Tetrahedron Vol. 40, No 2, PP. 269 to 292, 1984
Printed in Great Britain.

0040-4020/84 $5.00 + .00
Pergamon Press Ltd.

TETRAHEDRON REPORT NUMBER 158

DESIGN AND SYNTHESIS OF ARTIFICIAL ENZYMES

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(*Received in the UK* 18 *January* 1983)

CONTENTS

DESIGN OF ARTIFICIAL ENZYMES

Basic strategy of molecular design

Although enzymes may be prepared artificially, either by the "genetic engineering" or by stepwise synthesis, these methods are not topics of the present review. In this review, entirely artificial molecules will be discussed, which show the "function" exactly the same as or very similar to the functions of enzymes or other related proteins. Then, a question may arise how an entirely artificial molecule shows certain enzyme-like functions. The basic strategy of molecular design for entirely artificial enzymes ("*total functionalization*"[1]) is dismissed. "Translation" from biological to chemical concepts is necessary, which is possible only after making considerable simplification and generalization of otherwise complicated biological functions. This is further discussed in an example.

An enzyme has its own characteristic size and shape, which is primarily determined by its amino acid sequence. Therefore, activity of certain native enzymes is, in principle, determined by the total amino acid sequence and each amino acid residue must play some role in development of the whole enzyme activity. However, it is also true that substitution of single amino acid residue sometimes does not affect the enzyme activity seriously. It seems reasonable and satisfactory, in many cases, to assume that a small part of an enzyme molecule determines the substrate binding and another part determines the catalytic activity. Poor correlation between K_d and k_{cat} in enzyme catalyzed hydrolysis of a series of oligopeptide esters strongly supports the above "two-site" approximation (see Table 1). The two-site approximation is very helpful and convenient for designing appropriate molecular framework of entirely artificial enzymes. The strategy is schematically depicted in Fig. 1.

It should be noted that the two-site approximation does not hold in many enzyme reaction. Allosteric control, product inhibition, activation or induced disfit belongs to the remarkable exception to the oversimplified assumption and one should treat his molecular design with special cares when one of these sophisticated functions is attempted to mimick.

Table 1. $1/K_m$ and k_{cat} for hydrolysis of a series of similar substrates catalyzed by pepsin (pH = 4.0)[2]

peptide	$1/K_m$ (M^{-1})	k_2 (sec^{-1})
C_{bz}-Gly-His-Phe-Phe-OEt	1250	2.43
C_{bz}-His-Phe-Trp-OEt	4350	0.51
C_{bz}-His-Phe-Tyr-OEt	4350	0.16
C_{bz}-His-Tyr-Phe-OMe	1470	0.013
C_{bz}-His-Phe-Leu-OMe	1790	0.0025

Basic characteristics of enzyme action in its simplified and generalized form

As discussed in the previous section, simplification and generalization of enzyme function is important for our present purpose. In this section, classification of the generalized and simplified characteristics of enzyme action is listed.

(a) *Catalytic activity*

$$\text{A-B} + \text{E} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{A-B}\cdot\text{E} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \text{A}' + \text{B-E} \xrightarrow{k_3} \text{A}' + \text{B}' + \text{E}. \tag{1}$$

In Michaelis–Menthen type scheme (eqn 1), catalytic activity is expressed by the magnitude of k_2 and/or k_3. Corresponding thermodynamic quantities, $\Delta G_2(\Delta H_2, \Delta S_2)$ and/or $\Delta G_3(\Delta H_3, \Delta S_3)$ may be similarly used.

(b) *Binding capacity*. In eqn (1), the binding capacity is best expressed by $K_a = k_1/k_{-1}$ or its reciprocal $K_d = k_{-1}/k_1$, although biochemists often tend to use Michaelis constant $K_m = (k_{-1} + k_2)/k_1$, which is equal to K_d when k_2 is negligibly small.

(c) *Actual activity and selectivity obtained by combination of catalytic activity and binding capacity*. Apparent catalytic activity for i-th substrate;

$$v^i_{app}/E_0 = \underline{K}_a{}^i \cdot k_2{}^i \cdot k_3{}^i \cdot S_0{}^i, \text{ when } S_0{}^i \underline{K}_a{}^i \ll 1$$

or

$$v^i_{app} = \frac{S_0{}^i E_0 \underline{K}_a{}^i}{1 + S_0{}^i \underline{K}_a{}^i} k_2{}^i k_3{}^i,$$

$$= E_0 k_2{}^i k_3{}^i, \text{ when } S_0{}^i \underline{K}_a{}^i \gg 1$$

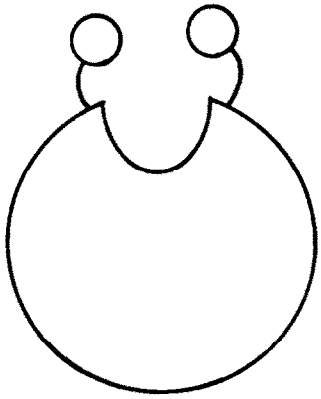


Fig. 1. Schematic representation of two-site approximation for design of artificial enzymes.

apparent selectivity between i-th and j-th substrate;

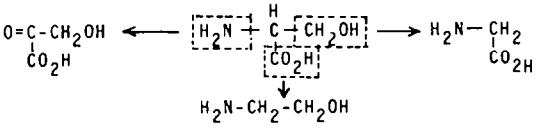
$\underline{K}_a^i/\underline{K}_a^i$ binding selectivity in simple competitive binding

or

k_{app}^i/k_{app}^j as apparent selectivity of catalysis.

Intermolecular selectivity (or specificity) may be taken for substrates of different sizes and shapes, nature of functional groups (or in general, recognition groups), and chirality of substrates; or intramolecular selectivity may be taken for regiochemistry, stereochemistry, nature of reacting group, reaction pathway.

The latter selectivity is best exemplified by the manifold degradation of certain amino acids shown in eqn 2.

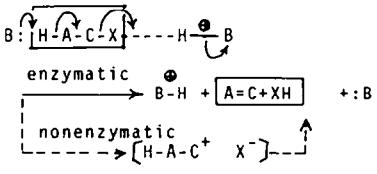


(2)

Pattern classification of rate enhancement or activity development mechanism in enzyme catalysis

Native enzymes use only several fundamental techniques for rate enhancement, and these limited number of techniques are commonly seen in different enzymes. Following are pattern clsssification of these enhancement mechanisms.

(a) *Acid-base catalysis*



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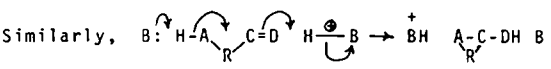

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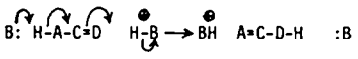


(5)

and many similar examples.

Acid-base catalysis often seen in enzymatic reactions has remarkably lowered potential barrier compared with corresponding nonenzymatic stepwise reaction (see Fig. 2). Typical examples of acid-base catalysis are listed in Table 2.

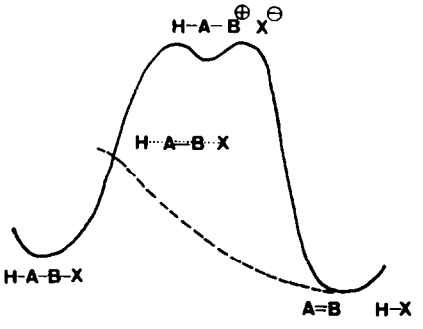
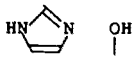
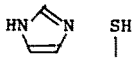


Fig. 2. General potential diagram of acid-base catalysis.

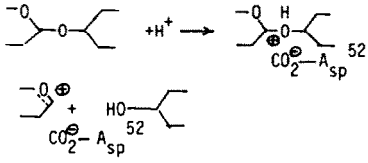
Table 2.

B	BH	enzyme
		ribonuclease
—	CO_2H	lysozyme
	—	chymotrypsin
	—	papain

(b) *Electrostatic stabilization or destabilization*

$$C^{\ominus}A\text{-}BD^{\oplus} \qquad c^{\ominus} \leftrightarrow \overset{\delta+}{A} \text{-----} \overset{\delta-}{B} \leftrightarrow \overset{\oplus}{D}. \tag{6}$$

Electrostatic stabilization of a transition state generally shown in eqn (6) accelerates the ionization of A-B. A typical example is rate enhancement in lysozyme catalysis (eqn 7).



(7)

However, significance of the electrostatic interaction is not necessarily limited to the rate enhancement but it may also contribute to molecular recognition in general. A well known example is the molecular recognition by acetylchlorine esterase (see Fig. 3), leading to the specific ester exchange at the ester carbonyl of the substrate, acetylchlorine.[4] Molecular recognition caused by electrostatic interaction further controls the geometry of the intermolecular interaction between substrates as seen in prenyl polymerase (Fig. 4). Head-to-tail orientation of prenyl- and isopentenyl

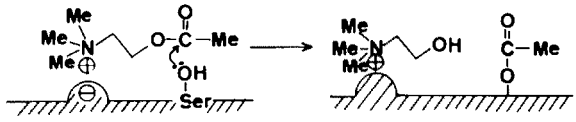


Fig. 3. Molecular recognition by acetylchlorine esterase.

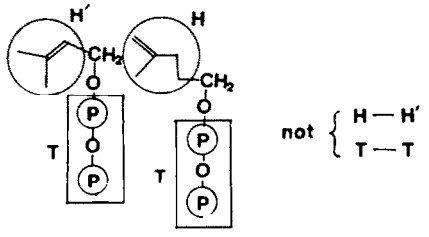


Fig. 4. Orientation of substrates.

pyrophosphate may be an important key step to induce exclusive $C^+ - \pi$ participation. The head-to-tail orientation may be provided by the electrostatic interaction in a hydrophobic microenvironment of the active site.[5]

(c) *Macrocyclic or pseudomacrocyclic effect*; *negative entropy site*. Certain elemental interaction such as electrostatic (involving hydrogen bonding or coordination) interaction may be easily amplified by bringing several interacting structural units together into a small region with appropriate arrangement

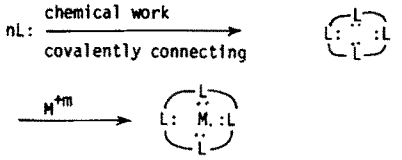
$$H_T = \sum_{i=1}^{n} \Delta H_i. \tag{8}$$

However, this very favorable enthalpy change is accompanied with unfavorable entropy loss due to loss of motional freedom of structural units and the entropy loss is also amplified by this "bringing together" process. As a result,

$$S_T = \sum_{i=1}^{n} \Delta S_i \tag{9}$$

multisite interaction does not necessarily lead to very strong binding.

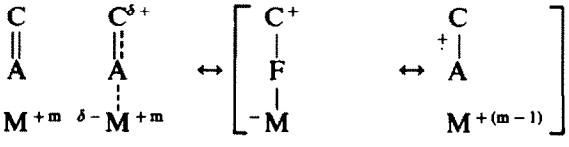
However, if this inevitable and considerable entropy loss is paid beforehand, multisite interaction must afford very strong host quest binding and this ideal situation may be brought by preparation of a covalently bound multisite host. In this case, a large entropy loss is involved in the (organic, inorganic or biological) synthesis step.



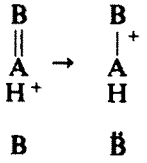
(10)

Typical examples are tetrapyrroles,[6] macrolides,[7] crown ethers[8] or cryptands[9] for amplified electrostatic, very often coordination interaction and cyclodextrins[10] or cyclophanes[11] for amplified hydrophobic interaction (see Fig. 5).

(d) *Specific coordination*. Very stable small molecules such as O_2, N_2, CO_2 and H_2O are frequently used in enzyme reactions as reagents or starting materials. These are readily available in almost infinite quantities but very difficult to activate. Various types of metalloenzymes are responsible for this particular activation through coordination. Metal coordination usually affects electron distribution of quest molecules considerably just like acid or base catalysis and moreover, it affects spin distribution, too, which is not seen in acid-base catalysis at all. Both effects activate small guest molecules easily. At



(11a)



for electron withdrawal from a quest

(11b)

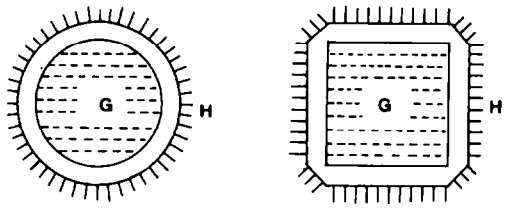
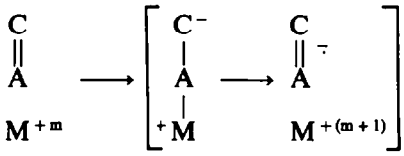


Fig. 5. Surface recognition by extended hydrophobic interaction.

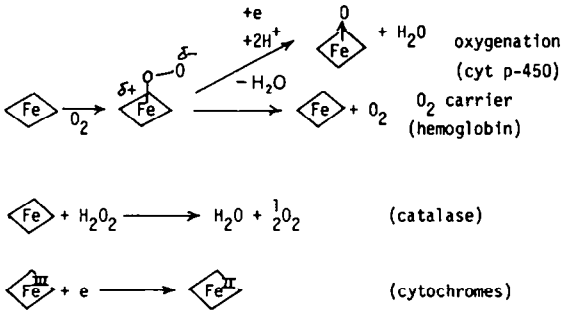
similarly, for electron donation,



(12)

the same time, a remarkable electrostatic stabilization at activated species is often provided by the metal iron. Typical examples of the small molecule activation by metalloenzymes are listed in Table 3.

Microenvironment around coordination centre is also very important for development of certain catalytic activity. Porphyrin iron complex (heme), for example, shows several entirely different functions depending on its microenvironment.[6]



(13)

Table 3.

metal center	guest to be activated	enzyme
S-Mo-S | | | S | (+ S-Fe-S) | S |	N_2	nitrogenase[12a]
Fe S	O_2	cytochrome P-450[12b]
N O N Zn N O N N O N	CO_2	carbonic anhydrase[12c]
$(protein \cdot Mn)_n$	H_2O	photosynthesis system II

Microenvironmental effect

As discussed in the previous section, microenvironment around an active site often determines or considerably modifies at least, catalytic activity or reaction-pathway. Remarkable environmental effect may be rather astonishing to organic chemists who are not familiar with biochemistry. Water, rather than the enzyme itself, is greatly responsible for the development of the microenvironmental effect because of its dielectric property and aggregating property.

Dielectric constant of bulk water is much higher than a nonpolar organic solvent as shown in Table 4. Apparently, a site surrounded by only hydrophobic side shains of amino acid residues (no water molecule incorporated) should have a much lower dielectric constant than bulk water and any electrostatic interaction (between point changes, charge-dipole, dipole–dipole etc) must be remarkably amplified as shown in eqn (14),

$$u = \frac{Z_1 Z_2}{\epsilon r} \tag{14}$$

where ϵ is a dielectric constant. Very strong association between trypsin and trypsin inhibitor is partly due to the enhanced electrostatic interaction in the hydrophobic contact region as schematically shown in Fig. 6.[13] In this particular example, not only direct interaction between positive and negative changes but also interaction between two positive charges bridged with a counter hydroxyl anion seems to be important.[14] In biopolymer binding, it is shown that the electrostatic interaction in the hydrophobic region has a special significance.

Self aggregation of water molecules makes thermodynamic state of a hydrophobic solute unusual and serious entropy destabilization takes place when a hydrophobic solute is introduced in water (see Fig. 7). Therefore, decrease of water assemblies is achieved by host-guest surface contact, playing an important role in driving the binding of a hydrophobic guest molecule to a hydrophobic sites of a host molecule[15] (see Fig. 5).

Amide grouping is also important in making its vicinity strongly polar, facilitating proton transport or electron transport. A well known example of the proton transport through α-helix is bacteriorhodopsin, which ejects proton from inside to outside on irradiation (See Fig. 8).[16] Another function of the amide grouping is its hydrogen bond forming capacity, by which a coenzyme is mediated. Hydrogenase, for example, has two units of cubic ion sulfur clusters in its

Table 4. Dielectric constant (25°)

H_2O	CH_3CONH_2	CH_3NO_2	CH_3OH	C_2H_5OH	C_6H_5N
78.5	59 (83°C)	36	32	24	12

CH_3CO_2H	$CHCl_3$	C_6H_6	0	0	$n\text{-}C_6H_{14}$
6	4.6	2.3	2.2		1.9

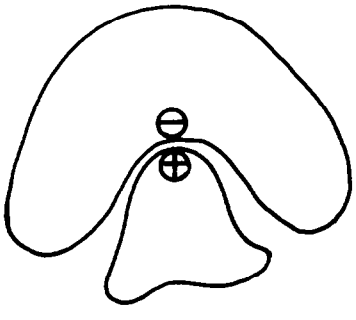


Fig. 6. Schematic representation of electrostatic interaction involved in trypsin–trypsin inhibitor association.

Thermodynamics of Methane Clathrate

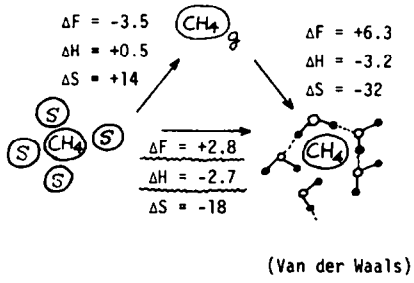


Fig. 7. Thermodynamic quantities for transfer of methane to aqueous phase.

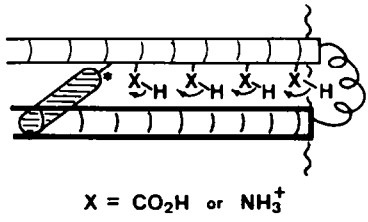


Fig. 8. Hypothetical mechanism of proton ejection from irradiated bacteriorhodopsin.

active site surrounded by amide groups (see Fig. 9). These hydrogen bonding markedly mediates the oxidation-reduction potential and as a consequence, hydrogenase has E° $-0.50 \sim -0.70$ SCE quite different from that of unbound Fe-S cluster, E° $-0.70 \sim -1.42$.

Rate enhancement caused by enzyme-substrate binding; approximation effect, induced fit and induced strain

When two (or more) different guest molecules are involved in a transition state of enzyme catalysis, these must be bound at the closest sites with appropriate orientation (see Fig. 10). Three body collision is not a favorable process in a statistical sense, but it is no more true for enzyme-substrates or host-guests association where the "soft-collision" or the long lived two body colliding complex should be expected. Therefore, "approximation effect" is necessary and important for understanding of some of enzyme catalyses and for making reasonable molecular design of certain sephisticated artificial enzyme.

Enzymes often change their conformation on the guest binding. Sometimes the conformation change leads to the better fit of substrate functional group with enzyme functional group and sometimes it drives guest structure to the structure of the transition state. In these cases, the overall phenomenon is called as "induced fit". Sometimes the conformation change induces a considerable strain in the enzyme-substrate complex, raising the potential of the starting state relative to the

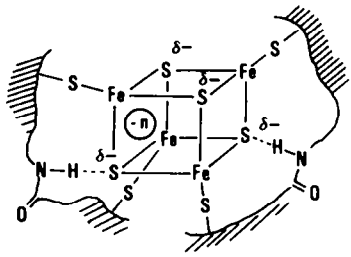


Fig. 9. Local structure of hydrogenase active site.

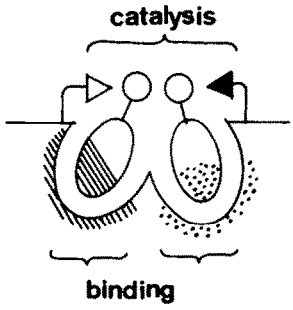


Fig. 10. Approximation effect.

transition state, where most of the induced strain is released due to bond lengthening. Thus the substrate binding leads to rate enhancement called as "induced strain" effect. This effect is not only limited to the rate enhancement but also provides specificity as shown in the lysozyme catalyzed sugar cleavage (see Fig. 11).[17]

Substrate specificities

As discussed in the previous section substrate specificity is one of the most important criteria of enzyme activity. In this section, strategy for specificity design is discussed.

(a) *General principle of host design.* By assuming a possible 3-dimensional structure of certain guest molecule, one can choose "recognition points" ($P_1 - P_i$) or surfaces ($S_1 - S_j$) properly. Then hypothetical "counter-recognition" points ($Q_1 - Q_i$) or surfaces ($T_1 - T_j$) are drawn and from a group of "counter-recognition elements", Q's and T's, several elements are taken and appropriately combined. Further addition of necessary structural units for connection of these elements, a total structure may be drawn (see Fig. 12).

Practical preparation of this hypothetical host molecule may be achieved either by (i) stepwise synthesis of total structure (Fig. 13a); or (ii) regio- and stereospecific functionalization on certain readily available host molecule (Fig. 13b).

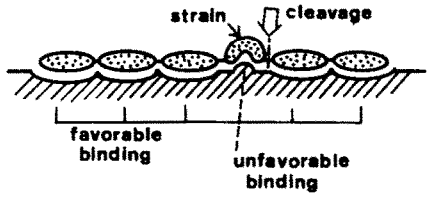


Fig. 11. Induced strain.

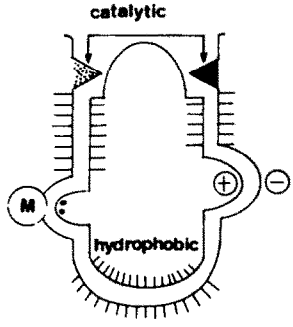


Fig. 12. General principle of host design.

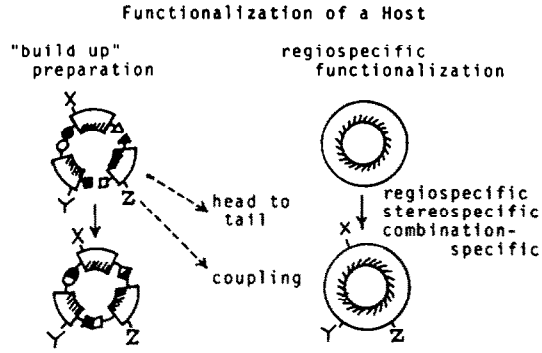


Fig. 13. Functionalization of a host.

(b) *General principle of guest design.* For certain host molecule, all possible recognition elements are taken and the structure of a hypothetical guest molecule is drawn in order to provide the "best fit" interaction. However, different from host design, satisfactory "screening" is possible in this case either via force field computation or via empirical investigation using a CPK molecular model, since recognition interaction is rather limited into small area and a number of possible structures (orientation, conformation) is not too large.

(c) *Multiple recognition.*[18] Host design or guest design described above is perfect or very satisfactory, at least, but requires laborious synthesis. In order to avoid the experimental work, it is convenient and practical to make simplified molecular designs. Thus one may take only a very limited number of the *recognition elements* (say, two or three) which seem most important among many participating elements. Prostaglandin $F_{2\alpha}$, for example, is recognized by its specific receptor mostly at 2 very important sites together with 2 moderately important sites and 2 additional weak recognition sites as shown in Fig. 14.[19] Therefore, a hypothetical artificial receptor having only specific recognition sites for CO_2H and OH groups at appropriate locations (double recognition model), prostaglandin $F_{2\alpha}$ may be bound to the artificial receptor specifically and strongly ($K_a \approx 2 \times 10^4$). Under ideal conditions, highly selective and reasonably strong binding of PG-$F_{2\alpha}$ may be achieved by using such a receptor as schematically shown in Fig. 15. Similarly, triple recognition models may be prepared as still convenient yet more sophisticated artificial receptors.

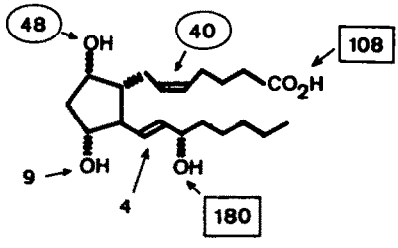


Fig. 14. Reduction of Ka for $PGF_{2\alpha}$·receptor complex via chemical modification.

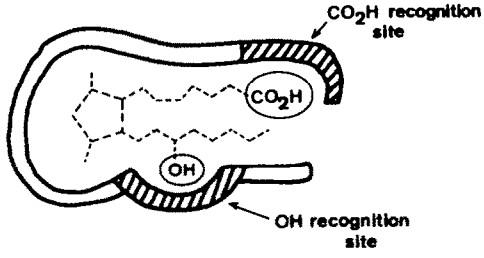


Fig. 15. Hypothetical artificial receptor for PG-$F_{2\alpha}$ via double recognition.

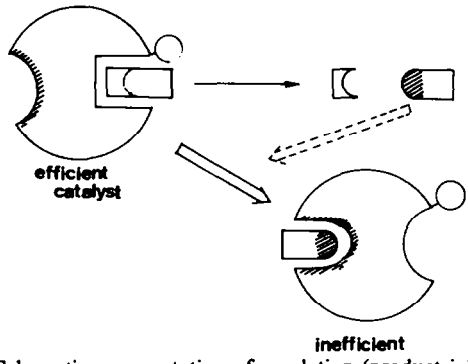


Fig. 16. Schematic representation of regulation (product inhibition).

(d) *Regulation and allosterism.* If enzyme catalysis is inhibited by the reaction product, the reaction rate becomes slow on accumulation of the product. Similarly, if enzyme catalysis is enhanced by the starting material (acting as an activator), the reaction rate becomes fast on excess loading of the starting material. In this way, reaction rate is regulated reasonably.

This type of regulation may be designed (see Fig. 15), although no successful example is available at present. More sophisticated regulation may be carried out by DNA mediation. Allosteric control of the enzyme activity is another important mechanism of regulation in nature. Two or more remote reaction sites of the same local structure are interacting with each other to control their reactivities. A reaction taking place at one site strongly mediates reactivity of the second (third, etc) site and a sigmoid-type reactivity profile is obtained as seen in haemoglobin-O_2 binding (see Fig. 17). No successful design of artificial allosteric enzymes is reported.

Fig. 17. Schematic representation of allosteric control.

EXAMPLES OF ARTIFICIAL ENZYMES

Artificial enzymes mimicking elemental nonmetal enzyme reactions

As discussed earlier, rather simple functions of native enzymes may be "mimicked" through the following general strategy; (mimicking enzyme function) = (equivalent micro-environment) + (isofunctional substitution). In this equation, isofunctional substitution means that a set of functional groups most appropriately orienting in an enzyme active site is substituted by one (or at most two) functional group acting by itself very similarly to a set of functional groups of an enzyme. This isofunctional substitution may be achieved by adjusting pK_a, nucleophilicity, steric repulsion, oxidation-reduction potential, O_2 affinity, etc of the artificial enzyme to those of certain native enzyme to be mimicked. The adjustment of catalytically important properties is rather easily made by careful consideration or prediction of electron density, spin density, orbital level, ligand field, steric interaction or solvent participation.

(a) *Ester hydrolysis.*

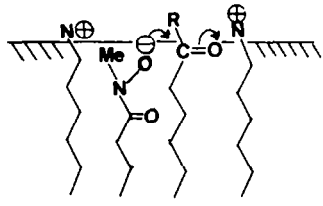
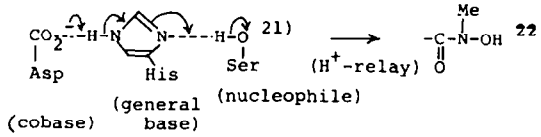


Fig. 18. Hydroxamate-CTAB micelle as a chymotrypsin model.

microenvironment: aprotic polar (due to strong electric field caused by N^+) at the reaction site and hydrophobic at the binding site.

Isofunctional substitution:

nucleophilicity, pK_a



Native enzyme	Artificial enzyme
lower pK_a, higher nucleophilicity of OH, most probably aprotic polar	much lower pK_a, much higher nucleophilicity than ordinary OH, aprotic polar
Electrostatic interaction: native enzyme	artificial enzyme
electrostatic stabilization of a tetrahedral anionic intermediate	electrostatic stabilization of a tetrahedral anionic intermediate

N; nucleophile,

The acylation rate constants observed for several substrates are shown in Table 5. Interestingly, a part of the enzyme action is satisfactorily mimicked by the use of these simple systems.

Deacylation activity is given again by the general base catalysis of His-57 in ester hydrolysis by chymotrypsin. This deacylation activity is successfully introduced into the hydroxamate micelle system through simple sophistication.

Compound 1 prepared for the purpose shows excellent deacylation activity as shown in Table 5.[23]

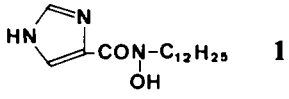


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Table 5. Acylation rate constants observed for hydroxamate micelles

micelle	substrate	$k_{cat} = k\Delta/K_{diss}$	ref.
$n\text{-}C_{11}H_{23}CONMeO^-$ $n\text{-}C_{16}H_{33}NMe_3Br$	$p\text{-}O_2N\phi OAc$	4830	20a
$n\text{-}C_{11}H_{23}CONMeO^-$ (alone)	$p\text{-}O_2N\phi OAc$	14.8	20a
$n\text{-}C_{12}H_{25}\text{-}N(OH)\text{-}CO\text{-}Imd$ $n\text{-}C_{16}H_{33}NMe_3Br$	$p\text{-}O_2N\phi OAc$	4380 deacylation 380 sec^{-1}	23
$n\text{-}C_{11}H_{23}CONMeO^-$ $n\text{-}C_{16}H_{33}NMe_3Br$	$p\text{-}O_2N\phi OCOCH_2Cl$	1.3×10^6	20b
chymotrypsin	$p\text{-}O_2N\phi OCOCH_2Cl$	1.0×10^6	

Substituted cyclodextrins as hydrolase models. Although micelle (reverse micelle or microemulsion) affords excellent microenvironment, it has only poor capacity of molecular shape or size recognition. Cyclodextrins, instead, only afford less hydrophobic environment in the cavity but it shows very high capacity of molecular (shape and size) recognition.[24] Thus cyclodextrins modified with appropriate functional group(s) are frequently used as artificial enzymes of excellent molecular recognition capacity.[25,26]

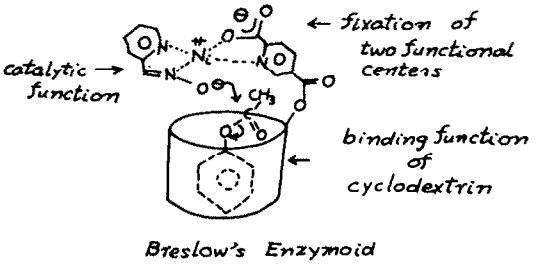


Fig. 19. Illustration of complex functionalization.

Microenvironment: Native chymotrypsin	Artificial enzyme (β-cyclodextrin)
Rather tight and rigid hydrophobic pocket for accommodation of aromatic side chains	Tight and rigid cyclodextrin cavity for accommodation of aromatic and aliphatic side chains
ca 10–12 × 5.5 × 4Å	*ca* 7.5 × 7.5 × 7Å

The molecular recognition capacity leads to the substrate shape and size specificities of native and artificial enzymes as shown in Table 6. Based on this concept, substrate (shape and size) specificity is readily controlled by simple consideration on the CPK molecular model. Typical examples are accessible control between p- and m- selective hydrolysis of esters by modified cyclodextrins (see Table 6).[27]

Precise molecular recognition is also important for the design of chiral recognition host. Two beautiful examples are shown in Fig. 20.

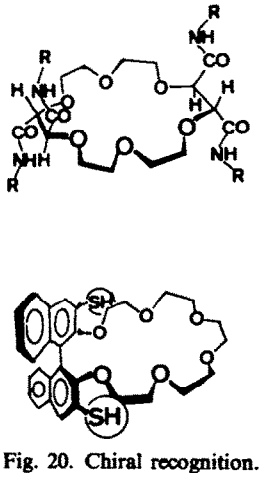
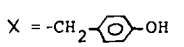
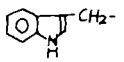
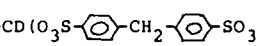


Fig. 20. Chiral recognition.

Table 6. Substrate specificity

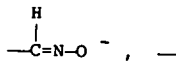
host	guest selectivity $(k_2 \cdot K_a)_{rel}$	
chymotrypsin substrate, $XCH(CO_2R)NHAC$	$1/K_m$	k_1
X = 	1400	193
	10000	51
$-CH_3$	1.6	1.3

p/m selectivity in hydrolysis of $O_2N\phi OA_c$ and related compound ; $\left(\frac{(k_2 K_a)_{CD}}{k_{H_2O}}\right)_p \Big/ \left(\frac{(k_2 K_a)_{CD}}{k_{H_2O}}\right)_m$

		ref.
β-cyclodextrin	p/m = 0.21	27a
β-CD-$OCH_2CON(OH)CH_2CH_2NMe_2$ (2ry)	p/m = 4.2	27b
β-CD-S-$CH_2CH_2CON(OH)Me$ (1ry)	=6.4	27c
β-CD  (1ry)	p/m = 8.3	28

Isofunctional substitution:

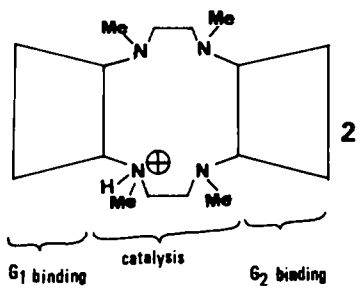
H^+ relay (native chymotrypsin) ⟶

 (low pK_a, high nucleophilicity)

(b) *Amide (peptide) formation.* Approximation: two closely located binding sites for ternary complex formation:

$$H + G_1 + G_2 \rightleftarrows H \cdot G_1 \cdot G_2$$

Compound **2** having two closely located binding sites is designed as an artificial enzyme catalyzing amide formation from carboxylic acid ester and amine.



Isofunctional substitution for activation of substrates:

enzyme, $H_2NCHRCO\text{-}O\text{-}P_i \rightarrow H_2NCHRCO\text{-}O\text{-}(t\text{-}RNA)$

artificial enzyme, $H_2N\text{-}CHR\text{-}CO\text{-}O\text{-}R$.

Another important function to be involved in the amide formation is product release from the active site. Usually more hydrophilic products are readily released from the active site but more hydrophobic products are more strongly bound to the active site. This trend makes product release very difficult, leading to undesired product inhibition. This problem is solved by "induced disfit" in the artificial enzyme catalysis, where amide grouping is too short to accommodate two hydrophobic moieties appropriately into two hydrophobic cavities of β-CD. A considerable part of the hydrophobic surface of the product amide is to be exposed to water in the hydrophilic catalytic site. This makes the product binding less favorable than substrate binding. As a result, the product amide is driven out of the cavity by the substrate. Thus, isofunctional substitution for the product release is complex mechanism in native enzyme system leading to relatively rigid, moderately separated binding sites for induced disfit of the product.

(c) *Amino transfer*. Since the coenzyme, pyridoxamine itself converts ketocarboxylic acids to corresponding amino acids,[16] a simple way to prepare artificial aminotransferase is to combine pyridoxamine and certain recognition site with a covalent bond or any other bonding. The first and excellent example of the artificial aminotransferase is presented by Breslow by combining pyridoxamine as a catalytic site and β-cyclodextrin as a recognition site (see Fig. 21).[31] The results are summarized in Table 7, indicating that moderate chiral selectivity is also provided by the "recognition site".

(d) C–C *Bond formation*. Among many possibilities known for C–C bond formation, ionic reactions are predominant. Therefore, microenvironment plays an important role to determine the reactivity and/or selectivity of enzymatic C–C bond formation reactions. Prenyl-polymerase,[5] for instance, has a hydrophobic active site, where very selective carbonium-π interaction takes place as shown in Fig. 22. This very strong π-participation is possible only in an aprotic environment since π-bond is not so strong for C^+ solvation in usual protic solvents (water, methanol, etc). At the same time deprotonation of the intermediate C^+-π complex is important, especially in a poorly solvating[32,33] aprotic solvent, in order to stabilize the transition state. This prenylpolymerase type activity is well prepared artificially by the use of parent β-cyclodextrin in vitamin K (analogs) synthesis.

Approximation: enzyme, E-G_1-G_2 ternary complex; artificial enzyme, H-G_1-G_2 ternary complex.

Microenvironment: enzyme, aprotic (hydrophobic) site; artificial enzyme, aprotic cavity.

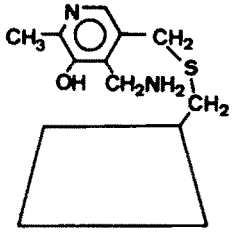


Fig. 21. Artificial aminotransferase.

Table 7. Aminotransfer reaction

R in $RCOCO_2H$ ⟶ $RCHNH_2CO_2H$
CH_3, $C_6H_5CH_2$, indolyl-CH_2,
$(O_2N)_2$indolyl-CH_2, $(O_2N)_2C_6H_3\text{-}C_6H_4CH_2$

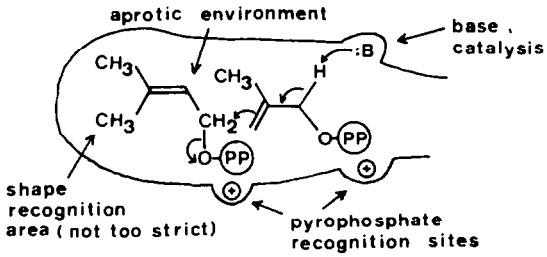


Fig. 22. Assumed mechanism of prenyl polymerization.

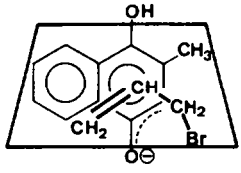


Fig. 23. Artificial vitamin-K synthetase.

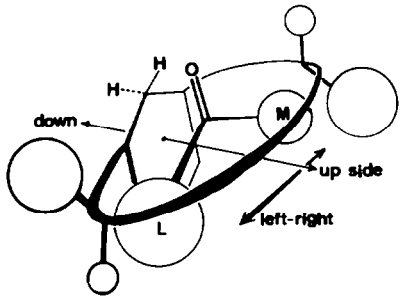
Table 8. Substrate specificity and receptor specificity exhibited by artificial receptor 9

	K_a (M^{-1})		
host guest	β-CD	β-CD-diamine·Zn	enhancement caused by Zn
adamantan-2-on-1-carboxylate	830	280000	330
adamantan-1-carboxylate	230	5300	23
cyclohexylamine	45	210	4.7
p-nitrophenolate	480	1200	2.5

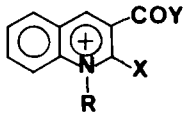
It is known from many X-ray crystallographic studies that any water molecule is not bound to the cavity of cyclodextrin-guest complexes.[34]

Isofunctional substitution: enzyme, acid-base; artificial enzyme, ArO^- + hydrogen bonding.

(e) *Dehydrogenase.* Chiral recognition: enzyme, (left hand-right hand side recognition) + (up side-down side recognition); artificial enzyme, (left hand-right hand side recognition) + (up side-down side recognition).



Isofunctional substitution: enzyme, NAD or NADP as a coenzyme artificial enzyme, quinolinium, 3, as an artificial coenzyme.



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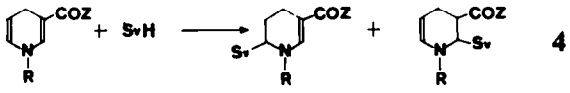
Although many NADH models are known for the fast and chiral reduction of carbonyl compounds, in a usual aprotic solvent,[36] they easily give solvent addition products, 4 and are not suitable catalysts in protic solvents. Quinolinium salts 3 or their reduced forms dihydroquinolines



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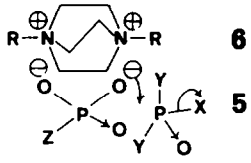
are much more resistant to the solvent addition and suitable for the mediated reduction of carbonyl compounds.[37] However, their reactivities are considerably lower than the coenzyme-enzyme system, where certain assisting groups must be important.[38]

Additional isofunctional substitution: enzyme, uncertain assisting group; artificial enzyme, $-CH_2-CH_2XH$ on amide N.[38]

(f) P–O–P *Bond formation.* P–O–P bond formation and breakdown is the most important chemical reaction involved in biological energy conversion or storage. However the detailed mechanism is not clear in this stage, though it is almost certain that there is more than one mechanism possible in native systems.

Isofunctional substitution and microenvironment: enzyme, uncertain $P-O^-$ activation; artificial enzyme, (hydrophobic) + (electrostatic).[39]



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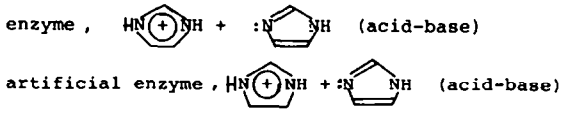
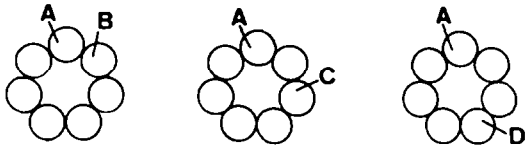
(g) P–O–P *Bond cleavage.* Isofunctional substitution:



Regiospecific ring opening of a cyclic pyrophosphate (a key intermediate involved in ribonucleic acid cleavage) may be resulted from relatively rigid "E" type orientation of imidazolium-imidazole pair. The requirement of "E" type orientation is fulfilled by the use of A, D-disubstitution on β-cyclodextrin[25] as shown in Fig. 24.[40] Similarly, artificial enzymes having other type orientations ("Z" and "R" types) may be prepared.[41]



A,B: "Z"-type orientation

A,C: "R"(rectangular) type orientation

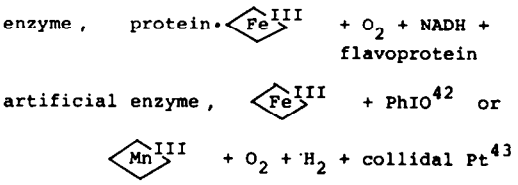
A,D: "E"-type orientation

Fig. 24. Regiospecific disubstituted β-cyclodextrins as artificial enzymes.

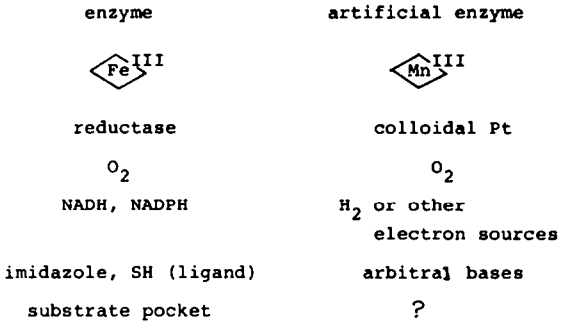
Artificial enzymes mimicking elemental metalloenzyme reactions

Metalloenzyme activity is mostly resulted from that of the central metal complex and the protein part (apoenzyme) affords certain unique environment as well as liqand(s) modifying the activity of the central metal complex.

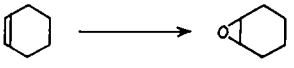
(a) *Cytochrome* P-450. Isofunctional substitution:



In the latter artificial enzyme system, isofunctional substitution may be further divided into following elemental substitutions,[43]



Olefins are very efficiently epoxidized with this artificial P-450 system;[43]



epoxide formed, mol/olefin consumed, mol; *ca* 100%, epoxide, mol/TPP·Mn used, mol; 6500%, epoxide, mol/Pt used, atom eqv; 29400%.

Other olefins converted to epoxides are 1-hexene, trans-2-octene, cis-2-octent, methylene-cyclohexane, 1-methylcyclohexene, 1, 2-dimethylcyclohexene.

(b) *Myoglobin* O_2 *binding*. Langmuir type O_2 binding is an example.

Isofunctional substitution: O_2 affinity, Fe^{II}·P for O_2 affinity (enzyme) leads to Fe^{II}·P (artificial enzyme).

Stabilization of O_2 adduct: O_2 pocket (enzyme)→picket fence for steric hindrance to haem-haem approach, preventing intermolecular electron transfer.[44,45]

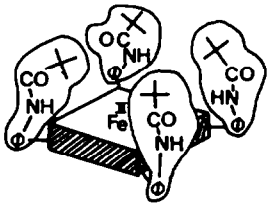
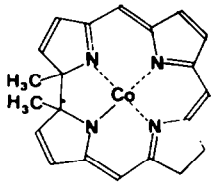


Fig. 25. Picket fence porphyrin.

(c) *Vitamin* B_{12} *activity.* Co·BCHC, 7, is used as a B_{12} model.



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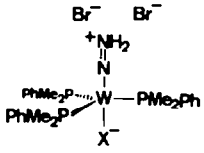
Isofunctional substitution: oxidation-reduction potential, vitamin B_{12} (corrin, ring size and conjugation)→BHDC. Co–C bond formation; enzyme, $BrCH_2C(COSEt)(CH_3)(CO_2Et) + NaBH_4 + L \cdot Co \rightarrow L \cdot Co\text{-}CH_2\text{-}C(CO_2Et)(CH_3)(COSEt)$.

Artificial enzyme; $L \cdot Co^I + RHal \rightarrow L \cdot Co\text{-}R$.[46]

(d) *Nitrogenase.* Nitrogenase has two important functions—namely, N_2 affinity and efficient electron supply. In order to reduce such a stable molecule as N_2, activation of N_2 by electron withdrawal as well as stabilization of a transition state (most likely negatively charged N_2 bound to the central metal) probably through protonation are necessary.



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Isofunctional substitution: N_2 affinity; enzyme, Mo-S cluster; artificial enzyme, W or V complex;[47] N_2 activation; enzyme, protonations? artificial enzyme, H_2SO_4 protonation. Electron supply; enzyme, Fe–S cluster, artificial enzyme, separate reduction.

Artificial receptors, carriers and channels

In addition to enzymes there are various kinds of proteins playing important roles in complicated biological phenomena. Among these, specific receptor or carrier function has been attempted to reproduce by the use of entirely artificial molecules and very recently, channel function also attracts attention of chemists. Interestingly, however, structure-function relationship is not yet clear for these native functional proteins. Some examples of the early trials based on purely chemical concept are shown below.

(a) *Chiral amino acid receptors or carriers.* As shown in Fig. 20(b), an artificial specific receptor is known, which has following functions very much similar to those of certain native specific carrier. Multiple recognition shown in Fig. 26 gives specificity toward nature of functional group and chiral specificity.

When the following functions are further added to an artificial receptor, one may prepare a specific carrier for amino acid. Hydrophobicity for ready incorporation into and fast transport across the lipid membrane, increased hydrophobicity on binding of an amino acid.

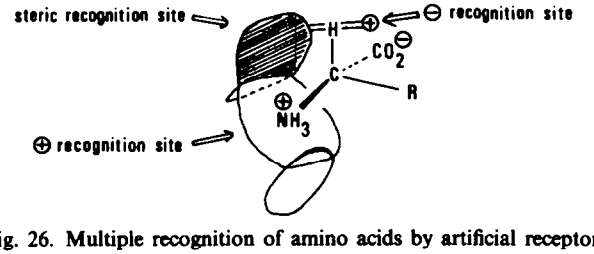


Fig. 26. Multiple recognition of amino acids by artificial receptors.

By applying necessary potential gradient, the artificial carrier transports amino acid across the artificial membrane.[48]

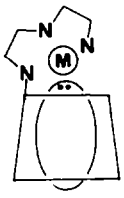
(b) *Specific receptors for hydrophobic anions*

Receptor function of metalloprotein may be expressed as

apohost + metal ion → holohost

hydrophobic recognition		hydrophobic recognition
+	coordination recognition	+
metallophiles		coordination recognition

Thus, an artificial receptor for double recognition is prepared, as exemplified by metal cap cyclodextrin, 9.[49] This receptor binds adamantan-2-one-1-carboxylate very strongly and exhibits a sharp spectrum of substrate specificity as shown in Table 8. From the Table one may notice the



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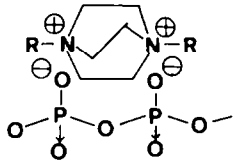
remarkable difference (up to 10^3) between apohost binding and holohost binding. This is a clear indication of *receptor specificity*.[49]

(c) *Pyrophosphate receptor.* Diammonium ion **6** has two cationic charges at fixed separation which is approximately equal to the distance between two anionic charges of two pyrophosphate oxygen atoms in the "Z" conformation (Fig. 27). As a consequence, **6** is a very specific and strong artificial receptor for pyrophosphates. As shown in Table 9, discriminative binding of pyro-



6

Fig. 27. DABCO Diammonium–pyrophosphate interaction.

Table 9. Relative equilibrium constants for extraction of nucleotides from water to $CHCL_3$-6

	pH 3.0	5.0	8.0
AMP	1	31	350
ADP[a]	45	610	2500
ATP[a]	7500	32000	111000
GMP	1	44	1600
GDP	280	670	8200
GTP	1100	8200	8200

phosphates against monophosphates is evident and moreover, triphosphates are more strongly bound than diphosphates by some reason which is not yet fully elucidated.

Since the present receptor molecule is highly hydrophobic (negligible solubility in water but reasonable solubility in usual monpolar solvents), 6 is also useful as a specific carrier molecule transporting pyrophosphates across the artificial membrane by applying appropriate chemical potential. A typical example of the pyrophosphate transport is shown in Fig. 28.

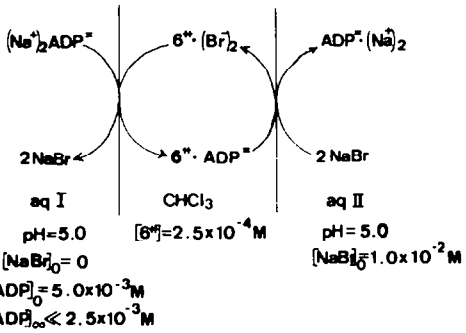
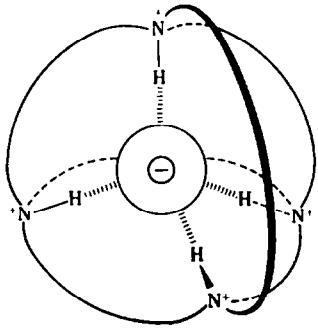


Fig. 28. Specific and active transport of pyrophosphates across artificial membrane.

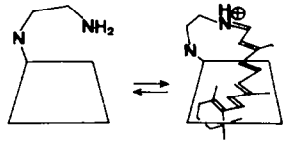
(d) *Spheric anion receptor.* Spheric anions are specifically recognized by the artificial host having positive charges which take the suitable positions surrounding the guest anion. Macrocyclic polyammoniums such as **10** are known to be strong and specific receptors.

Ammonium host having larger cavities bind larger anion selectively, as shown for a specific receptor for phosphate anion.[51]



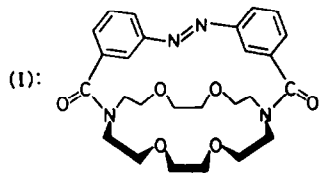
10

(e) *Retinal receptor.* Hydrophobic guest molecules are easily bound to an artificial receptor having a hydrophobic binding site, where the selectivity is simply related to the hydrophobicity of the guest and is not sensitive to the nature of functional groups. For the preparation of the artificial host molecule which is specific to hydrophobic guests bearing certain functional group, another recognition element for the functional group should be introduced. β-Cyclodextrin bearing an amino group **11** is an excellent receptor for retinal. Interestingly, the Schiff base formed from retinal and the receptor **11** has an absorption maximum at 497 nm, practically the same as native rhodopsin (bovine rhodopsin, [cis] 498 nm, bovine lumirhodopsin, [trans] 497 nm).



11

(f) *Photosensitive receptor.* Rhodopsin or bacteriorhodopsin generates potential gradient across the membrane when it is irradiated. Although the detailed mechanism is still uncertain, some attempts to mimic the response function via entirely different mechanisms were made. One of the interesting examples of this artificial photosensitive receptor **12** was prepared recently, exhibiting photoresponding Na^+ or K^+ release activity.[53]

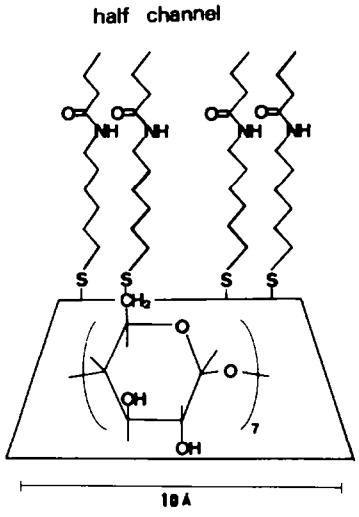


12

(g) *Artificial ion channel.* Since ion transport across the membrane is rather slow step, any artificial photosensitive receptor generates potential gradient much more slowly than the native rhodopsin system. Very rapid ion flow may be observed through very efficient ion channel. Thus, recently, preparation of artificial channels attracts some attention, although structure-function correlationship for native channel proteins has not yet been clarified.

Artificial channel must have following properties or characteristics: a part of channel must be hydrophobic to be incorporated into hydrophobic core of membrane; and a part of channel must be hydrophilic to be alligned along membrane surface.

Channel must have several ionophores inside the molecule at certain distances. A first artificial Co(II) channel **13** was prepared through which Co(II) is transported *ca* 30 times faster than though unfunctionalized membrane.[54]

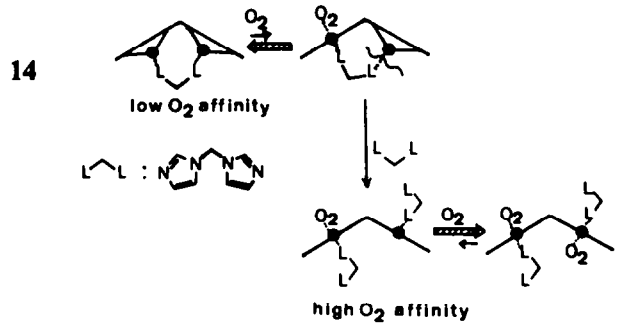


13

Artificial systems having complex and sophisticated functions

As discussed in the previous three sections 1–3, a wide variety of artificial enzymes are available at present. In this section, construction of complex and sophisticated functions by effective combination of artificial enzymes is discussed very briefly.

(a) *Artificial O_2 carrier.* Haemoglobin has very low O_2 affinity at low O_2 pressure but has normal (compared with myoglobin) O_2 affinity at high O_2 pressure, transferring O_2 effectively to myoglobin at low O_2 pressure tissue. This interesting O_2 carrier property is readily mimicked by the use of the artificial two-site cooperative molecule, 14.[55]



(b) *Artificial photosynthesis system.* Important events involved in bacteria-type photosynthesis may be effective charge separation on irradiation, effective electron transport, and effective dark reactions at both of oxidation and reduction end.

An artificial bacteria-type photosynthesis system was prepared by combination of the artificial molecules bearing individual function (see Fig. 29).[56]

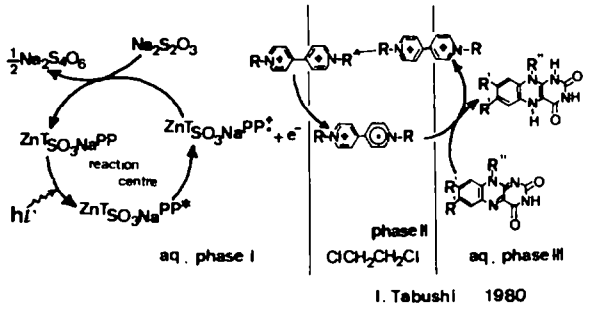


Fig. 29. Artificial bacteria-type photosynthesis system.

(c) *Artificial metabolizing system.* Artificial liposome modified with cyt-c_3 or alkylviologen on the membrane as an electron channel, and $K_3Fe(CN)_6$ in the interior as an electron acceptor can "metabolize" H_2 in the presence of colloidal Pt as an artificial hydrogenase, generating pH gradient as large as 4.0.[57]

CONCLUSION

Precise information on enzyme structure based on X-ray crystallographic studies and systematic understanding of reaction mechanism of catalysis allow us to make reasonable molecular design of a variety of artificial enzymes.

These artificial enzymes may be useful not only for acceleration, regulation or control of chemical reactions but also useful for more sophisticated purposes such as mediated transport or artificial cells.

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