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ARTIFICIAL REDOX ENZYMES

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A general method for synthesis of artificial redox enzymes has been offered. Some of the catalytic properties of these systems have been studied and presented.

Предложен общий метод синтеза искусственных окислительно-восстановительных ферментов. Изучены и представлены некоторые каталитические свойства этих систем.

Առաջարկվել է ընդհանուր մեթոդ արհեստական օքսիդա-վերականգնող ֆերմենտների սինթեզի համար: Ուսումնասիրվել և ներկայացվել են այդ սիստեմների որոշ կատալիտիկ հատկանիշները:

Introduction

Enzymes are proteins with catalytic activity that exhibit high specificity and large rate accelerations [1]. Although enzymes are large and complex molecules, their power to catalyze reactions can he attributed mainly to binding and catalysis. Binding is not only responsible for the specificity of the reaction but also by stereochemistry brings the substrate in close proximity and in the correct orientation to the active site. Other factors, such as the microscopic environment of the catalytic site and the stabilization of the transition state by hydrogen bonding, contribute to enzymatic activity, but binding and catalysis are the two essential features of all enzymes [2]. The field of artificial enzymes deals with the science of synthetically mimicking the exact nature of the binding subsite in terms of shape, size, and microscopic environment as well as mimicking the active site in terms of identity of groups, stereochemistry, interatomic distances of various groups, and the mechanism of action of the enzyme [3].

Binding: Binding of the substrate to the enzyme is an essential feature of all enzymatic reactions. Although covalent enzyme-substrate bonds are formed during some reactions, usually binding of the substrate to the active site of the enzyme involves non-covalent forces such as hydrophobic, van der Waals, or London dispersion forces, hydrogen bonding, and electrostatic interactions. Cumulative effects of such forces produce tight binding if the binding subsite is complementary to the structure of the substrate or if conformational changes occur in the enzyme during the binding process [4]. Cyclodextrins, consisting of 6, 7, or 8 units of α-1,4- linked D-glucopyranoses, have played an important role as binding sites in artificial enzymes. They are doughnut shaped molecules with secondary hydroxyl groups at the 2- and 3-positions of glucose units arranged in the more open end and primary hydroxyl groups at the 6-position at the other end. The interior of the cavity, consisting of a ring of C-H groups, a ring of glycosidic oxygen atoms, and another ring of C-H groups, is hydrophobic in nature, similar to the binding subsite of many enzymes. The inner diameter of the cavities are approximately 4.5 Å in α-cyclodextrin, 7.0 Å in β-cyclodextrin, and 8.5 Å in γ-cyclodextrin. α- and β-cyclodextrins fit an aromatic ring snugly with dissociation constants varying from 10^{-2} to 10^{-3} M, depending on the

substituent on the ring [5].

Catalysis: Enzymes use a variety of catalytic groups depending on the type of reaction that needs to be catalyzed [6]. These catalytic groups act intra-molecularly with the bound substrate to bring about large rate accelerations. The importance of intra-molecularity in enzymatic catalysis has been well established. There are several reviews which details the reasons for the large rate accelerations in intra-molecular reactions [7]. One of the widely used catalytic group for redox reactions is riboflavin. Flavoenzymes consisting of a riboflavin cofactor bound non-covalently to a substrate binding site play key roles in electron transport, respiration, photosynthesis, bioluminescence, and other oxidation-reduction systems [8,9,10].

Synergism of Binding and Catalytic Groups To Produce an Artificial Redox Enzyme: The design of the artificial redox enzyme consists of a cyclodextrin molecule acting as a binding site covalently attached to a flavin molecule acting as a catalytic site as shown in the Figure 1.

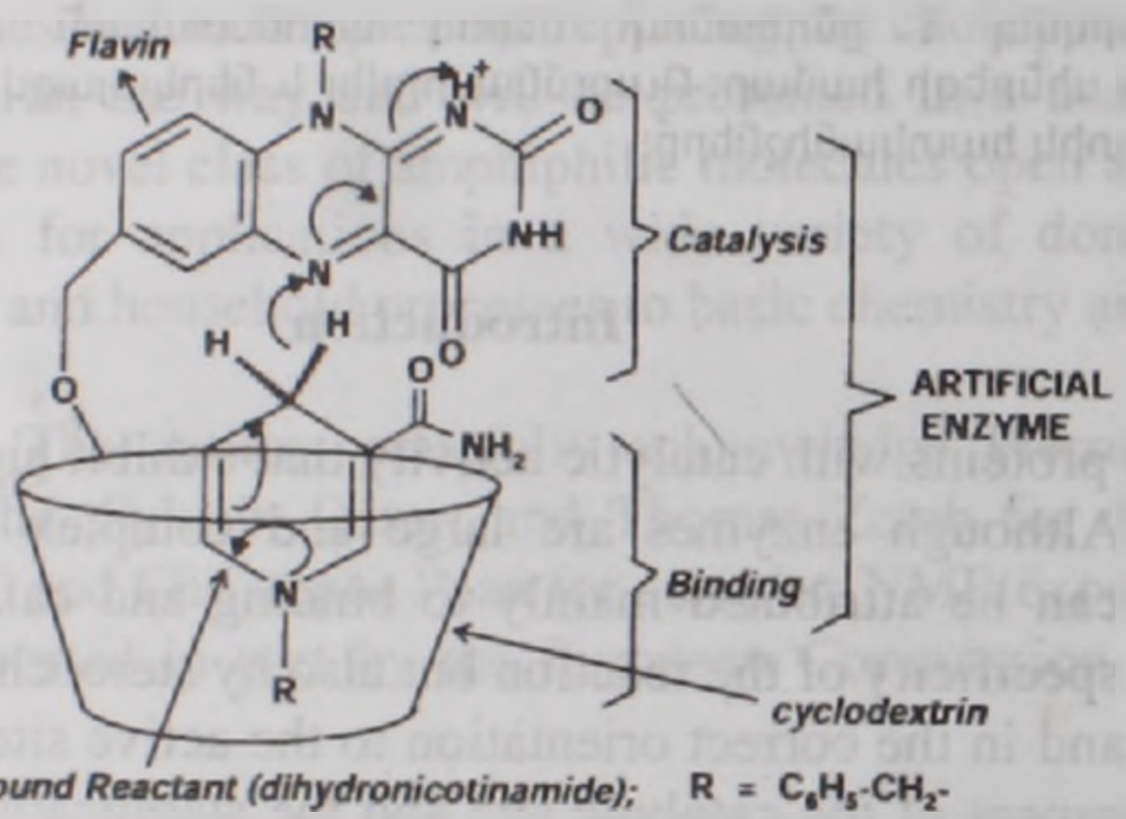


Figure 1. Schematic representation of an artificial redox enzyme.

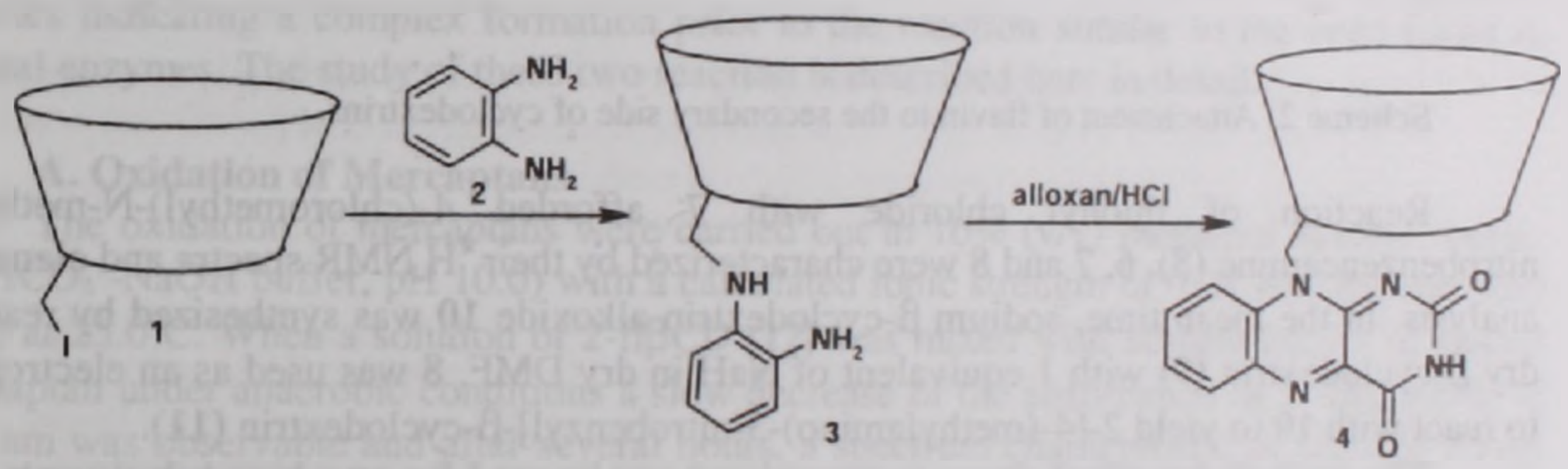
Synthesis of Artificial Redox Enzymes

Synthesis of artificial enzyme consists of attachment of a flavin derivative to cyclodextrins. Cyclodextrins have hydroxyl groups on its primary as well as secondary sides. Potentially, the flavin can be attached to either of these sides and the efficiency of the artificial enzyme depends on the orientation of the substrate bound in the cavity of cyclodextrin.

Attachment of Flavin to the Primary Side of Cyclodextrin. An approach for synthesis of artificial enzyme with flavin attached to the primary side of cyclodextrin involves synthesis of an o-phenylenediamine derivative of cyclodextrin and followed by its condensation with alloxan to give the desired flavocyclodextrin [11]. However, in the synthesis of flavins, the condensations of alloxan with o-phenylenediamine derivatives are carried out at high temperature [12] in the presence of acids such as sulfuric acid, hydrochloric acid, acetic acid, and boric acid [13]. Since cyclodextrins hydrolyze under acidic conditions at high temperature this approach can be successful only if the o-phenylenediamine derivative of cyclodextrin can be condensed with alloxan under conditions mild enough to minimize acid induced decomposition of cyclodextrin. Approximately one percent of the ring of p-cyclodextrin are hydrolyzed in 1.15N HCl at

60°C in 30 minutes [14]. Assuming that the rate of cleavage of mono-functionalized P-cyclodextrin is the same as that of the β-cyclodextrin, the amount of cyclodextrin hydrolyzed under these conditions can be tolerated in a reaction sequence.

Following Scheme 1, the reaction of 6-iodocyclodextrin (**1**) with a large excess of o-phenylenediamine (**2**) gave 6-(2-aminoanilino)-β-cyclodextrin (**3**). Upon work up, the TLC showed a single spot which had UV absorbance and charred upon H_2S0_4/heat treatment indicating the presence of both the phenylenediamine moiety as well as the cyclodextrin moiety in the product. ^1H and ^{13}C NMR spectra indicated that one o-phenylenediamine molecule was attached to the 6-position of β-cyclodextrin. The ^1H NMR spectrum showed all the peaks of β-cyclodextrin and multiplets of aromatic peaks of o-phenylenediamino moiety in the range between 6.3 - 6.6 ppm. The ^{13}C NMR peaks at 45.3, 70.0 and 84.5 ppm for C'6, C'5 and C'4 of the substituted glucose unit are shifted 15.1 and 2.0 ppm upfield and 2.8 ppm downfield respectively from the original peaks for C6, C5 and C4 of β-cyclodextrin indicating that the substiutent is at the 6-position [15]. However, the color of an aqueous solution of **3** turns dark brown when exposed to air for 1 day, suggesting the oxidation of the o-phenylenediamine moiety. Therefore, freshly prepared product was placed in vacuum for 1 hr at room temperature and then used immediately in the following reaction to avoid such oxidation.

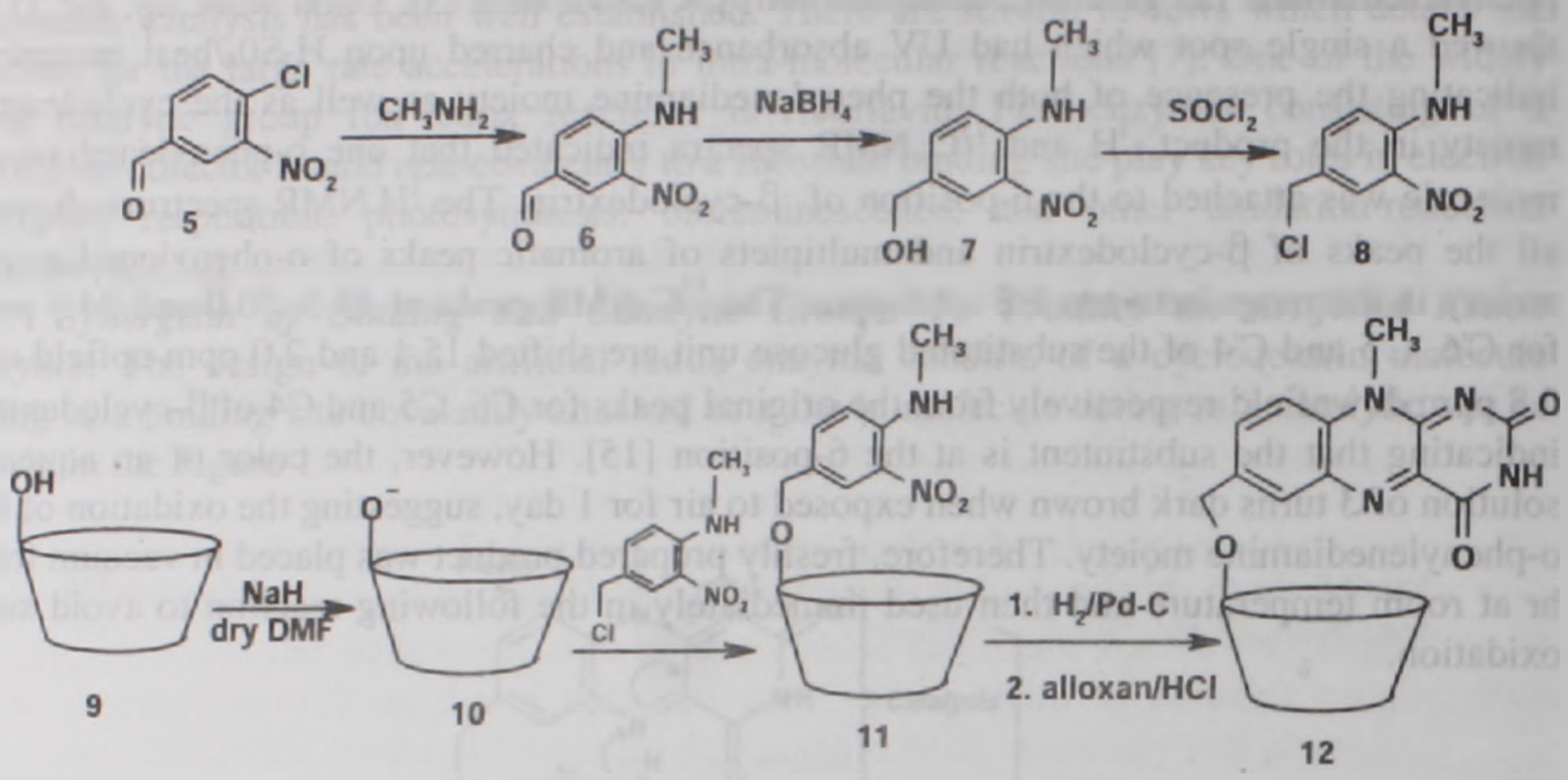


Scheme 1. Attachment of flavin to the primary side of cyclodextrin.

A mixture of **3** with alloxan monohydrate was dissolved in aqueous 1 N HCl and heated in a refluxing acetone bath for 15 minutes when TLC indicated the completion of more than 95% of the reaction. Reverse phase chromatography using C_{18} column and 10% aqueous acetonitrile as the eluent gave pure 6-(10-N-isoalloxazino)-β-cyclodextrin (**4**) as indicated by a single yellow TLC spot which charred on H_2S0_4/heat treatment and confirmed by ^1H and ^{13}C NMR and elemental analysis. The catalytic properties of this artifical redox enzyme (referred to as 6-flβCD) is reported in a later section.

Attachment of Flavin to the Secondary Side of Cyclodextrins. Binding studies have shown that the secondary side of cyclodextrin is more important than the primary side in enzyme mimic chemistry because the substrates generally bind to cyclodextrins with their functional groups oriented towards this side [16]. Thus, catalytic groups attached to the secondary side cyclodextrins should be more effective enzyme mimics. Conventional methods to modify this side of cyclodextrin give low yield of the product [17] and distort the cyclodextrin cavity [18]. Our approach for building organic models of flavoenzymes illustrated here by the synthesis of 2-(7α-O-7,10-dimethylisoalloxazino)-β-cyclodextrin (**12**)

(Scheme 2) overcomes these problems. 4-Chloro-3-nitrobenzaldehyde (**5**) was reacted with methylamine to yield 4-(methylamino)-3-nitrobenzaldehyde (**6**) which was then reduced by $NaBH_4$ to 4-(methylamino)-3-nitrobenzenemethanol (**7**).



Scheme 2. Attachment of flavin to the secondary side of cyclodextrins.

Reaction of thionyl chloride with 7 afforded 4-(chloromethyl)-N-methyl-2-nitrobenzeneamine (**8**). **6**, **7** and **8** were characterized by their ^1H NMR spectra and elemental analysis. In the mean time, sodium β-cyclodextrin alkoxide **10** was synthesized by reacting dry β-cyclodextrin (**9**) with 1 equivalent of NaH in dry DMF. **8** was used as an electrophile to react with **10** to yield 2-[4-(methylamino)-3-nitrobenzyl]-β-cyclodextrin (**11**).

The method described above is general and can be used for attaching substituents onto the secondary side of cyclodextrins without the stereochemical inversions and the ring distortion caused by the conventional methods for modification of the secondary side of cyclodextrin.

Compound **11** was hydrogenated and then condensed with alloxan monohydrate to yield **12**. The structure of **12** was elucidated by ^1H and ^{13}C NMR. The ^1H NMR in spectrum in D_2O exhibits all the normal peaks for β-cyclodextrin in the range of 4.4-3.0 ppm; and the peaks in the range of 7.2-7.45 can be assigned to aromatic protons of the flavin moiety by comparison with the spectrum of flavins [19]. The absence of any resonance at > 7.5 in ^1H NMR in D_2O and presence of a broad peak at 11.41 in the ^1H NMR in DMSO-d_6 indicate the presence of the acidic N-H(3) in this compound. In ^{13}C NMR (D_2O) the ten peaks at > 116 ppm can be assigned to the aromatic carbons of the flavin moiety in comparison to the spectrum of flavins [20]. These assignments are further supported by INEPT ^{13}C NMR in D_2O which shows three positive peaks corresponding to three methine carbons and seven negative peaks corresponding to quaternary carbons in the structure of the flavin moiety. The six normal peaks for the β-cyclodextrin moiety in ^{13}C NMR in D_2O of 14 can be assigned [21] as 60.1 (C6), 71.6 (C2), 71.9 (C5), 72.9 (C3), 80.93 (C4) and 101.6 (C1). The peak at 33.0 can be assigned to CH_3 because its chemical shift falls in the expected range for a methyl groups and it's appearance as a positive peak on INEPT ^{13}C NMR spectrum supports

the fact that it has three protons attached. The peaks at 79.8 and 99.6 can be assigned to C'2, and C'1 respectively of the substituted glucose unit because the flavin group, like the tosyl group, is an electron withdrawing group and when attached to the hydroxyl group at the 2-position of β-cyclodextrin, it will cause a large downfield chemical shift on C'2 and a significant upfield chemical shifts on C'1 of the substituted glucose unit15. These two peaks are shown as positive peaks in INEPT 13C NMR (D_2O) indicating that they correspond to methine carbons. The procedure described above is a general method for synthesis artificial redox enzymes. We have also synthesized an artificial enzymes based a-cyclodextrin using this method. The catalytic properties of these artifical redox enzyme (referred to as 2-flβCD and 2-flαCD) is reported in the following section.

Catalytic Reaction of Artificial Redox Enzymes

Flavoenzymes catalyze a variety of reactions using flavin co-enzyme as the catalytic group. Similarly, the artificial redox enzyme whose synthesis is described above catalyzes all these reaction since it has a flavin as the co-enzyme. Most of these reactions were found to follow a second order kinetics indicating that the reaction catalyzed is not of the substrate bound in the cavity. These reactions were comparable to reactions of riboflavin (ribloflavin is referred to as Rfl) However, oxidation of benzyl mercaptans to corresponding disulfide and photo-oxidation of benzyl alcohols to benzaldehyde were found to follow saturation kinetics indicating a complex formation prior to the reaction similar to the ones found in natural enzymes. The study of these two reaction is described here in detail.

A. Oxidation of Mercaptans.

The oxidation of mercaptans were carried out in 10% (v/v) methanol aqueous buffer ($NaHCO_3$ -NaOH buffer, pH 10.0) with a calculated ionic strength of 0.24 M (adjusted with KCl) at 25.0°C. When a solution of 2-flβCD **(12)** was mixed with 80-fold excess of benzyl mercaptan under anaerobic conditions a slow decrease of the absorbance of 2-flβCD **(12)** at 440 nm was observable and after several hours, a spectrum characteristic of reduced flavin remained. The spectrum of 2-flβCD can be completely restored by re-oxidation of the reaction mixture with air. Compared with the oxidation of mercaptans by flavins, the decomposition of flavins is so slow that it can be ignored. With excess benzyl mercaptan, the absorbance of 2-flβCD and Rfl as a function of time gave good first order plots under anaerobic conditions, but the reaction with 2-flβCD was faster than that with Rfl. When the concentration of benzyl mercaptan (still in excess) was varied a plot of the initial rates for the reaction of Rfl with benzyl mercaptan vs. the concentrations of mercaptan gave a straight line showing the reaction to be overall second order. The second order rate constant then can be obtained from this plot and shown in Table 1. In contrast to Rfl, similar plots for the reactions of 2-fl"CD and 2-flβCD with benzyl mercaptan showed the saturation kinetics. The K_{diss} and k_{cat} calculated from these plot s are listed in Table 1 [22].

The oxidations of substituted benzyl mercaptans and α-naphthyl mercaptan by Rfl and flavocyclodextrins were monitored by the decrease in absorbance at 440 nm due to flavin moiety. The kinetic measurements were performed in $NaHCO_3$ -NaOH buffer (pH 10.0) containing 30% (v/v) methanol at 25.0°C at a calculated ionic strength of 0.24 M. The oxidation of substituted benzyl mercaptans catalyzed by Rfl followed second order kinetics, while the saturation kinetics was observed for the oxidation catalyzed by flavocyclodextrins. The rate constants for these reactions are listed in Table 1.

Table 1. The Rate Constants for the Oxidation of Benzyl Mercaptan and Substituted Benzyl Mercaptans by Flavins*

	Substrate	Flavin	$K_{diss}x10^3$ (M)	$k_{cat}x10^3$ (s^{-1})	k_{cat}/K_{diss} ($s^{-1}M^{-1}$)	k_2x10^2 ($M^{-1}s^{-1}$)	$(k_{cat}/K_{diss})/k_2$***
1	Benzyl mercaptan**	2-flβCD	1.89± 0.23	1.11±0.13	0.587		53
2	Benzyl mercaptan**	2-flαCD	2.38±0.09	0.585±0.022	0.246		22
3	Benzyl mercaptan**	Rfl				1.11±0.06	
4	*p*-Chlorobenzyl mercaptan	2-flβCD	2.91±0.17	1.20±0.07	0.412		21
5	*p*-Chlorobenzyl mercaptan	6-flβCD				11.8±0.8	
6	*p*-Chlorobenzyl mercaptan	Rfl				1.93±0.1	
7	*o*-Chlorobenzyl mercaptan	2-flβCD	1.53±0.11	0.178±0.013	0.116		4
8	*o*-Chlorobenzyl mercaptan	Rfl				2.82±0.12	
9	*m*-Chlorobenzyl mercaptan	2-flβCD	8.85±1.02	3.53±0.40	0.400		16
10	*m*-Chlorobenzyl mercaptan	Rfl				2.45	
11	α-naphthyl mercaptan	2-flβCD				13.2±1.8	

* The kinetics measurements were carried out in $NaHCO_3$-NaOH buffer (pH 10.0) containing 30% methanol (v/v) at 25.0±0.1°C at calculated ionicstrength of 0.24 M.

** $NaHCO_3$-NaOH buffer containing 10% (v/v) methanol.

*** Second-order rate constants of the oxidation of mercaptans by Rfl.

The oxidations of benzenethiol, phenethyl mercaptan and cyclohexyl mercaptan by Rfl and flavocyclodextrins were also investigated in a similar manner. The oxidations either by Rfl or by flavocyclodextrins were either very slow, or did not proceed at all because the plots of absorbance vs. time for these reactions were almost the same as the decomposition of flavins.

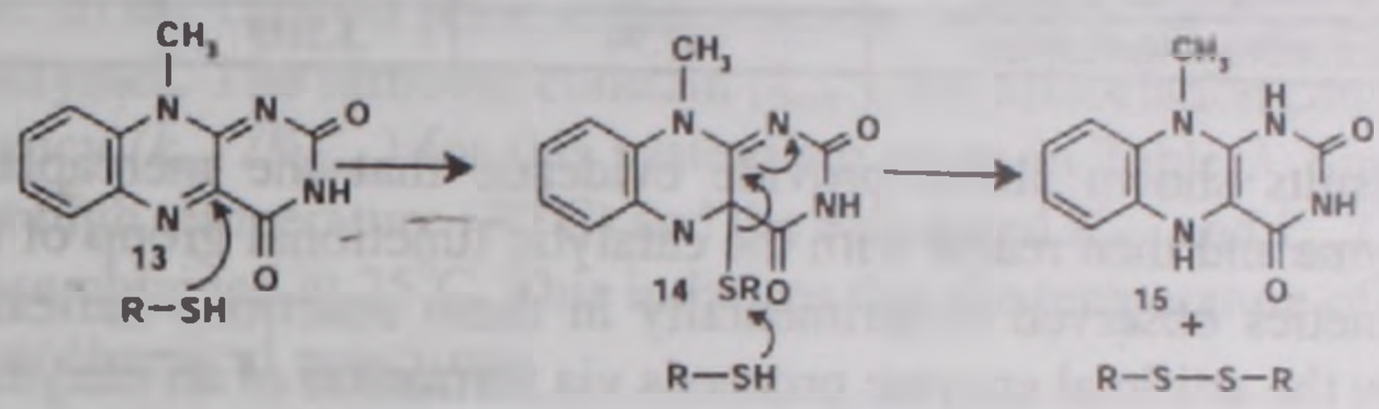
Table I gives all results for the oxidation of benzyl mercaptans by flavins. First of all, the oxidation of benzyl and substituted benzyl mercaptans by 2-flavocyclodextrins shows saturation kinetics, i.e., reactions proceed by the complex formation between 2-flavocyclodextrins and mercaptans. In contrast to 2-flavocyclodextrins, 6-flavo-β-cyclodextrin (6-flβCD) gives the second order kinetics in the oxidation of p-chlorobenzyl mercaptan (entry 5 in Table I), which is similar to the oxidation of mercaptans by Rfl. A computational chemistry study [23] of the conformations of the inclusion complexes between benzyl mercaptan and 2-flβCD indicates that conformation in which the thiol group is oriented towards the secondary side of cyclodextrin, is more stable than conformation where the thiol group is oriented towards the primary side of cyclodextrin.

The difference in energy between these two conformations is 44 kcal/mol. The preferred conformation 23 brings the thiol group of the substrate close to the catalytic functional group of 2-flavocyclodextrins. In the case of 6-flβCD, the complex formation brings the thiol group of the substrate away from the catalytic functional group. Therefore, the flavin moiety of 6-flβCD only reacts with unbound p-chlorobenzyl mercaptan.

Entries 1, 4 and 9 in Table I give the rate constants of the oxidation of benzyl mercaptan, *p*-chlorobenzyl mercaptan and An-chlorobenzyl mercaptan by 2-flβCD. The

stability of the complexes formed between benzyl mercaptans and 2-flβCD is directly related to the reaction rate. The more stable the complex (smaller K_{dis}), the higher the rate acceleration factor.

The oxidation of benzyl mercaptan by 2-flβCD (entry 1) is faster than by 2-flαCD (entry 2). Because the dissociation constants of these two reactions are close, the difference in rate is caused mainly by the difference of catalytic rate constant k_{cat}. Model studies for the oxidation of mercaptans to disulfides by oxidized flavins suggest that thiolate anions add to C(4a)-position of oxidized flavins, producing an adduct (**14**) as shown in scheme 3. The reaction of a second molecule of mercaptan with the adduct gives the disulfide and reduced flavin [24,25]. It has been shown that the rate-determining step in the oxidation of thiols by flavins is the formation of the C(4a) adduct.



Scheme 3. Mechanism for oxidation of mercaptans by flavins.

Therefore, the distance between C(4a) of catalytic functional group of the host and the sulfur atom of the thiol group of the guest [*d*6-C(4a)] (Figure 2) is very important for the catalytic reaction.

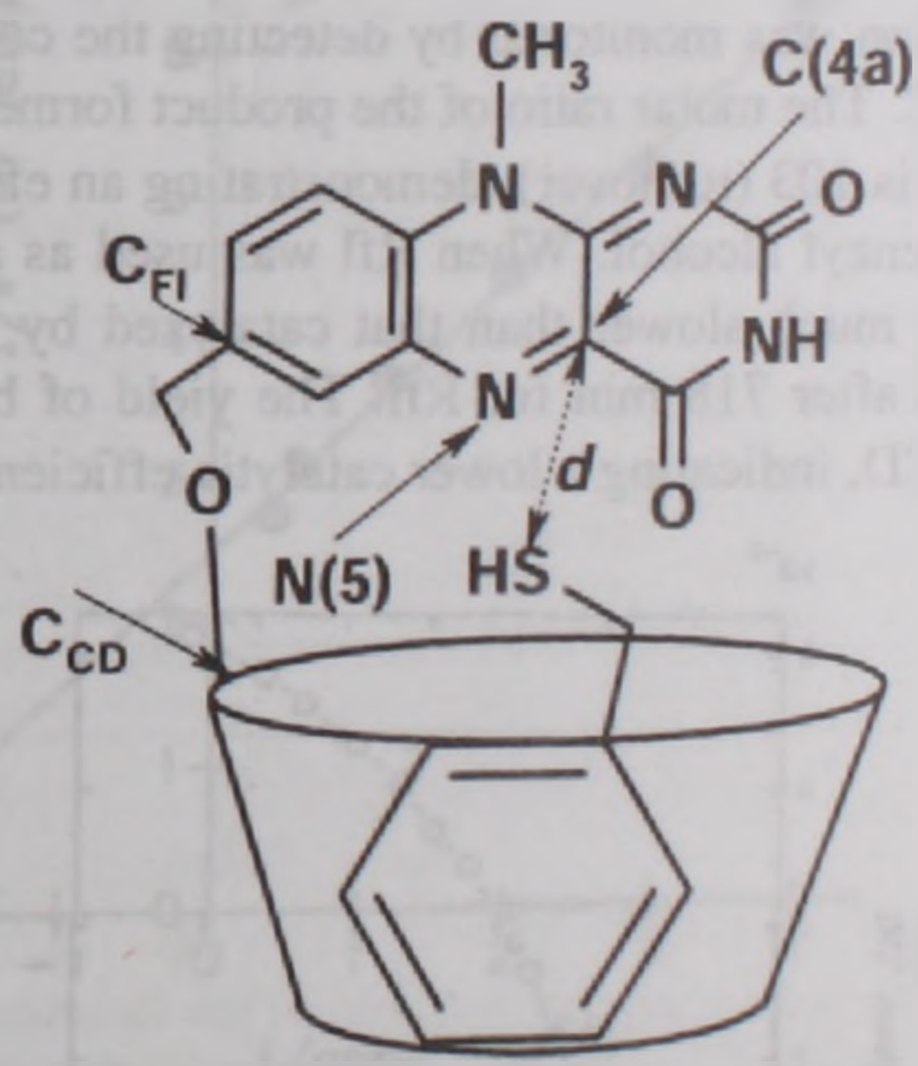


Figure 2. Important distances in the oxidation of mercaptans by flavins.

Because the cavity size of α-cyclodextrin and β-cyclodextrin differs, the *d*S-C(4a) will be different in the complexes of benzyl mercaptan with 2-flαCD and 2-flβCD. This difference is further transferred to the reaction rate. For the same reason, the oxidation of α-naphthyl mercaptan by 2-flβCD shows second order kinetics. Although α-naphthyl mercaptan can form a complex with β-cyclodextrin [26], the *d*S-C(4a) of the complex with

2-flβCD is too long to undergo reaction. The artificial enzyme 2-flβCD only reacts with unbound α-naphthyl mercaptan and the reaction follows the second order kinetics.

The reaction rates for the oxidation of different benzyl mercaptans by 2-flβCD can be alternately explained by the *d*S-C(4a) distance. Computational chemistry studies were performed to determine the relationship between the experimental catalytic rates and the dS-C(4a) distance. The results shown in Table 2 leads to the conclusion that the shorter the distance *d*S-C(4a), the faster the oxidation of the mercaptans by the artificial enzyme.

Table 2. The Relationship Between *d*S-C(4a) and the Catalytic Rates

Substrate	ΔE (Kcal/mol)	$d_{S\text{-}C(4a)}$ (Å)	k_{cat} xIO^{3} (s^{-1})
Benzyl mercaptan	-24.77	3.531	1.11
p-Chlorobenzyl mercaptan	-26.87	3.322	1.20
m-Chlorobenzyl mercaptan	-47.94	3.300	3.53

The results shown above provide evidence that the mercaptan first binds to the artificial enzyme and then reacts with the catalytic functional group of the artificial enzyme. Saturation kinetics observed experimentally in these reactions indicates that oxidation of mercaptans by the artificial enzyme proceeds via formation of an enzyme-substrate complex. This reaction path allows the artificial enzyme to catalyze the reaction 53 times faster than riboflavin.

B. Photo-oxidation of benzyl alcohols

Irradiation of an air-saturated aqueous solution containing 2-flβCD, benzyl alcohol, and $HClO_4$ with visible light of 360 nm < λ < 440 nm resulted in the formation of benzaldehyde. The reaction was monitored by detecting the concentration of benzaldehyde with reverse phase HPLC. The molar ratio of the product formed after 674 min to the initial amount of 2-flβCD used is 103 (turnover), demonstrating an efficient recycle of 2-flβCD in the photo-oxidation of benzyl alcohol. When Rfl was used as a photocatalyst instead of 2-flβCD, the reaction was much slower than that catalyzed by 2-flβCD (Figure 3). Only 6 turnovers were observed after 718 min for Rfl. The yield of benzaldehyde is lower in the case of Rfl than of 2-flβCD, indicating a lower catalytic efficiency of the former.

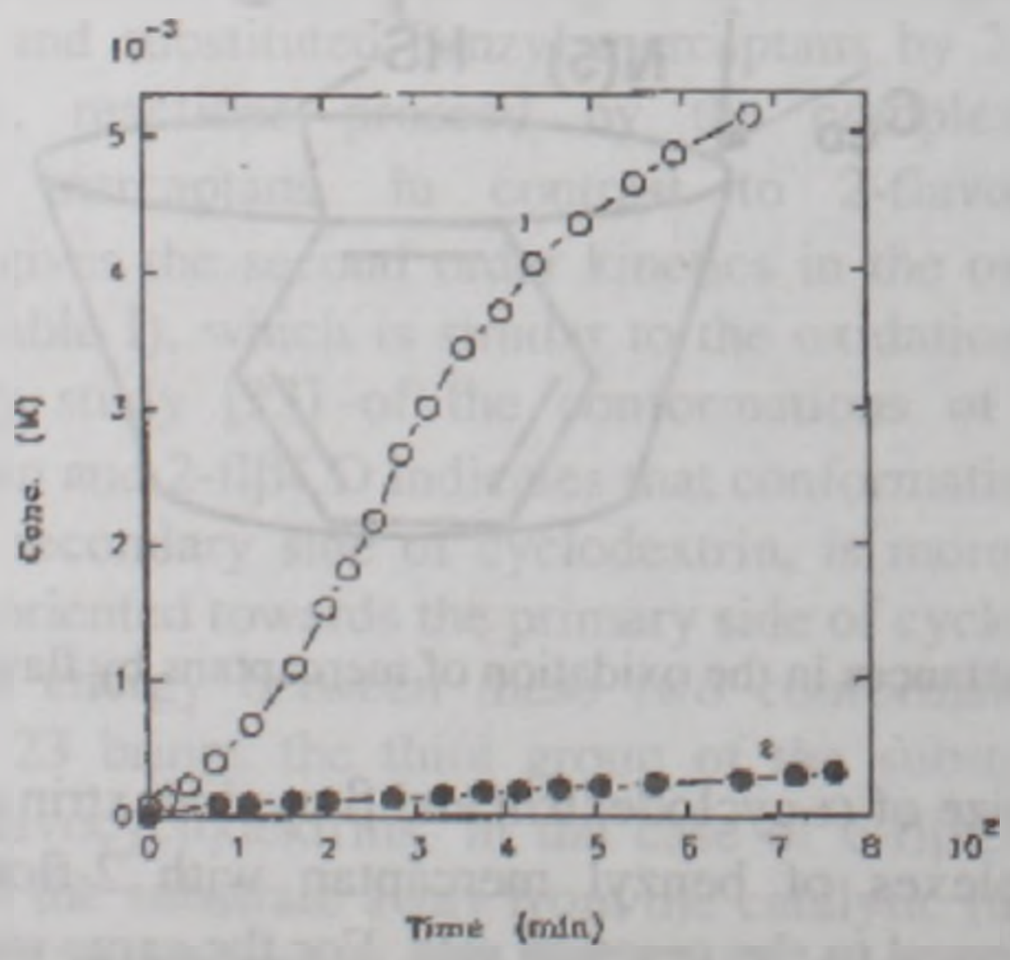


Figure 3. The Concentration of Benzaldehyde vs. Irradiation time for the Oxidation of Benzyl Alcohol by Oxygen Catalyzed by 1) 2-flβCD and 2) Rfl.

***Complex Formation between 2-FlβCD and p-t-Butylbenzyl Alcoho*l.** Cyclodextrins form inclusion complexes in solution with a variety of organic substances, and it is likely that increase in the rate of oxidation of substituted benzyl alcohols catalyzed by 2-flβCD compared with those catalyzed by Rfl involved the formation of 2-flβCD-benzyl alcohol complex. Efforts to get direct experimental evidences for the formation of complexes were made. The dependence of the catalytic action of flavins on the concentration of the substrate was examined. A plot of the initial rates vs substrate concentration for the oxidation of p-t-butylbenzyl alcohol catalyzed by Rfl gives a straight line with zero intercept, indicating a first order dependence on the substrate [27]. A similar plot for the same reaction catalyzed by the artificial enzyme [i.e., 2-flβCD] shows saturation kinetics. Lineweaver-Burk treatment of these data gives an excellent straight line with a slope of K_{diss}/V_{max} = 88.92 min and a Y intercept equal to $1/V_{max}$ = 2.46x105 M^{-1} min (Figure 4). This result indicates binding of the substrate to the catalyst prior to the reaction, which is similar to the reaction scheme followed by enzymes. The turnover constant (k_{cat}), the dissociation constant (K_{diss}), and the enzyme efficiency (k_{cat}/K_{diss}) for this system are given in Table 3. Similar studies were also performed at high temperature (70°C) and the calculated K_{diss} and k_{cat} (Table 3) are almost the same as those obtained at 25°C. This indicates that the temperature of the reaction is not important in photochemical reactions.

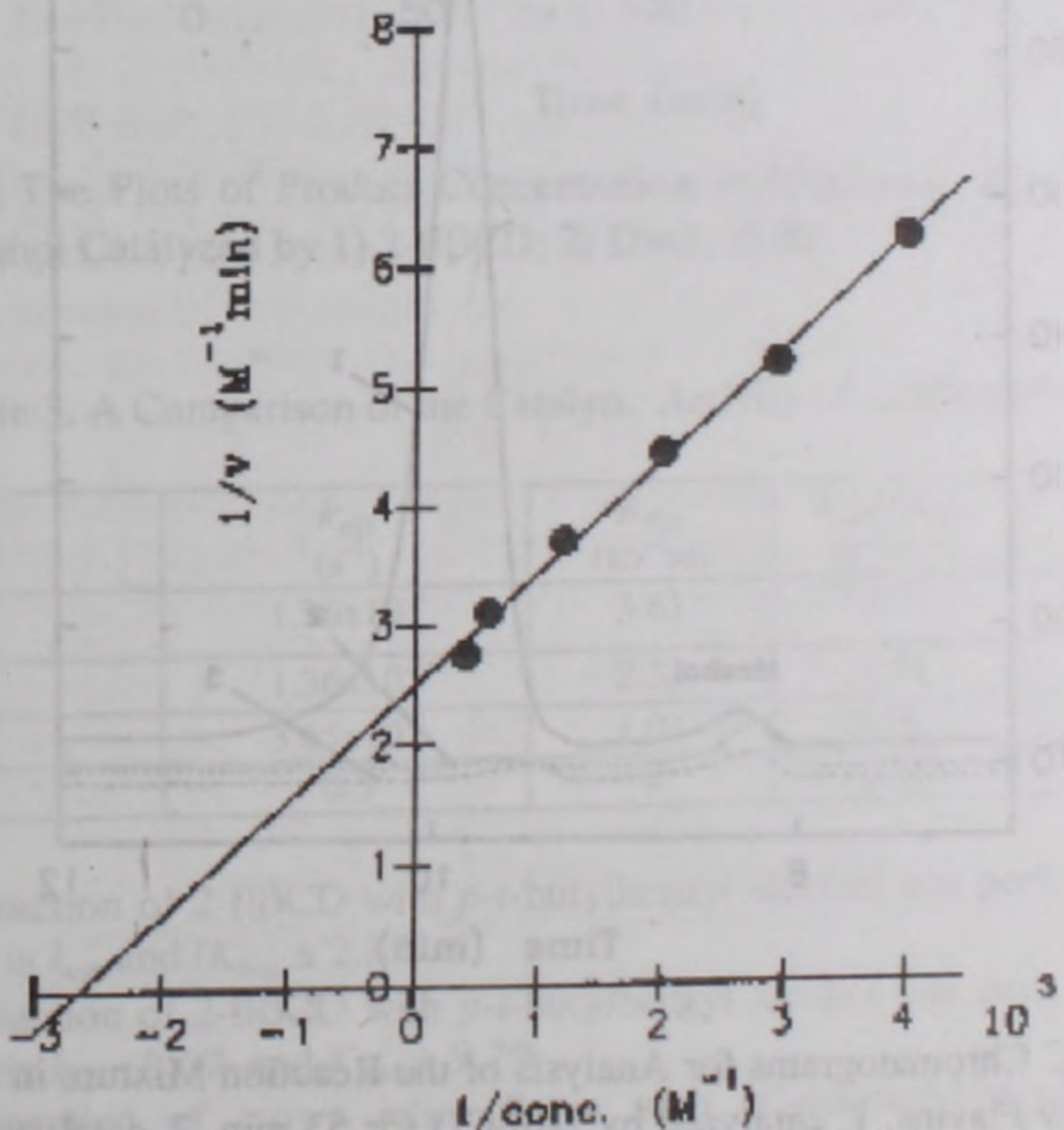


Figure 4. Double Reciprocal Plot of the Oxidation of p-t-Butylbenzyl Alcohol by the Artificial Enzyme.

The oxidation of p-t-butylbenzyl alcohol catalyzed by 2-flβCD was compared with that catalyzed by Rfl and 7,10-dimethylflavin (referred to as Dmfl) to determine the contribution of structural differences (the differences of redox potentials) between Rfl and 2-flβCD. The HPLC chromatograms of the reaction mixtures (Figure 5) and the plots of

product concentrations vs irradiation time (Figure 6) indicate that the oxidation catalyzed by 2-flβCD is much faster than those catalyzed by Rfl and Dmfl . Because the structure of the flavin moiety of 2-flβCD is identical with the structure of Dmfl, these results demonstrate clearly that the rate acceleration by 2-flβCD is due to complex formation. The differences in reaction rates caused by change in flavin structure (the difference of redox potentials) as in the cases of Rfl and Dmfl is very small (plots 2 and 3 in figure 6).

The oxidation of p-t-butylbenzyl alcohol under irradiation conditions catalyzed by 2-flαCD is only slightly slower than that catalyzed by 2-flβCD but is considerably faster than that catalyzed by Rfl and Dmfl. We interprets this by the fact that both 2-flαCD and 2-flβCD have binding sites (cyclodextrins) to form complexes with the substrate and thus this accelerates the reaction. The flavin moiety of 2-flαCD and 2-flβCD is identical, the difference of the reaction rates is caused by the different binding energies between 2-flαCD and 2-flβCD with the substrate. This observation further supports the conclusion that the rates of the oxidation of benzyl alcohols are accelerated by the complex formation between flavocyclodextrins and substrates.

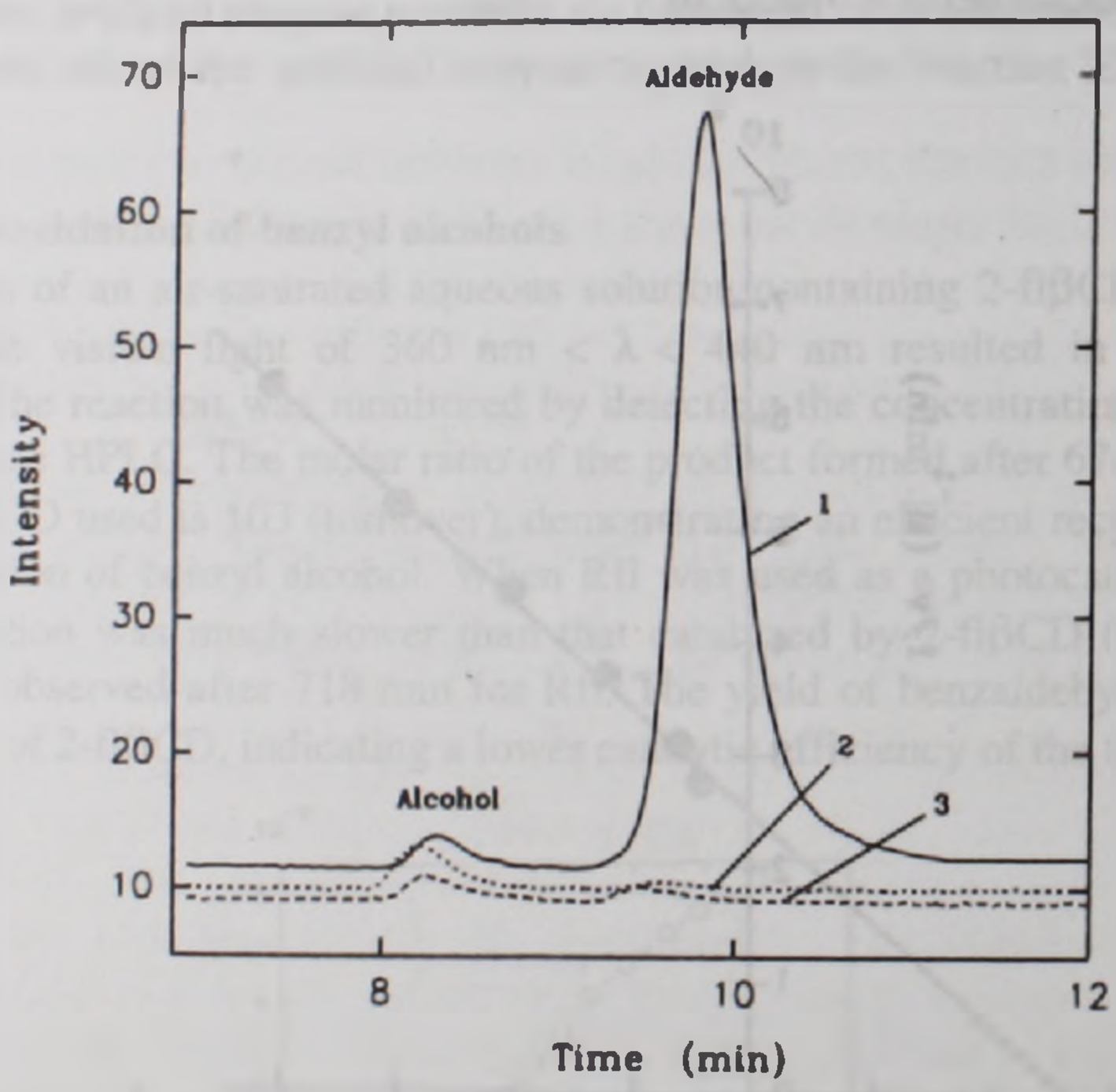


Figure 5. HPLC Chromatograms for Analysis of the Reaction Mixture in the Oxidation of p-t-Butylbenzyl Alcohol by Flavins. 1. catalyzed by 2-flβCD for 53 min. 2. catalyzed by Rfl for 51 min; 3. catalyzed by Dmfl for 76 min.

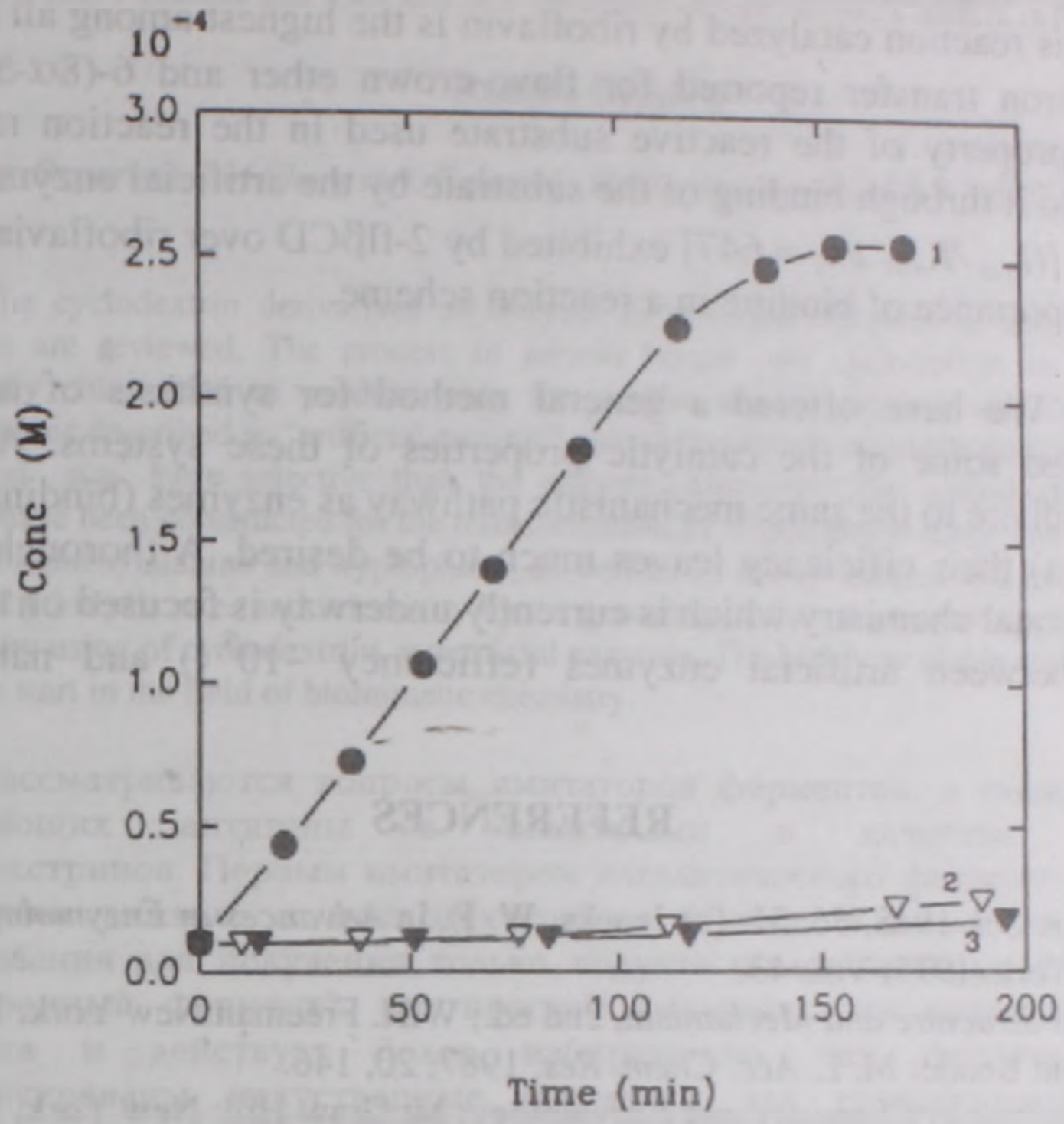


Figure 6. The Plots of Product Concentration vs Irradiation Time for the Oxidation of p-t-Butylbenzyl Alcohol Catalyzed by 1) 2-flβCD; 2) Dmfl; 3) Rfl.

Table 3. A Comparison of the Catalytic Activity of Artificial Redox Enzymes

Enzyme	k_{cat} (s^{-1})	K_{diss} (10^{-4}M)	$k_{cat}/<K_{diss}$ ($s^{-1}M^{-1}$)	k_2 ($s^{-1}M^{-1}$)	(k_{cat}/K_{diss}) $/k_2$
2-flβCD[a]	$1.36x10^{-3}$	3.61	3.77	$5.83x10^{-3}$	647
2-flβCD[b]	$1.36x10^{-3}$	2.32	5.86		
flavo-crown ether[c]	$3.65x10^{-5}$	1.01	0.361	$1.26x10^{-2}$	29
flavocyclodextr[d]	0.5	4	1250	117.1[e]	11

a. The reaction of 2-flβCD with *p-t*-butylbenzyl alcohol was performed at 25.0°C with error limits in k_{cat} and $/K_{diss}$ ± 2.8%.
b. The reaction of 2-flβCD with *p-t*-butylbenzyl alcohol was performed at 70.0°C with error limits in k_{cat} ±5.6% and K_{diss} ± 9.7%.
c. The reaction of crown ether flavin with N^1-dodecyl-l-(*p*-ammoniomethy)benzy)-l .4-dihydronicotinamide is from ref 23.
d. The reaction of 6-(8α-S-riboflavo)-α-cyclodextrin with 1-(1-hexyl)1.4-dihydronicotinamide is from ref 25.
e. pH7.0, 25°C.

It is interesting to compare this artificial enzyme with previously published non-proteinic enzyme models (Table 3): flavo-crown ether and 6-(8α-S-riboflavo)-α-cyclodextrin[28]. While the dissociation constants (K_{diss}) for all these systems are in the same

range, the turnover constant (k_{cat} for 2-flβCD is higher than flavo-crown ether. The turnover constant for 6-(8α-S-riboflavo)-α-cyclodextrin is the highest because the bimolecular rate constant (k_2) for this reaction catalyzed by riboflavin is the highest among all these systems. The efficient electron transfer reported for flavo-crown ether and 6-(8α-S-riboflavo)-α-cyclodextrin is a property of the reactive substrate used in the reaction rather than the advantage offered to it through binding of the substrate by the artificial enzyme. The highest acceleration factor [$(k_{cat}/K_{diss})/k_2 = 647$] exhibited by 2-flβCD over riboflavin offers a clear evidence for the importance of binding in a reaction scheme.

Conclusion: We have offered a general method for synthesis of artificial redox enzymes and studied some of the catalytic properties of these systems. Although these artificial enzymes adhere to the same mechanistic pathway as enzymes (binding the substrate prior to the reaction) their efficiency leaves much to be desired. A thorough investigation including computational chemistry which is currently underway is focused on the reasons for such a disparity between artificial enzymes (efficiency ~10^3) and natural enzymes (efficiency 10^{12}).

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