Perutz for the first time proposed the specific role for medium effect in conjunction with the crystal structure of lysozyme. Organic solvents have the advantage over water of providing a medium of low dielectric constant.

Therefore, strong electrical interactions occur between the reactants. The non-polar interior of enzymes provides the living cell with the equivalent of the organic solvents. The information has been utilized for explaining the crystal structures of many enzymes.
It is considered that medium effects vary according to the nature of the transition state of the enzyme catalyzed reaction and the interactions responsible for transition state stabilization. A charged transition state is stabilized by solvation in an aqueous solvent and destabilized by a hydrophobic, aprotic environment resembling a classical aprotic organic solvent. Enzymes are more versatile than ordinary solvents and may at close but distinct sites, strip part of a substrate or transition state of water molecules resembling organic solvent while simultaneously providing interactions with polar, charged or protic groups at least as efficiently as solvent water at other parts. Therefore, a significant difference between the solvent molecules constituting the active site of enzymes and bulk solvents used by organic chemists is in the potential for diversity and spatial localization of their medium effects.

The effect of the medium at the active site of enzymes differ significantly from bulk solvents, both in their diversity (presence of more than one type of solvent) and in their spatial arrangement.
Medium effects have been studied using simple catalytic reaction with synzyme having systematic alkylation of a polymeric scaffold bearing amine groups that catalyzes the Kemp elimination of benzisoxazoles. It has been shown that catalysis by these synzymes occurs primarily by specific, localized enzyme-like medium effects. These effects differ significantly from the non-specific medium effects (desolvation activation) exhibited by the solvents. Ligand binding studies indicated that the synzyme active sites provide localized micro-environments having a combination of hydrophobic and apolar regions on one hand and dipolar, protic and positively charged on the other. Such localized micro-environments are not available in bulk solvents. A leaving group (Bronsted) analysis indicates that compared to solvent catalysis, the efficiency of synzyme catalysis shows little sensitivity to leaving group pKa. It has been shown that the interfaces of protein surfaces and their hydrophobic cores provide a micro-environment that is intrinsically active and may serve as a basis for further evolutionary improvements to give proficient and selective enzymes.
Metallo-synzymes

Metal ion complexes of functionalised 1,10-phenanthroline derivatives are also studied as model systems for hydrolytic metallo-enzymes. Amphiphilic metallo-complexes incorporated into micelles or vesicles and water soluble complexes in pure aqueous buffer solutions are found to be catalytically active in the hydrolysis of activated carboxylic and phosphate esters. It has been found that mixed micellar systems containing Zn +2 and Cu +2 complexes of lipophilic ligands with 1,10-phenanthroline or pyridine group as chelating moiety and a pendant N- alkylated imidazole group are efficient catalysts in the hydrolysis of p-nitrophenyl picolinate and diphenyl p-nitrophenyl phosphate. Kinetic studies of the hydrolysis of p-nitrophenyl picolinate indicated that catalysis proceeds by preliminary formation of a reactive ternary complex composed of metal ion, ligand, and substrate.
These synzymes operate via a metal hydroxide-ion catalyzed mechanism and exhibit turn over behavior while retaining their full activity. The catalytic role of a hydroxymethyl group covalently bound to the 1,10-phenanthroline ligand in the vicinity of the reaction center and the effect of incorporation of the ligand into micelles are shown. The Zn+2 complex of the lipophilic ligand bearing the hydroxymethyl group is shown to be 25 times more active in the hydrolysis of p-nitrophenyl picolinate than the lipophilic metallo-complex lacking this group. Under turn over conditions, the hydroxymethyl containing metallo-catalyst displays a kinetically biphasic behavior characteristic for an acylation-deacylation mechanism. The acylation of the hydroxymethyl group is found to be 133 times faster than deacylation.
Metal ion complexes of lipophilic 1,10 phenanthroline containing (S)-2-(hydroxymethyl) pyrrolidine at the \( \alpha \)- position are highly active and enantioselective in the hydrolysis of \( p \)-nitrophenyl esters of N-protected phenylalanine.

Metal ion complexes of 1,10 –phenanthroline having two long alkyl chains and a nucleophilic hydroxymethyl group, (S)-2-(hydroxymethyl)pyrrolidine or ephedrine group at the \( \alpha \)-position incorporated in C18C12 vesicles are catalytically active toward \( p \)-nitrophenyl picolinate and show activity and enantioselectivity towards \( p \)-nitrophenyl esters of N-protected leucine as the substrate. In mixed metallo-vesicles, the amphiphilic ligand is anchored in the core of the bilayer membrane by the alkyl chains, whereas the chelated head group protrudes into the aqueous interface. The metallo-complexes appear to be active in both the exo- and endovesicular side of the bilayer and the fluidity of the vesicle membrane has no effect on the enantioselectivity.
Biomimetic Chemistry

The design of synzymes is done by taking the advantage of Biomimetic Chemistry. It is considered that molecules of low molecular weights are likely to reproduce the function of the enzyme. Therefore, first synthesis of biomimetic ligands is carried out, and thereafter, preparation of similar complexes of the active sites and evaluation of their in vitro and in vivo activity is done.

Synzyme for superoxide dismutase has been constructed using Biomimetic chemistry. Superoxide molecule being a metastable molecule and being dismutant spontaneously in medium protic (aqueous), evaluation of the activity of superoxide dismutase is a tough task. Design of ligands and complexes functional or structural mimes of metalloproteins has been done by the scientists and application of the design to the modeling of the superoxide dismutases to iron or manganese has been taken.
First, synthesis of small metal complexes of transition likely to reproduce the activity of superoxide dismutase is done. The intermediate species implied in the dismutation of superoxide are identified. Species metal superoxide for example, or derived species like dimmers di μ-oxo can be isolated and characterized. Thereafter, search is done to find the methods allowing an effective dismutation of superoxide in order to prepare molecules with therapeutic aim. Many clinical studies showed the protective role of superoxide dismutases with respect to pathologies implying superoxide. The use of enzymes presents a certain number of disadvantages like cost, immuno-reactivity etc which can be counteracted by replacing them by small complexes. Besides, compounds capable of more generally interacting with oxygen and their derivatives of reductions to make catalysis of oxidation are obtained.
Two metals namely iron and manganese known to be present with the active site of superoxide dismutases may be selected. Manganese has the advantage of least toxicity among the metals (iron, manganese, copper, nickel) known to intervene naturally in the dismutation of the superoxide.

Synthesis of the ligands is done having structural approach similar to environmental metal to the active site of superoxide dismutases with iron and manganese. A metal in a geometry bipyramid trigonale, two histidines and an aspartate constituting the median plane, the third histidine, more remote and a water molecule occupying the two apical positions. A ligand for central architecture having an amine tripodale of the type TREN (sorting (aminoethyl) amine) is synthesized and is studied in detail. This family of ligands has been divided into two large sub-families: Polyamines tripodaux ligands polydentes encumbered and tripod ligands tetradentes more open implying at the Lewis bases of the amine type and carboxylate type. The isolated complexes in solid phase of the original structures are infinite propellers or chiral complexes.

**Software, SPARTAN**, has been used to estimate the geometry of ligands and to optimize chemical structure to support chelation before carrying out synthesis.
Evaluation of Superoxide dismutase activity in these Complexes

In direct studies, anhydrous medium (dissolved KO2, anhydrous dimethyl sulfoxide (DMSO) ) with follow up by traditional cyclic voltammetry (dissolved KO2 or production of superoxide per reduction to the electrode of dissolved dioxygene), to highlight directly derived metal superoxides or species is used. Having complexes isolated in anhydrous conditions without water fixed on metal and of complexes isolated in water, with two water molecules fixed on metal, importance of water related to metal in the catalysis of dismutation of superoxide could be highlighted.
http://www.lsbu.ac.uk/biology/enztech/artificial.html  Artificial Enzymes.
http://library.wur.nl/wda/abstracts/ab1702.html
http://64.233.179.104/translate_c?hl=en&sl=fr&u=http://www/icmo.u-psud.fr/w-lcbb/
www.umist.ac.uk/departments/chemistry/intranet/teaching/projects/abstracts/omalley-403.pdf
Abzymes are also categorized as artificial enzymes or synzymes. If antibodies act as enzymes, these are named as ‘Abzymes’. The abzymes are also called ‘Catmab’ that means catalytic monoclonal antibodies (monoclonal antibodies with catalytic activity). Abzymes are mostly artificial enzymes, however, are also found in humans with asthma as antivasoactive intestinal peptide autoantibodies and in patients with the autoimmune disease systemic lupus erythematosus where these bind and hydrolyze DNA.

The vasoactive intestinal peptidase activity has been shown to be present on Bence-Jones proteins. Bence-Jones proteins are monoclonal human light chains found in the urine of persons with multiple myeloma. Other protease activities are characterized for cleaving throglobulin in the serum of persons with Hashimoto thyroiditis or for hydrolyzing factor VIII in hemophilia patients infused with homologous factor VIII. The DNA hydrolyzing activity may be correlated to the presence of high levels of anti-topoisomerase I antibodies in the serum of the patients.
What is Enzyme Catalysis?

Natural enzymes bind the substrate(s) (biomolecules) and subsequently catalyze their conversion to new products. According to the transition state theory, enzymes catalyze the reaction by stabilizing the chemical intermediate between substrates and products (also called the transition state). Formation of the transition state is energetically unfavorable without enzyme that provides the activation energy to push the reaction through the transition state. Ultimately, enzyme catalysis is the acceleration of the reaction rate. The transition state of a reaction is difficult to study due to no finite lifetime. It is considered that an enzyme is complementary to the transition state and virtually is a requirement for catalysis. There are experimental evidences in support of complementarily between the enzyme and the transition state. If so, some functional groups in the substrate and in the enzyme must interact preferentially with the transition state rather than the ES complex. Altering these groups should result in no or little effect on ES complex formation and kinetic parameters should not change. However, altering these groups should have much effect on the overall rate of the reaction since bound substrate lacks potential binding interactions required to lower the activation energy.
What are Abzymes?

Abzymes are important to perform specific actions on DNA. These are selected against transition state analogues. Abzymes have a low \( K_m \) indicating that they bind a target molecule. However, abzymes also show low \( V_{\text{max}} \) indicating a slow reaction rate. If an antibody is developed to a stable molecule like an unstable intermediate of another reaction, the developed antibody enzymatically binds to and stabilize the intermediate state and thus catalyzes the reaction.

Antibodies molecules are produced by the immune system to bind and neutralize antigens (foreign molecules to the body). Foreign proteins of bacteria and viruses, some small chemical molecules called haptens act as antigens. The purpose of production antibodies is defense (protection from harm against the foreign intruder). The human body is capable of producing antibodies to virtually any encountered antigen. Each antibody binds its own unique target like a key fitting in a lock.
Immunoglobulin as transition state analogues

Structurally antibodies (immunoglobulin G-type) are Y shaped molecules. An antibody molecule is consisted of two identical heterodimers joined together by disulfide bonds. Each heterodimer is consisted of a short peptide (light chain) and a longer peptide (heavy chain). The light and heavy chains are joined together by disulfide bonds. One end of the antibody molecule has conserved regions that are formed by the interface of two of the two heavy chains, and are also called constant domains. These constant domains have similar amino acid compositions in most of the immunoglobulins-(G type) irrespective of the structure of the antigen to which these bind. The other end of the antibody molecule is variable in structure and amino acid sequence. These variable domains bind the antigens specifically. The interface of two of the heavy and light chain variable regions forms a single deep pocket that is the antigen binding site and changes as per the shape of the antigen. Each immunoglobulin G molecule has two identical antigen binding sites. There are certain regions within the variable regions that are called complementarity determining regions. Amino acids within these regions specifically contact the antigen through non-covalent forces.
If an antibody binds to a transition state molecule, it is expected to catalyze a corresponding chemical reaction by forcing substrates into transition state geometry. However, it is very difficult to raise an antibody against an unstable chemical. Therefore, compounds that look alike are used for raising the antibody and these compounds are called as ‘transition state analogs’. The selected transition state analog must be more stable than the transition state molecule itself but should mimic its three dimensional structure. On injection of transition state analog in the blood stream of an animal, it acts as hapten and elicits antibody production. Afterwards, antibodies are isolated from the blood and screened by experimental assays to check whether it catalyzes the desired reaction.
In 1974, Niels Jerne gave a theory regarding the immune system as a network of interacting idiotypes. He postulated the idiotypic network that for each immunoglobulin (Ab1) generated against an antigenic determinant, there existed a complementary antibody (Ab2) directed against the idiotypic determinants of Ab1. When the idiotypic determinant superimpose with the binding site of Ab1, some of the Ab2 may mimic the antigen’s determinants and are designed as ‘internal images’ of the original antigen.

First an antibody (Ab1) is raised that recognizes the active site of an enzyme so that the combining site of Ab1 has structural features complementary to that of the enzyme. A second set of antibodies (Ab2) is raised against Ab1 combining site. Among these second antibodies (also called anti-idiotypic antibodies), some of them may represent a structural internal image of the original enzymatic site and some others may exhibit catalytic activity. Using this procedure, antibodies with esterase and amidase are characterized. These abzymes have efficient catalytic activities with a relaxed specificity compared to the model (natural) enzyme.
Examples of Abzymes

In 1986, Peter Schulz and Richard Lerner generated abzymes that catalyzed the hydrolysis of an ester bond (using p-nitrobenzoate as the substrate). The mechanism involves the nucleophilic attack of the oxygen atom of water on the carbonyl atom in p-nitrobenzoate. The interaction generates a transition state with a tetrahedral geometry. A stable analog was generated by replacing the carbon at the center of the tetrahedron with phosphate for use as hapten. The raised antibody catalyzed the reaction and rate of the reaction as determined by measuring Vmax and Km, were found to be much higher than the corresponding uncatalyzed reaction. Since 1986, more than 100 different abzymes have been constructed that catalyze different reactions. These reactions include hydrolysis of chemical bonds (esters, carbonates, amides, phosphates etc), stereospecific synthesis of compounds as well as reactions of isomerization, decarboxylation, oxidation and reduction etc.
In 1989, scientists produced an abzyme catalyzing the hydrolysis of a glycine-phenyl alanine bond. The antibodies were produced against a hapten that induced the selective recognition of the glycine-phenyl alanine sequence and also generation of residues capable to complex a metal ion. After complexing with zinc, this antibody exhibited good catalytic activity. It has been found that antibodies raised against different metallo-porphyrins may mimic metallo-proteins. The heme-abzymes are able to catalyze peroxidase like activities and may be used for enantio-selective oxidation of molecules.

In 2000, Scott Taylor Laboratory succeeded in producing an abzyme capable of catalyzing hydrolysis of an N-methyl carbamate that was raised against a phosphoramidate transition state analog. This abzyme is shown to be capable of triggering activation of bis-carbamate prodrugs of type 2 by an electron relay mechanism. It has been speculated that the above mentioned approach to prodrug activation will help in solving the problem of side effects often associated with most cancer chemo-therapeutic regimens.
The same group (Scott Taylor group) also obtained an abzyme that catalyzed hydrolysis of a phosphonate ester by raising antibodies to strained cyclic phosphinate transition state analog. They are engaged in developing abzymes capable of carrying detoxification of organophosphorus poisons and for studying the role of strain in biocatalysis. The group is also engaged in developing inhibitors of protein tyrosine phosphatases and estrone sulfatase since inhibitors of these enzymes can be exploited for treatment of diabetes and breast cancer.

Gouverneur’s laboratory is engaged in designing and preparing new reagents for halogenation including reagents that mimic haloperoxidases. They are applying concept of electrophilic fluorodesilylation to the preparation of organofluorine compounds including its application to biologically active molecules such as fluorinated nucleosides and fluorinated analogues of HIV protease inhibitors.
Neustroev’s laboratory is engaged in studying abzymes having amylolytic activity. They found various immunoglobulins from sera of patients with autoimmune diseases and human milk having amylolytic activity (activity capable of breaking $\alpha$-1,4-D-glucosyl linkages of maltosaccharides, starch, glycogen and several other artificial substrates). Pure IgM fractions have been isolated from several tens of analyzed patients with clinically definite diagnostics of multiple sclerosis (MS) and systemic lupus erythematosus (SLE), and these fractions showed more than three times amylolytic activity compared to healthy donors. Similarly, average values for the specific amylolytic activity of IgGs and sIgAs from human milk were five times less than of IgMs from autoimmune patients.
Evidences have been produced to prove that the amylolytic activity of abzymes was their intrinsic property and was not due to any enzyme contamination. Fab fragments derived from IgM and IgG fraction of human abzymes displayed the same level of amylolytic activity. Values of Michaelis constant (Km) of the abzyme mediated hydrolysis of different α-1,4- D-maltooligosaccharides and α-1,4- D-maltooligosaccharides with chromogenic and fluorescent label at the reducing end were in the range of 1-2 to 0.01 mM. Abzymes from different donors showed catalytic heterogeneity in Km and also showed different mode of action in the hydrolysis of natural and artificial substrates. For example, one part of amylolytic immunoglobulins showed exo-amylase activity whereas others showed α- glucosidase activity yielding glucose and also capable of cleaving p-nitrophenyl - α-D- glucopyranoside.
The Scientists are engaged to improve abzymes through the use of protein engineering so that their activities may even be more than the natural enzymes. They are successful in cloning genes coding for immunoglobulin G molecules. The millions of gene products from an immunized animal are screened for the production of antibodies having desired catalytic activity. Once a gene product having desired catalytic activity is found, the same can be produced in bacteria in large amount and it is called as ‘recombinant antibody’.

That’s way, an antibody gene may be immortalized for further study. The gene encoding a recombinant antibody may be mutated selectively or randomly for improving the activity of the original antibody. Generally complementarity determining regions (CDRs) have been selected for mutational studies since amino acids present within CDRs directly contact the antigen. After mutational studies, screening is again done to select improved molecules.
The immune system produces high affinity selective receptors by screening a large library of antibody combining sites. Diversity is generated from the association of variable, diversity, and joining segments followed by somatic hypermutation of the germline antibody genes to optimize antibody-antigen recognition. High resolution structural studies for the molecular interactions may help to analyze the relationship between active site geometry, hapten structure and catalytic efficiency at the atomic level. The resolution of an increasing number of tri-dimensional structures of abzymes brings a better understanding of the appearance and evolution of the catalytic function not only in antibody binding sites but also in enzyme active sites. The study must provide basis for optimizing antibody activity through both directed mutagenesis and improvements in hapten design.
Applications of Abzymes

The abzymes are found to have potential role in the therapeutics. Abzymes have been constructed capable of cleaving cocaine molecule at specific bonds causing detoxification of cocaine. Due to similarity between the hydrolysis of cocaine and hydrolysis of p-nitrobezoate, both reactions occur through tetrahedral intermediates and the transition state analogs mimic this geometry. Anti-cocaine abzymes (abzymes capable of cleaving cocaine molecules) may be used to treat patients who are addicted to cocaine. These abzymes may also be used to reverse the lethal effects of a cocaine overdose.
The Scientists are engaged in exploiting the use of abzyme technology for specific targeting of cancer cells for destruction. Cancer cells have specific determinants called as tumor cell antigens on their surface that are lacking in normal cells. If specific antibodies are raised capable of specific binding these tumor cell antigens, cancer drugs can be delivered directly to the tumor. Abzymes with two distinct antigen binding sites are being tested. One site binds with high affinity to tumor cell antigen whereas the second site catalyzes the cleavage of a prodrug. The prodrug is a non-toxic precursor of a cytotoxic drug. First, the antibody is administered to a patient, it binds the tumor cells with high affinity. Afterwards, prodrug is injected in the blood that becomes activated in the vicinity of the targeted antibody. By this approach, tumor cells are selectively destroyed sparing the healthy cells from destruction due to the toxic effect of cancer drugs. The scientists are also engaged in using abzymes to inactivate viruses. Abzymes have been isolated that cleave viral coat proteins of human immunodeficiency virus. Abzymes have also been developed capable of catalyzing destruction of viral genes.