

# Hydrophobic and Ionic Interactions in the Ester Hydrolysis by Imidazole-Containing Polymers

Iwhan Cho and Jae-Sup Shin

*Department of Chemistry, Korea Advanced Institute of Science and Technology, Seoul 131, Korea  
(Received December 18, 1981)*

*N*-Methacryloyl-*L*-histidine and *N*-methacryloyl-*L*-histidine methyl ester were synthesized and polymerized to obtain polymeric catalysts with different functions. In the presence of each of these polymers the solvolytic reactions of *p*-nitrophenyl acetate (PNPA), 3-nitro-4-acetoxybenzoic acid (NABA), 3-acetoxy-*N*-trimethylanilinium iodide (ANTI) and 3-nitro-4-decanoyloxybenzoic acid (NDBA) were performed in 20% aqueous ethanol. For the purpose of comparison the low molecular weight analogs (LMWA's), *L*-histidine, *L*-histidine methyl ester and *N*-acetyl-*L*-histidine were also subjected to catalyze the solvolyses of above substrates. In the solvolysis of PNPA the polymeric catalysts exhibited lower activities than the LMWA's. However the ionic substrates, NABA and ANTI were solvolyzed at anomalous rate by polymeric catalyst, indicating that electrostatic effects are operative in the catalysis by polymers. Furthermore in the solvolysis of hydrophobic monomer NDBA, polymeric catalysts exhibited highly enhanced activities compared with the LMWA's implying that hydrophobic interaction can be the most important contribution to the high catalytic activity of imidazole-containing polymers.

## Introduction

Looking into the labyrinth of enzymatic actions, a number of investigations have adopted model systems for studies. In the mechanisms of reactions involving hydrolytic enzyme systems the intramolecular multiple catalysis involving imidazole functions in hydrophobic environment has often been enunciated. And this type of catalysis is considered to be closely related with the unique efficiency and specificity of the enzymes. On this premises it seems justifiable to investigate the intramolecular cooperative catalysis of multifunctional catalysis systems involving imidazole in hydrophobic surroundings employing simplified model imidazole-containing polymers. In the past many investigations had adopted various imidazole-derivatives and-polymers for model enzyme studies with mixed results.<sup>1-3</sup>

In the course of our study on the polymeric catalysts it was necessary to prepare imidazole-containing polymers with different functions and to investigate the catalytic activities towards the solvolyses of a series of activated phenyl esters. Poly(*N*-methacryloyl-*L*-histidine) (Ic) and poly(*N*-methacryloyl-*L*-histidine methyl ester) (IIc) were synthesized and their catalytic activities for the solvolyses of phenyl esters of different characters—neutral, anionic, cationic and hydrophobic—were examined. The structures of substrates employed are shown below. We have also prepared the low molecular weight analogs (LMWA's) from histidine and their ester-hydrolytic activities were examined for the purpose of comparison. We have obtained some interesting results which can shed some new light on or at least confirm the extent of contribution of ionic interaction-promoted catalysis in hydrophobic environment to the activity of the simplified polymeric model enzyme systems. Results of first phase work in this study are presented in the present report.

## Experimental

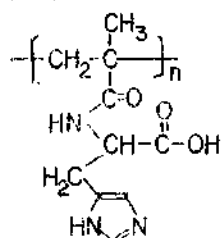
*N*-Methacryloyl-*L*-histidine. This compound was prepared according to the procedure of Okamoto<sup>4</sup>. Yield; 38 %.

*N*-Methacryloyl-*L*-histidine methyl ester. *L*-Histidine methyl ester dihydrochloride (0.12 mol) was dissolved in 2 *N* NaOH solution (0.39 mol) and to it was added at 0°C with vigorous stirring a solution of methacryloyl chloride (0.15 mol) in dichloromethane (60 ml). The reaction mixture was then brought to pH 2 with dilute hydrochloric acid and by-product methacrylic acid was removed by extraction with diethyl ether. After adjusting back to pH 7 water was evaporated under vacuum controlling the temperature below 20°C. The product was then extracted with ethanol. Upon the removal of ethanol under vacuum, the product crystallized. This procedure was repeated two more times. Yield; 35%.

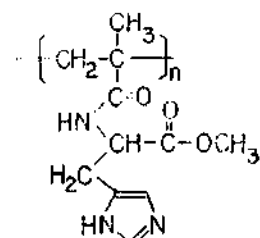
Poly(*N*-methacryloyl-*L*-histidine) (Ic). *N*-Methacryloyl-*L*-histidine (0.02 mol) and azobis(isobutyronitrile) (AIBN, 0.4 mole %) in ethanol was introduced into a polymerization tube. The solution was then degassed under vacuum and tube was sealed. During polymerization the polymer precipitated. After 48 hr at 65°C, the tube was opened and the content were added to a large volume of ethanol and stirred for 1 hr. The polymer was isolated on sintered glass funnel and was washed with ethanol again. Thus prepared polymer was dried in vacuum oven. The polymer was very hygroscopic. Yield; 74 %  $\eta_{inh}=0.26$  dl/g (1 *N* HCl 30°C).

Poly(*N*-methacryloyl-*L*-histidine methyl ester) (IIc). The polymerization procedure was same as that described above, and 0.018 mol of *N*-methacryloyl-*L*-histidine methyl ester and AIBN (0.4 mole %) were used. This polymer obtained was also very hygroscopic. Yield; 71 %  $\eta_{inh}=0.33$  dl/g (1 *N* HCl, 30°C).

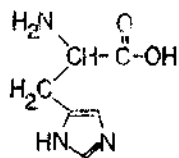
3-Nitro-4-acetoxybenzoic Acid (NABA). NABA was prepared by the procedure of Overberger *et al.*<sup>5</sup> The crude



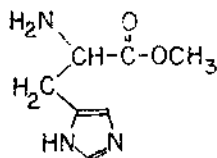
Poly(N-methacryloyl-L-histidine) (Ic)



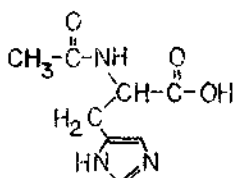
Poly(N-methacryloyl-L-histidine methyl ester) (IIc)



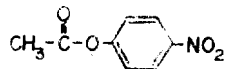
L-Histidine (III)



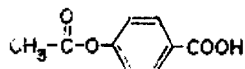
L-Histidine methyl ester (IVc)



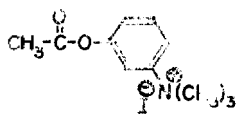
N-Acetyl-histidine (Vc)



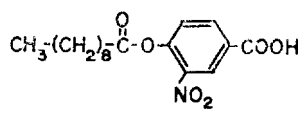
p-Nitrophenyl acetate (PNPA)



3-Nitro-4-acetoxybenzoic acid (NABA)



3-Acetoxy-N-trimethylanilinium iodide (ANTI)



3-Nitro-4-decanoyloxybenzoic acid (NDBA)

product was recrystallized from benzene. m.p. 152–153 °C.

**3-Acetoxy-N-trimethylanilinium Iodide (ANTI).** ANTI was prepared by the procedure of Overberger *et al.*<sup>6</sup> The crude product was twice recrystallized from nitromethane. Yield; 95 %, mp 209–210 °C.

**3-Nitro-4-decanoyloxybenzoic Acid (NDBA).** NDBA was prepared by the procedure of Overberger *et al.*<sup>7</sup> The crude product could be purified by two recrystallizations from cyclohexane and then finally from methanol. mp 71–72 °C.

**Kinetic measurements of Solvolytic Reactions.** Solvolysis rates were measured in 80% water–20% ethanol solution buffered with 0.02 M tris(hydroxymethyl) aminomethane and hydrochloric acid with sufficient potassium chloride to adjust the ionic strength to 0.02. In all cases, a 10-fold molar excess of catalyst over substrate was used. The reactions were followed spectrophotometrically at the absorption maximum of the corresponding phenols: at 400 nm for PNPA; at 410 nm for NABA and NDBA; and at 277 nm for ANTI. All experiments were carried out at 25 °C. Kinetic data were treated as pseudo first-order by plotting  $(A_{\infty} - A_t)$  values vs. time. The slope of the plot ( $k_{\text{meas}}$ ) was corrected by subtracting the blank rate to obtain the observed rate constant  $k_{\text{obs}}$ . The second-order rate constant  $k_{\text{cat}}$  was then calculated by dividing  $k_{\text{obs}}$  by the catalyst concentration. Thus obtained

$k_{\text{cat}}$  values are to be called apparent catalytic constants.

$$k_{\text{cat}} = k_{\text{obs}} / [\text{catalyst}]$$

## Results and Discussion

**Solvolysis of p-Nitrophenyl Acetate (PNPA).** For the comparison with the previously investigated imidazole catalyst systems the neutral PNPA was solvolyzed by various imidazole-containing polymers and LMWA's at two different pH 7.08 and 8.03. The results are summarized in Table 1.

The results in Table 1 show that apparent catalytic rate constants for polymeric catalysts were lower than those for the LMWA's. These results are in contrast with those observed with poly[4(5)-vinylimidazole] and imidazole in the solvolyses of the same PNPA<sup>5</sup>. The enhanced catalytic activity of poly[4(5)-vinylimidazole] has been attributed to bifunctional cooperative actions by neighboring imidazole groups on the polymer. However no such effects were observed in the solvolysis of PNPA by polymers Ic and IIc. It seems probable that the imidazole groups of poly[4(5)-vinylimidazole] are in close proximity, thus the bifunctional catalysis becoming possible. This is obviously not the case for presently investigated polymers Ic and IIc. If no bifunctional catalysis is expected, polymeric catalysts would suffer more steric hindrance in their reaction with substrates, exhibiting lower activity.

**Solvolysees of Charged Substrates, NABA and ANTI.**

The solvolyses of negatively charged substrate, NABA and positively charged substrate, ANTI catalyzed by various

TABLE 1: Apparent Catalytic Rate Constants ( $k_{\text{cat}}$ ) for the Solvolyses<sup>a</sup> of p-Nitrophenyl Acetate (PNPA) by Various Imidazole-Containing Polymers and Their Low Molecular Weight Analogs ( $k_{\text{cat}}$ , 1/mole min)

pH	Catalyst <sup>b</sup>				
	Ic	IIc	IIIc	IVc	Vc
7.08	1.3	1.0	1.2	2.4	1.9
8.03	2.1	1.4	7.2	9.0	7.5

<sup>a</sup> The concentration of catalysts were all  $5 \times 10^{-4}$  mole/l (in imidazole). The concentration of substrate was  $5 \times 10^{-5}$  mole/l.

<sup>b</sup> Ic: Poly(N-methacryloyl-L-histidine); IIc: Poly(N-methacryloyl-L-histidine methyl ester); IIIc: L-Histidine; IVc: L-Histidine methyl ester; Vc: N-Acetyl-L-histidine.

TABLE 2: Apparent Catalytic Rate Constants ( $k_{\text{cat}}$ ) for the Solvolyses<sup>a</sup> of 3-Nitro-4-acetoxybenzoic Acid (NABA) and 3-Acetoxy-N-trimethylanilinium Iodide (ANTI) by Various Imidazole-Containing Polymers and Their Low Molecular Weight Analogs ( $k_{\text{cat}}$ , 1/mole min)

Substrate	pH	Catalyst				
		Ic	IIc	IIIc	IVc	Vc
NABA	8.03	1.8	8.4	10.8	12.7	9.7
ANTI	8.78	16.7	6.9	4.8	4.2	5.0

<sup>a</sup> See Table 1 for catalysts. The concentration of catalysts were  $5 \times 10^{-4}$  mole/l (in imidazole). The concentration of substrates were all  $5 \times 10^{-5}$  mole/l.

imidazole-containing polymers and their LMWA's were performed. The results are summarized in Table 2.

Interesting to note in Table 2 is the catalytic effects of negatively charged polymeric catalyst Ic toward the solvolyses of charged substrates. Ic exhibited enhanced activity for the solvolysis of positively charged ANTI but retarded activity for negatively charged NABA. The retardation in catalysis is significant even when compared with its activity for the neutral substrate. The enhanced and retarded catalytic activity of Ic toward charged substrates are the obvious consequence of favorable and unfavorable ionic interactions between catalyst and substrates. Also interesting to observe is that this type of ionic interactions are not operative or insignificant if there are any in the case of LMWA's. We are not certain why this type of ionic interactions are observable only with polymeric catalysts.

*Solvolyses of 3-Nitro-4-decanoyloxybenzoic Acid.* In order to see the extent of hydrophobic interaction operative in the presently investigated system, the solvolyses of the hydrophobic substrate, NDBA were carried out in the presence of polymer catalysts and LMWA's at pH 8.03 and the results are shown in Table 3.

When the acyl group of substrate NABA becomes larger,

**TABLE 3: Apparent Catalytic Rate Constants ( $k_{cat}$ ) for the Solvolyses\* of 3-Nitro-4-decanoyloxybenzoic Acid (NDBA) by Various Imidazole-Containing Polymers and Their Low Molecular Weight Analogs (pH 8.03) ( $k_{cat}$ , 1/mole min)**

Catalyst	Ic	IIc	IIIc	IVc	Vc
Catalytic rate constant	47.5	61.3	2.9	5.8	4.0

\* See Table 1 for catalysts. The concentration of catalysts were all  $5 \times 10^{-4}$  mole/l (in imidazole). The concentration of substrate was  $5 \times 10^{-5}$  mole/l.

the steric hindrance in the nucleophilic reaction at the carbonyl group will increase. At the same time hydrophobicity of the substrate will also increase. In the solvolysis of NDBA by LMWA's such as IIIc, IVc and Vc, the apparent catalytic rate constants were found to be smaller than those for NABA, indicating increase in steric hindrance. In spite of the large increase in steric hindrance polymeric catalysts and IIc exhibited highly enhanced catalytic activity toward NDBA, and it indicates that the polymethacrylate backbones possess significant hydrophobic character. For the rate constants presented in Table 3 it becomes conclusive that in the presently studied polymer catalyst systems the contribution of hydrophobic interactions are far greater than that of ionic interactions.

We are now in the process of construction of a system in which ionic interactions are combined with hydrophobic interactions to maximize the catalytic activity of polymeric imidazoles, and the results will be published at later date.

### References

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