Then, the reaction between two molecules of genetic zwitterion occurs, in which the phosphonium ring of one molecule is opened by a nucleophilic attack of the anion of the other molecule according to the mode of the Arbuzov reaction involving an oxidation-reduction. The propagation proceeds via the successive attack of the genetic zwitterion **10** onto dimeric zwitterion **11** ($p = 1$) to form a macrozwitterion of an alternating copolymer **3**.

The ^{31}P NMR spectrum of the reaction mixture at the early stage of copolymerization showed signals at δ +61.9 and +20.1, which may be ascribed to the phosphorus atoms of the terminal phosphonium ion and phosphonate anion of intermediates **10** and/or **11**.

The more detailed studies including kinetics and mechanism of the present copolymerization are now in progress.

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Registry No. 1, 4546-13-8; (1)(**2a**) (alternating copolymer), 123168-40-1; (1)(**2b**) (alternating copolymer), 123168-39-8; (1)(**2c**) (alternating copolymer), 123168-41-2; **4b**, 123183-65-3; **7**, 1638-86-4; **8**, 123168-38-7.

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A Holoenzyme Model of Thiamine Dependent Enzymes: Polymer Catalyst Supported Thiazolium Salt

Intense attention has been focused on studies of enzyme and coenzyme model reactions. A coenzyme, the most important site of a holoenzyme, acts as the active catalytic site of enzymic reaction by using its own functional group. Therefore, in a few cases, a coenzyme has catalytic activity even without an apoenzyme, and a biomimic compound, which has a functional group similar to that of a coenzyme, has catalytic activity comparable to that of a coenzyme. From this viewpoint, many chemists have studied the model reaction of coenzyme-dependent enzymes in detail,[1] and it has become evident that the apoenzyme not only plays a role in the support of the coenzyme but also participates actively in the stereospecificity and substrate specificity of enzymic reaction. In the heme protein, the heme, which is isolated from the supporting protein, loses its activity in vivo;[2] i.e., the apoenzyme plays a role as a protector of the active site.

Table I
Results of Acyloin Condensations[a]

cat.	yield, %	cat.	yield, %
MPTI	4.5	PTS^+(14)-DVB(5)	29.9
PTS^+(14)	21.0	PTS^+(9)-NVP(89)	4.7
PTS^+(69)	13.7		

[a] [cat.] = 0.036 mmol and [furfural] = 3.6 mmol; in phosphate buffer, 3.3 mL (pH 8.0), at 60 °C for 40 h.

Since Breslow[3] reported that the thiazolium salt unit is an active site of thiamine pyrophosphate, which is the coenzyme for a number of important biochemical reactions including decarboxylation and acyloin condensation, many chemists have investigated the catalytic activity of thiazolium salt in various reactions.[4] It was reported that in an aqueous system a thiazolium salt changed into an open-chain compound and lost its activity because of an attack by hydroxide ions, even though naturally occurring thiamine-dependent enzymes are known to be active in a hydrophilic environment.[5] In vivo an apoenzyme may protect an active site, i.e., a thiazolium salt unit, from the attack of hydroxide ions. Therefore, many model reactions have been performed in a nonaqueous system. Concerning the aqueous system, Tagaki et al.[6] obtained a highly active thiazolium salt catalyst when acyloin condensation was carried out in the micellar system. Recently, Breslow et al.[7] synthesized a thiazolium salt with a γ-cyclodextrin and reported that the catalyst had high catalytic activity in the dimethyl sulfoxide/H_2O mixed solvent. They emphasized the importance of the incorporation of a substrate for this reaction.

To the best of our knowledge, no study has investigated the role of synthetic polymer catalysts, which are used as a model compound of a holoenzyme by considering the protection of an active site. In the present study, we conducted acyloin condensation of furfural in buffer solution using a thiazolium salt polymer whose synthesis was reported in our previous investigation.[8] It became clear that the hydrophobic environment generated by the polymer main chain around the active site was important in maintaining the catalytic activity in the aqueous system.

The thiazolium salt polymer catalysts were synthesized by quaternization of homopoly(4-(4-vinylphenyl)thiazole) (VPT) and abbreviated as $PTS^+(x\%)$ in which x stands for the mol % of quaternized thiazole units. The acyloin condensation of furfural was done in phosphate buffer (pH 8.0, $\mu = 0.194$) at 60 °C. An excess of substrates (100 times the amount of catalyst) was reacted in a sealed, degassed tube, in which the system was heterogeneous throughout the reaction.

After the reaction, the reaction mixture was poured into MeOH. The precipitated polymer catalyst was filtered and the filtrate was analyzed and quantified by high-performance liquid chromatography (JASCO TriRoter-V chromatograph) with benzophenone as an internal standard. The results are shown in Table I. A low molecular weight analogue, *N*-methyl-4-phenylthiazolium iodide (MPTI), which gave a quantitative yield in MeOH,[8] had very low activity in the aqueous system. However, a polymeric catalyst, PTS^+(14), had a higher catalytic activity than MPTI in the aqueous system. Moreover, the insoluble cross-linked polymer catalyst PTS^+(14)-DVB(5) (containing 5 mol % of divinylbenzene) had the highest catalytic activity. It is thought that the hydrophobic environment formed by polymer main chains protects the active site from an attack by hydroxide ions. This assumption is supported by the fact that the more hydrophilic polymer catalysts PTS^+(69) and PTS^+(9)-NVP(89), which were synthesized by quaternization of the copolymer

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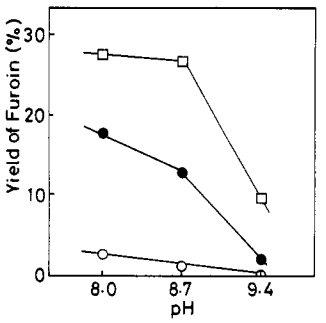

Figure 1. Dependence of furoin yield on pH in condensation reaction of furfural catalyzed by thiazolium salts ([cat.] = 3.6×10^{-2} mmol; [furfural] = 3.6 mmol; (○) MPTI, (●) $PTS^+(14)$, (□) $PTS^+(14)$–DVB(5); pH 8.0, phosphate buffer; pH 8.7, tris buffer; pH 9.4, ammonium buffer).

Table II
Results of Acyloin Condensations by Recovered Catalysts[a]

cat.	yield, % 1st	2nd[a]	inc[b]	inc 1st
MPTI	4.5		0.6	0.1
$PTS^+(14)$	21.0	0.0		
$PTS^+(14)$–DVB(5)	29.9	2.6	17.6	0.6
$PTS^+(15)$–DVB(21)	27.5	3.8	28.0	1.0

[a] [cat.] = 0.036 mmol; [furfural] = 3.6 mmol; in phosphate buffer, 3.3 mL (pH 8.0) at 60 °C for 40 h. [b] Recovered polymer catalyst. [c] Incubated in phosphate buffer (pH 8.0) at 60 °C for 40 h before use.

of VPT with *N*-vinylpyrrolidone (NVP), has less catalytic activity.

It was reported that a thiamine pyrophosphate lost catalytic activity at a high pH (pH >8).[5] We examined the pH dependence of catalytic activity for each catalyst (Figure 1). MPTI had less activity at a high pH, but the polymer catalyst $PTS^+(14)$ had appreciably greater activity at pH 8.7 as compared with the result at pH 8.0. The protection of the active site in the cross-linked polymer catalyst was greater in a more basic system (pH 9.4) in which even the $PTS^+(14)$ catalyst had less activity. We conclude that the environment around the active site becomes more hydrophobic because of the cross-linked polymer chain.

It is desirable to recover and to reuse the catalyst. Although recovering a low molecular weight catalyst is difficult, $PTS^+(14)$ can be easily recovered from the reaction mixture by the precipitation into an excess of methanol. These results are shown in Table II. The recovered polymer catalyst was used again after being washed with ether and dried, and the amount of reused polymer catalyst was the same as in the first test in spite of the loss of the active site. In methanol, it was found that the reused thiazolium salt polymer had sufficient activity.[8] Contrary to our expectations, however, in the buffer solution (pH 8.0) the recovered $PTS^+(14)$ catalyst lost all activity, and even the cross-linked PTS^+–DVB catalyst had scarcely any activity.

IR spectra of the hydrophilic polymer catalyst $PTS^+(69)$ and the recovered $PTS^+(69)$ (reacted at pH 8.4, 60 °C, 40 h) are shown in Figure 2. In an IR spectrum of the recovered polymer (Figure 2c), the characteristic absorption of an open-chain aldehyde appeared at 1670 cm^{-1}. In this figure, a spectrum of the incubated thiazolium salt polymer (Figure 2b; incubated in phosphate buffer (pH 8.4) at 60 °C for 55 h without substrate) closely resembled that of the recovered polymer catalyst. It was confirmed that the absorption at 1670 cm^{-1} was not that of a substrate (furfural) remaining in the polymer domain. Because the

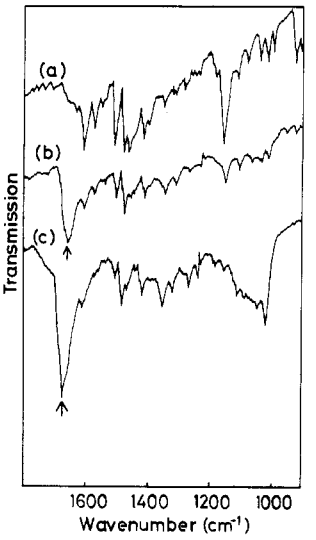

Figure 2. IR spectra of (a) $PTS^+(69)$, (b) incubated $PTS^+(69)$, and (c) recovered $PTS^+(69)$.

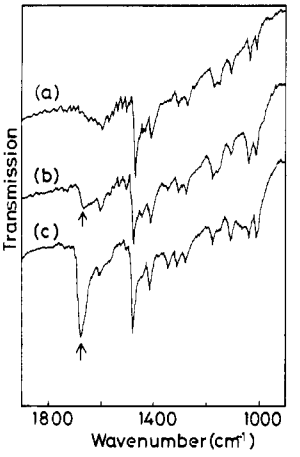

Figure 3. IR spectra of (a) $PTS^+(15)$–DVB(21), (b) incubated catalyst catalyst, and (c) recovered catalyst.

thiazolium salt polymer catalysts were hydrolyzed to the open-chain compound under the present reaction conditions, the recovered $PTS^+(14)$ lost its catalytic activity. Table II shows the activity of the incubated thiazolium salt catalyst that was incubated in phosphate buffer (pH 8.0) at 60 °C for 40 h without substrates before use. As expected, MPTI had less catalytic activity, but the activity loss of $PTS^+(14)$–DVB(5) was small. Further, a higher cross-linked catalyst $PTS^+(15)$–DVB(21) was completely unaffected by the incubation. This claim was supported by a factor shown in the IR spectra of $PTS^+(15)$–DVB(21) catalysts (Figure 3). The recovered polymer (reacted at pH 8.7, 60 °C for 18 h) exhibited a characteristic absorption of the open-chain compound at 1670 cm^{-1} (Figure 3c). However, in an IR spectrum of the incubated polymer (at pH 8.7, 60 °C for 55 h; Figure 3b) that absorption scarcely appeared. The difference between the activity of the recovered polymer catalyst and that of the incubated polymer catalyst may be due to a change in the number of active sites. Since the polymer catalyst was swollen by furfural during the reaction, the active site was easily hydrolyzed and catalytic activity decreased. However, the polymer did not swell in the incubation system; therefore the incubated polymer catalyst retained a certain amount of the active site.

In conclusion, the thiazolium salt polymer catalyst had higher catalytic activity in the acyloin condensation of furfural in buffer solution than its low molecular weight

analogue. The cross-linking of the thiazolium salt polymer catalyst was found to be useful in protecting an active thiazolium salt unit from attack by hydroxide ions at high pH, in the same way as thiamine pyrophosphate in vivo is protected by the surrounding apoenzyme. The polymer catalyst has higher activity than the low molecular weight analogue not only because of the protection of the active site but also because the hydrophobic substrate is preferentially adsorbed to the polymer domain by hydrophobic interaction.

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CORRECTIONS

Wesley Memeger, Jr.: Novel Thermotropic Main-Chain Polyhydrocarbons and Block Copoly(hydrocarbon–azomethines). Volume 22, Number 4, April 1989, p 1577.

1. The temperature in the legend to Figure 5 should read 260 instead of 270 °C.
2. The PMT of polymer D in Table I should read 220 instead of 300 °C.

V. Pavone, E. Benedetti,* V. Barone, B. Di Blasio, F. Lelj, C. Pedone, A. Santini, M. Crisma, G. M. Bonora, and C. Toniolo*: Structural Versatility of Peptides from $C^{\alpha,\alpha}$-Dialkylated Glycines. A Conformational Energy Computation and X-ray Diffraction Study of Homopeptides from 1-Aminocyclohexane-1-carboxylic Acid. Volume 21, Number 7, July 1988, p 2064.

In Table II, the bond distances $C^{\alpha}{}_i$–$C^{\beta 1}{}_i$, $C^{\alpha}{}_i$–$C^{\beta 2}{}_i$, $C^{\beta 1}{}_i$–$C^{\gamma 1}{}_i$, $C^{\beta 2}{}_i$–$C^{\gamma 2}{}_i$, $C^{\gamma 1}{}_i$–C^{δ}, and $C^{\gamma 2}{}_i$–C^{δ} given for *p*-BrBz(Acc^6)$_4$O-*t*-Bu, res 2, should be 1.560 (10), 1.546 (10), 1.360 (11), 1.358 (12), 1.540 (14), and 1.534 (13) Å, respectively.