Macromolecules

Volume 23, Number 13 June 25, 1990

Preparation and Enzymatic Activity of Poly[(*N*-acylimino)ethylene]-Modified Catalase

Masatoshi Miyamoto, Kensuke Naka, Makoto Shiozaki, Yoshiki Chujo, and Takeo Saegusa*

Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan

Received August 4, 1989; Revised Manuscript Received December 19, 1989

ABSTRACT: New amphiphilic polymeric modifiers, i.e., carboxylic acid terminated poly[(*N*-acetylimino)-ethylene] (**3a**) and poly[(*N*-propionylimino)ethylene] (**3b**), were prepared, each of which was coupled with bovine liver catalase [H_2O_2:H_2O_2 oxidoreductase; EC 1.11.1.6] by using a carbodiimide. The catalytic activity of the resulting polymer–enzyme hybrid was measured in an aqueous solution, and the influences of the molecular weight of the modifier and of the extent of modification on the enzymatic activity were examined. The activity of the hybrid was also assayed in benzene and in chloroform. It was significant to find that the **3b**-modified catalase showed an excellent catalytic activity in benzene in comparison with a poly(ethylene glycol) (PEG) modified catalase. The rate of decomposition of H_2O_2 catalyzed by the **3b**-modified catalase was about 20 times higher than the PEG-modified one.

Introduction

Many studies have been carried out since the 1950s to apply enzymes to organic syntheses because they achieve amazing rate enhancements for the reactions they promote and catalyze regiospecific and stereospecific reactions.[1] In quite recent years, considerable interest has been paid to using them in nonaqueous media since most organic reactions occur in organic solutions and, moreover, the absence of water often alters the intrinsic catalytic nature of enzymes as in the case of lipase, which catalyzes an ester synthesis in nonaqueous media.[2] Although most enzymes are unstable in organic solvents, some routes to use enzymes in nonaqueous media have been reported, which contain, for example, the usage of immobilized enzymes,[3] chemically modified enzymes,[4] or enzyme–lipid complexes,[5] the reaction in a reverse micelle,[6] and simply, the heterogeneous reaction of an enzyme itself in organic media.[2,7] Among these approaches, the chemical modification of enzyme using a polymeric modifier is considered to be the most suitable system to evaluate the catalytic nature of the enzyme in organic media because the resulting polymer–enzyme hybrid becomes soluble in organic media, and, hence, it catalyzes the reaction in a homogeneous system. It is also interesting that the environment around the enzyme can be controlled relatively easily by changing the extent of modification as well as by the selection of the modifier.

Recently, Inada et al. reported the preparation of poly(ethylene glycol) (PEG) modified enzymes, which were soluble in organic solvents, and catalyzed chemical reactions in them.[4] At present PEG is the sole well-examined polymeric modifier although the amphiphilic property of polymeric reagents seems to be very important for controlling the stability and the solubility of modified enzymes in nonaqueous media.

We have investigated the amphiphilic property of poly[(*N*-acylimino)ethylene]s (PAI's), which are prepared by the ring-opening polymerization of 2-oxazolines (**1**).[8] In a series of these polymers, the hydrophilicity/hydrophobicity is easily controlled by selecting their acyl group, i.e., 2-alkyl substituent of the oxazoline monomer. The introduction of a reactive functional group at each of its polymer ends for the coupling reaction with an enzyme or for the block copolymerization between different PAI's to control its amphiphilic property is also achieved easily since the polymerization of **1** proceeds in a living mechanism.[9,10] These facts are of a great advantage in the design of a novel polymeric modifier with an appropriate amphiphilic property.

In the present paper is described the preparation of the carboxyl-terminated poly[(*N*-acylimino)ethylene] (**3**) and the chemical modification of an enzyme with **3**. Bovine liver catalase (**2**), which could be obtained in a highly pure form from a commercial source, was selected as the target enzyme to be modified. The enzymatic activity of the resulting polymer–enzyme hybrid was also mea-

0024-9297/90/2223-3201$02.50/0

Scheme I

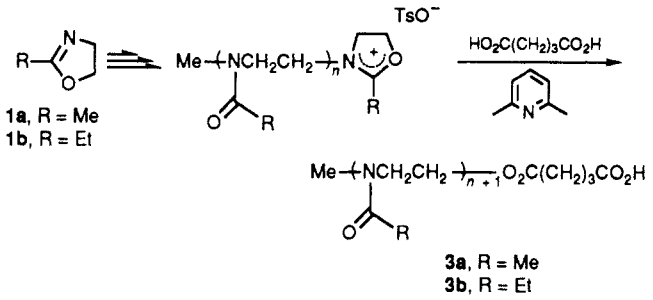


Table I
Preparation of Carboxyl-Terminated Poly[(*N*-acylimino)ethylene][a]

run no.	1	[M]/[I]	product 3	M_n[b]	M_w/M_n[b]	F_n[c]
1	**1a**	10.6	**3a**-1	840	1.13	1.05
2	**1a**	18	**3a**-2	2000	1.18	1.00
3	**1a**	107	**3a**-3	10600	1.16	1.06
4	**1b**	34	**3b**-1	3500	1.04	0.61
5	**1b**	91	**3b**-2	6500	1.04	0.68

[a] Polymerization was carried out by using methyl tosylate as initiator in CH_3NO_2 at 70 °C for 48 h. [b] Determined by GPC measurement. Each of the calibration curves was obtained by using standard samples of **2a** and **2b**, respectively. [c] Average of the extent of functionalization per molecule, determined by alkali titration.

sured in an aqueous solution and in organic media. In addition, the effects of the molecular weight of **3** and of the extent of modification of the resulting hybrid on the enzymatic activity were examined. The enzymatic activity of the hybrid was compared with that of a PEG-modified **2** to confirm the advantages of PAI as a polymeric modifier.[4b]

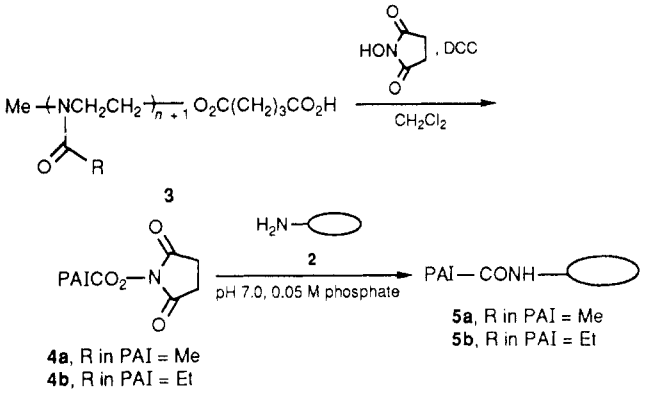
Results and Discussion

Preparation of Amphiphilic Polymeric Modifier. The polymeric modifiers were prepared according to a preceding paper.[11] A carboxylic acid functional group was introduced to the polymer by quenching its living propagating end with a large excess amount of glutaric acid in the presence of 2,6-lutidine to form a half-ester-type product (Scheme I).

The molecular weight and the extent of functionalization of the resulting carboxylated polymeric modifiers, **3**, prepared from 2-methyl- (**1a**) or 2-ethyl-2-oxazolines (**1b**) were summarized in Table I. The molecular weights of the parent polymers, poly[(*N*-acetylimino)ethylene] (**3a**) and poly[(*N*-propionylimino)ethylene] (**3b**), were roughly controlled by changing the feed ratio of the initiator to the monomer. The extent of functionalization (F_n) of **3** was calculated based on their molecular weight, which were determined by GPC with PAI calibration, together with the amount of terminal carboxylic acid groups determined by titration. Although the quantitative half-esterification with glutaric acid has not been achieved for **3b**, they were used in the following reactions since a large excess amount of **3** was used for the chemical modification.

Synthesis of Poly[(*N*-acylimino)ethylene]-Modified Catalase. Bovine liver catalase (**2**) consists of four subunits and contains 108 lysine residues (molecular weight = 242 000).[12] It has been known that the reaction site of catalase is the heme and a part of the histidine residue, and the lysine residues do not participate in the reaction.[12b] To couple with amino groups of the lysine residues of **2**, **3** was first converted to an activated ester terminated polymer, **4**, by the reaction with

Scheme II



dicyclohexylcarbodiimide and *N*-hydroxysuccinimide in dichloromethane. Then, a large excess amount of **4** was reacted with **2** in a 0.05 M phosphate buffer (pH 7.0), and the mixture was kept overnight at 25 °C (Scheme II).

The unreacted modifier was removed by dialyzation against distilled water using ultrafiltration. After lyophilization, pale green powder was obtained. The yields of the modified catalase were in a range of 11–86%. Any particular relationship between the yield of the hybrid and its structure or the reaction conditions was not observed. It was assumed that the low yields in some cases were due to the absorption of the product on a membrane filter for ultrafiltration during the purification process. A PEG-type hybrid (M_n of PEG = 5000) as well as an acetylated catalase was also prepared in a similar manner (see the Experimental Section).

The extent of modification of the amino groups, which was calculated by the determination of the unreacted amino residue according to the method using 2,4,6-trinitrobenzenesulfonic acid,[13] could be roughly controlled by the molar ratio [**4**]/[Lys] as shown in Table II, and it reached about a half by repeating the reaction twice. However, no further modification could be achieved even by increasing the molar ratio [**4**]/[Lys] or by repeating the reaction, additionally. It is considerable that there is a certain amount of internal amino groups in catalase, which are difficult to react with the polymeric modifier owing to the steric hindrance around the amino groups.

To confirm the above assumption, the modification of **2** with acetic acid was carried out by an analogous procedure. The extent of modification reached to 57% by using a 20-fold amount of acetic acid to the lysine residues. This value is much higher than any of those of **5** with a similar [**4**]/[Lys] ratio, i.e., run nos. 3, 7, 9, and 11 in Table II.

Although a water-soluble carbodiimide has been usually used for coupling reactions in an aqueous solution, a trial to employ 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride brought about no substantial improvement on the efficiency for the condensation between **2** and **3**.

In Figure 1, the GPC charts of **5b**-2 (23)[14] (curve B) and **5b**-2 (53) (curve C) were compared with that of **2** (curve A). Both curves of **5b**-2 are unimodal, and they are shifted to a higher molecular region with an increase in the extent of modification. Figure 1 clearly shows these hybrid contained neither an unreacted polymeric modifier (curve D) nor **2**. Obviously, the polymeric modifier was covalently attached to catalase without causing the dissociation of catalase into subunits.

Catalase contents in the hybrid were determined by the biuret method and by the absorption peak at 279

Table II
Preparation of Poly[(N-acylimino)ethylene]-Modified Catalase

run no.	modifier	[4]/[Lys]	product: 5 or 7 (EM[a] %)	product: yield, mol %	product: $M_n \times 10^{-5}$ [b]	product: polymer content, wt %
1	**3a**-1	5.0	**5a**-1 (8)	13	2.49	2.9
2	**3a**-1	9.6	**5a**-1 (20)	87	2.60	6.9
3	**3a**-1	25.8	**5a**-1 (38)	16	2.76	12.4
4	**3a**-2	4.8	**5a**-2 (11)	57	2.66	8.9
5	**3a**-2	10.0	**5a**-2 (15)	11	2.74	11.8
6	**3a**-2	32.9	**5a**-2 (44)	54	3.37	28.2
7	**3a**-3	20.0	**5a**-3 (27)	86	5.54	56.3
8	**3a**-3	20.0/20.0[c]	**5a**-3 (42)	57	7.27	66.7
9	**3b**-1	21.4	**5b**-1 (38)	69	3.87	37.4
10	**3b**-1	20.0/20.0[c]	**5b**-1 (53)	48	4.44	45.4
11	**3b**-2	23.3	**5b**-2 (23)	76	4.05	40.3
12	**3b**-2	20.0/20.0[c]	**5b**-2 (53)	32	6.18	60.8
13	**3b**-2	22.9/22.9[c]	**5b**-2 (59)	77	6.61	63.4
14	6[d]	20.0	**7** (35)	83	4.31	43.9
15	acetic acid	22.1	8 (57)	100	2.45	

[a] The extent of modification of lysyl amino group. Determined by using 2,4,6-trinitrobenzenesulfonic acid. [b] Calculated value from F_n and the molecular weight of **3**. [c] The modification process was repeated twice without isolating the first step product. [d] Prepared from monomethoxypoly(ethylene glycol) (M_n = 5000).

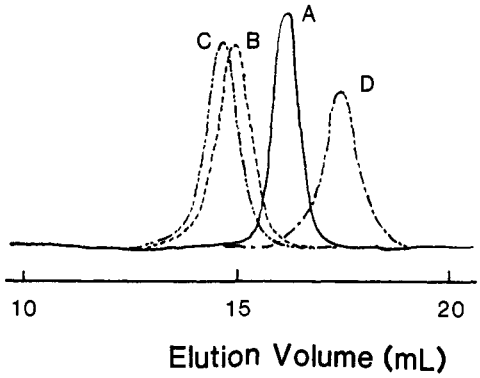


Figure 1. Gel permeation chromatograms of **2** (A), **5b**-2 (23) (B), **5b**-2 (53) (C), and **3b**-2 (D) [Wakobeads G-50 columns (2 × 30 cm), 0.05 M phosphate buffer (pH 7.0), 1.0 mL/min, with UV detection monitored by 220 nm].

nm derived from aromatic amino acid residues. These two values agreed well with each other and were also in good accordance with the calculated one based on the concentration of heme in the hybrid, which was determined by the strong absorption peak of heme at 406 nm. The above results were taken to conclude that catalase did not liberate heme by these modified reactions. The same conclusion was set for the other **5b** samples as well as for the series of **5a**.

Assessment of Enzyme Activity in Aqueous Medium. The activity of **3a**-modified catalase, **5a**, was assayed at 30 ± 0.01 °C in a phosphate buffer solution (pH 7.0) containing ca. 20 mM of H_2O_2. The consumption of hydrogen peroxide was calorimetrically determined by the reaction with titanium sulfate.[18] In order to compare the effect of the polymeric modifier on catalase functions, the activity of **5** was calculated per nanomole of the hybrid. Prior to the evaluation of the enzymatic activity of the resulting hybrid, the following two facts were confirmed; i.e., the aqueous solution of **3a** showed no detectable consumption of hydrogen peroxide in the absence of catalase within the time scale of the enzyme assay, and PAI did not absorb hydrogen peroxide in the buffer, at all. Figure 2 shows the enzymatic activities of **5a** against the extent of modification of the amino groups. The enzymatic activity of **5a** decreases with an increase in the extent of modification, especially, in the cases of **5a**-1. Obviously, the inactivation of **5a** with an increase in the extent of modification strongly depends on the molecular weight of the modifier. In the

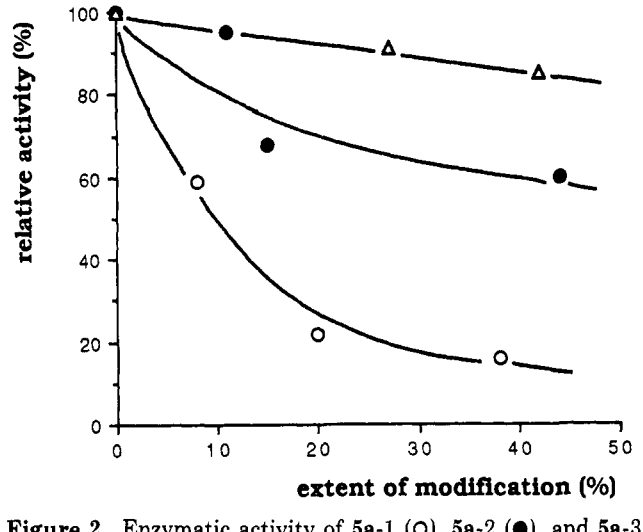


Figure 2. Enzymatic activity of **5a**-1 (○), **5a**-2 (●), and **5a**-3 (△) in aqueous solution (0.05 M phosphate, pH 7.0) at 30 ± 0.01 °C against the extent of modification of amino groups in catalase.

case of **5a**-3, 90% of the original activity remained even by 43% of modification, although the relative activity of **5a**-1 was decreased to less than a quarter only by 20% of modification. A similar effect of the molecular weight of the modifier on the enzymatic activity has been reported in the case of PEG-modified alkaline phosphatase.[19] In the case of **5b**, on the contrary, the drop of the enzymatic activity was hardly recognized even when 59% of the lysine residues were modified with **3b**-2, and more than 90% of the original activity was kept by using **3b**-1 with 53% of modification (Figure 3).

Two assumptions are considerable to explain the inactivation of the hybrid: (1) The break of hydrogen bonding by the chemical modification brings about a conformational change of the hyperstructure of the enzyme. (2) The chemical modification of the lysyl group near the reaction site of the enzyme prevents the approach of the substrate into the reaction site, sterically. We have no distinct evidence to choose between the two. Another explanation that some interaction between the polymers themselves or between the polymer and the enzyme causes a conformational change of the enzyme can be omitted because the acetylated catalase (8) showed a poor activity, i.e., 25% of the original activity. The above observations can be tentatively explained by the fact that as

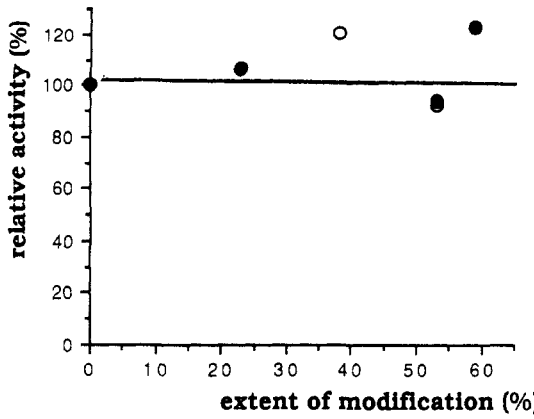


Figure 3. Enzymatic activity of **5b**-1 (○) and **5b**-2 (●) in aqueous solution (0.05 M phosphate, pH 7.0) at 30 ± 0.01 °C against the extent of modification of amino groups in catalase.

the molecular weight of **3a** becomes lower, **3a** reacts with the lysyl amino groups, which located inside of **2** and participated in hydrogen bonding or placed near the active center, more easily, and it results in the inactivation of catalase.

The reason for the high enzymatic activity of **5b** is also not clear. In the cases of **3b**, the sterically bulkier propionyl substituents may prevent the penetration of **3b** into the inside of enzyme regardless of its molecular weight. The steric bulkiness around the terminal carboxylic group would be caused by a relatively shrunken conformation of **3b** in an aqueous solution in comparison with that of **3a** since the former is less hydrophilic than the latter. The shrunken conformation of **3b** was confirmed from GPC measurements. From calibration curves of **3a** and **3b** for the TSK-G4000SW column (a pH 7 phosphate buffer eluent), it was estimated that elution volumes of **3a** and **3b** samples with M_n = 5000 were 10.8 and 11.6 mL, respectively. The apparent molecular weights of both samples based on a calibration curve of globular protein standards were calculated as 22 000 for **3a** and 11 000 for **3b**.

To confirm these hypotheses, many further investigations with varying the type and the molecular weight of modifier, the enzyme, and the substrate would be required.

Enzymatic Activity in Organic Solvents. In a previous report,[15] it has been found that organic solvents, being not miscible with water, are preferable as nonaqueous media for an enzymatic reaction, and benzene has been most frequently selected. To make the comparison between the enzymatic activity of **5** and that of the PEG-type one in an earlier work easy, benzene was chosen as solvent in the present study, too. Chloroform was also selected because of its relatively high solubility to organic substrates. Prior to determination of the enzymatic activity of **5**, their solubility in these solvents was measured. In 4 mL of the solvent was kept 0.20 mg of the hybrid at 20 °C. After filtration the concentration of the hybrid was determined on the basis of the UV absorption of the soret band (at 405 nm) and of carbonyl groups (at 200 nm). The results are summarized in Table III. The hybrid modified with **3a** of relatively low molecular weight, **5a**-1 and **5a**-2, was not soluble in both solvents. In the case of **5a**-3, it became soluble in chloroform but not in benzene. On the other hand, the **5b** samples were well soluble in both of the solvents. These differences in the solubility of the modifiers are well understood considering the fact that **3a** is soluble in chloroform, but not in benzene, and **3b** is soluble in both solvents. Neither a remarkable conformation change nor a liberation of heme occurred in these solvents since absorptions of the soret band of **5** in both media were very similar to that in the aqueous solution.

Table III
Solubility of Polymer–Enzyme Hybrid in Organic Media

hybrid (EM,[a] %)	M_n of modifier	solubility,[b] ×10⁸ mmol/mL in chloroform	in benzene
5a-3 (27)	10600	*c*	*c*
5a-3 (42)	10600	1.1	*c*
5b-1 (38)	3500	6.0	2.5
5b-2 (53)	6500	12.5	3.8
7 (35)	5000	11.3	3.8

[a] The extent of modification of lysyl amino group. [b] Determined by the absorption spectrum of supernatant liquid. [c] Practically insoluble in the solvent.

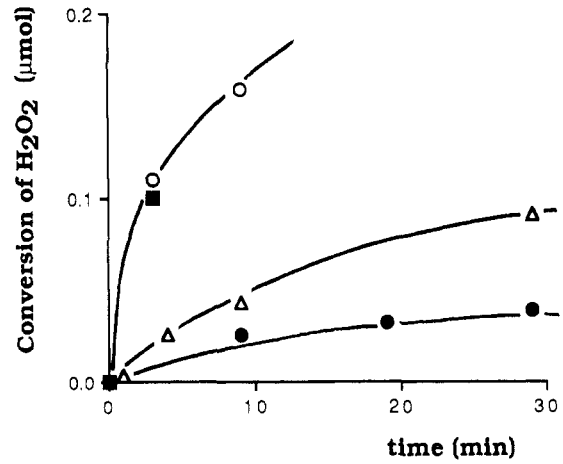


Figure 4. Time–conversion curves of hydrogen peroxide catalyzed by **2** (●), **5a**-3 (42) (△), **5b**-2 (53) (○), and **7** (■) in chloroform at 30 ± 0.01 °C.

Considering the results of the activity measurement in the aqueous medium, **5a**-3 and **5b**-2 were selected as the samples for the enzymatic assay in organic media. At first, the enzymatic activity of the hybrids was checked in a heterogeneous chloroform medium to compare their activity with that of unmodified catalase, **2**, which is completely insoluble in chloroform. Under the experimental condition (0.1 mg/4 mL of solvent), the amounts of the chloroform-soluble parts of the samples were measured as 14 wt % {for **5a**-3 (42)}, 50 wt % {for **5b**-2 (53)}, and 46 wt % {for **7**}.

For the enzyme assay in organic media, water-saturated (pH 7.0 phosphate buffer) solvents containing ca. 0.2 mM of hydrogen peroxide were used. Figure 4 depicts the time course of conversion of hydrogen peroxide per nanomole of the enzyme at 30 °C. It is clearly shown that the catalytic activity of **5a**-3 (42) to convert hydrogen peroxide was about 2 times higher than that of **2**, and that of **5b**-2 (53) was 10 times as high. The improvements on catalytic activity seem to be mainly caused by the better solubility of the hybrid than that of **2** in chloroform.

A more detailed study on the enzymatic assay of **5b** was further carried out in homogeneous organic media to cancel problems arising from the different solubility between the samples. In these measurements, 0.2 mg of the sample was added to 4 mL of the solvent, and after filtration the concentration of the hybrid in the solution was determined by UV spectroscopy. Table IV summarizes the enzymatic activities of **5b**-1, **5b**-2, and **7** in chloroform and in benzene.

In chloroform, each of the **5b** samples gives slightly better values than **7**. Namely, the values for **5b**-1 (38)

Table IV
Enzymic Activity of Polymer–Enzyme Hybrid in Organic Media

hybrid	M_n of modifier	activity,[a] unit/nmol/min in chloroform	activity,[a] unit/nmol/min in benzene
5b-1 (38)	3500	7.7	130
5b-2 (23)	6500	*b*	120
5b-2 (53)	6500	4.8	110
7 (35)	5000	2.4	4.8

[a] $[H_2O_2]_0$ = ca. 0.3 mM, at 30 °C. [b] Did not examine.

and **5b**-2 (53) are, respectively, 3 and 2 times as much as that for **7**.

On the other hand, the activities of **5b** were much enhanced in a homogeneous benzene solution. Although the rate of decomposition of hydrogen peroxide catalyzed by **7** in benzene is only twice as fast as that in chloroform, the rates for **5b** are about 20 times higher than the corresponding rates in chloroform. As a result, the **5b** samples are at least 20 times more active than the PEG-type hybrid, **7**, in benzene. This excellent activity of **5b** in benzene would be ascribed to a stronger hydrogen-bonding ability of **5b** between an amide oxygen and water in comparison with that of PEG between ether oxygen and water.

Although further detailed and systematical experiments should be required to find some distinct relationship between the enzymatic activity and the molecular weight of the modifier as well as between the former and the extent of modification, the preliminary results described in the present paper have given an important fact that the environment around the enzyme, which strongly depends on the properties of polymeric modifier, highly influences the enzyme functions.

Experimental Section

Materials. Carboxylic acid terminated poly[(*N*-acetylimino)ethylene] and poly[(*N*-propionylimino)ethylene] (**3**) were prepared according to the preceding paper.[11] Monomethoxypoly(ethylene glycol) (M_n = 5000) was purchased from Aldrich Chemical Co. and was carboxymethylated according to the literature.[16] Bovin liver catalase (H_2O_2:H_2O_2 oxidoreductase; EC1.11.1.6) was obtained from Sigma Chemical Co. and was purified as follows: To 10 mL of 0.05 M phosphate buffer (pH 7.0) was added 0.5 mL of catalase suspension (500 mg/11 mL of water), and a clear supernatant solution was obtained by centrifugation at 2000 rpm for 10 min. It was dialyzed for 2 h against 200 mL of 0.05 M phosphate buffer (pH 7.0) by using Spectra pore 4 dialysis membrane while changing the buffer three times. The amount of catalase was determined by the biuret method and the absorption peak at 405 nm.[17] Chloroform and benzene (analytical grades) for enzymatic reaction media were dried by the conventional method and were distilled under nitrogen.

Instrumentation. The UV spectroscopy was recorded on a Hitachi Model 200-20 spectrophotometer. GPC analyses were performed by using a Wakobeads G-50 column (30 cm × 2) for catalase and the hybrids or a TSK-GEL G4000SW column for **3** in 0.05 M, pH 7.0, phosphate buffer at room temperature, a Shodex AC-803 column for **3b** in chloroform at room temperature and a TSK-GEL G2500H column for **3a** in DMF containing 0.4% triethylamine at 50 °C.

Preparation of *N*-Succinimide Activated Ester of Polymeric Modifier (4). In 10 mL of dry dichloromethane was dissolved 2.0 g (3.1×10^{-1} mmol) of carboxylic acid terminated poly[(*N*-propionylimino)ethylene] (M_n = 6500), **3b**-2. To the solution was added 0.08 g of *N*-hydroxysuccinimide (7.0×10^{-1} mmol) and 0.15 g of dicyclohexylcarbodiimide (7.0×10^{-1} mmol). After the solution stood overnight at room temperature, precipitated dicyclohexylurea was removed by filtration. The filtrate was poured into 50 mL of diethyl ether to precipitate a polymeric product, which was purified further by repeated reprecipitation from dichloromethane to diethyl ether. After drying in vacuo, a white solid polymer, **4b**, was obtained quantitatively.

General Procedure for the Chemical Modification of Catalase. The modifications were carried out at 25 ± 1 °C. To 13 mg of catalase (5.2×10^{-5} mmol) in 18 mL of 0.05 M phosphate buffer (pH 7.0) was added 1.3 g of **4b** (2.0×10^{-4} mol) with stirring. After standing overnight, the unreacted modifier was removed by dialysis against distilled water using an Amicon Diaflo PM-30 membrane. After lyophilization, 15 mg of a pale greenish powder was obtained. The extent of modification of polymeric modifier was determined by measuring the amount of unreacted amino groups in catalase by the method using 2,4,6-trinitrobenzenesulfonic acid[11] and was found to be 23%. The same procedure was also applied for the synthesis of the PEG-modified catalase, **7**.

Enzyme assay was carried out as follows. An enzyme solution (0.2 mL; 8×10^{-10} M) was added to 5.8 mL of 0.05 M phosphate buffer solution (pH 7.0), which contained ca. 20 mM H_2O_2, and the reaction mixture was incubated at 30 ± 0.01 °C. A small portion of the solution was drawn out at every 2 min, and the consumption of hydrogen peroxide was calorimetrically determined by the reaction with titanium sulfate according to the method reported by Pobiner.[18] The slope of the time–conversion curve at t = 1 min was measured, from which the activity was calculated. For the enzyme assay in organic solvents, a 30% aqueous hydrogen peroxide solution (0.1 mL) was shaken with 50 mL of a dry organic solvent, and the organic phase was used as the substrate solution, which contained ca. 0.2 mM of hydrogen peroxide. With 4 mL of the solution was mixed 0.2 mg of the hybrid and a homogeneous enzyme solution was obtained by filtration. The concentration of the hybrid was determined on the basis of the UV absorption of the soret band at 406 nm. The enzymatic activity was measured by a similar procedure to that in the aqueous medium.

References and Notes

(1) Porter, R., Clark, S., Eds. *Enzymes in Organic Synthesis*; Pitman Press: London, U.K., 1985.
(2) Kirchner, G.; Scollar, M. P.; Kilbanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072.
(3) Fukui, S.; Tanaka, A. *Annu. Rev. Microbiol.* **1982**, *26*, 145.
(4) (a) Takahashi, K.; Nishimura, T.; Saito, Y.; Inada, Y. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 261. (b) Takahashi, K.; Ajima, A.; Yoshimoto, T.; Inada, Y. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 761. (c) Takahashi, K.; Ajima, A.; Yoshimoto, T.; Okada, M.; Matsushima, A.; Tamaura, Y.; Inada, Y. *J. Org. Chem.* **1985**, *50*, 3414. (d) Lee, H.; Takahashi, K.; Kodera, Y.; Ohwada, K.; Tsuzuki, T.; Matsushima, A.; Inada, Y. *Biotechnol. Lett.* **1988**, *10*, 403. (e) Baillargeon, M. W.; Sonnet, P. E. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1812.
(5) Okahata, Y.; Ijiro, K. *J. Chem. Soc., Chem. Commun.* **1988**, 1392.
(6) Luisi, P. L. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 439.
(7) Zaks, A.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 3194.
(8) Kobayashi, S.; Saegusa, T. In *Ring-opening Polymerization*; Ivin, K. J., Saegusa, T., Eds.; Elsevier Applied Science Publishers: Essex, U.K., 1984; Vol. 2, p 761.
(9) Kobayashi, S.; Kaku, M.; Sawada, S.; Saegusa, T. *Polym. Bull.* **1985**, *13*, 447.
(10) Kobayashi, S.; Shoda, S.; Masuda, E.; Shimano, Y. *Polym. Prepn., Jpn. (Engl. Ed.)* **1987**, *36*, E341.
(11) Miyamoto, M.; Naka, K.; Tokumizu, M.; Saegusa, T. *Macromolecules* **1989**, *22*, 1604.
(12) (a) Schroeder, W. A.; Shelton, J. R.; Shelton, J. B.; Robberson, B.; Apell, G. *Arch. Biochem. Biophys.* **1969**, *131*, 653. (b) Schonbaum, G. R.; Chance, B. In *The Enzymes*; Boyer, R. D., Eds.; Academic Press: New York, 1976; Vol. 13, p 363.
(13) Snyder, S. L.; Sobocinski, P. Z. *Anal. Biochem.* **1975**, *64*, 284.
(14) The extent of modification is shown in parentheses.
(15) Laane, C.; Boeren, S.; Vos, K.; Veeger, C. *Biotechnol. Bioeng.* **1987**, *30*, 81.
(16) Buckmann, A. F.; Morr, M.; Johansson, G. *Makromol. Chem.* **1981**, *182*, 1379.
(17) Aebi, H. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Eds.; Verlag Chemie and Academic Press: Weinheim and New York and London, 1974; p 673.
(18) Pobiner, H. *Anal. Chem.* **1961**, *33*, 1423.
(19) Yoshinaga, K.; Shafer, S. G.; Harris, J. M. *J. Bioact. Compat. Polym.* **1987**, *2*, 49.