Artificial Enzymes

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Contents

I. Introduction

All living things in nature maintain their internal metabolic balances quite well when they are in healthy conditions. In other words, metabolic materials are in equilibria in each living creature primarily in a collaboration of biological catalyses by numerous enzymes. Enzymes are sophisticated proteins having catalytic groups and often require specific cofactors or coenzymes for catalytic performance. If we look at enzymic functions from physicochemical viewpoints rather than biological ones, catalytically active amino acid residues of enzyme proteins as well as coenzyme factors are buried in hydrophobic and water-lacking reaction sites furnished by enzyme proteins and well separated from the bulk aqueous phase needed to attain thermodynamic stabilities.

In consideration of such physicochemical roles of enzyme proteins, we are allowed to use man-made materials for construction of artificial enzymes that are capable of simulating catalytic functions demonstrated by enzyme proteins. Two types of such artificial enzymes or apoenzymes, macrocyclic compounds and molecular assemblies, are cited in this article as those which can provide specific microenvironments for substrate-binding and subsequent catalysis in aqueous media. Those microenvironmental properties are primarily due to hydrophobic effects generated by the macrocyclic internal cavity and the internal domain of molecular assemblies in aqueous media, and other noncovalent intermolecular interactions, such as electrostatic, charge-transfer, and hydrogen-bonding modes, between a substrate and an apoenzyme model are greatly enhanced in such microenvironments.

In this review article, primarily we summarize recent studies on functional simulation of holoenzymes requiring coenzyme factors, such as vitamin B_1, B_2, B_6, and B_{12} as well as hemes and NADH. Most of those coenzymes are soluble in aqueous media when separated from the corresponding apoproteins and, consequently, cannot be readily incorporated into hydrophobic microenvironments provided by the artificial systems. Under such circumstances, the present authors have elaborated on modified cofactors, vitamin B_6 and B_{12} in particular, so that a hydrophobic property is donated to those coenzymes and the resulting modified ones are readily incorporated into the apoenzyme models. In addition, catalytic functions of hydrolases, which do not require a coenzyme factor, were also simulated by cyclophanes, cyclodextrins, micelles, and bilayer membranes in aqueous media, even though there are other mimicking reactions carried out in organic solvents.

Modified proteins which are derived by mutagenic treatments of natural enzymes are often called artificial enzymes. It must be emphasized here that artificial enzymes described in this article are not directly related to protein structures but capable of carrying out functional simulation of enzymic catalysis in the overall reaction schemes.

II. Artificial Apoenzymes

A. Macrocyclic Compounds

Naturally occurring apoenzymes are known to play important roles in specific molecular recognition toward substrates and furnish sophisticated reaction sites for enzymic catalysis. Such characteristic features of supramolecules are generated essentially through the formation of specific enzyme−substrate complexes as key intermediates. In order to simulate such functions of naturally occurring supramolecules, macrocyclic compounds exhibiting guest-inclusion capability, such as cyclodextrins[1,2] and cyclophanes,[3−7] have been developed as enzyme mimics.

Cyclodextrins are cyclic glucose oligomers with cylindrical shapes having the primary hydroxyl groups at the more restricted rim of the cylinder. The three most common cyclodextrins are α-, β-, and γ-species,

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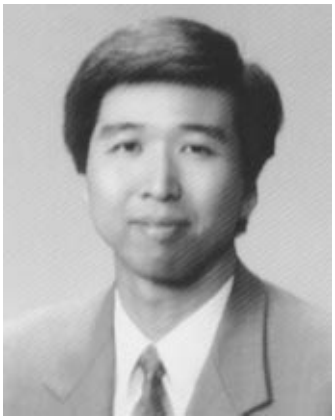
Yukito Murakami, born in 1931 in Yamaguchi Prefecture, Japan, completed his undergraduate study and graduate study for his Master's degree at Kyushu University in 1954 and 1956, respectively. He subsequently moved to the United States for his advanced graduate study and earned his Ph.D. degree in 1959 under the supervision of Professor Arthur E. Martell at Clark University. He was appointed Associate Professor in the Department of Organic Synthesis (now Department of Chemical Science and Technology), Faculty of Engineering at Kyushu University in 1959, and promoted to Professor in 1969. He held a joint appointment at the Institute for Fundamental Research in Organic Chemistry of the same university for two years (1993–1995). He became Professor Emeritus of Kyushu University in 1995. He served as Chairman of the Second International Symposium on Bioorganic Chemistry held in Fukuoka, Japan, in 1993, and also as President of the Division of the Biofunctional Chemistry of the Chemical Society of Japan (1987–1989). His research interests in recent years cover supramolecular organic and inorganic chemistry in connection with biochemical functions.



Jun-ichi Kikuchi, born in 1954 in Ehime Prefecture, Japan, completed his undergraduate study at Nagasaki University in 1977. He received his Master's and Ph.D. degrees from Kyushu University in 1979 and 1982, respectively, under the supervision of Professor Yukito Murakami. He subsequently became Research Associate in 1982 in the laboratory of Professor Junzo Sunamoto at Nagasaki University. In the following year, he moved to Kyushu University and spent five years as Research Associate in the laboratory of Professor Yukito Murakami. He was then appointed Associate Professor in the Department of Applied Chemistry, Faculty of Science and Engineering at Saga University. Since 1993 he has been Associate Professor in the Institute for Fundamental Research in Organic Chemistry at Kyushu University. His research interests are in the areas of supramolecular, biofunctional, and physical organic chemistry based on organic synthesis.

which are composed of six, seven, and eight glucopyranose units, respectively (Chart 1). α-, β-, and γ-cyclodextrins provide internal cavities with inner diameters of ∼4.5, ∼7.0, and ∼8.5 Å, respectively. The internal cavities are apolar relative to the bulk aqueous phase, so that cyclodextrins are capable of forming inclusion complexes with various hydrophobic guest molecules in aqueous media. During the period from 1970 through the beginning of the 1980s, numerous successful studies of natural and modified

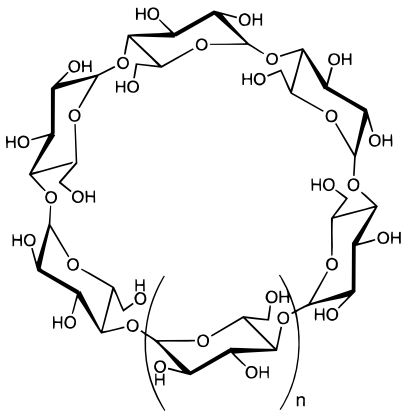
Yoshio Hisaeda, born in 1956 in Ehime Prefecture, Japan, completed his undergraduate study and graduate study for his Master's degree at Kyushu University in 1979 and 1981, respectively. He subsequently became Research Associate and received his Ph.D. degree in 1986 under the supervision of Professor Yukito Murakami at Kyushu University. He was appointed Associate Professor in the Department of Organic Synthesis (now Department of Chemical Science and Technology), Faculty of Engineering at Kyushu University in 1988 and promoted to Professor in 1995. He spent a year (1993–1994) with Professor Jonathan L. Sessler at the University of Texas at Austin as a Visiting Professor. His research interests are concerned with bioinorganic and bioorganic chemistry as well as electroorganic chemistry based on metal coordination chemistry and organic synthesis.

Osamu Hayashida, born in 1965 in Nagasaki, Japan, completed his undergraduate study and graduate study for his Master's degree at Kyushu University in 1988 and 1990, respectively. He subsequently became Research Associate and received his Ph.D. degree in 1994 under the supervision of Professor Yukito Murakami in the Department of Organic Synthesis (now Department of Chemical Science and Technology), Faculty of Engineering at Kyushu University. His research activity is focused on the development of macrocyclic compounds and supramolecular assemblies capable of performing functional simulation of naturally occurring receptors in aqueous media.

cyclodextrins as enzyme models were reported by the research groups of Bender,[1,2] Breslow,[8−10] Tabushi,[11] and others.[12,13] Modified cyclodextrins were developed by introducing catalytically active groups selectively into the primary or the secondary hydroxyl groups. For example, selective modification of one of the secondary hydroxyl groups was carried out by the reaction of *p*-toluenesulfonyl chloride with cyclodextrins in pH 11 buffer. On the other hand, a monotosylated cyclodextrin at the primary hydroxyl group was obtained when the reaction was carried out in pyridine. Various functionalized cyclodextrins bearing a coenzyme factor, such as a thiazolium salt,[10,14] isoalloxazine,[15−17] and pyridoxamine,[9,10,18,19] were prepared by the replacement of a tosyl group with other functional groups.

On the other hand, cyclophanes with a sizable internal cavity show the following characteristic

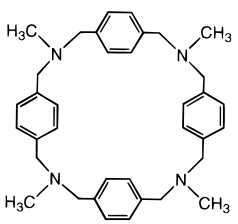
Chart 1



n = 1 α-CD
n = 2 β-CD
n = 3 γ-CD

properties. First, the most important driving force for molecular recognition in aqueous media is the hydrophobic interaction, and cyclophanes are typical hosts capable of providing such hydrophobic binding sites. Second, the macrocyclic cavity generally provides a stable binding site which is scarcely affected by external factors such as pH, temperature, and ionic strength. Third, a wide synthetic variation of the macrocycles can be achieved, relative to the limited range of structural modifications of semiartificial cyclodextrins, so that an appropriate recognition site with regard to size, shape, and microenvironmental property is provided for a target guest molecule. In addition, distinct molecular discrimination can become operative by the introduction of functional groups into appropriate sites of cyclophanes giving additional noncovalent interactions, such as electrostatic, hydrogen-bonding, charge-transfer, and metal-coordination interactions.

The rigid 2,11,20,29-tetraaza[3.3.3.3]paracyclophane ring has been frequently used as a fundamental molecular skeleton for functionalized macrocyclic hosts. An *N*,*N*′,*N*″,*N*‴-tetramethyl derivative (**1**) was first prepared by Yoshino et al.[20] and found to form 1:1 adducts with various organic solvent molecules as confirmed by X-ray crystallography.[21,22] A cyclophane bearing diphenylmethane moieties **2**, prepared by Koga et al.,[23] is particularly attractive because durene, a guest molecule, was accommodated in its cavity as confirmed by X-ray structural analysis.



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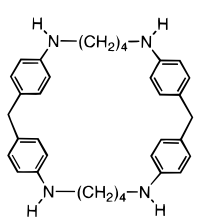

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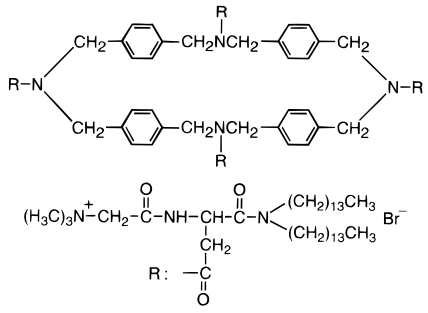
There are many enzymes which require a coenzyme factor, and enzyme proteins primarily provide specific microenvironments for reactions of coenzymes concerned. In such cases, a reaction proceeds through formation of a ternary complex composed of an apoenzyme, a coenzyme, and a substrate. In order to simulate the coenzyme-dependent enzymic reactions by employing cyclophanes capable of forming substrate complexes in a 1:1 molar ratio of host to guest, a coenzyme factor must be covalently introduced into the macrocycle. Various cyclophanes bearing coenzyme factors such as a thiazolium salt,[24] isoalloxazine,[25] and pyridoxamine[26] were developed.

The active sites of enzymes generally provide hydrophobic microenvironments where an incorporated substrate molecule and catalytic groups are free from hydration. Such environmental conditions are quite different from those provided by the bulk aqueous phase, and functional groups tend to exercise their catalytic performance at full efficiency due to tight interactions between the naked reacting species. Accordingly, it is desirable for water-soluble cyclophanes as inclusion catalysts to provide hydrophobic binding sites that can be well desolvated upon guest incorporation. It must be noted here that hydrophobicity and hydrophilicity are conflicting concepts. Therefore, hydrophobic sites must be reasonably separated from hydrophilic groups that are required for giving water solubility to cyclophanes, so that the hydrophobic efficiency remains maintained for guest incorporation. However, hydrophobic cavities of simple and monocyclic cyclophane derivatives are too small and shallow to allow effective desolvation. Thus, some modification of a macrocyclic skeleton is required to give a three-dimensionally extended hydrophobic space. Several macrocyclic hosts have been prepared along this line.

Murakami et al.[27,28] prepared octopus cyclophanes composed of a macrocyclic tetraaza[3.3.3.3]paracyclophane ring, four double alkyl chains, and L-aspartate residues interposed between them (**3** and **4**). These hosts exhibit the following unique func-



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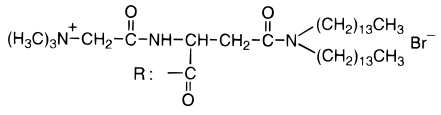

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tions with regard to molecular recognition in aqueous media. (1) The host molecules provide cavities that are deep and hydrophobic enough to incorporate hydrophobic guests of various bulkiness through an induced-fit mechanism originating from the flexible character of the alkyl branches. (2) Electrostatic and charge-transfer interactions in addition to hydrophobic interactions come into play in the host−guest complexation process, so that the binding of guest molecules is enhanced. (3) Formation of both 1:1 and 1:2 host−guest complexes is favored so long as electrostatic repulsion between guest molecules is not

operative in the hydrophobic cavity. (4) The hydrophobic cage provided by the octopus cyclophanes is highly apolar and acts to suppress the molecular motion of guests. Thus, octopus cyclophanes can be used as apoenzyme models for effective simulation of enzymatic functions.

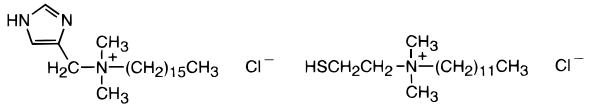
B. Molecular Assemblies

Macrocyclic compounds as stated above are enzyme models each being capable of providing a substrate binding cavity constituted with a rigid macrocyclic skeleton. It is quite important to indicate another type of enzyme models which provide a substrate binding site as a result of self-aggregation of functional elements through noncovalent intermolecular interactions in solution. Micelles are cited as traditional molecular assemblies and can be classified into two categories with regard to the nature of their cores, hydrophobic or hydrophilic. Reversed micelles formed with various surfactants in apolar solvents in the presence of small amounts of water molecules are the latter type of molecular assemblies and have been extensively studied on their characteristic features of cores.[29,30] However, simulation of the enzymatic functions especially related to formation of the enzyme−substrate complex has been carried out by utilizing the former type of molecular assemblies which furnish hydrophobic binding sites in aqueous media.

Aqueous micelles are typical and simple aggregates for such purpose and numerous applications as enzyme models have been accumulated prior to 1980.[29,31] In general, molecular structures characteristic of micelle-forming amphiphiles are composed of a polar head moiety, a long hydrophobic chain, and in some cases an additional functional group for effective catalysis. Cationic amphiphiles having an imidazolyl (**5**)[32] or a thiol moiety (**6**)[33] in the polar head are typical examples. While micelles provide hydrophobic reaction sites effective for acceleration of various enzyme-mimetic reactions, those aggregates are generally soft and difficult to achieve conformational fixation of substrates for regio- and stereospecific reactions. Peptide surfactants bearing amino acid residue(s), such as **7**, developed by Murakami et al. may improve such a weak aspect of micellar aggregates, since the amphiphiles tend to form tight aggregates relative to those formed with simple surfactants, originating from the intramicellar hydrogen-bonding interaction.[34−37]

It is well recognized that incorporation of substrate species into aqueous micelles proceeds through hydrophobic, electrostatic, and charge-transfer interactions. On the other hand, the hydrogen-bonding interaction between a substrate and a catalyst seems to be also effective for stereoselective catalysis in the hydrophobic micellar phases. Recently, Nowick et al. clearly demonstrated that a hydrogen-bonding interaction between two hydrophobic species, having a thymine and an adenine moiety individually as a pair, is effective in a micellar phase formed with dodecyl sulfate.[38]

Another type of well-known molecular aggregates in aqueous media is bilayer membranes. Preparation and characterization of liposomes formed with natural phospholipids have been well established.[39] However, utilization of the liposomes for simulation of enzymatic functions, especially in acid−base catalysis, seems to encounter difficulties due to their chemical and morphological instabilities. For example, Sunamoto et al. found that liposomal membranes formed with egg lecithin or dipalmitoylphosphatidylcholine were chemically damaged when a hydrophobic imidazole derivative **5** was incorporated.[40] Thus, bilayer membranes composed of synthetic amphiphiles are more favorable candidates for enzyme mimics.



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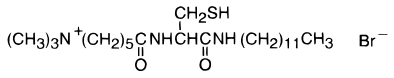

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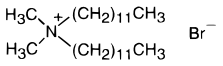
Since the first report on a totally synthetic bilayer membrane of didodecyldimethylammonium bromide (**8**) by Kunitake and Okahata in 1977,[41] numerous bilayer-forming lipids were designed and synthesized up to the present time.[29,42−48] While the synthetic lipids are readily capable of attaining high chemical stability by appropriate molecular design, morphological stabilization of the aggregates is more important and generally rather difficult.



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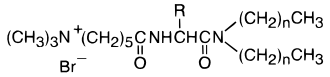
To attain such morphological stability of the synthetic bilayer membrane, versatile approaches have been adopted[49−52] to polymerize bilayer components and such studies have been reviewed by Ringsdorf et al.[53] On the other hand, the morphological stability of bilayer aggregates can be attained through noncovalent intermolecular interactions among lipid molecules. Murakami et al. developed peptide lipids **9** having α-amino acid residue(s) interposed between a polar head moiety and a hydrophobic double-chain segment through amide linkages.[46,54−56] Aggregate



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structures of the peptide lipids are markedly stabilized by intermolecular hydrogen-bonding interactions between amino acid residues that become effective in the hydrophobic membrane domain. The physicochemical stability of single-walled vesicles formed with the cationic peptide lipids is much larger than that of liposomal membranes formed with naturally occurring phospholipids under comparable conditions. In addition, molecular design of lipids to adopt various types of molecular assemblies, such as micelles, bilayer membranes, nonbilayer aggregates of inverted hexagonal, or inverted cubic phase, became feasible by employing the peptide lipids.[54−60]

In general, the bilayer membrane is superior to the aqueous micelle with respect to aggregate rigidity

and stereochemical orientation of component molecules, so that substrate specificity, catalytic activity, and reaction selectivity are more readily enhanced. In addition, phase states, gel and liquid crystal, and phase separation behavior among the component species can be utilized to control the catalytic reactions.

III. Constitution and Catalytic Functions of Artificial Enzymes

A. Vitamin B_1 Functions

Thiamine pyrophosphate (**10**, TPP) is a coenzyme for a number of important biological reactions in carbohydrate metabolism.[61,62] TPP-dependent enzymes catalyze (i) nonoxidative decarboxylation of α-keto acids (pyruvate decarboxylase), (ii) oxidative decarboxylation of α-keto acids (pyruvate oxidase), and (iii) interconversion of carbohydrates via ketol transfer (transketolase). The flavin-dependent pyruvate oxidase transforms pyruvate into acetyl phospahte via reaction sequence shown in Scheme 1.[63−66] The decarboxylation of pyruvate produces an

Scheme 1

Pyruvate + TPP–E–FAD_{ox} ⇌ Oxyethyl-TPP–E–FAD_{ox} + CO_2

Oxyethyl-TPP–E–FAD_{ox} ⇌ Acetyl-TPP–E–FAD_{red}

Acetyl-TPP–E–FAD_{red} + O_2 ⇌ Acetyl-TPP–E–FAD_{ox} + H_2O_2

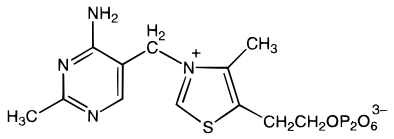
Acetyl-TPP–E–FAD_{ox} + P_i ⇌ Acetyl phosphate + TPP–E–FAD_{ox}

E : Apoenzyme

FAD : Flavin adenine dinucleotide

P_i : Inorganic phosphate

active aldehyde **11**, which is oxidized by the flavin coenzyme to give an acetylthiazolium intermediate. The intermediate is subject to phosphorolysis to afford acetyl phaosphate.



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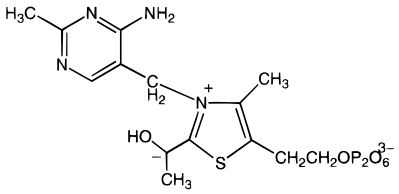

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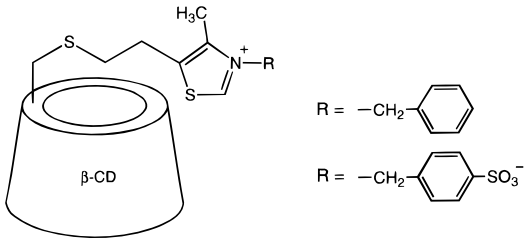
The catalytic function of TPP comes mainly from direct participation of the thiazolium ring, and simple thiazolium derivatives promote formation and cleavage of a carbon−carbon bond in the absence of the corresponding apoenzyme even though both reactivity and selectivity are significantly reduced.[67] TPP is bound noncovalently to apoenzymes in biological reactions, and the resulting holoenzymes demonstrate specific substrate recognition, marked rate enhancement, and appropriate selection of reaction pathways. In order to mimic catalytic performance by such holoenzymes, various thiazolium derivatives were prepared, which are covalently or noncovalently bound to artificial host molecules bearing guest-binding sites such as cyclodextrin derivatives,[10,14,68] micelles,[69−72] and cyclophanes.[73,74]

Breslow et al.[10,14,68] prepared mimicking models having a substrate-binding site provided by β- or γ-cyclodextrin to which a thiazolium moiety is covalently bound as a reaction site (**12** and **13**). The



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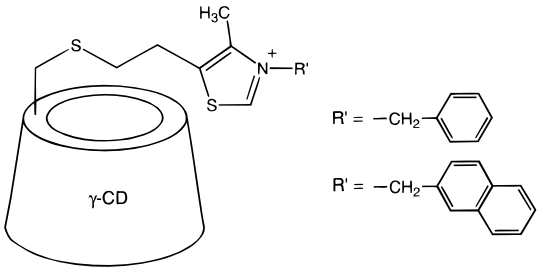

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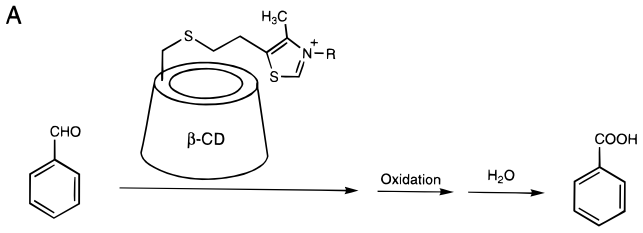
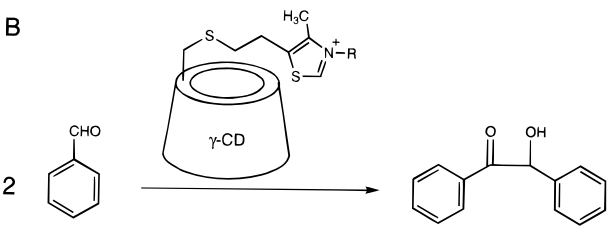
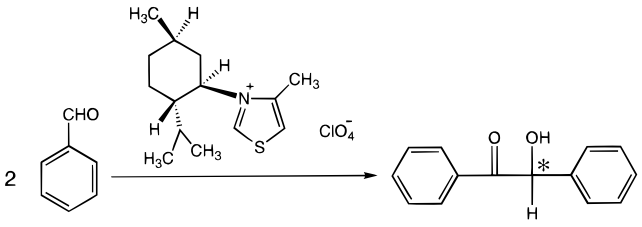
thiazolium derivatives bearing β-cyclodextrin group (**12**), were found to bind preferentially a benzaldehyde molecule and activate it for oxidation, as shown in Scheme 2A. For example, the modified β-cyclodextrin having a benzylthiazolium moiety showed a rate enhancement by approximately 2-fold for the oxidation reaction relative to the rate with a simple thiazolium salt bearing an *N*-benzyl group. However, the β-cyclodextrin cavity is too narrow to hold two benzaldehyde molecules, so that the benzoin condensation is inhibited when the substrate is incorporated into the β-cyclodextrin cavity. On the other hand, the larger cavity of γ-cyclodextrin (**13**) is capable of binding two benzaldehyde molecules simultaneously. The second-order rate constant for benzoin condensation mediated by **13** was about 7-fold greater than

Scheme 2



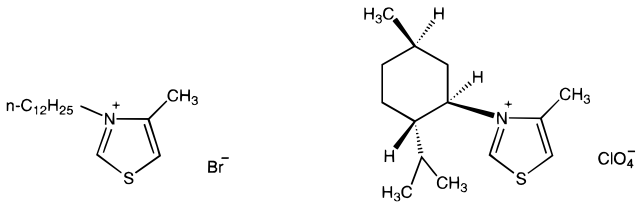


Scheme 3



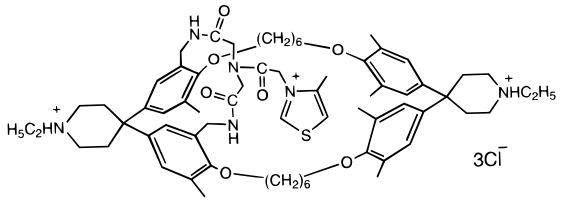
that by a simple thiazolium salt bearing an *N*-benzyl group, even though this is not a biomimetic reaction (Scheme 2B).

Tagaki et al. prepared a thiazolium salt bearing an alkyl chain such as *N*-dodecylthiazolium bromide (**14**) which forms micelles (CMC, 3.3×10^{-3} M in H_2O).[69,70] The H/D exchange rate at the 2-position of **14** was 16-fold faster than that of a nonmicelle-forming 3-methyl analog. The high acidity of the 2-H proton of thiazolium ring promoted an acyloin condensation of aldehydes, such as benzaldehyde and furfural, upon micelle formation. They also prepared an optically active thiazolium salt bearing a (1α,2β,5α)-5-methyl-2-(1-methylethyl)cyclohexyl group (**15**) and examined an asymmetric benzoin condensation in aqueous media, which resulted in formation of an enantiomer with optical purity of 35% (Scheme 3).



14 **15**

Diederich et al. studied benzoin condensation mediated by a thiazolium salt covalently linked to a cyclophane **16**.[73−75] The equilibrium in benzoin condensation was favorable for formation of benzoin in methanol, and **16** catalyzed benzoin condensation with turnover behavior more effectively than a nonmacrocyclic thiazolium derivative **17** as shown in Scheme 4A. Moreover, **16** catalyzed the reverse



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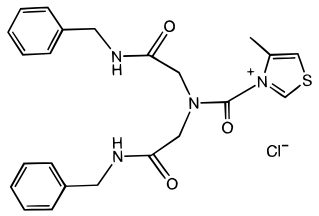

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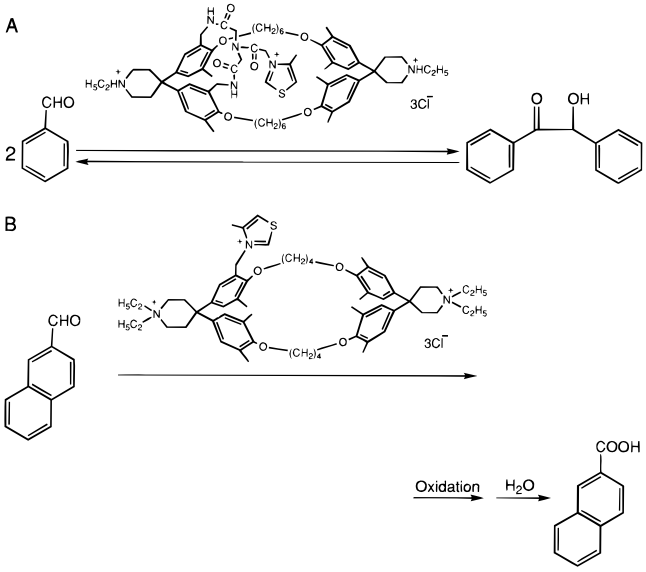
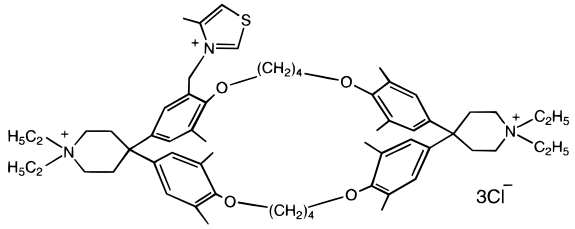
reaction to give benzaldehyde from benzoin in Me_2SO, even though the equilibrium is still in favor of benzoin formation. A micropolarity effect of the cavity of **16** was observed on the acidity of the 2-H

Scheme 4



proton of the thiazolium ring on the basis of H/D exchange experiments. They suggested that the rate enhancement comes from a micropolarity effect on pK_a (K_a stands for an acid dissociation constant) change for example, even though the reaction was not carried out in aqueous media.

They also synthesized another thiazolium cyclophane **18** as a catalyst for oxidation of aromatic aldehydes to the corresponding carboxylic acids in the presence of potassium ferricyanide in Me_2SO−aqueous phosphate buffer (60:40 v/v) (Scheme 4B). A saturation kinetics was observed for the reactions catalyzed by **18**; with regard to oxidation of 2-naphthaldehyde, $K_M = 5.4$ mM, $V_{max} = 7.5 \times 10^{-6}$ M s^{-1}, and $k_{cat} = 0.90$ min^{-1}. By contrast, the rate in the presence of nonmacrocyclic derivative **19** increased in a linear fashion as the substrate concentration increased. The apparent bimolecular rate constant, k_{cat}/K_M, for 2-naphthaldehyde oxidation mediated by **18** was 75-fold greater than the second-order rate constant catalyzed by **19**. The results clearly indicate that the substrate-binding ability of enzyme models is apparently important for rate acceleration.



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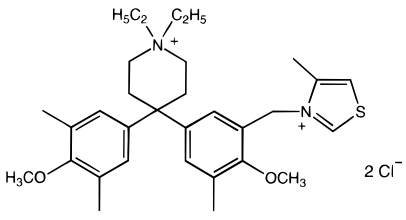
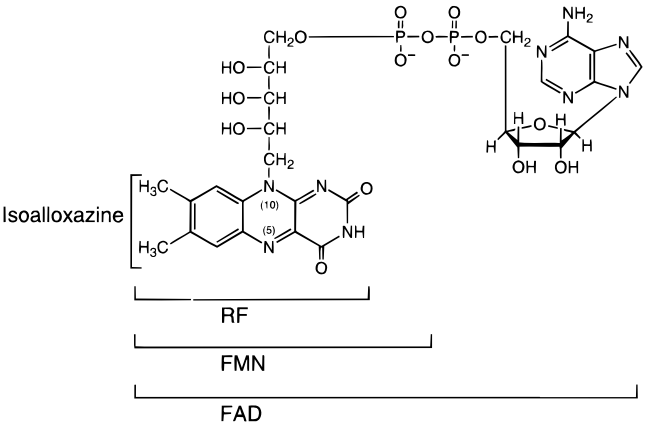


19

B. Vitamin B_2 Functions

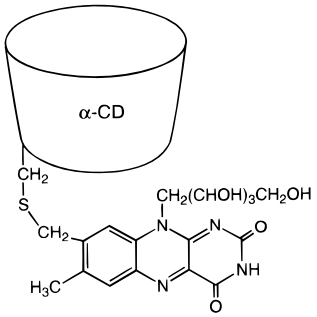
Flavoenzymes contain a coenzyme riboflavin (RF, Chart 2), often as flavin mononucleotide (FMN) or

Chart 2



flavin adenine dinucleotide (FAD), and catalyze various electron-transfer reactions via either single electron or simultaneous two-electron transfer mechanism.[76−79] In the process of catalyzing electron-transfer reactions, the flavin moiety itself is reduced upon accepting electrons from a substrate and reoxidized via electron transfer to another acceptor. RF is known to be tightly bound or, in some cases, even covalently attached to the active sites of apoenzymes.[79] An apoprotein around RF is responsible for substrate selectivity and kinetic and thermodynamic controls of the electron-transfer process. In order to simulate such functions of holoenzymes in aqueous media, various flavin derivatives, which are covalently bound to artificial guest-binding segments such as cyclodextrins[15,17,80,81] and cyclophanes,[25,82] were prepared.

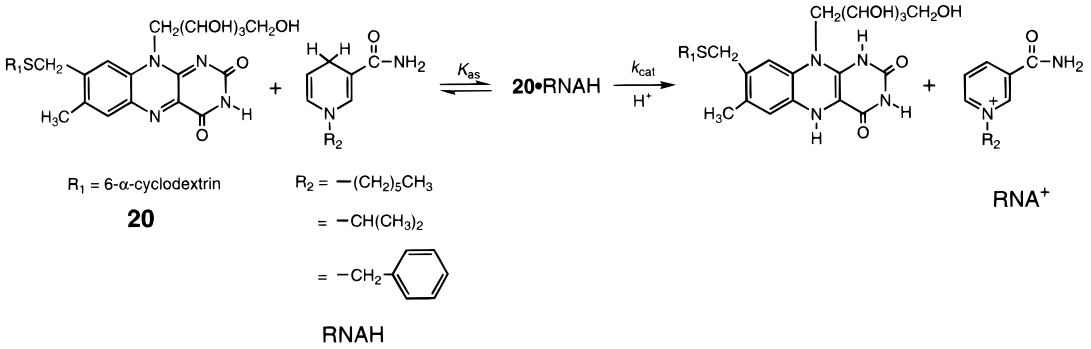
Tabushi et al. prepared a flavo-α-cyclodextrin in which the flavin segment is bound to the primary rim of α-cyclodextrin (**20**) and investigated its electron-transfer activity toward *N*-alkyldihydronicotinamides (RNAH) such as benzyl-, isopropyl-, and *n*-hexyl-NAH in an aqueous buffer, as shown in Scheme 5.[15] Mediator **20** was confirmed to bind RNAH with large association constants (K_{as}) of 1050, 260, and 2500 M^{-1} for benzyl-, isopropyl-, and *n*-hexyl-NAH, respectively, although smaller than that of an artificial flavoprotein, 8α-*S*-flavopapain,[82] with benzyl-NAH (K_{as} = 10300 M^{-1}). The most interesting aspect with regard to the efficiency of **20** is a large rate constant for the electron transfer. A rate constant for the electron-transfer reaction from benzyl-NAH to the flavin moiety of **20** was 90-fold larger than that for a combination of 8α-*S*-flavopapain with benzyl-NAH. The overall efficiencies of **20** in the dihydroflavin production, as evaluated by $k_{cat}K_{as}$, were 63, 94, and 1200 M^{-1} with benzyl-, isopropyl-, and *n*-hexyl-NAH, respectively; the first value being larger than that for 8α-*S*-flavopapain with benzyl-NAH by 56 times.



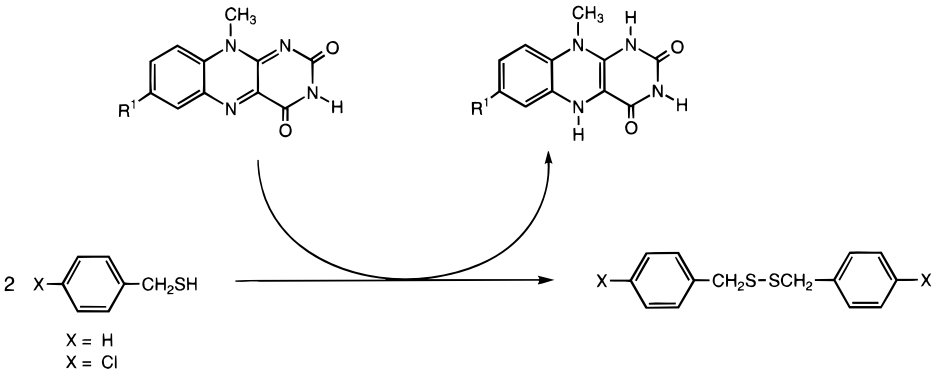
20

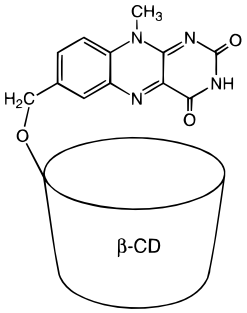
D'Souza et al. prepared flavoenzyme models in which a flavin segment is covalently attached to either the secondary rim (**21**) or the primary rim (**22**) of β-cyclodextrin and investigated their catalytic properties in oxidation of thiols such as (*p*-chlorophenyl)methanethiol and phenylmethanethiol (Scheme 6).[17,80] A saturation kinetics was observed for oxida-

Scheme 5



Scheme 6





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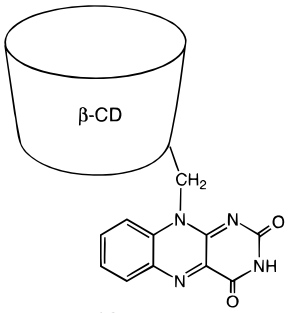


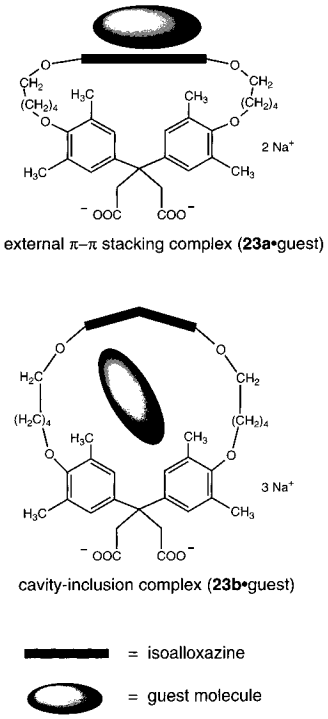
22

tion of the thiols by **21**, indicating that the reaction proceeds via formation of an artificial enzyme complex with the substrate. The apparent rate constants, k_{cat}/K_M, for oxidation of (*p*-chlorophenyl)-methanethiol and phenylmethanethiol mediated by **21** were 21 and 53 times greater, respectively, than the second-order rate constants catalyzed by RF alone in aqueous media. On the other hand, **22** exercised a second-order kinetics in the oxidation of (*p*-chlorophenyl)methanethiol in a manner similar to that observed for oxidation of the identical thiol by RF alone in aqueous media. A computer-aided molecular modeling study of the inclusion complexes revealed that a configuration that allows the thiol group to be oriented toward the secondary rim of cyclodextrin is more stable than an alternative one that allows the thiol group to be oriented toward the primary rim. With regard to the former configuration, the thiol group of the substrate is placed close to the flavin segment of **21**. On the other hand, the complex formation with **22** brings the thiol group apart from the catalytic group, and **22** is forced to react only with the unbound (*p*-chlorophenyl)methanethiol as a consequence.

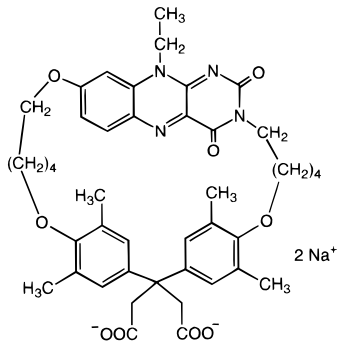
The isoalloxazine segment of the coenzyme riboflavin in the oxidized form assumes a planar geometry, whereas the dihydroisoalloxazine segment in the reduced state takes a butterfly shape bent around the axis connecting two nitrogens N-5 and N-10 of the central ring by ~30° angles.[83,84] The difference in shape of the isoalloxazine segment between the oxidized and reduced states is expected to affect the substrate recognition ability. Various artificial flavoenzymes bearing a binding site have been designed to investigate the geometric effect of the isoalloxazine segment, as caused by the oxidation state, on substrate binding.[25,81]

Seward and Diederich synthesized **23** by incorporating an isoalloxazine moiety into the macrocyclic skeleton as a model for an active site of flavoenzymes, and characterized the structural and electrochemical properties of **23** in its oxidized and reduced states.[25,81] In aqueous solution, **23a** was readily and quantitatively reduced to **23b** by sodium hydroborate. The oxidized host **23a** was regenerated quantitatively from **23b** by treatment with molecular oxygen in solution. ^{1}H NMR titration with **23a** and naphthalene guests, such as 6-hydroxy-2-naphthonitrile, showed that **23a** formed external $\pi-\pi$ stacking complexes with the guests that are stabilized by ΔG of 3−4 kcal mol^{-1}, as shown schematically in Scheme 7. On the other hand, the reduced host **23b** included the guests in its cavity. These cavity-inclusion

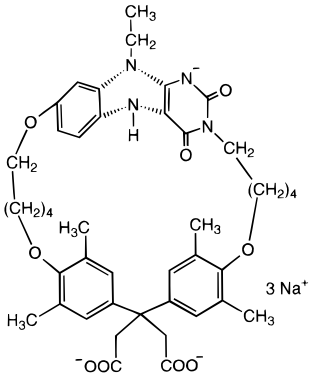
Scheme 7



complexes were confirmed to be similar in stability to the external $\pi-\pi$ stacking complexes formed by the oxidized host. However, catalytic properties of **23** remain to be investigated.



23a

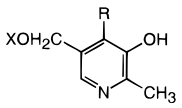


23b

C. Vitamin B_6 Functions

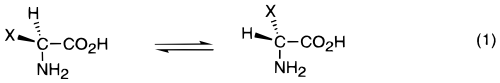
Natural enzymes which require a cofactor called pyridoxal 5′-phosphate (PLP), a representative spe-

Chart 3

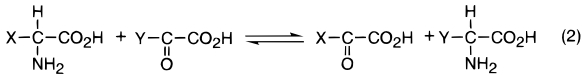


PLP : R = CHO, X = PO_3H_2
PL : R = CHO, X = H
PMP : R = CH_2NH_2, X = PO_3H_2
PM : R = CH_2NH_2, X = H

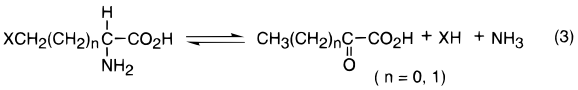
cies of the vitamin B_6 family (Chart 3), catalyze various reactions of α-amino acid substrates as follows (refer to eqs 1−6): (i) racemization of a chiral



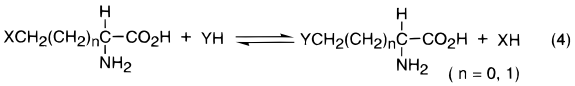
(1)



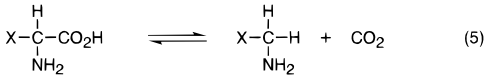
(2)



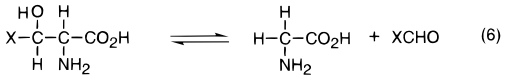
(3)



(4)



(5)



(6)

center at the α-carbon atom; (ii) transamination, the interconversion between an α-amino acid and an α-keto acid; (iii) elimination of an electronegative group at the β- or γ-position to give the corresponding α-keto acid and ammonia; (iv) replacement of an electronegative group at the β- or γ-position with another substituent; (v) decarboxylation at the α-carbon atom; (vi) aldolase-type reaction accompanied with a C−C bond cleavage at the α,β-position.

At the active site of a vitamin B_6-dependent enzyme, the aldehyde moiety of PLP forms the internal aldimine Schiff base with an ϵ-amino group of a lysine residue covalently involved in an apoprotein. Various noncovalent interactions also contribute to conformational fixation of the coenzyme at the catalytic site. An external aldimine Schiff base, derived by transimination of the internal aldimine with a substrate, is known to be a common intermediate for all the vitamin B_6-dependent enzymic reactions (Scheme 8). One of the three bonds around the α-carbon atom of an amino acid moiety in the intermediate then undergoes cleavage as effected by an electron sink effect of the conjugated pyridine segment. Racemization, transamination, β- (or γ-) elimination, and β- (or γ-) replacement reactions proceed through the C−H bond cleavage, whereas decarboxylation and dealdolation occur through the C−C bond scission.

Model studies on the vitamin B_6-dependent enzymes have a long history extended over a half century, originating from pioneering work by Snell and his co-workers, and metal ions coordinated to the intermediate Schiff base derived from an amino acid are capable of promoting these reactions nonenzymatically in the absence of an apoprotein.[85,86] However, simulation of remarkable catalytic functions performed by holoenzymes, such as drastic rate enhancement, high substrate specificity, and distinct reaction selectivity, cannot be attained without considering microenvironments provided by apoproteins.

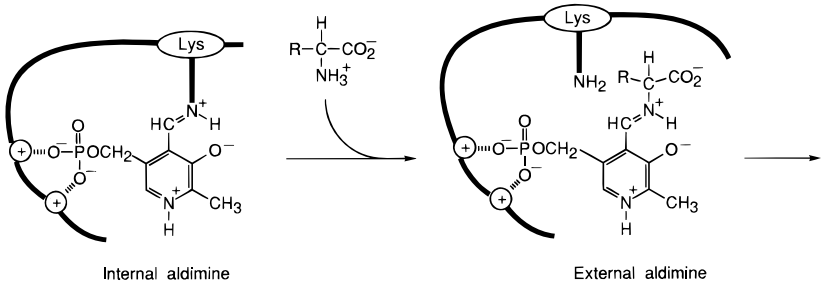
1. Transamination Reactions

The biological transamination of an α-amino acid with an α-keto acid, as catalyzed by the vitamin B_6-dependent aminotransferase, proceeds through a shuttle mechanism involving reversible isomerization between two pairs of aldimine Schiff bases [ASB(AA1) and ASB(AA2)] and the corresponding ketimine Schiff bases [KSB(KA1) and KSB(KA2)], as shown in Scheme 9.

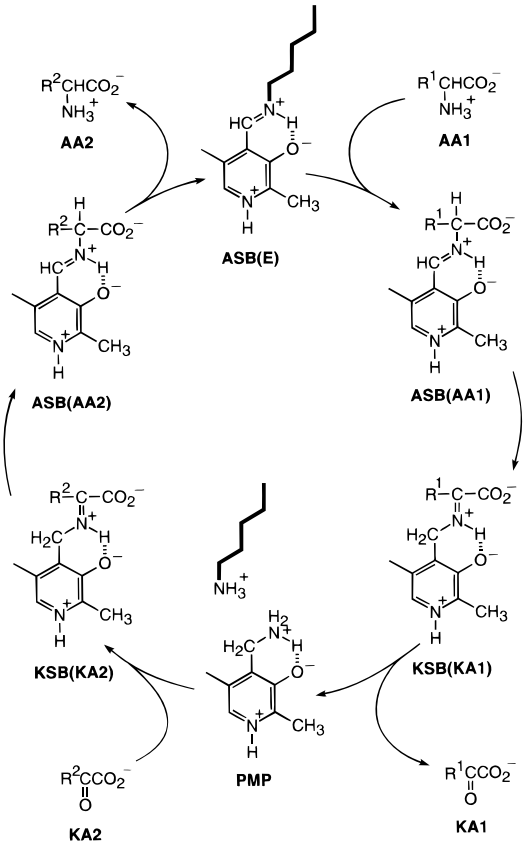
From a viewpoint of biomimetic chemistry, recent interest in relation to this reaction resides in stereoselective formation of α-amino acids from the corresponding α-keto acids. Thus, several approaches have been reported by employing chiral vitamin B_6 analogs in order to control the stereochemical course of the half-transamination reaction, the reaction between an α-keto acid and a pyridoxamine derivative to afford the corresponding α-amino acid and pyridoxal derivative, respectively.

It is well known that stereoselective interactions within metal complexes can be used to perform asymmetric synthesis. In this regard, Bernauer et al. examined the half-transamination reaction between pyridoxamine (PM) and α-keto acids in the

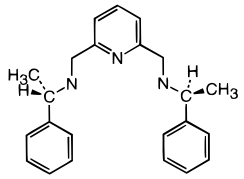
Scheme 8



Scheme 9



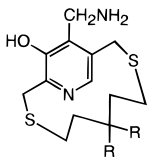
presence of a Cu(II) complex of C_2 symmetry with a terdentate ligand **24**.[87,88] In a weakly acidic solution,



24

optically active α-amino acids with (*R*)-configuration were formed preferentially, and the maximum enantiomeric excesses (ee) were 80, 54, 48, and 29% for phenylalanine, alanine, leucine, and valine, respectively. They proposed that the enantioselectivity comes from formation of an intermediate Cu(II)−ketimine complex formed with PM, an α-keto acid, and **24**. However, the relatively high ee values were only obtained at the initial reaction stage, and the ee values drastically decreased due to an undesirable racemization reaction occurred subsequently.

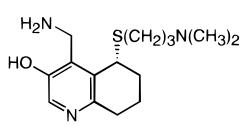
Kuzuhara et al. synthesized a chiral vitamin B_6 analog having a nonbranched ansa chain[89] between 2′- and 5′-positions (**25a**).[90,91] In the presence of Zn-



25a: R = H
25b: R = CH_3

(II) ions [a 1.3:1 molar ratio of Zn(II) to (*S*)-**25a**] in organic solvents such as methanol, *tert*-butyl alcohol, acetonitrile, and nitromethane at room temperature, they observed stereoselective half-transamination between (*S*)-**25** and phenylpyruvic acid. The reaction gave phenylalanine in a fairly good yield but in a low enantiomeric excess ranging from 6 to 26%, the sign of optical rotation being dependent on the solvent used. They also prepared another chiral pyridoxamine derivative bearing a branched ansa chain (**25b**),[92] and revealed that the enantioselective potency of **25b** overcomes that of **25a** in the half-transamination reaction with various α-keto acids.[93] When the molar ratio of Zn(II) to a chiral pyridoxamine analog was reduced to 0.5:1, the enantiomeric excess of the products remarkably increased particularly in methanol. Thus, D- and L-leucine were formed with an ee value as high as 95% by the reaction of the corresponding α-keto acid with (*S*)- and (*R*)-**25b**, respectively. By employing the above chiral pyridoxamine analog, it became possible to synthesize nonnatural α-amino acids with a high enantiomeric purity form the corresponding α-keto acids.[94,95] On the basis of kinetic analysis of the above-mentioned half-transamination reaction, Kuzuhara et al. once proposed a mechanism for the asymmetric induction via kinetically controlled stereoselective protonation to the carboanion attached to an octahedral Zn(II) chelate intermediate.[96,97] However, recently they touched upon some suspect about their own proposal.[98]

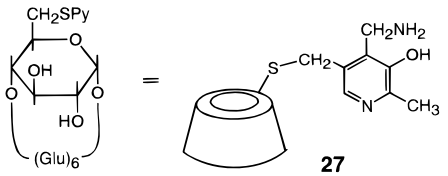
Breslow et al. also revealed that, on the basis of general-base catalysis, an asymmetric half-transamination reaction was effectively mediated by a bicyclic pyridoxamine derivative carrying an oriented catalytic side arm (**26**).[18,99] The reaction was carried out in methanol at 30 °C in the presence of Zn(II) ions. Rates for conversion of the ketimine Schiff base, formed with **26** and α-ketovaleric acid, indolepyruvic acid, or pyruvic acid, to the aldimine Schiff base were enhanced 20−30 times relative to those carried out in the presence of the corresponding pyridoxamine derivatives without the catalytic side arm. With α-ketovaleric acid, **26** underwent transamination to afford D-norvaline with 90% ee. A similar preference was seen in the formation of tryptophan and alanine from indolepyruvic acid and pyruvic acid, respectively. Racemization of the products scarcely took place in competition with their asymmetric synthesis. A compound, with a propylthio group at the same stereochemical position as the aminothiol side arm in **26**, produced a 1.5:1 excess of L-norvaline, in contrast to the large preference for D-amino acids with **26**. Thus, extremely preferential protonation seems to take place on the *si* face when the catalytic side arm is present as in compound **26**.



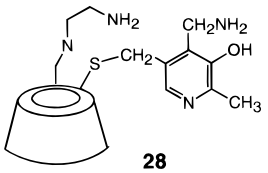
26

Several vitamin B_6-dependent holoenzyme models, capable of providing a substrate binding site and performing molecular recognition, have been developed. Breslow et al. prepared holoenzyme mimics by joining a vitamin B_6 unit to a *β*-cyclodextrin skeleton.[9,10,18,100−103] For example, a compound (**27**),

in which a pyridoxamine moiety is attached to the primary rim of β-cyclodextrin with an intervening thioether linkage, showed marked substrate specificity in the half-transamination reaction in 2.7 M phosphate buffer at pH 9.3 and 26 °C. A substrate binding ability was provided by the macrocyclic cavity, and **27** accelerated the reaction with indolepyruvic acid and phenylpyruvic acid by 12- and 15-fold, respectively, relative to those reactions of the same substrates with the simple pyridoxamine.[18] Tryptophan and phenylalanine formed by the reaction with **27** showed preferable formation of the L-enantiomers at L/D ratios of 2:1 and 5:1, respectively, since **27** provided an optically active hydrophobic cavity for those substrates. While a compound with a pyridoxamine moiety attached to the secondary rim of β-cyclodextrin was also effective in rate enhancement, this model showed a 1.8:1 D/L preference for the formation of D-enantiomer of tryptophan. On these grounds, Tabushi et al. developed A-(modified B_6)-B-[(ω-aminoethyl)amino]-β-cyclodextrin (**28**) as a potent artificial aminotransferase.[104] The



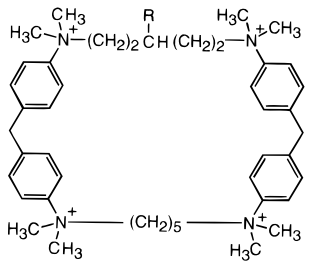
27

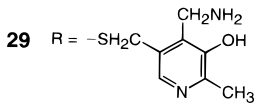


28

catalysis by **28** resulted in formation of aromatic L-α-amino acids, such as phenylalanine, tryptophan, and phenylglycine with high chiral selection (90−96% ee). In addition, the prototropy rate ratio observed with **28** against the simple pyridoxamine was ca. 2000 in the phenylalanine formation. However, the true catalytic cycle, exhibiting amino group transfer from an α-amino acid to an α-keto acid, has not been successful with such modified cyclodextrins.

As for cyclophanes having a sizable internal cavity, Breslow et al. prepared an aminotransferase mimic having a substrate-binding site composed of a tetraaza[1.7.1.7]paracyclophane ring and a covalently bound pyridoxamine moiety (**29**).[26] In 2.7 M phosphate buffer at pH 9.3 and 26 °C, **29** accelerated the





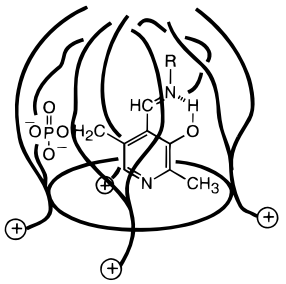
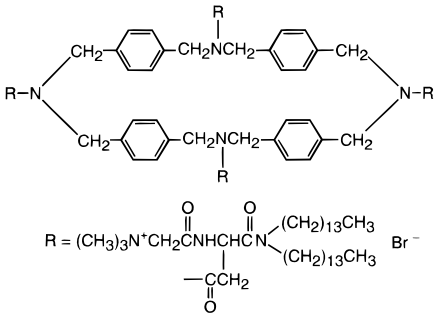


Figure 1. Schematic representation of the ternary complex composed of a substrate, PLP, and the octopus cyclophane as an apoenzyme model. (Reprinted from ref 27. Copyright 1989 Kluwer Academic Publishers.)

conversion of aromatic α-keto acids bearing good hydrophobic segments to the corresponding α-amino acids by more than 1 order of magnitude as compared with the rates for reactions catalyzed by pyridoxamine; the acceleration factors were 31 and 12 for phenylpyruvic acid and indolepyruvic acid, respectively, while the factor was 6 for α-ketovaleric acid. It must be noted here that all the above aminotransferase models were able to perform only the half-transamination reaction and the true catalytic cycle was not observed.

With an aim of synthesizing a novel artificial enzyme system, Murakami et al. used an octopus cyclophane (**30**) as a functional model of apoproteins and constituted an artificial holoenzyme by incorporating PLP noncovalently into the host cavity.[27] Alkylamines having various hydrophobic chains were employed as substrates in place of α-amino acids for evaluation of the hydrophobic effect on the Schiff base-forming equilibrium. The Schiff base formation constant was markedly dependent on the chain length of a substrate in the presence of **30**, indicating that the octopus cyclophane can be utilized as an effective apoenzyme model capable of forming a ternary complex (Figure 1).



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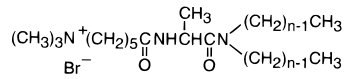
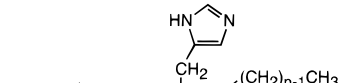
It is well known that the specific microenvironments provided by surfactant micelles in aqueous media are effective for simulation of catalytic functions in the hydrophobic active sites of enzymes. Kondo et al. reported that *N*-dodecylpyridoxal chloride forms a Schiff base with hydrophobic α-amino acids, such as tryptophan, phenylalanine, phenylglycine, and *S*-benzylcysteine, in the cationic micelle of hexadecyltrimethylammonium chloride with large formation constants, and that the resulting Schiff bases underwent transamination to give the corresponding α-keto acids effectively even in the absence of metal ions in aqueous media at 30.0 °C.[105−108]

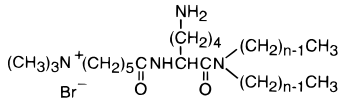
Chart 4



31 (n = 12, 14, 16)



32 (n = 12, 14, 16)



33 (n = 14, 16)

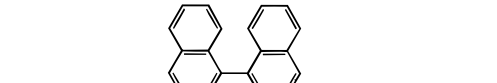


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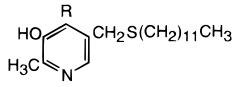
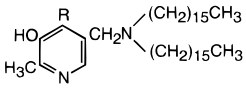
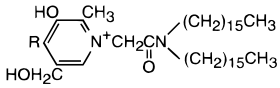
Chart 5



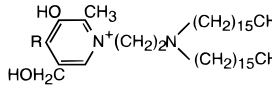
35a (R = CHO)
35b (R = CH_2NH_2)



36a (R = CHO)
36b (R = CH_2NH_2)



37a (R = CHO)
37b (R = CH_2NH_2)



38a (R = CHO)
38b (R = CH_2NH_2)

Murakami et al. constituted catalytic bilayer membranes as artificial vitamin B_6-dependent enzymes in combinations of peptide lipids (Chart 4), hydrophobic vitamin B_6 derivatives (Chart 5), and metal ions.[109] The vitamin B_6 moiety is placed in the so-called hydrogen-belt domain interposed between the polar surface region and the hydrophobic domain composed of double-chain segments within the bilayer assembly. Marked substrate discrimination by the artificial holoenzyme is performed through hydrophobic, electrostatic, and metal-coordination interactions between substrates and the catalytic site.

Catalytic behavior of the functionalized bilayer membrane was first examined in detail with regard to the half-transamination reaction, the transformation of pyruvic acid to alanine. The molecular assembly was constituted in combination of a peptide lipid having an L-alanine (**31**) or an L-histidine residue (**32**), a pyridoxamine derivative bearing a single chain (**35b**) or a double chain (**36b**), and Cu(II) ions in aqueous media at pH 7 and 30.0 °C.[110−112] The reaction proceeded through fast equilibrated formation of the Cu(II) complex of a ketimine Schiff base derived from pyruvic acid and the pyridoxamine

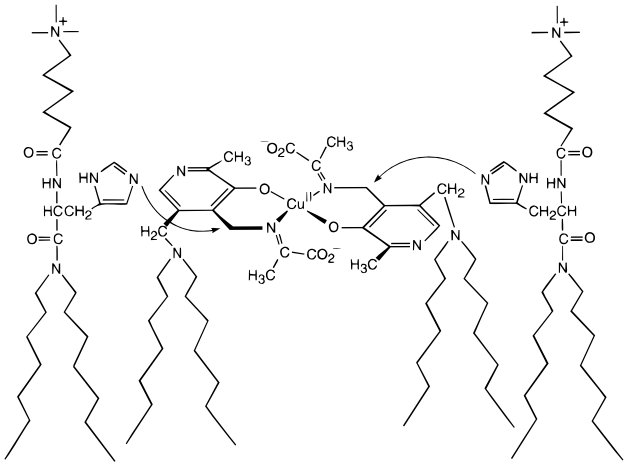


Figure 2. Bifunctional assistance in the transamination with pyruvate via formation of the 2:1 ketimine−Cu(II) chelate in the **32** ($n = 16$) vesicle. (Reprinted from ref 56. Copyright 1991 Springer-Verlag.)

derivative, followed by slower conversion into the corresponding aldimine Cu(II) chelate. The observed rate constant for the isomerization process showed significant dependence on the Cu(II) ion concentration, reflecting formation of the 2:1 and 1:1 [ketimine−Cu(II)] chelate species. The reactivity of the 2:1 chelate was at least 1 order of magnitude larger than that of the 1:1 chelate in each reaction system, and the effective charge on the Cu(II) atom seems to give out much effect on the reactivity. The efficient hydrophobic interaction of the two double-chain segments of the 2:1 ketimine−Cu(II) chelate derived from **36b** with the hydrophobic vesicular domain results in anchoring the pyridoxamine moieties tightly in the intramembrane region, and consequently mobility of the pyridoxamine moieties is much repressed relative to that of the corresponding complex of **35b**. The specific rate constant evaluated for the 2:1 ketimine−Cu(II) chelate in the **32**($n = 16$)−**36b** system was 4 times larger than that for the same complex in the **31**($n = 16$)−**36b** system under comparable conditions. Accordingly, the holoenzyme model system composed of **32**($n = 16$), **36b** and Cu(II) ions which is capable of performing both tight anchoring of the vitamin B_6 moiety in the intramembrane region and catalytic assistance of the imidazolyl group gives the largest reactivity in the transamination among those combinations (Figure 2).

The other half-transamination, transformation of an α-amino acid to the corresponding α-keto acid, was also examined kinetically by employing the catalytic bilayer membranes composed of a peptide lipid (**31** or **32**) and a hydrophobic pyridoxal derivative (**35a**, **36a**, or **37a**).[113,114] In the absence of metal ions, the reaction of L-phenylalanine proceeded through fast equilibrated formation of the aldimine Schiff base with the pyridoxal derivative (**37a**), followed by much slower conversion into the pyridoxamine derivative (**37b**) and β-phenylpyruvic acid. The rate-determining step in the overall reaction is the isomerization of the aldimine to the corresponding ketimine, since the accumulation of the ketimine species was not observed to any detectable extent during the reaction.

Addition of metal ions to an equilibrium mixture of the aldimine Schiff base, **37a**, and L-phenylalanine caused acceleration of the half-transamination reac-

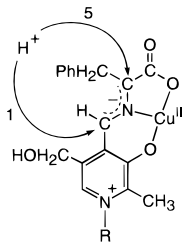


Figure 3. Protonation susceptibilities of two reaction sites in the Cu(II)−carboanion chelate formed during the half-transamination of L-phenylalanine with **37a** in the **31** (n = 16) vesicle. (Reprinted from ref 114. Copyright 1986 The Royal Society of Chemistry.)

tion. The catalytic activity of Cu(II) ions is by far the highest among those of metal ions employed; acceleration factors relative to the metal-free system in the **31**(n = 16)−**37a** vesicle at pH 10 and 30.0 °C are 6000, 37, 25, and 25 for Cu(II), Fe(III), Al(III), and Zn(II) ions, respectively. The Cu(II) complex of the carboanion intermediate, derived from the Cu(II)−aldimine chelate by α-hydrogen removal, was observed by means of electronic absorption spectroscopy during the reaction in the vesicular system. On the other hand, the corresponding metal−carboanion chelates were not detected in the reaction systems mediated by other metal ions. The result strongly indicates that the square-planar coordination geometry around the metal ion is important for effective electron delocalization in the intermediate carboanion species. When **35a** or **36a** was used in place of **37a**, the transamination reactivity with L-phenylalanine was extremely low even in the presence of Cu(II) ions. Thus, quaternization of the pyridyl nitrogen makes effective contribution to change in the rate-determining step and consequently to acceleration of the isomerization. In addition, the higher reactivity in the **32** (n = 16) vesicle relative to that in the **31** (n = 16) vesicle was also observed, as originated from catalytic assistance of the imidazolyl group. Detailed kinetic analysis of the reactions carried out in the vesicular system indicates that the transformation of the Cu(II)−carboanion chelate into the Cu(II)−ketimine chelate is a kinetically unfavorable process in comparison with the competing formation of the Cu(II)−aldimine chelate from the same carboanion species (Figure 3). Thus, an acceleration of the hydrolysis of the Cu(II)−ketimine species is inevitably important for the effective isomerization in the direction from the aldimine chelate to the ketimine, and the molecular assemblies described here are valid for such catalysis.

Two critical requirements at least need to be fulfilled in order to set up an artificial enzyme that is effective for the transamination reaction between an α-amino acid and an α-keto acid; acceleration of the reversible isomerization between the aldimine and ketimine Schiff bases and substrate selectivity in the Schiff base formation equilibria. The bilayer vesicle constituted with **32** (n = 16), **37a**, and Cu(II) ions satisfies these requirements for catalytic performance. Murakami et al. was first to observe that the transamination reaction of L-Phe with pyruvic acid proceeded with turnover behavior of the catalyst system in an aqueous medium at pH 5 and 30.0 °C (Figure 4).[115] The active site of the artificial aminotransferase can be easily modified by changing the peptide lipid or the coenzyme component.[116] When **37a** was replaced by **36b**, the transamination proceeded at a kinetic rate comparable to that in the vesicular system involving **37a** under the reaction conditions that the pyridyl nitrogen was positively charged. The turnover behavior was also observed by replacement of **32** with a peptide lipid bearing an L-lysine residue (**33**). However, such turnover behavior was not observed in a vesicular system composed of **31**, **37a**, and Cu(II) ions; a micellar system formed with hexadecyltrimethylammonium bromide (CTAB), **37a**, and Cu(II) ions; and an aqueous system involving *N*-methylpyridoxal (or pyridoxal) and Cu(II) ions without amphiphiles, under the mild reaction conditions.

In order to clarify the mechanistic implication in substrate selectivity exercised by the bilayer-type artificial aminotransferase, its catalytic activity toward various sets of substrates was examined in the single-walled covesicle composed of **32** (n = 16), **36b**, and Cu(II) ions (Table 1).[117,118] With regard to the transamination reaction of various DL-α-amino acids, having different alkyl chains, with pyruvic acid, the reactivity increased sensitively along with increase in hydrophobicity of the amino acid. For a combination of L-phenylalanine and a slightly hydrophobic α-keto acid, 2-oxobutanoic acid, the reactivity was approximately one-third of that for a combination of

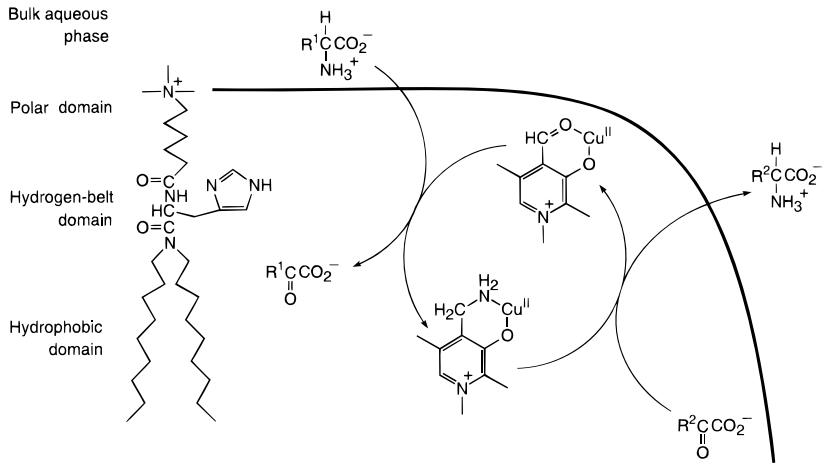


Figure 4. Schematic representation for transamination cycle with the catalytic bilayer membrane composed of peptide lipid **32** (n = 16), hydrophobic vitamin B_6 **37a**, and Cu(II) ions: R^1, hydrophobic group; R^2, hydrophilic group.

Table 1. Substrate Selectivity of an Artificial Transaminase Formed with 32 (n = 16), 36b, and Cu(II) Ions at 30.0 °C[a,118]

α-amino acid derivative [$R^1CH(NH_2)CO_2R^2$]			α-keto acid (R^3COCO_2H)	relative
R^1	R^2	chirality	R^3:	reactivity[b]
$CH_3(CH_2)_5$	H	DL	CH_3	83
$CH_3(CH_2)_3$	H	DL	CH_3	23
$CH_3(CH_2)_2$	H	DL	CH_3	7.7
CH_3CH_2	H	DL	CH_3	1.8
$PhCH_2$	H	D	CH_3	42
$PhCH_2$	H	L	CH_3	31
$PhCH_2$	H	L	CH_3CH_2	11
$PhCH_2$	CH_2Ph	L	CH_3	6.0
$PhCH_2$	CH_3	L	CH_3	5.6
$PhCH_2O_2CCH_2$	H	L	CH_3	100[c]
$PhCH_2O_2CCH_2$	CH_2Ph	L	CH_3	9.6
HO_2CCH_2	H	L	CH_3	5.0
HO_2CCH_2	CH_2Ph	L	CH_3	1.6
$PhCH_2O_2C(CH_2)_2$	H	L	CH_3	56
$PhCH_2O_2C(CH_2)_2$	CH_2Ph	L	CH_3	7.7
$HO_2C(CH_2)_2$	H	L	CH_3	2.6

[a] In an aqueous acetate buffer (25 mM, μ = 0.10 with KCl) at pH 5.0. Concentrations (in mM): α-amino acid derivatives, 5.0; α-keto acids, 5.0; **36b**, 0.05; **32** (n = 16), 1.0; $Cu(ClO_4)_2$, 0.05. [b] Evaluated on the basis of reaction times for one turnover. [c] 5 h/turnover.

L-phenylalanine and pyruvic acid. Thus, the present vesicular system requires a combination of a hydrophobic α-amino acid and a hydrophilic α-keto acid to achieve high catalytic performance. In addition, the substrate selectivity evidently comes from the molecular recognition exercised in the course of formation of the Cu(II)−aldimine chelate, and the reaction step for formation of the Cu(II)−ketimine chelate is not responsible for the selectivity in the overall reaction cycle.

The substrate selectivity was also much affected by modification of the α-carboxyl moiety of an α-amino acid. The transamination reactivity of L-phenylalanine with pyruvic acid was much depressed upon conversion of the α-carboxylate group into its methyl or benzyl ester, in spite of increased hydrophobicity of the α-amino acid species. This seems to reflect the fact that the stability of the Cu(II) complex of the terdentate aldimine Schiff base formed with the former α-amino acid and **36a** is much enhanced relative to that of the Schiff base chelate derived from the latter amino acid ester. Consequently, the substrate selectivity is of thermodynamic origin; the higher stability the aldimine Schiff base chelate attains, the greater overall reactivity is observed. The substrate selectivity in the transamination reactions of various L-aspartate and L-glutamate analogs with pyruvic acid supported this mechanistic implication.

Murakami et al. also examined enantioselectivity by the catalytic bilayer membranes in the transamination reaction.[118−121] The enantioselective formation of D-α-amino acids in the half-transamination reaction of α-keto acids with **36b** was observed by employing the bilayer covesicle composed of **31** (n = 16) having an L-alanine residue and a peptide lipid (**34**) bearing both a chiral (*S*)-binaphthol moiety as a bidentate ligand and an L-alanine residue in the presence of Cu(II) ions at pH 7.0 and 30.0 °C; the observed ee values were 40, 34, 24, and 15% for valine, leucine, phenylalanine, and alanine, respectively.[120] Since the racemic valine was obtained in the absence of **34**, the chiral reaction site provided by the bilayer aggregate formed with **31** alone is insufficient for the enantioselective transamination. The results indicate that the observed enantioselectivity is attained via formation of an intermediate ternary complex formed with the ketimine Schiff base of **36b** with a bulky α-keto acid, **34**, and Cu(II) ions. In addition, replacement of Cu(II) ions with Zn(II) or Al(III) ions resulted in disappearance of the enantioselectivity, reflecting the importance of coordination geometry of the intermediate complex. It seems, however, that the enantioselective catalysis in the overall transamination cannot be attained by this bilayer system directly, because the system lacks a functional group for the acid−base catalysis which is required to exhibit the turnover behavior.

Murakami et al. also found that the bilayer vesicle composed of a peptide lipid [**32** (n = 6)] having an L-histidine residue, **36b**, and Cu(II) ions exhibits the enantioselectivity in the half-transamination of α-keto acids to give the corresponding D-α-amino acids at pH 5.0 and 30.0 °C.[119] However, the observed ee values were not so high: 20, 15, 0, and 0% for valine, leucine, phenylalanine, and alanine, respectively. The imidazolyl group of **32** in the bilayer membrane is mainly present as the nonprotonated form under the pH conditions employed, because the pK_a value was evaluated to be 4.5. In addition, the enantioselectivity performed by the bilayer vesicle disappeared as pH was lowered to 3.5. Thus, it seems that an important role of the imidazolyl group of the lipid is to act as a chiral ligand for the intermediate Cu(II) chelate as well as to perform enantioselective acid−base catalysis. They examined the enantioselectivity of the overall transamination by employing the vesicular system. Although the amino group transfer from D-phenylalanine to pyruvate was accelerated to a 33% extent relative to that from L-phenylalanine to pyruvate, enantioselective formation of alanine was not detected.

On these grounds, a catalytic bilayer membrane was developed recently[121] as a real artificial aminotransferase, showing both high enantioselectivity and turnover behavior. The catalyst was constructed with a peptide lipid bearing an L-lysisne residue [**33** (n = 16)], a hydrophobic pyridoxal derivative quaternized at the pyridyl nitrogen (**38a**), and Cu(II) ions. Such a bilayer assembly exhibited turnover behavior for the transamination between L-phenylalanine and pyruvic acid to form D-alanine with an enantiomeric excess as high as 90% at pH 7.0 and 30.0 °C. The highest enantioselectivity observed for this system comes from the following supramolecular assistance: (i) In these bilayer systems, phenylalanine as one of the substrates is responsible for inducing the opposite chirality in alanine. (ii) The protonated ϵ-amino group of the L-lysine residue involved in **33** enhanced the formation of D-alanine much more favorably than that of L-alanine; the pK_a value of the ϵ-amino group was evaluated to be 9.3. (iii) Orderly arrangement of assembly species in the bilayer membrane is essential for the enantioselective catalysis. (iv) The stereochemical control is sensitive to the orientation of the pyridoxal moiety in the bilayer membrane. (v) The presence of a chiral phenylalanine is indispensable to perform the enantioselective transamination. The key reaction step

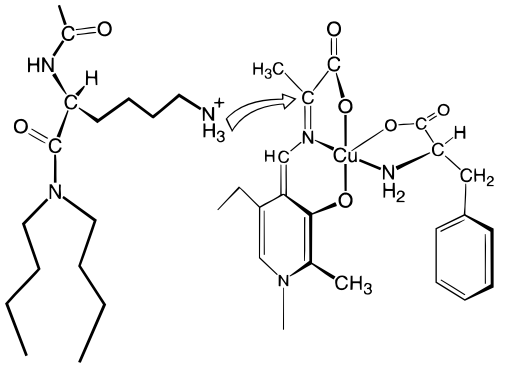


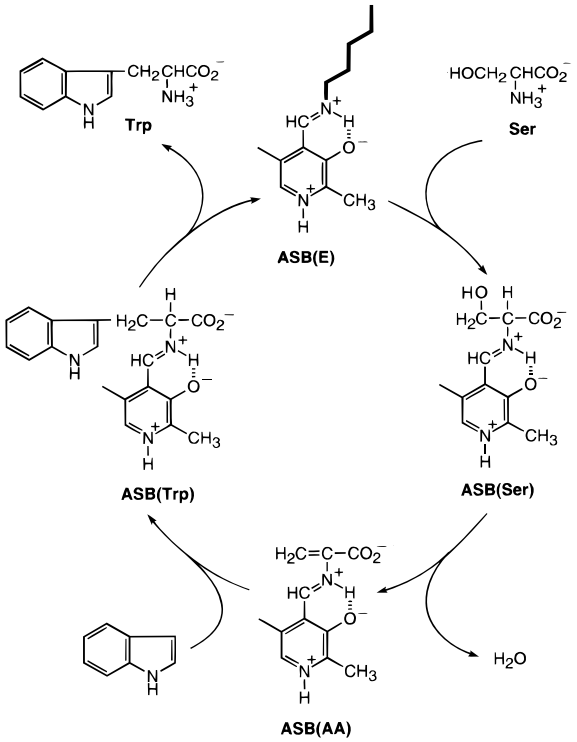
Figure 5. Protonation of the quinoid−Cu(II) complex, showing *si* face attack, to yield the corresponding aldimine Schiff base complex with D-alanine. (Reprinted from ref 121. Copyright 1995 American Chemical Society.)

in forming a chiral alanine is stereoselective protonation of the quinoid−Cu(II) chelate, which is the unstable intermediate present in the course of transformation from the ketimine Schiff base chelate to the aldimine Schiff base chelate. They proposed a five-coodinated Cu(II) complex as a key intermediate in the stereoselective protonation (Figure 5), in which the L-phenylalanine molecule present in excess over **38a** acts as a chiral bidentate ligand to be bound to the quinoid−Cu(II) complex, the amino nitrogen and the carboxylate oxygen being coordinated to the Cu(II) ion at the fourth basal site and the axial position, respectively. In addition, the hydrophobic phenyl group of the coordinated L-phenylalanine must be oriented in the hydrophobic inner membrane domain in a manner parallel to the lipid alkyl chains. As a result, the ϵ-ammonium group of the lysine residue of **33** acts as a proton source to protonate the quinoid−Cu(II) complex preferentially from the less hindered *si* face of the imino carbon to afford the aldimine chelate.

2. β-Replacement Reactions

The β-replacement reaction of an α-amino acid with a nucleophile is very attractive from the viewpoint of synthetic organic chemistry, because various β-substituted alanines may be prepared from a simple α-amino acid, such as serine, and nucleophiles. A reaction catalyzed by tryptophan synthase, formation of tryptophan from serine and indole, is one of the most well-known β-replacement reactions. In the β-subunit of tryptophan synthase, the reaction proceeds via several steps shown in Scheme 10. The aldimine Schiff base [ASB(Ser)], derived by transimination of the enzyme-bound PLP [ASB(E)] with serine, is a common intermediate in the vitamin B_6-catalyzed reactions. In order to achieve tryptophan formation, an effective nucleophilic attack of indole on the β-elimination product, the Schiff base of PLP with 2-aminoacrylate [ASB(AA)], formed by dehydration of ASB(Ser), must be followed by release of the product from the aldimine Schiff base of tryptophan [ASB(Trp)]. When ASB(AA) is hydrolyzed prior to the attack by indole, the reaction results in β-elimination to give pyruvate, ammonia, and ASB(E). Accordingly, it is required for an effective artificial tryptophan synthase to perform preferential attack by indole toward the intermediate ASB(AA) species rather than its hydrolysis.

Scheme 10



Breslow et al. prepared a pyridoxal-bound β-cyclodextrin from the corresponding pyridoxamine-cyclodextrin conjugate (**27**) by the half-transamination reaction.[122] They found that this pyridoxal derivative produced 3−5 times more tryptophan when it (at 21 mM) was incubated with 10 mM indole, 51 mM β-chloroalanine, and 2.5 mM $Al_2(SO_4)_3$ for 30 min at pH 5.2 and 100 °C, relative to the reaction in which the pyridoxal derivative was replaced by pyridoxal. However, the yield of tryptophan was only a few percent. As expected, this kinetic advantage disappeared at higher (40−80 mM) concentrations of indole due to saturation of the binding site. Furthermore, L-tryptophan was produced in ca. 10% excess relative to the D-enantiomer when 10 mM indole was used.

Murakami et al. found that the catalytic bilayer membrane effective as an artificial aminotransferase also behaved as an artificial tryptophan synthase which produces tryptophan derivatives by β-replacement of serine (5 mM) with indoles (5 mM) under mild conditions at pH 5 and 30 °C.[123,124] An extent of the β-replacement catalyzed by pyridoxal (0.025 mM) in homogeneous aqueous solution was extremely low, even in the presence of Cu(II) ions; yields of tryptophan based on pyridoxal for 210 h of incubation were 2.9 and 8.0% for metal-free and Cu(II)-catalyzed systems, respectively. On the other hand, the bilayer vesicle formed with **31** ($n = 16$) and **37a** drastically accelerated the reaction; the reactivity was enhanced by 51-fold (r_{rel}) relative to that of pyridoxal in homogeneous aqueous solution. The imidazolyl group of **32** ($n = 16$) enhanced the reaction further, r_{rel} being 130. The Cu(II) ion is catalytically effective for the reaction carried out in all vesicular systems. As a whole, the bilayer catalyst composed of **32** ($n = 16$), **37a**, and Cu(II) ions exhibited the highest activity for the β-replacement reaction, showing turnover behavior, r_{rel} being 180.

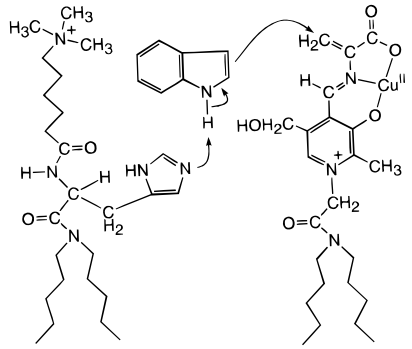


Figure 6. Key step of the β-replacement reaction catalyzed by the bilayer-type artificial tryptophan synthase composed of **32**, **37a**, and Cu(II) ions.

This potent catalyst system must exercise catalytic functions of three kinds cooperatively to achieve the best performance: (i) a microenvironmental effect of hydrophobic nature provided by the bilayer vesicle, which affords effective incorporation of indole molecules and elimination of water molecules in the reaction site, (ii) a general acid−base catalysis by the imidazolyl group of the peptide lipid, and (iii) a coordination effect by Cu(II) ions. When **37a** was replaced by **36a** in the vesicular system, the catalytic efficiency of the latter in the β-replacement was comparable to that of the former at pH 5.0 but much depressed at pH 9.5. Since the pK_a value for the pyridyl nitrogen of the Schiff base, derived from **36a** and α-amino acid, in the vesicle lies in the 6−7 range, the pyridyl nitrogen of **36a** is not protonated at pH 9.5. This means the pyridyl moiety of **37a**, bearing a positive charge on the nitrogen atom, acts to promote the β-replacement reaction as an electron sink. Accordingly, a possible activation mechanism in the key reaction step of the β-replacement catalyzed by the supramolecular catalyst composed of **32** (n = 16), **37a**, and Cu(II) ions can be provided as shown in Figure 6.

As for the β-replacement reaction carried out in the **32**(n = 16)−**37a** vesicular system, L-serine and its hydrophobic benzyl ester derivative were comparable to each other in reactivity. Thus, it is clear that the formation of an aldimine Schiff base of **37a** with the substrate α-amino acid is not referred to the rate-determining step. On the other hand, marked substrate specificity was observed for indole derivatives; relative reactivities for the reactions of L-serine with 5-hydroxyindole, 5-methylindole, 5-methoxyindole, and indole were 40, 18, 13, and 1, respectively. Such selectivity for indole derivatives primarily reflects differences in their nucleophilicity.

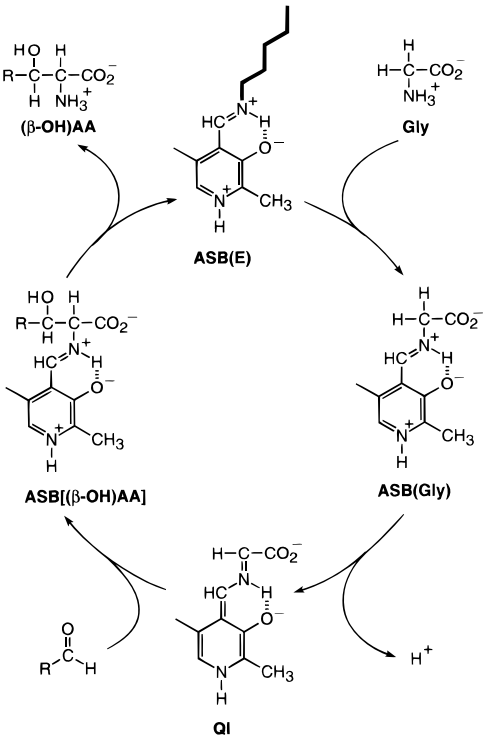
When **37a** was replaced by **38a** in the **32** (n = 16) vesicle, the β-replacement also proceeded effectively in the presence of Cu(II) ions. The modified artificial tryptophan synthase showed marked enantioselectivity in the β-replacement reaction.[125] The formation of D-tryptophan prevails over that of the corresponding L-form in 50−55% ee regardless of chirality of the substrate, serine. On the other hand, any detectable enantioselectivity was not observed when the **31** (n = 16) vesicle was used in place of the **32** (n = 16) vesicle. Such results mean that the imidazolyl group of the L-histidyl residue, introduced covalently into the peptide lipid exercises stereospecific acid catalysis in the protonation of the prochiral carboanion intermediate to afford the aldimine Schiff base of **38a** with tryptophan.

The enantioselectivity was also observed when the β-replacement reaction was mediated by the covesicle formed with **38a**, **31** (n = 16), and an additional peptide lipid having (*S*)-binaphthol and (*S*)-alanyl moieties (**34**) in the presence of Cu(II) ions.[126,127] The binaphthol moiety of the lipid acts as a chiral ligand toward the intermediate Schiff base chelate to control the enantioselectivity. The formation of L-tryptophan prevails over that of the corresponding D-form by ca. 30% ee.

3. Aldolase-type Reactions

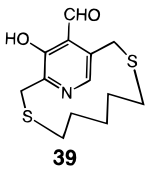
Serine hydroxymethyltransferase is an aldolase requiring PLP and catalyzes interconversion between glycine and various β-hydroxy-α-amino acids, such as serine, threonine, and β-phenylserine, via formation of a quinoid intermediate (QI) derived from the enzyme-bound Schiff base of PLP with the amino acid substrate (Scheme 11). In a nonenzymatic system,

Scheme 11



the aldolase-type reaction of β-hydroxy-α-amino acids to give glycine and aldehyde occurs readily at high pH in aqueous solution in the presence of pyridoxal and metal ions.[85] The reverse reaction of the aldolase-type reaction is of interest in view of asymmetric synthesis of α-amino acids via C−C bond formation.

Kuzuhara et al. synthesized a Zn(II) complex of a Schiff base produced from glycine and a chiral vitamin B_6 analog having an ansa chain between 2′- and 5′-positions (**39**), the corresponding pyridoxal derivative of **25a**.[128,129] They found that the aldolase-



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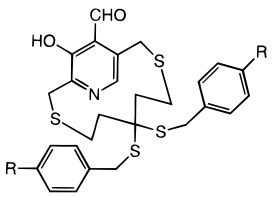
type reaction of the complex with either acetaldehyde or propionaldehyde proceeded in carbonate buffer (pH 10.0)−methanol (75:35 v/v) at 10−27 °C to afford the corresponding β-hydroxy-α-amino acid in a range of 27−77% enantiomeric excess. The results show the following specific features: (i) *Erythro* isomers are 1.2−1.8 times dominant over *threo* ones. (ii) As the reaction temperature is lowered, the enantiomeric excess increases on one hand and the chemical yield is reduced on the other. (iii) The chemical yield and the enantiomeric selectivity of reaction product are not remarkably different between acetaldehyde and propionaldehyde when mediated by the Zn(II) chelate of **39**. (iv) The use of (*S*)-enantiomer of the pyridoxal derivative in the reaction affords the (*S*)-amino acid in excess and *vice versa*, and the enantiomeric excess decreases as the reaction time goes longer. Accordingly, the reaction occurred on the same face as was occupied by the ansa chain.

Recently, Breslow et al. also confirmed such enantioselectivity in the aldolase-type reaction of acetaldehyde with glycine as mediated by **39** and **40a** in the presence of Zn(II) ions in methanol−water (1:1 v/v) at 35 °C.[130] In addition, they prepared a chiral



40a : R = H
40b : R = $CH_2N(CH_3)_2$

cyclophane derivative of pyridoxal (**40b**) which has amino groups oriented specifically over one face of the cofactor. The (*S*)-isomer of this compound mediated the formation of threonine and *allo*-threonine from glycine and acetaldehyde with enantioinductions that were a function of pH, reversing the chiral selectivity from low to high pH; the ee values at pH 5.0 and 10.0 were −63.4% and +48.8% for (*R*)-threonine and −75.3% and +23.5% for (*R*)-*allo*-threonine, respectively. Explanations were advanced for this stereochemical reversal and for the otherwise surprising preference of the former compounds to react on the more hindered face of the pyridoxal. They suggested that the reaction intermediate is geometrically distorted by the transannular chain and that this distortion leads to reaction on the face that carries the chain due to a stereoelectronic effect (Figure 7). They also considered that the stereochemical reversal observed with **40b**, as controlled by pH, may reflect catalysis by the protonated form (Figure 8), but a coordination effect of metal ions

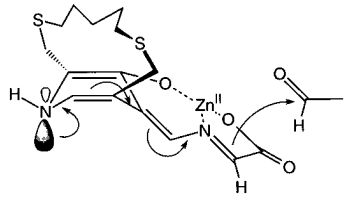


Figure 7. A stereoelectronic explanation of the preferential stereochemical course for the aldolase-type reaction of glycine with acetaldehyde as mediated by Zn(II)−**39** chelate. (Reprinted from ref 130. Copyright 1994 American Chemical Society.)

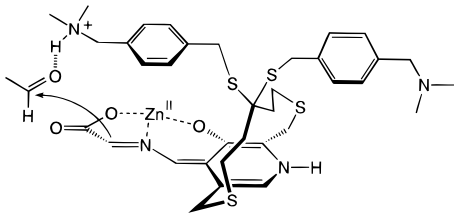


Figure 8. Catalysis by the protonated amine for the stereoselective aldolase-type reaction of glycine with acetaldehyde as mediated by Zn(II)−**40b** chelate at low pH. (Reprinted from ref 130. Copyright 1994 American Chemical Society.)

bound to the protonation-free form cannot be excluded.

Murakami et al. evaluated the catalytic efficiency of the bilayer-type artificial enzyme for the aldolase-type reaction.[131,132] Formation of β-phenylserine from glycine and benzaldehyde was found to proceed effectively by cooperative catalysis of a pyridoxal derivative (**36a**) and Zn(II) ions in the bilayer vesicle formed with **31** at pH 7.0 and 30.0 °C. The *threo* isomer was dominantly produced over the corresponding *erythro* form. The diastereoselectivity for the reaction in various reaction media increased in the following order, reflecting molecular organization of the reaction site matrix: homogeneous aqueous media (22−27% de), a small bilayer vesicle in the liquid-crystalline state (30% de), a large bilayer vesicle in the liquid-crystalline state (42% de), and a bilayer vesicle in the gel state regardless of vesicular size (54−56% de).

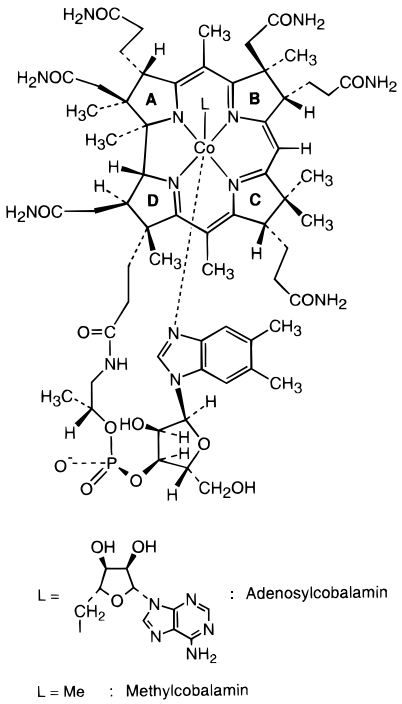
They carried out modification of the active site of the artificial holoenzyme by employing various combinations of peptide lipids, hydrophobic pyridoxal derivatives, and metal ions and clarified their catalytic activities and enantioselectivities. A marked enantioselectivity in favor of *threo*-β-phenylserine as the main product was observed in the covesicle of **31** ($n = 16$) and **34** in combination with **36a** and Cu(II) ions; the ee value for formation of (2*S*,3*R*)-β-phenylserine over its enantiomeric (2*R*,3*S*)-isomer was 58% at the initial stage of the reaction. Although the ee value gradually decreased along progress of the reaction, a relatively high enantioselectivity was retained at least over several hours. Such asymmetric induction comes from catalytic assistance of the chiral binaphthol moiety of **34** in the key reaction step, in a manner analogous to those observed for the transamination and the β-replacenent reactions. The enantioselective formation of *threo*-β-phenylserine was also mediated by another bilayer assembly formed with **31** ($n = 16$), **34**, and **38a** in the presence of Cu(II) ions; the (2*R*,3*S*)-isomer was dominant over the (2*S*,3*R*)-species in a 13% ee after 3 h of incubation. The opposite enantioselectivity performed by the second supramolecular assembly, as compared with that for the **36a** system, reflects a different stereochemical environment around the Cu(II)−quinoid intermediate that allows the attack of benzaldehyde.

D. Vitamin B_{12} Functions

1. Enzymic Reactions

Vitamin B_{12} is a cobalt complex coordinated with a tetrapyrrole ring system, namely corrin, and linked to a 5,6-dimethylbenzimidazole moiety as a heterocyclic base (Chart 6). There are two B_{12} active forms: 5′-deoxyadenosylcobalamin and methylcobal-

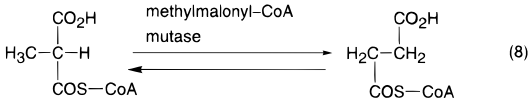
Chart 6



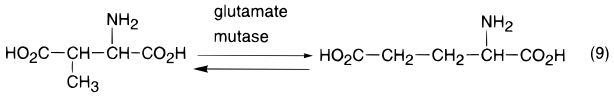
amin. Vitamin B_{12}-dependent enzymes are known to catalyze two types of reactions: rearrangements as exemplified by methylmalonyl-CoA mutase and methylation by methionine synthetase. The rearrangement reactions involve the intramolecular exchange of a functional group (X) and a hydrogen atom between neighboring carbon atoms (refer to eq 7).[133,134]

(7)

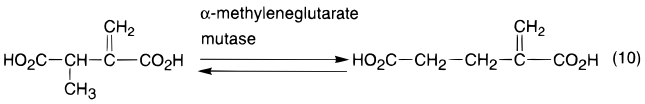
These reactions have attracted much attention because of their novel nature from the viewpoints of organic and organometallic chemistry. Carbon-skeleton rearrangement reactions, mediated by methylmalonyl-CoA mutase, glutamate mutase, and α-methyleneglutarate mutase are shown by eqs 8−10.



(8)



(9)



(10)

Even though the real reaction mechanisms involved in the carbon-skeleton rearrangements have not been clarified up to the present time, radical mechanisms are considered to be the most plausible ones on the basis of ESR studies for methylmalonyl-CoA mutase,[135] glutamate mutase,[136] and α-methyleneglutarate mutase.[137] A general feature of the radical mechanism is illustrated in Figure 9:[138] the 5′-deoxyadenosyl moiety bound to cobalamin (vitamin B_{12}) undergoes homolytic cleavage to give cobalamin

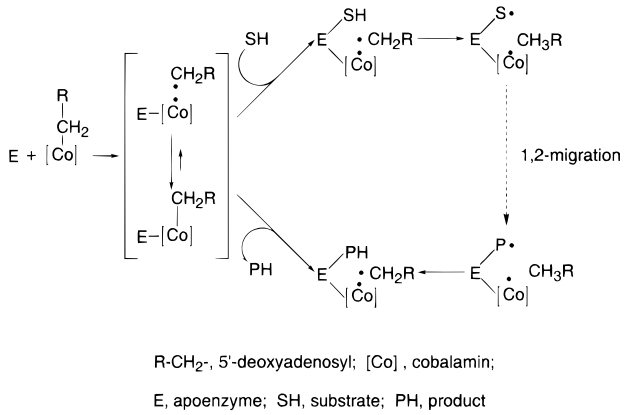


Figure 9. A general feature of the radical mechanism for rearrangement reactions mediated by the 5′-deoxyadenosylcobalamin-dependent enzyme. (Reprinted from ref 138. Copyright 1979 American Chemical Society.)

in the Co^{II} state and the 5′-deoxyadenosyl radical upon incorporation of a substrate into a specific microenvironment provided by the corresponding apoprotein; the 5′-deoxyadenosyl radical abstracts a hydrogen atom from the incorporated substrate to afford deoxyadenosine and the substrate radical in the active site of apoprotein; the substrate radical is eventually isomerized via 1,2-migration of a functional group to form the corresponding product radical; the product radical abstracts a hydrogen atom from deoxyadenosine placed in its vicinity; and the deoxyadenosyl radical is then bound to cobalamin to recover the original coenzyme state.

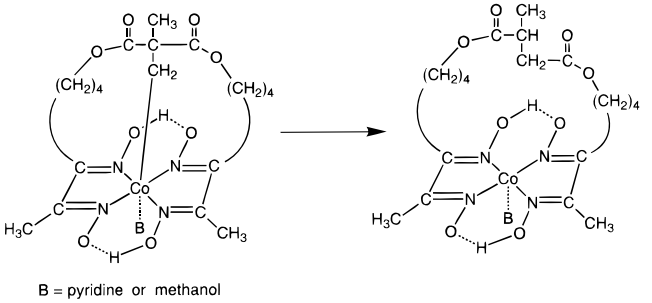
2. Role of Cobalt Complex

As for the role of the cobalt species, Halpern et al.[139] proposed a reversible free radical carrier mechanism; coenzyme B_{12} is referred simply to a source of the 5′-deoxyadenosyl free radical that acts to generate a substrate radical by abstracting a hydrogen atom from the substrate to initiate the reaction, and behaves as a reversible free radical carrier. They excluded possible participation of the cobalt species in the rearrangement process. In connection with the biological reaction, a free radical rearrangement reaction, promoting 1,2-migration of a thioester group in a substrate radical generated by reaction of the corresponding bromide with *n*-Bu_3SnH and 2,2′-azobisisobutyronitrile, was investigated in methanol at relatively high temperatures (60−114 °C) as a model reaction for methylmalonyl-CoA mutase; the rearrangement product was obtained in a relatively low yield (1−9%).[140,141] On the other hand, thermal decomposition of the dimethyl thiomalonate complex of vitamin B_{12} was carried out in aqueous media (pH 8−9) in the dark at room temperature, and the rearrangement product was obtained in a good yield (50−70%).[142−144] In addition, there is some experimental evidence indicating that a thioester group in ligands coordinated to a cobalt complex more readily migrates to the adjacent carbon as compared with the same group involved in the corresponding free radicals.[145−147] Therefore, it is not reasonable to exclude the possible participation of the cobalt species in the course of 1,2-migration reactions, even though free radicals are spontaneously converted into the corresponding isomerization products to a certain extent. Since the participation mode of the cobalt complex has not yet been clarified for the rearrangement reaction occurring in biological systems, this is also a challenging subject of investigation.

3. Model Reactions with Apoenzyme Functions

It is obvious that an apoprotein plays an important role in such radical reactions as mediated by vitamin B_{12}-dependent enzymes.[148,149] The model reactions, which were designed in consideration of the role of apoenzymes, are as follows. The first example of the active site around adenosylcobalamin was demonstrated by Rétey et al.[150−153] They synthesized a number of intramolecularly bridged cobaloximes, simulating the stereochemical conditions at the active site of methylmalonyl-CoA mutase (Scheme 12). The

Scheme 12



substrate is anchored covalently with two methylene bridges to a planar part of the complex. They have succeeded in achieving the rearrangement reaction to obtain the desired product in a good yield (82%). This result indicates an importance of the role of apoproteins.

Fendler et al. tried to utilize micelles as an apoenzyme model for vitamin B_{12} in aqueous media by employing sodium bis(2-ethylhexyl)sulfosuccinate (Aerosol-OT), hexadecyltrimethylammonium bromide (CATB), and sodium dodecyl sulfate (SDS) as surfactants.[154−156] They reported micellar effects on the rates of methyl transfer from methylcobalamin to the mercury ion, but did not carry out the isomerization reaction to mimic adenosylcobalamin-dependent enzymic reactions. Since they used cobalamin itself, the cobalt complex was not incorporated into the micelles; vitamin B_{12} would be placed in the micellar surface due to an electrostatic interaction as shown in Figure 10.

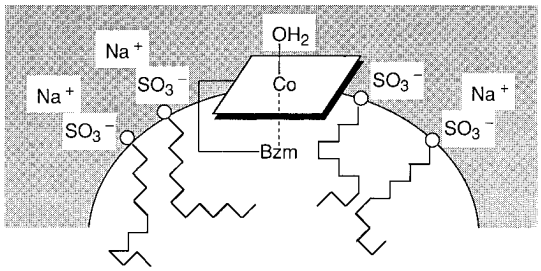
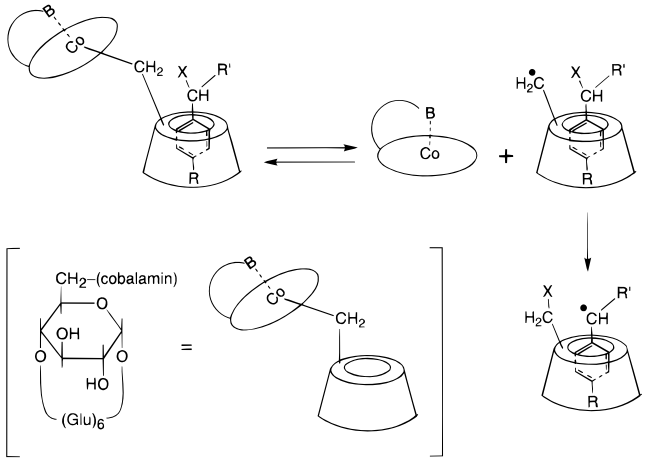


Figure 10. Schematic representation for the interaction between SDS micelles and cobalamin. (Reprinted from ref 155. Copyright 1977 American Chemical Society.)

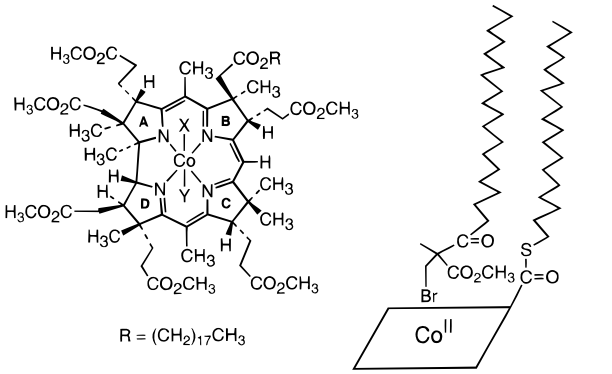
Breslow et al. prepared a cyclodextrin-bound B_{12}, in which cobalamin is directly linked to the primary carbon of β-cyclodextrin by a cobalt−carbon bond (Scheme 13).[157] They expected that a hydrophobic substrate is incorporated into the cyclodextrin cavity in water, so that the cyclodextrinyl radical may undergo an intracomplex atom transfer to generate a substrate radical as shown in Scheme 13. Even though they did not mimic all steps of the B_{12}-dependent rearrangement reaction, this is an interesting example showing that a substrate and B_{12} are bound together in a receptor site.

Scheme 13



As a model of the B_{12} coenzyme−apoenzyme complex (Chart 7), which provides a catalytic site and

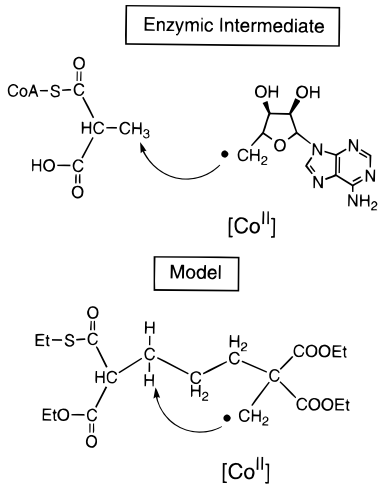
Chart 7



an anchor group with a substrate recognizing subunit, Keese et al. investigated properties of a vitamin B_{12} derivative modified with a C_{18}-alkyl side chain, as a catalyst for the rearrangement of a methylmalonyl moiety bearing a complementary substituent to the corresponding succinate analog in both protic and polar-aprotic solvents under electrochemical conditions.[158] The rearrangement of the methylmalonyl radical to the succinyl one became an efficient process when the reaction was carried out in a protic solvent, indicating that a supramolecular feature, such as preassociation of an appropriately modified substrate with the Co complex, becomes effective under such reaction conditions.

Methylmalonyl-CoA mutase catalyzes the interconversion between methylmalonyl-CoA and succinyl-CoA, in which the thioester group migrates to the methyl carbon following hydrogen abstraction. Dowd et al. reported mimicking reactions in which both hydrogen abstraction and group migration occur within the same model (Chart 8).[159] It is interesting to note that the abstraction of hydrogen from an unactivated carbon is coupled with the group migration. As for the abstraction of hydrogen from an unactivated carbon, Schrauzer et al. developed a new method for synthesis of organo-cobalamins by utilizing both molecular oxygen and vanadium(III) ions as oxidizing and reducing reagents, respectively.[160−163] Furthermore, Murakami et al. have developed a catalytic cycle by coupling a B_{12} artificial enzyme with the Schrauzer's methodology.[164−166]

Chart 8



4. Construction of Artificial B_{12} Enzyme

Various cobalt complexes have been synthesized as model complexes of vitamin B_{12}.[167] However, most of those complexes cannot be qualified as favorable model complexes in the following aspects: (i) Redox behavior of the central cobalt, which is mainly controlled by basicity of an equatorial ligand, must be similar to that for the naturally occurring vitamin B_{12}. (ii) Electronic properties must be equivalent to those of the natural B_{12}, which are provided by the corrin ring with eight double bonds and a direct bond between rings A and D. (iii) The steric effects, which are caused by a methyl moiety and a hydrogen atom at C1 and C19 positions in the corrin ring, respectively, and by four propionamides and three acetamides placed at the α- and β-peripheral sites, respectively, must be retained by model complexes. The naturally occurring apoproteins, which provide relevant reaction sites for vitamin B_{12}, are considered to perform additional important roles that lead to desolvation and close association of reacting species.[148,149] On this ground, Murakami et al. have been interested in the catalytic activity of vitamin B_{12} in hydrophobic microenvironments in order to simulate the catalytic functions of holoenzymes concerned.[168−170] Under such circumstances, they have prepared hydrophobic vitamin B_{12} derivatives which have ester groups in place of the peripheral amide moieties of natural vitamin B_{12} (Chart 9). These modified cobalt complexes satisfy all the above requirements and are readily soluble in a wide range of organic solvents.

Murakami et al. reported a cyclophane-type B_{12} artificial enzyme which can be constructed with a

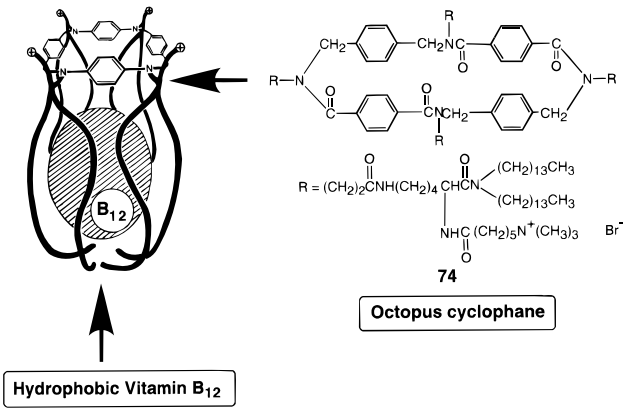


Figure 11. Construction of a cyclophane-type artificial vitamin B_{12} enzyme.

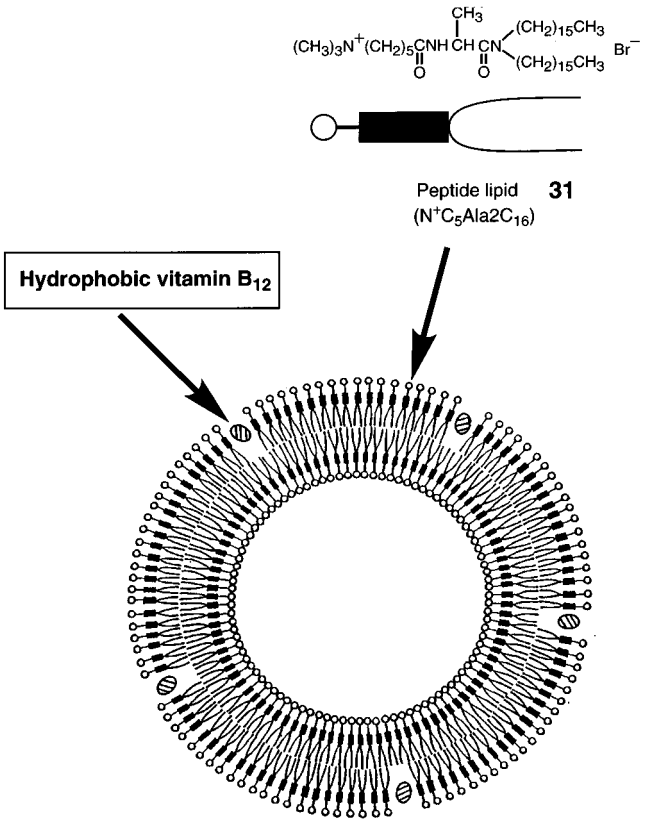
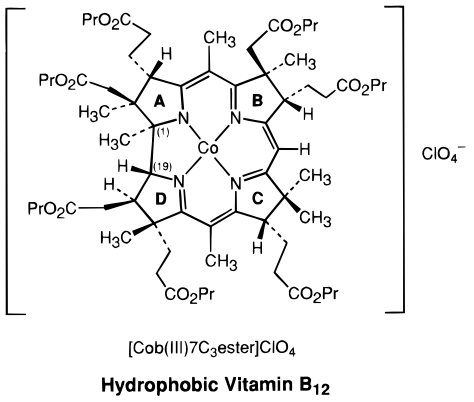


Figure 12. Construction of a vesicle-type artificial vitamin B_{12} enzyme.

Chart 9



combination of an octopus cyclophane and a hydorophobic vitamin B_{12} (Figure 11).[147,164,165,171] The hydrophobic vitamin B_{12} is noncovalently incorporated into the octopus cyclophane in a 1:1 molar ratio by a hydrophobic interaction. In addition, a vesicle-type B_{12} artificial enzyme was constructed in noncovalent combination of a single-walled bilayer vesicle composed of $N^+C_5Ala2C_{16}$ with the hydrophobic vitamin B_{12} (Figure 12).[166,173−177] The morphological stability of the synthetic bilayer vesicle is not perturbed by noncovalent incorporation of the hydorophobic vitamin B_{12}.[166,170]

5. Artificial Methylmalonyl-CoA Mutase

Methylmalonyl-CoA mutase catalyzes the interconversion between methylmalonyl-CoA and succinyl-CoA. With regard to a characteristic aspect of the substrate, methylmalonyl-CoA, it has two electron-withdrawing groups on the same carbon atom. Therefore, chemical species with such structural characteristics are referred to appropriate substrate models,

and successful model reactions have been reported by Murakami et al.[147,165,166,171−173] Substrate species were linked to the hydrophobic vitamin B_{12} at one axial site of the nuclear cobalt, and the resulting alkylated complexes were noncovalently fixed in the bilayer membrane domain in aqueous media and irradiated with a 500 W tungsten lamp to result in homolytic cleavage of the Co−C bond. It must be noted here that the natural enzyme mediates the homolytic Co−C bond cleavage in the dark, and the corresponding apoenzyme is responsible for such action. However, we have not been successful in obtaining appropriate models that are capable of performing such action in the dark. Under such circumstances, Murakami and his associates have been adopting photolysis conditions to generate substrate radicals.

Some typical experimental results are shown in eqs 11−14 in comparison with the data obtained for the reactions in homogeneous solutions. The apparent

(11)

Methanol	70 %	Trace	8 %
Benzene	65 %	Trace	10 %
Vesicle	25 %	Trace	63 %
Cyclophane	23 %	Trace	65 %

(12)

Methanol	87 %	0 %	0 %
Benzene	78 %	Trace	Trace
Vesicle	41 %	23 %	18 %
Cyclophane	40 %	21 %	19 %

(13)

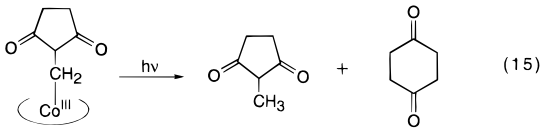
Methanol	88 %	0 %
Benzene	82 %	1.3 %
Vesicle	75 %	9 %
Cyclophane	75 %	13 %

(14)

Methanol	83 %	0 %	0 %
Benzene	80 %	Trace	Trace
Vesicle	61 %	4 %	13 %
Cyclophane	60 %	4 %	14 %

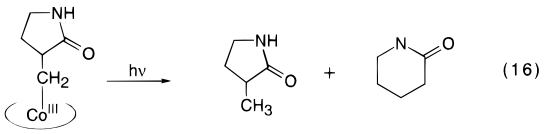
migratory aptitude of electron-withdrawing groups was observed to increase as follows: CN ≈ $CO_2C_2H_5$ < $COCH_3$. The rearrangement reactions, which hardly take place in homogeneous solutions, proceed readily in the vesicle due to a cage effect generated by the apoenzyme model. Such microenvironmental effects provided by the bilayer membrane becomes more pronounced when the hydrophobic vitamin B_{12} is covalently bound to the lipid species.[178−182] A simplified artificial vitamin B_{12} holoenzyme has been constructed with a combination of bilayer membrane and a Costa-type model complex in a similar manner.[183,184]

Even though naturally occurring vitamin B_{12}-dependent enzymes do not catalyze ring-expansion reactions, such reactions are mediated by this artificial enzyme under experimental conditions comparable to those applied to the above-mentioned reactions. As shown in eqs 15 and 16, the ring-expansion reactions are much enhanced in the vesicular phase or the cyclophane cavity.[164,175]



(15)

Methanol	69 %	19 %
Benzene	57 %	28 %
Vesicle	19 %	67 %
Cyclophane	15 %	73 %



(16)

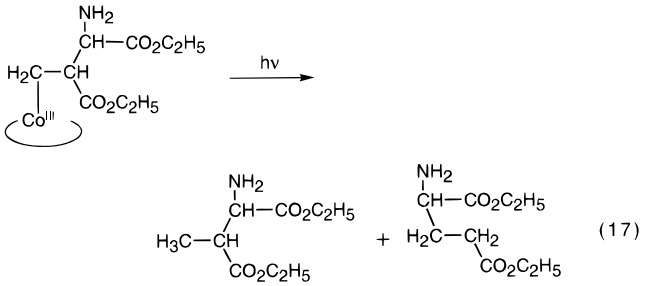
Methanol	83 %	3 %
Benzene	76 %	9 %
Vesicle	63 %	24 %
Cyclophane	61 %	26 %

6. Artificial Glutamate Mutase

Glutamate mutase mediates interconversion between methylaspartic acid and glutamic acid. A model system with apoenzyme functions has been reported by Murakami et al.[165,166,174,177] Diethyl methylasparate was linked to a hydrophobic vitamin B_{12}, and the resulting alkylated complex underwent homolytic cleavage of the Co−C bond upon irradiation with visible light. It is apparent from eq 17 that the isomerization reaction takes place only in the apoenzyme models.

The overall reaction sequence mediated by the artificial methylmalonyl-CoA and glutamate mutases in the single-walled bilayer vesicle is illustrated in Figure 13: a substrate ($CRXYCH_2$−) bound to a hydrophobic vitamin B_{12} undergoes the homolytic Co−C cleavage upon irradiation with visible light, the generated substrate radical is converted into the product radical, and the product radical abstracts a hydrogen atom from its vicinity to give the final product.

A question arises as to the hydrogen source for the formation of the product species, the final step in the overall reaction sequence. Does a hydrogen atom come from a bulk aqueous phase or else from molec-



Methanol	83 %	0 %
Benzene	78 %	Trace
Vesicle	66 %	14 %
Cyclophane	68 %	15 %

(17)

ular species constituting an artificial enzyme? In order to clarify this query, product analyses have been performed for the photochemical cleavage of a hydrophobic vitamin B_{12}, bearing a benzyl moiety at one axial site of the nuclear cobalt, in various deuterated media under anaerobic conditions.[185] The results were obtained as follows: (i) the solvent acts as a hydrogen source in CD_3OD to give the deuterated toluene in a 80% yield; (ii) bibenzyl is exclusively obtained in C_6D_6 which does not act as a hydrogen source; (iii) the major product in the vesicular phase with D_2O as a bulk solvent is the non-deuterated toluene. The observation indicates that a hydrogen atom is taken from the hydrophobic vitamin B_{12} or the lipid molecule and not from bulk water, so that the vesicular domain is well shielded from the bulk aqueous phase and lacks water molecules.

It became necessary to answer the next question as to which functional group migrates preferentially to the neighboring carbon atom, the glycyl group or the carboxylic ester, in a reaction mediated by the artificial glutamate mutase. It became clear by utilizing a deuterated substrate that the glycyl group migrates predominantly in a manner as observed for the corresponding enzymic reaction.[177] Dowd et al. observed that the β-methylaspartate−glutamate rearrangement took place readily via formation of a ketimine Schiff base derivative of diethyl β-methylaspartate, as mediated by vitamin B_{12}.[186,187] On this basis, they suggested the possible formation of such a Schiff base intermediate in the corresponding enzymic reaction. However, the glycyl group can migrate without the Schiff base formation in the apoenzyme model, even though the yield is relatively low under the conditions.

7. Turnover Catalysis

All the above reactions are stoichiometric rather than catalytic, even though the substrate species undergo isomerization. In order to improve this situation, Murakami et al. coupled an effective process for activation of substrates with the catalytic mediator composed of [Cob(II)7C_3ester]ClO_4 and the N^+C_5Ala2C_{16} vesicle.[166] An appropriate amount of [Cob(II)7C_3ester]ClO_4 and a large excess of vanadium trichloride were dissolved in an aqueous medium containing the N^+C_5Ala2C_{16} vesicle and a sufficient excess of a substrate. The solution was irradiated with a 500 W tungsten lamp at 20 °C under aerobic conditions. The overall reaction cycle is shown in Figure 14. A substrate is activated by vanadium(III) ions and molecular oxygen,[160−163] and the resulting radical species undergoes coupling with [Cob(II)-7C_3ester]$^+$ to afford the corresponding alkylated complex. The alkylated complex is subjected to homolytic cleavage to give the original substrate and the isomerized products; the former being subjected to catalysis. Similar reactions proceed in the cyclophane-type artificial enzyme.[164,165]

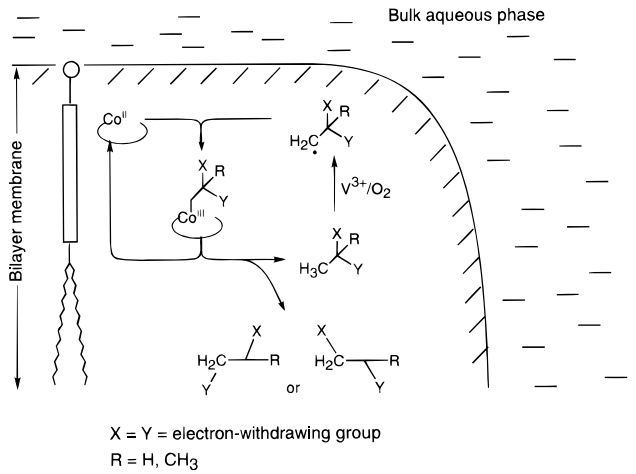


Figure 14. Schematic representation of catalytic carbon-skeleton reactions in the bilayer vesicle. (Reprinted from ref 166. Copyright 1991 The Royal Society of Chemistry.)

The following aspects became apparent from their investigations: (i) A combination of vanadium trichloride and atmospheric oxygen abstracts a hydrogen

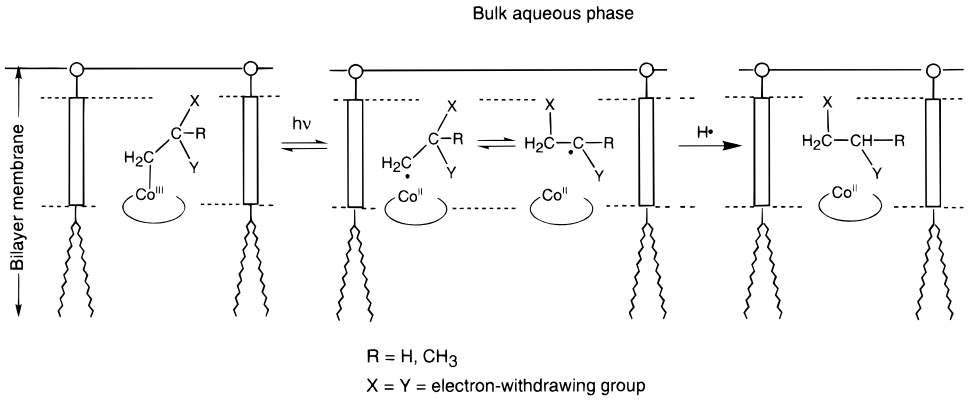


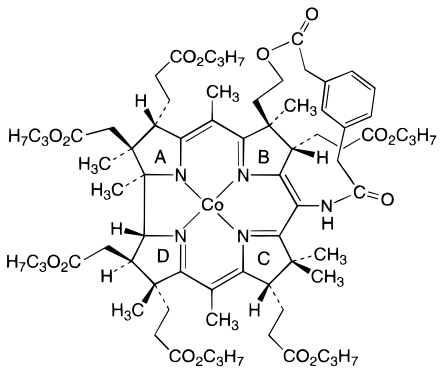
Figure 13. Schematic representation for the photochemical carbon-skeleton rearrangement of an alkyl ligand bound to the hydrophobic vitamin B_{12}. (Reprinted from ref 166. Copyright 1991 The Royal Society of Chemistry.)

atom from the terminal methyl group of a substrate species to form the corresponding radical species, which then undergoes reaction with [Cob(II)7C_3-ester]$^+$ to form the Co−C bond. (ii) Since both suppression of molecular motion and desolvation effects operate on chemical species incorporated into the bilayer vesicle, [Cob(II)7C_3ester]$^+$ and the substrate radical, which are produced by homolytic cleavage of the Co−C bond upon photolysis, must form a tight pair. Under such conditions, the nuclear cobalt acts to promote the 1,2-migration of electron-withdrawing groups. This artificial holoenzyme is expected to be applied to other nonenzymatic reactions that undergo similar reaction mechanisms.

8. Enantioselective Reactions

An important aspect of catalysis performed by vitamin B_{12}-dependent enzymes is chiral recognition toward substrate species. The structural properties of B_{12} and the role of apoenzyme seem to be important to show such functions. Schrauzer et al. investigated the asymmetric alkyaltion of Cob(II)alamin with DL-alanine in the presence of V^{3+} and oxygen radicals, and obtained the alanine-bound cobalamin in a 12% ee of the D-alanine-bound complex.[160] Ogoshi et al. studied the alkylation of Cob(I)alamin with prochiral 1-acetyl-1-alkylcyclopropanes that induces an asymmetric center in the resulting alkyl ligands, 24−33% ee.[188] Golding and his co-workers carried out the alkylation of Cob(I)alamin with *tert*-butyloxirane and methyloxirane, affording preferentially (*R*)-alkyl-bound products in 62 and 50% ee, respectively.[189] It seems such a structural framework of the corrin moiety does not achieve high enantioselectivity in nonenzymatic reactions. Under such circumstances, the corrin moiety needs to be modified so as to provide an asymmetric reaction site on the β-axial site for the alkylation. Murakami et al. have investigated the enantioselective alkylation of hydrophobic vitamin B_{12} derivatives, which bear a chiral binaphthyl moiety, with various racemic 3-bromo-2-methylpropionic esters in methanol, and the (*S*)-enantioselectivity as high as 65% ee was reported.[190,191] Furthermore, they have prepared a novel hydrophobic vitamin B_{12} (strapped hydrophobic vitamin B_{12}; Chart 10) modified by introducing a 1,3-phenylenediacetyl moiety into the peripheral site around the corrin's B ring, and the highest (*S*)-selectivity (75% ee) was reported with the strapped hydrophobic vitamin B_{12}.[192] The enantioselective reaction in an apoenzyme model is a future subject of interest yet to be carried out.

Chart 10

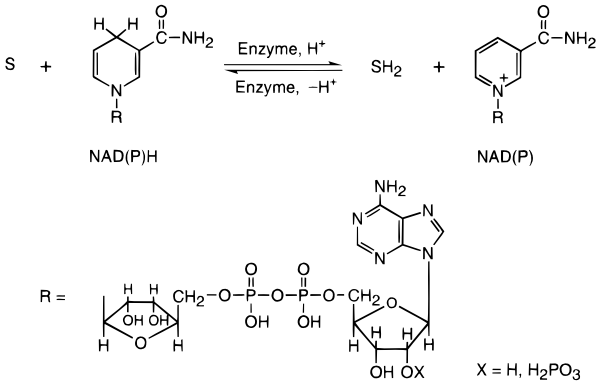


Strapped Hydrophobic Vitamin B_{12}

E. NADH Functions

Nicotinamide adenine dinucleotide (NAD), its reduced form (NADH), and their phosphate esters (NADP and NADPH, respectively) are well-known coenzymes as the pyridine nucleotides which cover a wide range of redox reactions in biological systems. As shown in Scheme 14, NAD(P)H reduces various substrates while it is converted to the corresponding oxidized species, NAD(P).

Scheme 14

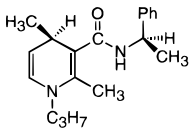


Recent studies on chemistry of the coenzyme NADH have been mainly focused on the following three aspects: details in mechanism of the hydride transfer, the stereoselective reduction, and activation of the reaction.

In order to understand the mechanism of the enzyme-catalyzed reactions of NADH with substrates, the nonenzymatic hydride-transfer reactions from NADH or its model compounds to substrates have been extensively studied. However, there still remains controversy with regard to the hydride-transfer process; whether the hydride transfer occurs in one step or consists of overall transfer of two electrons and a proton in an e^-−H^+−e^- sequence through an intermediate charge-transfer complex.[193−196]

In the active site of the NADH-dependent enzymes either the 4-*pro*-(*R*) or 4-*pro*-(*S*) hydrogen of the 1,4-dihydronicotinamide ring is used properly in the reaction of a substrate to achieve stereoselective reduction. Such stereochemical control is attributable to the chiral ternary complex formed with an apoprotein, a coenzyme, and a substrate. Much effort has been paid to develop NADH mimics being capable of exhibiting high stereospecificity. Ohno et al. synthesized a NADH model with a chiral center at the C-4 position of a 1,4-dihydropyridine ring by replacement of one of the two hydrogen atoms with a methyl group and an additional chiral center at the C-3 moiety (**41**).[197] In the presence of Mg(II) ions,

this NADH model exerted high enantiospecificity in the reduction of certain carbonyl compounds such as α-keto esters and trifluoroacetophenones; methyl benzoylformate was reduced by (*R*,*R*)-**41** to give the (*R*)-mandelate with an enantiomeric excess of 98% in acetonitrile at room temperature. Ohno et al. prepared other NADH analogs each having a chiral center at the C-4 position of the 1,4-dihydropyridine ring and have been investigating the mechanism of stereochemical control.[198−201] They proposed the formation of an intermediate ternary complex in which the Mg(II) ion is located between the substrate and the dihydropyridine and coordinated to both species. Vekemans et al. clarified that a C-4 substituted 1,4-dihydropyridine derivative with achiral carboxamide substituent at C-3 position (**42**) also performed enantioselective reduction of carbonyl substrates in acetonitrile in the presence of Mg(II) ions.[202] Methyl benzoylformate was reduced by (*R*)-



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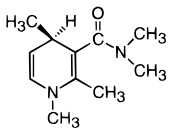


42

42 to afford the (*R*)-mandelate in a 95% ee at −25 °C, whereas the ee value decreased to 66% at 25 °C. The enantioselectivity was reversed by employing (*S*)-**42**. The results imply that the configuration at the C-4 position of the dihydronicotinamide ring determines the enantioselectivity in the reduction of the substrate and that stereochemical nature of the C-3 moiety additionally influences the enantioselectivity. They proposed a structure for the intermediate ternary complex formed during the reaction (Figure 15), which is intrinsically analogous to the molecular arrangement proposed by Ohno et al. as mentioned above.

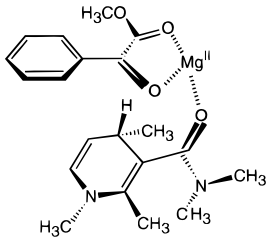
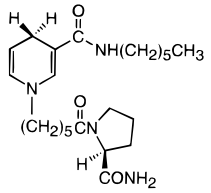


Figure 15. Proposed reaction species for the enantioselective reduction performed by **42** with Mg(II) ions. (Reprinted from ref 202. Copyright 1989 The Royal Society of Chemistry.)

Inoue et al. introduced an L-prolinamide residue as a chiral auxiliary at the N-1 or C-3 position of 1,4-dihydronicotinamide.[203−205] An optical yield as high as 90% was observed in the reduction of ethyl benzoylformate by employing mono- (**43**) and bis-NADH analogs (**44**) in the presence of Mg(II) ions. They assumed that the remote asymmetric induction was due to a stereospecific blockage of one of the diastereotopic faces in the 1,4-dihydronicotinamide by an interaction of the amide functions with Mg(II)



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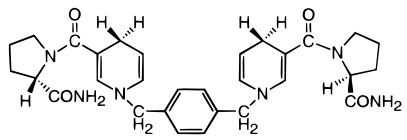
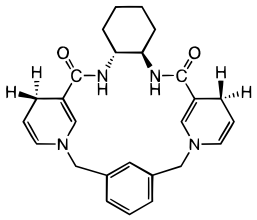
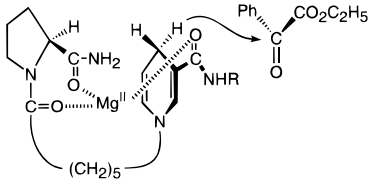


44

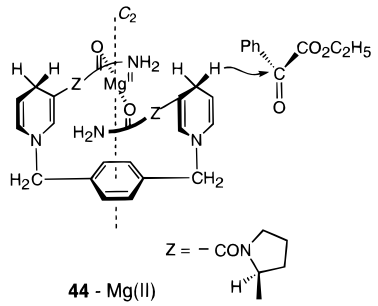


45

ions (Figure 16). Skog and Wennerström prepared a C_2-symmetric macrocyclic bis-NADH analog (**45**), in which hydride transfer occurs in an antarafacial manner.[206] On reduction of methyl benzoylformate with the (*R*,*R*)-form of **45** in dichloromethane in the presence of Mg(II) ions in a temperature range −25 through +25 °C, (*R*)-methyl mandelate was formed in 95% ee.



43 - Mg(II)



44 - Mg(II)

Figure 16. Proposed reaction species for the enantioselective reduction performed by **43** and **44** with Mg(II) ions. (Reprinted from refs 205 and 203. Copyright 1985 the Weizmann Science Press of Israel and 1981 American Chemical Society, respectively.)

Chiral NADH models exhibiting high enantiospecificity were also reported by Bourguignon et al.,[207−211] Davies et al.,[212,213] Gelbard et al.,[214] Iwata et al.,[215] and Meyers et al.,[216−218] and the stereoselective reduction of benzoylformates to the corresponding mandelates were recently reviewed by Davies et al.[219]

As mentioned above, we were able to reproduce stereospecificity of the NADH-dependent enzymes for certain substrates by employing chiral NADH mimics and metal ions. However, reaction rates for such model reactions are extremely low in comparison with those for the natural enzymes. On this ground, several approaches have been made to develop potent NADH mimics with high catalytic efficiency.

In order to manipulate the reactivity of 1,4-dihydronicotinamide by giving the molecule a multicenter

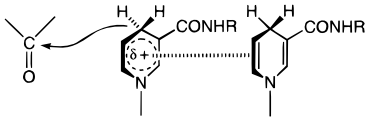
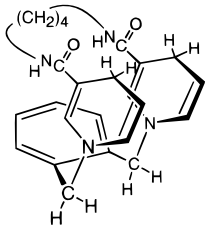


Figure 17. Electronic interaction between two dihydronicotinamide moieties which stabilizes a transition state of the reduction of a carbonyl substrate in apolar environment.

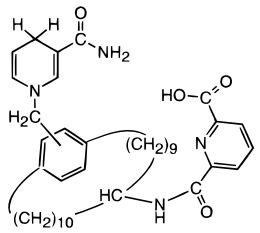
character, Murakami et al. prepared a series of closely related bis(dihydronicotinamide)s.[220,221] The reactivity in dichloromethane was subjected to change depending upon their molecular structures in the reduction of hexachloroacetone. An effective charge-transfer interaction, which emerges from a favorable face-to-face arrangement of the two dihydronicotinamide rings in the transition state of reduction, is responsible for the kinetic enhancement (Figure 17). Such an interaction is favored in less polar solvents such as dichloromethane and chloroform but not in a polar solvent such as acetonitrile which stabilizes the charge-localized transition state. Thus, a cyclic dimer **46** in which the two nicotinamides attain such a favorable conformation already in the ground state, was most reactive among 18 dihydronicotinamides employed. On the other hand, Hirobe et al. prepared



46

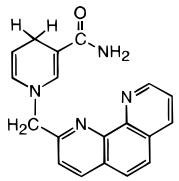
N-benzyl-1,4-dihydronicotinamides having 2-methyl, 6-methyl, and 2,6-dimethyl substituents and investigated their reactivities toward hexachloroacetone.[222] The reaction rates were especially enhanced for the 2,6-dimethyl derivative due to steric and electronic effects.

Several NADH models with the aim of simulating the holoenzyme functions have been reported. Murakami et al. synthesized a [20]paracyclophane bearing 1,4-dihydronicotinamide and 2-pyridinecarboxylic acid moieties (**47**) as an alcohol dehydrogenase model, in which activation of a substrate was expected owing to a substrate−Zn(II) interaction.[223,224] The kinetic

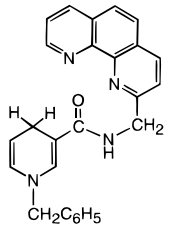


47

behavior in the reduction of a carbonyl substrate, hexachloroacetone, analyzed on the basis of the Zn(II)-coordination modes revealed that a Zn(II) complex of **47** in which the dihydronicotinamide moiety is free from metal coordination exercised a much enhanced activity, while a Zn(II) complex of **47** in which both the dihydronicotinamide and the pyridinecarboxylic acid moieties are simultaneously coordinated to the same Zn(II) ion showed a decreased reactivity relative to the metal-free **47**. Engbersen et al. prepared two phenanthroline-linked 1,4-dihydronicotinamides (**48a** and **48b**) as alcohol dehydrogenase models.[225] In the reduction of 2-pyridinecar-



48a



48b

boxaldehyde (PyCHO) in acetonitrile−chloroform (95:5 v/v) at 25 °C, **48a** was 4 times more reactive than a simple NADH model, 1-benzyl-1,4-dihydronicotinamide, in the presence of 1 equiv of Zn(II) ions. On the basis of detailed kinetic analysis, they concluded that the larger reactivity was due to formation of an active ternary complex composed of **48a**, PyCHO, and Zn(II) ions as shown in Figure 18. On the other hand, addition of more than one equivalent of the metal ions was required for the reaction of PyCHO with **48b**, and the reaction proceeded much more slowly than that with **48a**, presumably due to a geometrical arrangement of the corresponding ternary complex which does not allow an efficient hydride transfer from the dihydronicotinamide to the substrate.

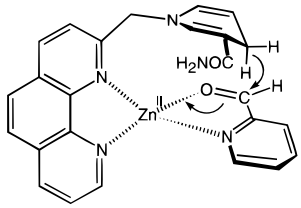
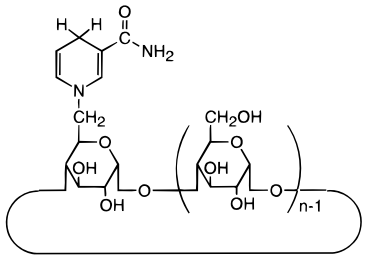


Figure 18. A reactive ternary complex composed of **48a**, a substrate, and Zn(II) ions. (Reprinted from ref 225. Copyright 1990 American Chemical Society.)

Toda et al. introduced a dihydronicotinamide moiety covalently into the C-6 position of a glucose ring of α- and *β*-cyclodextrins (**49**).[226] They examined

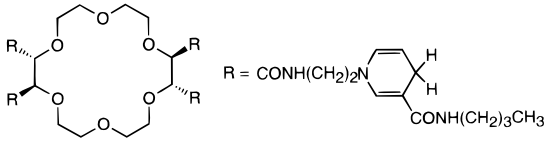


49 (n = 6, 7)

kinetic behavior in the reduction of ninhydrin by the NADH models bearing a hydrophobic cavity in aqueous media at pH 7.0 and 25 °C. Plots of the pseudo-first-order rate constant vs the substrate concentration showed saturation behavior reflecting formation of an intermediate inclusion complex. Dissociation constants (K_M) and rate constants (k_{cat}) as evaluated on the basis of the Michaelis−Menten-type analysis

were 3.5×10^{-2} M and 1.0×10^{-1} s^{-1} for **49** ($n = 6$) and 4.3×10^{-3} M and 4.8×10^{-2} s^{-1} for **49** ($n = 7$), respectively. The k_{cat}/K_M values indicated a large rate enhancement (15- and 50-fold, respectively) for **49** ($n = 6$) and **49** ($n = 7$) as compared with those obtained in the presence of NADH.

On the other hand, Behr and Lehn previously reported that binding of pyridinium substrates to a crown ether bearing the dihydronicotinamide moieties **50** led to enhancement of the hydrogen-transfer rate from the dihydropyridine moiety to a pyridinium within the intermediate complex in acetonitrile at 23 °C.[227] Kellogg et al. synthesized a series of chiral bridged macrocyclic 1,4-dihydropyridines of the crown ether-type and examined the potentiality of these compounds in enantioselective reductions.[228] For example, in the reduction of ethyl benzoylformate by **51** having L-valine residues in the macrocyclic skeleton, (*S*)-mandelate was obtained with an enantiomeric excess of 90% in acetonotrile−chloroform (2:1 v/v) at room temperature in the presence of Mg(II) ions.



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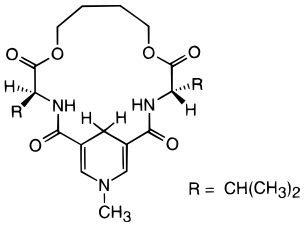
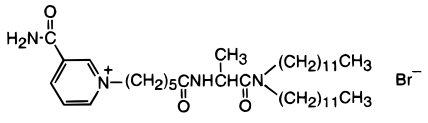


51

Murakami et al. studied the NADH-dependent holoenzyme models by employing synthetic bilayer membranes.[229,230] A peptide lipid bearing the nicotinamide moiety as a polar head (**52**) forms stable single-walled vesicles in aqueous media upon sonication and is subject to molecular organization that is quite favorable for formation of charge-transfer complexes with electron donors such as 3-indolylacetic acid. The remarkably large formation constant observed for the present vesicular system ($K = 10^3$−10^4 M^{-1}), as compared with those for micellar ($K = 10^1$ M^{-1}) and polymer systems ($K = 10^2$ M^{-1}), is not simply due to high local nicotinamide concentrations but reflects the tight side-by-side arrangement of the amphiphile molecules provided by their hydrophobic and hydrogen-belt interactions as well as by an electrostatic interaction (Figure 19). The high orientation of nicotinamide head groups in the tight vesicles of **52** is in favor of forming the 2:1 (electron acceptor−electron donor) ternary complex with effective contact among interacting species upon desolvation.



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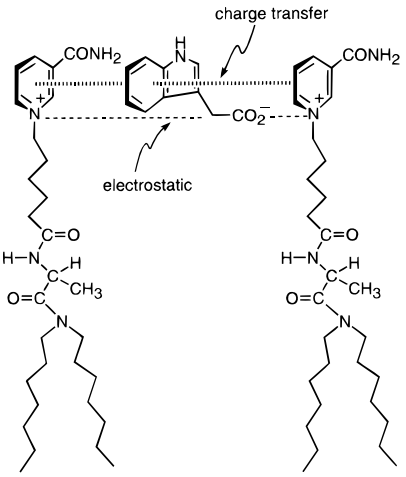
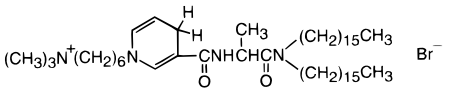


Figure 19. Enhancement of charge-transfer interaction between an indole ring as an electron donor and nicotinamide rings as electron acceptors in the bilayer membrane formed with **52**.

The charge-transfer interaction is also effective in the vesicular system composed of **53**, the 1,4-dihydronicotinamide moiety being the charge-transfer donor. In order to evaluate the reactivity of the



53

present vesicle, the following substrates were used: cationic *N*-methylacridinium chloride and Malachite Green; nonionic trifluoroacetophenone; anionic 2,6-dichloroindophenol and sodium 2,4,6-trinitrobenzenesulfonate. The reaction was carried out in an aqueous phosphate buffer (10 mM) at pH 7.1 and 15.0 °C containing 1% (v/v) ethanol. In general, the reaction rate for reduction with NADH models is greatly influenced by the nature of microenvironment where the reaction takes place. Prior to the kinetic analysis of the substrate binding process with the membrane and the subsequent reduction, a reference reaction was examined by employing *N*-propyl-1,4-dihydronicotinamide in a homogeneous solution composed of ethanol−water (1:1 v/v) which provides the polarity equivalent to that in the reaction site placed in the hydrogen-belt domain of the **53** vesicle. As for the reduction of the cationic and nonionic substrates, each substrate underwent reaction at an identical rate regardless of the nature of media, ethanol−water or membrane. This implies that the electrostatic repulsion by the positively charged surface of the membrane toward the cationic substrates inhibits the effective incorporation of the substrates into the membrane while the hydrophobic interaction between the hydrophobic domain of the membrane and the nonionic substrate prevents the attainment of the effective substrate concentration in the hydrogen-belt domain.

As for the reduction of the anionic substrates, however, the marked rate acceleration was observed in the membrane system. The reaction rates for the reduction of 2,6-dichloroindophenol and 2,4,6-trinitrobenzenesulfonate in the bilayer membrane were enhanced by 72- and 1.1×10^5-fold, respectively, relative to those in ethanol−water (1:1 v/v). The kinetic analysis carried out for the latter substrate

on the basis of the pseudo-phase concept showed that the high reactivity in the bilayer membrane is gained primarily by the effective incorporation of the substrate into the hydrogen-belt domain through electrostatic, hydrogen-bonding, and charge-transfer interactions and partly by the proximity effect which becomes valid between the 1,4-dihydronicotinamide moiety and the bound substrate molecule.

F. Cytochrome P-450 Functions

Cytochrome P-450-dependent monooxygenases are membrane-bound enzymes that catalyze a great variety of reactions, among which epoxidation of alkenes by molecular oxygen is cited as a typical example.[231] The active site of the enzymes involves Fe(III) protoporphyrin IX and an axial thiolate ligand. After the Fe(III) complex is reduced to the Fe(II) state, this site binds and cleaves molecular oxygen, and subsequently water and an oxoiron(V) complex are formally formed. The latter species transfers its oxygen atom to a substrate molecule. Electrons required in the process are provided by NADPH through mediation of a flavoprotein. We limit here our review to catalytic simulation of the enzyme by appropriate models prepared under consideration of apoenzyme functions, although a great number of model reactions have been reported without such functions.[232−235]

Nolte et al. constructed synthetic model systems of cytochrome P-450, which incorporate all possible features of the natural enzyme system, in combination of (i) a membraneously bound metalloporphyrin, (ii) an axial ligand, (iii) an electron donor, (iv) an electron carrier, and (v) a membrane system which holds components within its bilayer or within its inner aqueous compartment.[236,237] Polymerized vesicles, prepared from [tetrakis[4-(hexadecyloxy)-phenyl]porphyrinato]manganese(III) acetate (**54**) and an isocyano surfactant (**55**) by adding nickel capronate, act as a microreactor (Figure 20).[236] Pt(II) ions were placed in their inner aqueous compartments and reduced to a colloidal state by bubbling molecular hydrogen through the dispersion. They carried out the epoxidation of water-soluble and water-insoluble alkenes (2,5-dihydrofuran and styrene, respectively) by the use of Methylene Blue as an electron carrier. When 2,5-dihydrofuran was used as a substrate, 3,4-epoxytetrahydrofuran and a ring-opened compound were detected. As for styrene, only a ring-opened product was detected. Therefore, catalytic systems that mimic enzyme functions can be designed by using polymerized vesicles, although turnover numbers for these substrates are relatively small, 8 and 1.3, respectively, for 2,5-dihydrofuran and styrene.

They also designed a novel bimetallic model system for cytochrome P-450.[237] The system was composed of vesicles containing α-(acetato)[5,10,15,20-tetrakis-(2,6-dichlorophenyl)porphyrinato]manganese(III) (**56**), *N*-methylimidazole as an axial ligand, and an amphiphilic Rh(III) complex (**57**) in combination with sodium formate as an electron donor as shown in Figure 21. They found that the membrane system showed a dramatic efficiency in mimicking the enzymic catalysis. They used two different amphiphiles, the positively charged dimethyldioctadecylammonium chloride (DODAC) and the negatively charged dihexadecylphosphate (DHP), for preparation of bilayer vesicles. Various substrates were epoxidized by the catalytic system prepared in combination with the DHP vesicle, and the turnover numbers were found to be quite high. When α-pinene and styrene were used as substrates, α-pinene oxide and styrene oxide were obtained in turnover numbers of 360 and 55 per hour, respectively. On the other hand, when the DHP vesicle was replaced by the DODAC vesicle,

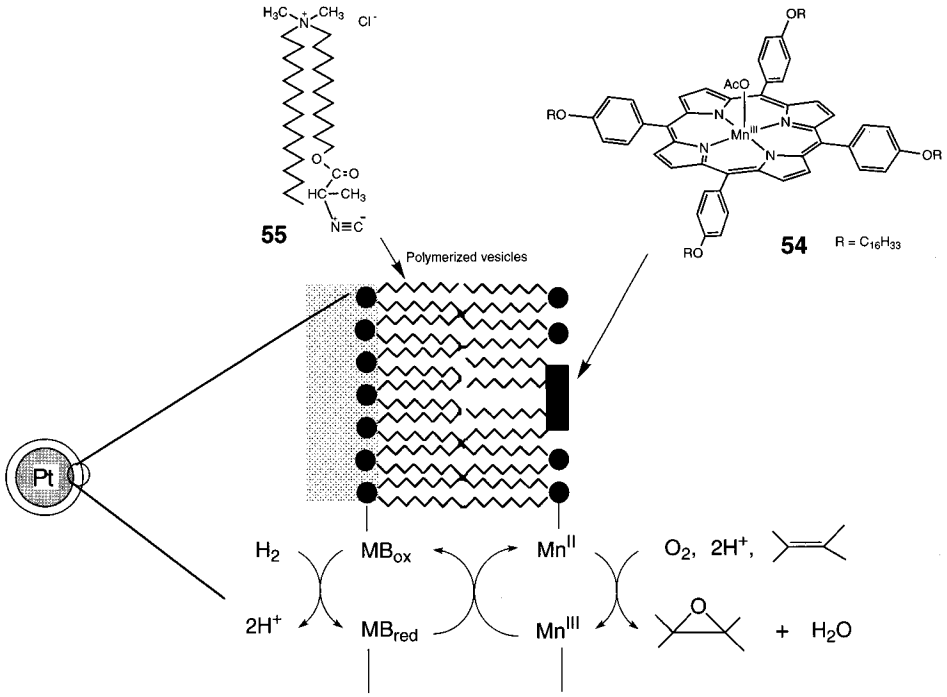


Figure 20. Polymerized vesicle as a microreactor. MB_{ox} and MB_{red} stand for the oxidized and reduced forms of Methylene Blue, respectively. (Reprinted from ref 236. Copyright 1986 American Chemical Society.)

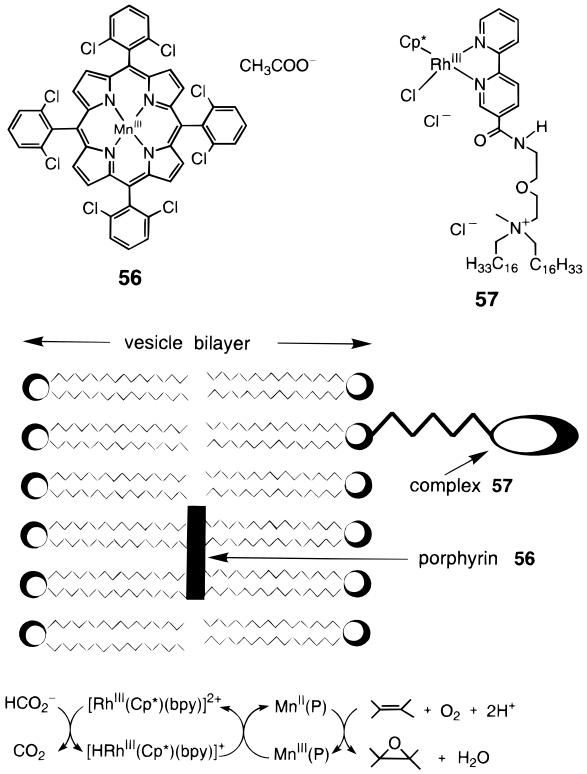


Figure 21. Schematic representation of the cytochrome P-450 mimic and the catalyzed reaction. (Reprinted from ref 237. Copyright 1994 VCH Verlagsgesellschaft mbH.)

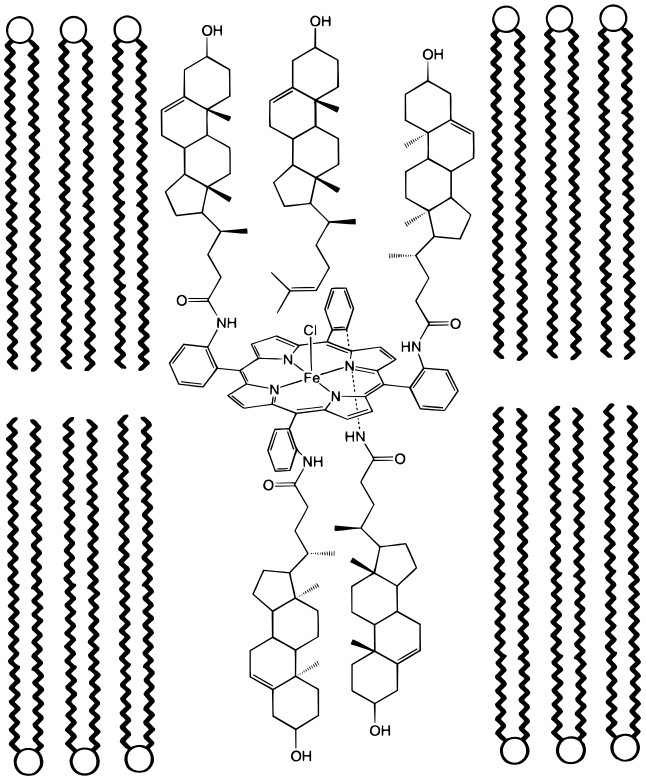


Figure 22. Molecular bilayer assembly of steroidal metalloporphyrin, desmosterol, and phospholipid. (Reprinted from ref 238. Copyright 1989 American Chemical Society.)

the epoxidation did not tale place. The concentration of protons was presumably too low to allow formation of the catalytically active oxomanganese(V) species in the positively charged interface. This bimetallic membrane-bound P-450 model system catalyzed the epoxidation of alkenes in good turnover numbers. The next target of research by using such an efficient artificial system is to obtain good substrate selectivity and enantioselectivity.

Groves et al. investigated the microenvironmental role performed by membranes in the overall enzymatic process.[238,239] They designed and characterized a membrane-spanning porphyrin that associates with phospholipid bilayers and catalyzes the oxygenation of sterols and polyunsaturated fatty acids with remarkable regioselectivity. They constructed a bilayer-type assembly of a steroidal metalloporphyrin, desmosterol, and a phospholipid such as dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) as shown in Figure 22.[238] The steroidal metalloporphyrin was found to be in a well-defined and highly ordered microenvironment within the bilayer membrane. Fe(III) and Mn(III) steroidal porphyrins were used as catalysts for regioselective epoxidation and hydroxylation. Diolefinic sterols were epoxidized exclusively at the side chain. As for epoxidation of linoleic acid, the double bond closer to the hydrophobic terminus (the $C^{12}-C^{13}$ bond) was preferentially epoxidized relative to the double bond closer to the hydrophilic terminus (the C^9-C^{10} bond) at a ratio of 2:1 in the bilayer membrane. The selectivity in epoxide formation (12,13-expoxide/9,-10-epoxide) was raised to 9/1 when rigidity of the bilayer assembly increased by the addition of 20 mol % cholesterol. For these reactions, iodosylbenzene was used for the formation of high-valent metal−oxo species.

They developed a multicomponent vesicular assembly in which electrons derived from the enzymic decarboxylation of pyruvic acid serve to reduce a synthetic, membrane-spanning Mn(III) cholesteryl porphyrin as shown in Figure 23 and to mediate subsequent oxygen activation and transfer.[239] They studied the enzymic reduction of the steroidal manganese porphyrin in the presence of molecular oxygen and ethylbenzene, resulting in formation of acetophenone. The reaction was postulated to proceed as follows (Figure 23): (1) binding of pyruvate oxidase to the vesicles, (2) oxidative decarboxylation of pyruvic acid with the concomitant reduction of enzyme-bound FAD to $FADH_2$, (3) electron transfer from $FADH_2$ to AmFl, (4) reduction of Mn(III) to Mn(II) by the reduced AmFl, and (5) binding and reductive activation of molecular oxygen to produce a high-valent manganese oxo species responsible for hydrocarbon oxidation.

Nango et al. also used a bilayer membrane for functional simulation of the cytochrome P-450 catalysis.[240] The ring hydroxylation of phenylalanine derivatives and toluene with H_2O_2 was catalyzed by halogenated manganese porphyrins in the DPPC vesicle. They compared the reactivity in CH_2Cl_2/H_2O and that in the phospholipid bilayer and indicated that the hydroxylation was enhanced in the DPPC vesicle when imidazole was present.

Water-soluble polymers are often taken for granted as typical apoenzyme models. Limited studies have been reported on the reactivity of polymer-bound porphyrins as a cytochrome P-450 model.[241−243] Hirobe et al. synthesized porphinatoiron(III) covalently

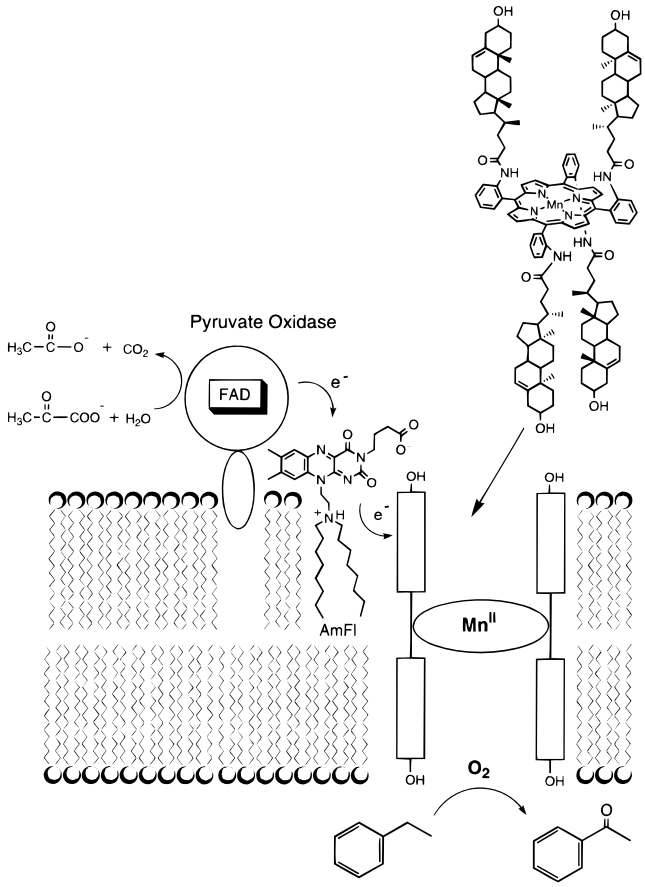
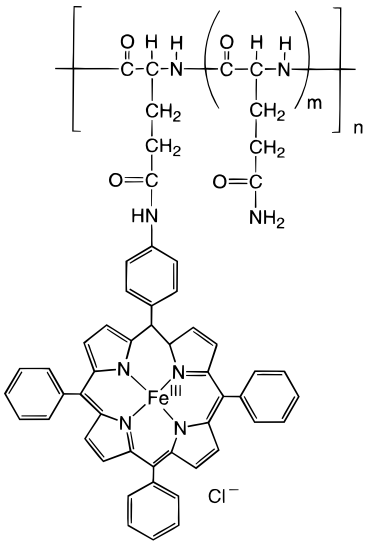


Figure 23. Schematic representation of biocompatible catalysis in a synthetic multicomponent redox membrane containing pyruvate, pyruvate oxidase, amphiphilic flavin (AmF1), steroidal manganeseporphyrin, ethylbenzene, and DPPC. (Reprinted from ref 239. Copyright 1990 American Chemical Society.)

bound to a polypeptide (**58**) and examined its reactivity as a cytochrome P-450 model.[244] With such a

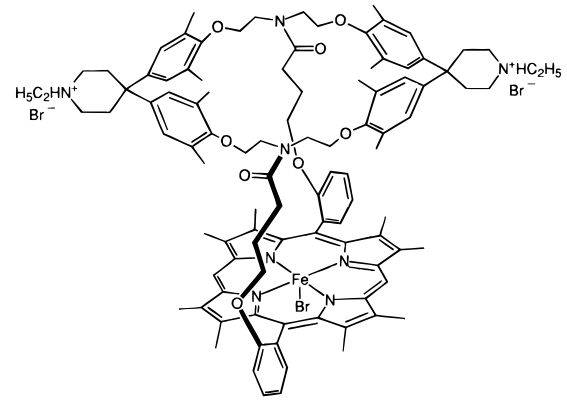


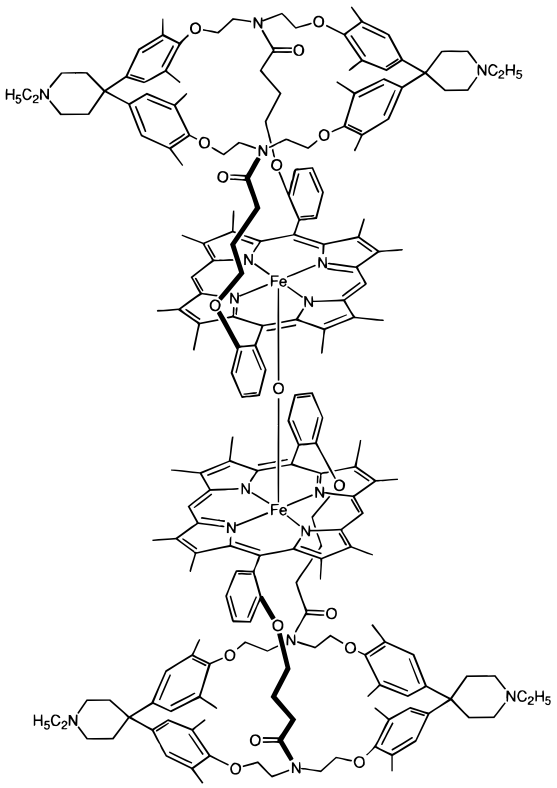
58

model compound, the formation of a μ-oxo dimer of the porphinatoiron was inhibited, and the polypeptide chain around the porphinatoiron modified the reactivity. This polymer catalyzed the hydroxylation of aniline with H_2O_2 effectively and also catalyzed the monooxygenase-type oxidation of olefins in combination with the $O_2-NaBH_4-Me_4NOH$ system more selectively than the corresponding polymer-free porphinatoiron. The polymer-free porphinatoiron oxidized 2-phenylpropene to give the corresponding hydroxylated product, 2-phenyl-1-propanol, in a 41% yield and the coupled product, 2,3-dimethyl-2,3-diphenylbutane, in a 46% yield. On the other hand, the polypeptide-bound porphinatoiron largely suppressed coupling of the olefin (12%) and gave a better yield of 2-phenyl-2-propanol (71%). A similar reactivity was observed when 1,1-diphenylethylene was used as a substrate.

Diederich et al. designed a porphyrin-bridged cyclophane as a cytochrome P-450 model.[245] They prepared the porphyrin−cyclophane having a porphyrin attached to an apolar cyclophane skeleton with two straps. They also synthesized its metal complexes and a μ-oxo dimer (Chart 11). These compounds are freely soluble in protic solvents such as methanol and CF_3CH_2OH, and the Fe(III) derivatives were active catalysts as cytochrome P-450 mimics in those protic environments. In the presence of iodosylbenzene as an oxygen-transfer agent, the

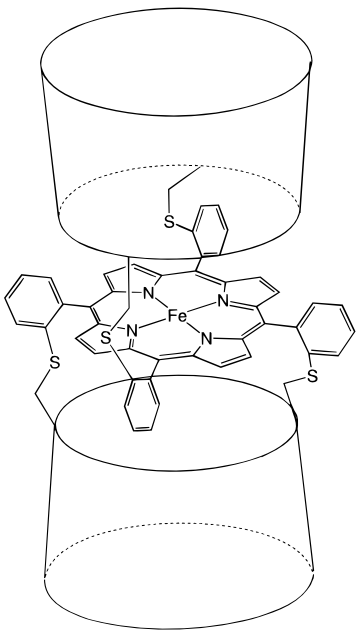
Chart 11





Fe(III) derivative catalyzed oxidation of the productively bound acenaphthylene to acenaphthen-1-one in CF_3CH_2OH. The reaction was suggested to take place in the cyclophane cavity rather than on the open side of the porphyrin. Phenanthrene acted as a competitive inhibitor, possibly as a result of strong but unproductive binding. The μ-oxo dimer, which allows substrate approach to the Fe center only through complexation in the cyclophane cavity, showed a high catalytic activity in the oxidation of acenaphthylene in methanol. In this medium, the dimer was stable enough to suppress dissociation into the two monomers. A major problem encountered in all reactions in alcoholic solvents is the poor yield of the desired product; even in the absence of the iron−porphyrin catalyst, a large portion of the initial acenaphthylene reacts, presumably together with iodosylbenzene and solvent, to give polymeric materials. The nature of this undesirable side-reaction channel, which is not observed with simple Fe(III) tetraphenylporphyrinates in an aprotic solvent like CH_2Cl_2, remains unclear. As an interesting mimic of cytochrome P-450 aromatase activity, isotetralin was converted with turnover behavior by the iron porphyrin-bridged cyclophane to 1,4-dihydronaphthalene and naphthalene in CF_3CH_2OH using iodosylbenzene as an oxygen-transfer agent.

Cyclodextrin is cited as another macrocycle that is capable of acting as a typical apoenzyme model. Ogoshi et al. prepared water-soluble porphyrins sandwiched with cyclodextrin skeletons, which have two binding pockets provided by cyclodextrins (**59**).[246,247] They studied epoxidation of hydrophobic



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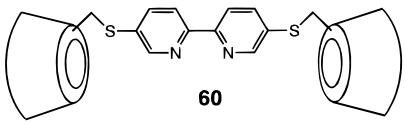
alkenes in an aqueous phosphate buffer using iodosylbenzene as an oxygen source and the cyclodextrin-sandwiched porphyrinatoiron as the catalyst.[248] The epoxidation of cyclohexene was found to proceed effectively, while only a trace amount of cyclohexene oxide was detected with a simple water-soluble porphinatoiron, tetrakis(*p*-sulfonatophenyl)porphinatoiron, as the catalyst. This catalytic effect exercised by the sandwiched porphyrin complex in the epoxidation may be originated from the effective binding of an alkene in the cyclodextrin cavities. Stabilization of an oxene generated in the aqueous media by bulky and hydrophobic cyclodextrin moieties is another possible origin of the effect.

G. Hydrolase Functions

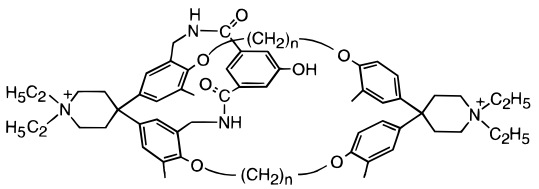
The simulation of hydrolase functions has been extensively studied by utilizing enzyme mimics of various structural types since the mechanisms of these reactions became known. Studies on artificial hydrolases by employing functionalized cyclodextrin derivatives were reviewed by Breslow[249] and Tabushi.[11] Recently, Breslow and Zhang prepared a β-cyclodextrin dimer (**60**) which can bind metal ions with its linker segment.[250,251] With ester substrates



60

that are bound to **60** with the two cyclodextrin cavities so as to place the ester group next to the metal ion, catalytic hydrolysis was observed to take place with good turnover and very high rate acceleration. The mechanism apparently involves attack by metal hydroxide species in a manner as observed with many metalloenzymes. For example, in an aqueous solution at pH 7.0 and 37 °C, the rate of hydrolysis of *p*-nitrophenyl 3-adamantylpropionate by **60** in the presence of Cu(II) ions was 220 000-fold faster than that of uncatalyzed hydrolysis. Furthermore, with excess substrate, at least 50 turnovers were seen in the hydrolysis process. They also found that oxidative hydrolysis of phosphate di- and triesters were much accelerated by cooperative assistance of hydrogen peroxide, La(III) ions, and **60**. The cleavage rate of bis(*p*-nitrophenyl) phosphate in the presence of an excess amount of hydrogen peroxide at low La(III) concentrations, pH 7.0, and 25 °C, was accelerated by 9.7×10^4 times in the absence of **60** and 300×10^4 times in its presence relative to the uncatalyzed reaction. At higher La(III) concentrations, **60** gave no acceleration effect and became even a weak inhibitor. As for a phosphate triester, methyl bis(*p*-nitrophenyl) phosphate, there was no observable catalysis by hydrogen peroxide and La(III) ions. However, the hydrolysis of the triester was 384 times accelerated by cooperative catalytic assistance of **60**, hydrogen peroxide, and La(III) ions, at rather high La(III) concentrations.

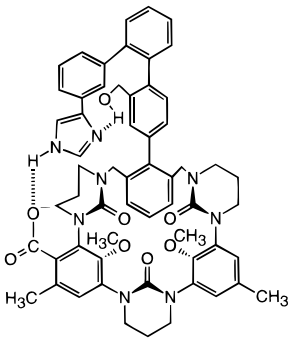
Murakami et al.[4] and Tabushi et al.[3] have developed various types of cyclophanes with a sizable hydrophobic cavity as fully synthetic hydrolase mimics and have reviewed them previously. Diederich et al. prepared cyclophanes possessing the phenolic hydroxyl group as a nucleophile positioned in a well-defined way atop the macrocyclic binding site (**61**).[252]



61a (n = 4)
61b (n = 6)

In phosphate buffer at pH 8.0 and 20 °C, the larger macrocycle (**61b**) accelerated the cleavage of 4-nitro-1-naphthyl acetate relative to the reaction mediated by the smaller one (**61a**) even though both reactions were promoted relative to the reaction in the same buffer without mediators. The reactions catalyzed by the two very similar hosts differ entirely in their kinetics. A Michaelis−Menten-type saturation kinetics was only observed when mediated by **61b**, whereas the cleavage of the ester in the presence of **61a** strictly followed second-order kinetics. On the basis of apparent second-order rate constants, the reaction catalyzed by **61b** was 22 times faster than the reaction in the presence of **61a**.

With an aim at mimicking the catalytic function of α-chymotrypsin, Cram et al. challenged the synthesis of an ultimate target host (**62**) having an arrangement of functional groups roughly identical with that in the enzyme.[253] Unfortunately, however,



62

they have not obtained the final target compound up to the present time. They prepared a precursor of **62** lacking only the carboxylate group and evaluated its potency as a transacylase mimic. In pyridine−chloroform, a substrate, the salt of *p*-nitrophenyl alaninate, instantaneously acylated the imidazolyl group of the precursor host via formation of an intermediate host−guest complex, and subsequently an acyl transfer reaction from the imidazolyl group to the phenolic hydroxyl group took place slowly. Although the mechanism for their reaction is different from that proposed for α-chymotrypsin catalysis, the reactivity for acylation of the imidazolyl group of the host was higher by a factor of 10^{10} than that for the corresponding reaction with 4-phenylimidazole.

Sanders et al. created a cyclic porphyrin trimer (**63**) in which convergent binding sites are positioned in such a way that substrate molecules can be held in close proximity through metal-coordination interactions.[254] An acyl transfer reaction of *N*-acetylimidazole with 4-(hydroxymethyl)pyridine in dry toluene solution at 70 °C was effectively catalyzed by **63**, showing a turnover behavior, via formation of a tetrahedral intermediate doubly bound to the inside cavity of the trimer (Figure 24); **63** increased the initial rate by some 16-fold relative to the uncatalyzed reaction.

On the other hand, artificial receptors, capable of recognizing a guest species through multiple hydro-

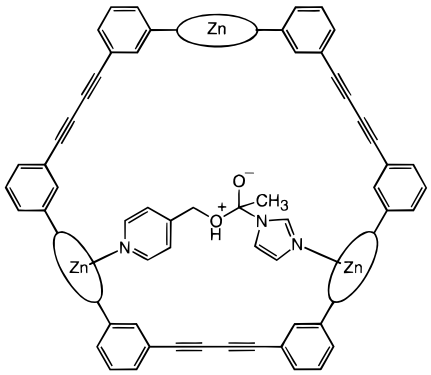
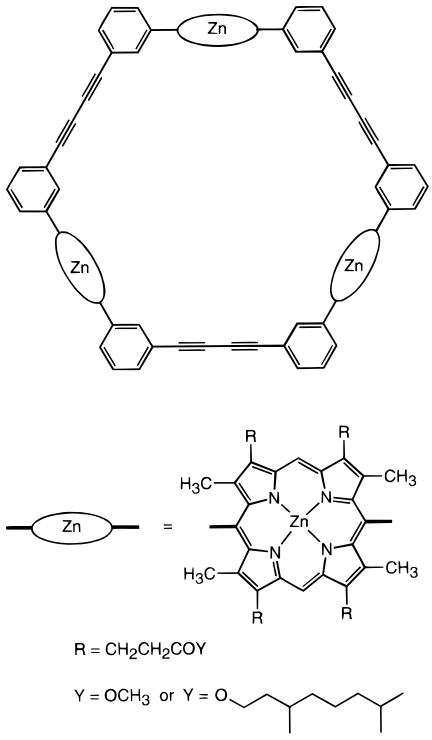
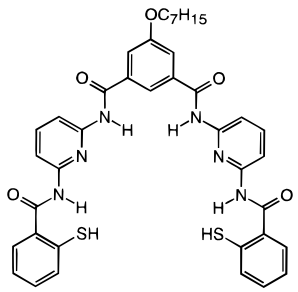


Figure 24. Tetrahedral intermediate doubly bound to the inside cavity of **63**. (Reprinted from ref 254. Copyright 1994 American Chemical Society.)



63

gen-bonding interactions with highly directional character, were utilized as enzyme mimics. Such a receptor (**64**) prepared by Tecilla and Hamilton strongly bound a barbiturate ester through six hydrogen bonds (Figure 25) and accelerated the ester cleavage in dichloromethane at 25 °C by the thiol nucleophile covalently involved in the receptor and placed close to the carbonyl group of the guest; the rate acceleration was more than 10^4 as compared with the reaction by a simple thiol.[255]



64

Anslyn et al. synthesized a bis(alkylguanidinium) receptor (**65**) as a staphylococcal nuclease mimic and

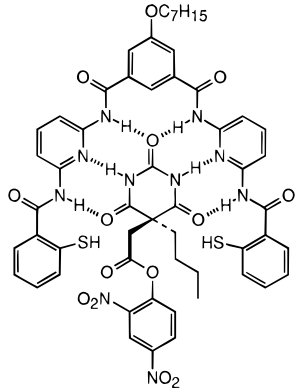
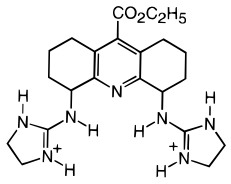
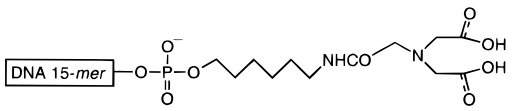


Figure 25. Effective binding of a barbiturate ester by **64**. (Reprinted from ref 255. Copyright 1990 The Royal Society of Chemistry.)

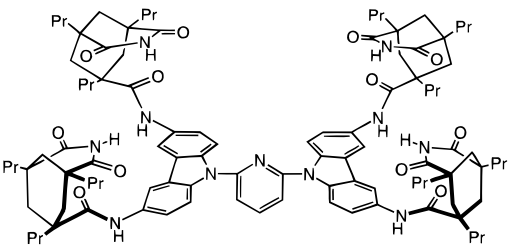
found that **65** increased the imidazole-catalyzed mRNA hydrolysis by a factor of 20 in aqueous media at pH 7.05 and 37 °C.[256,257] Analogous nuclease mimics that possess two guanidinium-like moieties were also reported by Göbel et al.[258] and Hamilton et al.[259] Komiyama et al. found that the sequence-selective hydrolysis of RNA proceeded by employing Lu(III) complexes bound to a DNA oligomer (**66**).[260] Rebek et al. examined a template effect furnished by synthetic receptors derived from Kemp's triacid in aminolysis of *p*-nitrophenyl ester with aminoadenosine.[261] They observed a receptor (**67**) accelerated the reaction 160-fold via formation of a termolecular complex composed of the receptor and two kinds of the substrates.



65

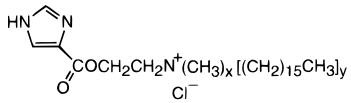


66



67

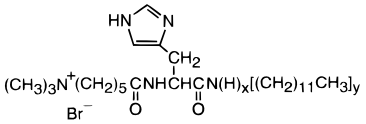
It is well known that micelles and bilayer membranes provide appropriate hydrophobic reaction sites in aqueous media for mimicking hydrolase catalysis.[29] Moss and Kim studied catalytic efficiency of double- and single-chain amphiphiles (**68a** and **68b**, respectively), each having an imidazolyl group in the polar head moiety, in the cleavage of *p*-nitrophenyl esters in aqueous media.[262] They claimed that an enhanced reactivity of the vesicle-forming



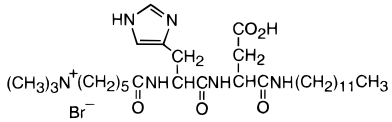
68a (x = 1, y = 2)
68b (x = 2, y = 1)

68b relative to the micelle-forming **68a** is originated from greater binding ability of the former toward the substrates. In addition, the molecular orientation of the catalytic group relative to the substrate is more critically controlled in the bilayer membrane in comparison with the micellar aggregate.

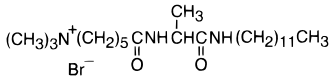
Murakami et al. prepared cationic peptide surfactants bearing a histidine (**69a**) and both histidine and aspartate residues (**70**) and examined their catalytic behavior in the hydrolysis of *p*-nitrophenyl carboxylates in comicellar phases formed with a peptide surfactant having an alanine residue (**71**) in aqueous media at 30.0 °C.[37] The hydrolysis of *p*-nitrophenyl



69a (x = 1, y = 1)
69b (x = 0, y = 2)



70



71

hexanoate in those comicelles was much enhanced relative to the corresponding uncatalyzed reaction; the rate acceleration at pH 7.3 was 28- and 41-fold for the **69a**−**71** and the **70**−**71** comicelles, respectively, at highest concentration of each catalyst. It is noteworthy that the reactions in the present tight micellar system predominantly underwent general-base catalysis by the imidazolyl group over its normal nucleophilic catalysis; the contribution of the former mechanism was ca. 65% in both comicelles. In addition, the hydrolytic activity of a comicelle composed of **69a**, **71**, and an additional peptide surfactant having an aspartate residue in place of the alanine residue of **71** was lower than that of the **70**−**71** comicelle, and similar to that of the **69a**−**71** comicelle. On the basis of these results and their pH−rate profiles, they proposed a reaction mechanism for catalysis by the **70**−**71** comicelle, shown in Figure 26, which bears a close resemblance to that predicted for the hydrolysis catalyzed by the triad system of serine proteases.

Murakami et al. reported that a single-walled bilayer vesicle, formed with a synthetic peptide lipid (**69b**) having an L-histidine residue, interposed between a polar head moiety and a hydrophobic double-chain segment, catalyzed the enantioselective hydrolysis of an amino acid ester, *p*-nitrophenyl *N*-(benzyloxycarbonyl)-L(D)-phenylalaninate.[263] Both reactivity and enantioselectivity in the bilayer vesicle

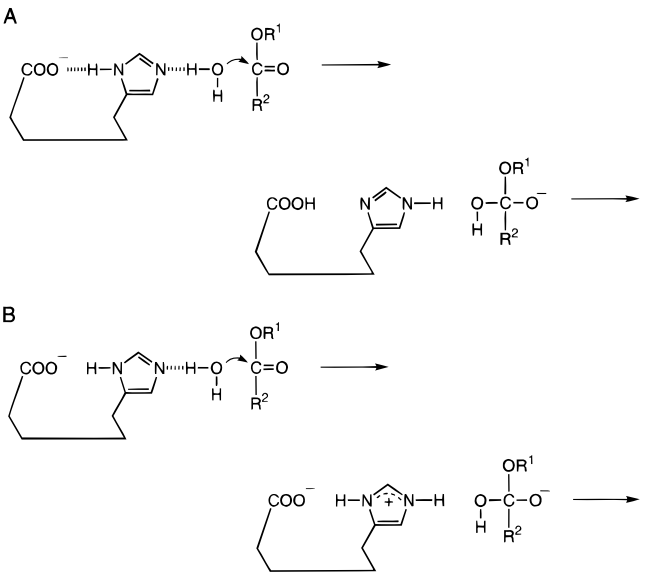
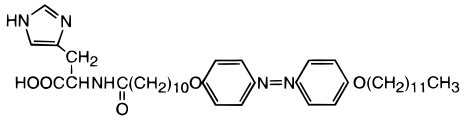


Figure 26. Two plausible reaction mechanisms proposed for hydrolysis of *p*-nitrophenyl ester as catalyzed by the **70**−**71** comicelle: (A) two proton migration; and (B) one proton migration. (Reprinted from ref 37. Copyright 1981 American Chemical Society.)

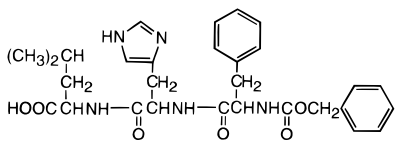
were larger than those in the micellar system formed with the corresponding single-chain amphiphile (**69a**). The enantioselectivity values, k_{obsd}(L)/k_{obsd}(D), were 2.5 and 3.3 at 4 °C and 2.7 and 4.4 at 20 °C for the aggregates of **69a** and **69b**, respectively, in phosphate buffer at pH 6.55. The hydrolysis of the L-form at 20 °C was accelerated by factors of 5 690 and 22 100 by the catalysis of **69a** and **69b**, respectively, as compared with the reactivity in the absence of the catalyst. Such advantages of the bilayer system as compared with the micellar system presumably comes from orderly molecular arrangement performed in the bilayer membrane.

Kunitake et al. prepared an azobenzene-containing amphiphile (**72**) that contains the L-histidine head and examined the hydrolysis of a *p*-nitrophenyl ester in the dialkylammonium bilayer matrix.[264] They observed the reaction control by phase separation of the membrane which was induced by either change of the medium pH or complexation of the catalyst with Cu(II) ions.



72

Ueoka et al. observed large enantioselectivity in the hydrolysis of *p*-nitrophenyl *n*-dodecanoyl-D(L)-phenylalaninate with an LLL-tripeptide catalyst (**73**) in a coaggregate system, which was composed of double- and single-chain amphiphiles.[265−268] The enantioselectivity is very sensitive to molecular packing modes



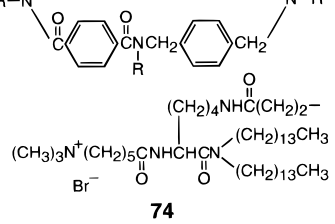
73

of the matrix molecules in the aggregates, which comes from composition of the amphiphiles, the ionic strength of medium, and the reaction temperature. In a particular case, they observed perfect enantiospecificity for the L-form in a vesicular system composed of 59 mol % ditetradecyldimethylammonium bromide and 41 mol % hexadecyltrimethylammonium bromide in 20 mM Tris/20 mM KCl buffer at pH 7.6 and 30 °C. Such remarkable enantioselective catalysis was also observed in specific coaggregates composed of a cationic double-chain surfactant and an anionic or a nonionic single-chain surfactant.

H. Application to Nonenzymatic Reactions

One of the goals for development of the artificial enzymes is utilization of them as catalysts for reactions which cannot be mediated by natural enzymes.

Murakami et al. examined the photoreaction of 2-azidobiphenyl to afford carbazole in the presence of an octopus cyclophane (**74**).[269] Both 2-azidobiphenyl and pyrene, a sensitizer, were simultaneously incorporated into the cyclophane cage through the hydrophobic interaction in aqueous solution, and the pyrene-sensitized photoreaction of the former species was enhanced in the ternary complex (Figure 27); the reaction rate was increased by 6.7-fold upon addition of 0.1 mM of **74** (2-fold molar excess to the substrate and the sensitizer) in 4% v/v ethanol−water at 20 °C.



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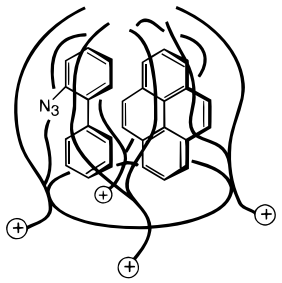


Figure 27. Schematic representation of the reactive ternary complex composed of **74**, 2-azidobiphenyl, and pyrene molecules. (Reprinted from ref 269. Copyright 1988 The Royal Society of Chemistry.)

Kelly et al. prepared a reaction template (**75**) which was designed to use hydrogen-bonding interactions to bind two substrates simultaneously, resulting in the formation of a ternary complex.[270] They studied the S_N2 alkylation of an amine by an alkyl halide in $CDCl_3$ at 25 °C and showed that **75** accelerated the reaction by 12-fold through the formation of a ternary

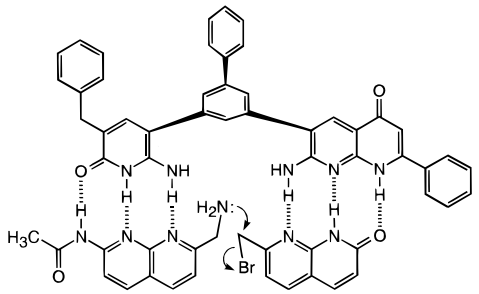
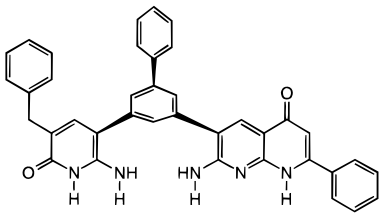


Figure 28. Schematic representation of the reactive ternary complex composed of **75** and two kinds of substrates. (Reprinted from ref 270. Copyright 1990 American Chemical Society.)

complex as derived by complemental hydrogen-bonding interactions (Figure 28).



75

Mock et al.[271] investigated the 1,3-dipolar cycloaddition of an alkyne, propargylamine or *N-tert*-butylpropargylamine, to an azide, azidoethylamine or *N-tert*-butylazidoethylamine, to afford the corresponding triazole derivative as catalyzed by a polycyclic molecular receptor, cucurbituril (**76**), in aqueous formic acid at 40 °C. They found that a catalytic amount of **76** markedly accelerated (ca. 10^5-fold) the reaction regiospecifically, yielding only the 3,5-disubstituted triazole as a product. This stereospecificity was attributed to the formation of a transient ternary complex between **76** and the substrates (Figure 29). Simultaneous binding of both substrates

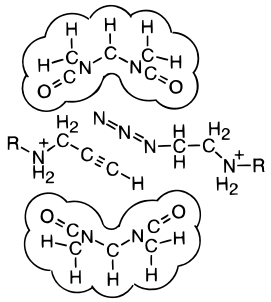
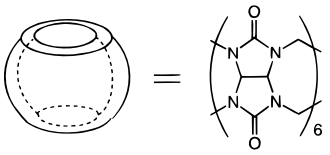


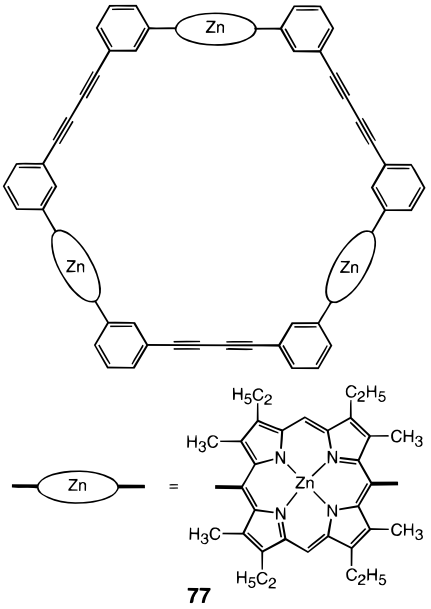
Figure 29. Conjectured cross-sectional representation of the reactive ternary complex composed **76** and two kinds of substrates. (Reprinted from ref 271. Copyright 1989 American Chemical Society.)

aligns the reactive groups within the core of **76** so as to facilitate production of the triazole. The reaction was claimed to be enhanced by strain-induced compression between these substrates incorporated into the three-dimensionally restricted cavity.



76

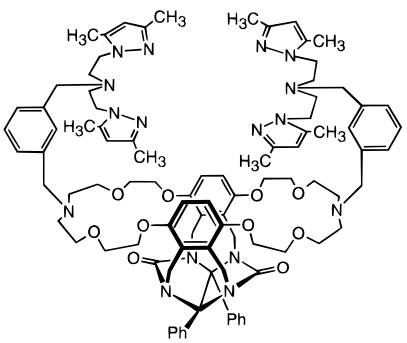
Sanders et al. employed a porphyrin trimer (**77**) as an enzyme mimic and examined the reversible Diels−Alder reaction between a furan-derived diene and a maleimide-derived dienophile, both having a pyridine moiety as a ligand to Zn(II) ions.[272−274] In



77

the absence of any host, the reaction afforded two products, the kinetically favored *endo*-adduct and the thermodynamically favored *exo*-adduct in $C_2H_2Cl_4$. Addition of one equivalent amount of the trimer to the two reactants resulted in acceleration of the forward Diels−Alder reaction by ca. 1000-fold at 30 °C and 200-fold at 60 °C, yielding the *exo* adduct as the only detectable product. On the basis of kinetic analysis of the reaction, they claimed that the observed positive templating effect is partly due to concentration of the diene and the dienophile within the host cavity and partly due to a reduced activation energy caused by preliminary binding of the reacting species.

Nolte et al. synthesized a novel metallohost containing a substrate binding site and two Cu(II) ions held by two bis pyrazole ligand sets (**78**).[275] The host



78

cavity is capable of binding dihydroxybenzene guests; association constants in chloroform were in the range of 2000−3000 M^{-1}. In the presence of benzylic alcohols the Cu(II) centers of the metallohost were reduced to Cu(I). During this process the alcohols were oxidized to aldehydes. Benzylic alcohols possessing phenolic hydroxyl functions are extremely effective in the reduction, because they are bound to

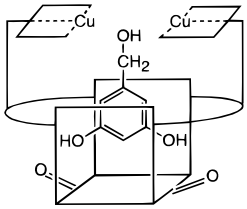


Figure 30. Proposed orientation of 3,5-dihydroxybenzyl alcohol in the cavity of $Cu(II)_2$−**78**. (Reprinted from ref 275. Copyright 1994 American Chemical Society.)

the host cavity and are oriented in the right direction for interaction with the copper centers (Figure 30); a rate enhancement by at least 4 orders of magnitude for the oxidation reaction as compared to the rate with benzyl alcohols lacking the hydroxyl group.

IV. Future Perspective

It became apparent from the foregoing discussions that microenvironmental properties are quite influential in the catalytic activities of coenzyme factors. In order to simulate catalytic functions of holoenzymes, structural modifications of coenzyme factors need to be accompanied with collaboration of microenvironmental effects provided by apoprotein models. When we design apoprotein models, these models must furnish hydrophobic microenvironments in their internal domains, so that the reaction sites are reasonably separated from the bulk aqueous phase. Such hydrophobic microenvironments seem to act to repress molecular motion of reacting species incorporated there and to make them bare due to desolvation. Furthermore, noncovalent intermolecular interactions, such as electrostatic, charge-transfer, and hydrogen-bonding modes, become further pronounced under the hydrophobic and water-lacking microenvironments. In this regard, our guiding principle for designing apoprotein models, which emphasizes the importance of hydrophobicity in the first place, has been approved in the light of accumulated research accomplishments summarized here. However, with further efforts, more elaborated apoprotein models will be able to enhance the catalytic activities of coenzyme factors to greater extents and the resulting artificial holoenzymes will become favorable for formation of desired enantiomers.

This review article primarily summarizes catalytic performance of artificial enzymes individually. However, many enzymic catalyses are involved in each living body and are in efficient equilibria among them to maintain material balances. The next target of our studies on artificial enzymes is to establish multicatalytic systems through functional alignments of artificial enzymes in a sequential manner. Such a multicatalytic system may be also designed in a combination of artificial enzymes with natural ones and the resulting system may even enhance the catalytic activity of each artificial enzyme involved in the system as a consequence.

We are expecting that there is a bright perspective on future development of efficient artificial enzymes, which will lead to establishment of a revolutionary concept in catalysis.

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