PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/16403

Please be advised that this information was generated on 2019-04-19 and may be subject to change.
Poly(aminomethylenes). 10. Esterolytic catalysis by poly(carbylhistidine) and poly(carbylhistamine)*

J. M. van der Eijk, Ch. F. Gusdorf, R. J. M. Nolte and W. Drenth

Laboratory for Organic Chemistry of the University at Utrecht, Croesestraat 79, 3522 AD Utrecht, The Netherlands
(Received November 27th, 1978)

Abstract. The catalytic activities in the hydrolysis of 4-nitro- and 2,4-dinitrophenyl acetate were determined for a polymer of 3-(4-imidazolyl)-2-isocyanopropanoic acid (Ia) and of 2-(4-imidazolyl)-1-isocyanoethane (Ib). A detailed kinetic analysis has been performed. The reaction starts with acylation of the imidazole groups followed by deacylation. With excess of substrate the second step is rate-determining and the system shows burst kinetics behaviour. The rate of acylation of Ia can be analyzed by a two term equation; the major term involves the COOH groups. A transition state is proposed in which COOH stabilizes the negative charge developing on carbonyl oxygen. This system is approximately 400 times more active than e.g. histidine. The data for Ib reveal that the polymer imidazole groups are approximately six times more active than e.g. the imidazole group of histamine. This phenomenon is ascribed to a cooperative effect of the polymer imidazoles.

The deacylation is general base catalysed. Its rate constants are almost equal to those of acetylimidazole. For comparison, the hydrolysis of the charged substrates 4-acetoxy-3-nitrobenzoic acid anion and 3-acetoxy-N,N,N-trimethylanilinium iodide by Ia and Ib has been included.

Introduction

Histidyl imidazole is involved in the catalytic action of several hydrolytic enzymes. In order to understand its role, monomeric and polymeric model systems have been studied. Cooperative effects of imidazole and other catalytic functions like carboxyl and hydroxyl groups have given insight into the factors which govern the high efficiency of enzymes.

In this paper we describe the esterolytic catalytic activity of poly(aminomethylenes), \( \text{R-}N=C^\text{n} \), which contain imidazole groups in their side chains R. Two factors have been decisive for choosing poly(aminomethylenes) as supports for imidazole. Firstly, these polymers have a rigid rod structure and thus, provide a rather well defined microenvironment for catalysis. Most polymeric supports for catalysts do not fulfill this condition because they are random coils like vinyl polymers or networks like poly(ethyl-imide). Secondly, the poly(aminomethylene) rigid rods are tightly coiled helices. Left-handed and right-handed helices can be obtained through resolution or by the stereoselective polymerization of one enantiomer of a chiral monomer. Because of their chirality enantiomers of poly(aminomethylenes) are attractive model systems for the enantiospecific action of enzymes. So far, little attention has been paid to this aspect of enzymatic catalysis in polymeric model systems.

The purpose of the present investigation was to study the catalytic activity of poly(aminomethylene) anchored imidazole towards achiral activated esters. This study was deemed to be necessary before directing ourselves to enantioselective esterolysis experiments. The latter experiments will be the subject of a subsequent paper. Two polymers have been used: poly(carbylhistidine), Ia, and poly(carbylhistamine), Ib. (Their official names are in Ref. 12.) Syntheses have been described in a previous paper.13

* Dedicated to Prof. Dr. E. Havinga on the occasion of his 70th birthday.

2 F. Schneider, Angew. Chem. 90, 616 (1978).
Results

Hydrolysis of 4-nitro- and 2,4-dinitrophenyl acetate. In our first series of experiments the esterolytic activities of both polymers, 1a and 1b, and for comparison, also of l-histidine, 2a, and histamine, 2b, towards 4-nitrophenyl acetate (PNPA) and 2,4-dinitrophenyl acetate (DNPA) were measured under conditions of excess of imidazole groups at 25.00°C in 29 vol. % aqueous ethanol; [Cat.] > [Substrate]. Acetate, phosphate or Tris buffers were added and the ionic strength was kept constant at 0.02 mol/l.

Rates were determined by following the increase in absorption at 400 or 360 nm. These absorptions increase because of the release of 4-nitrophenolate and 2,4-dinitrophenolate ions, respectively. All experiments obeyed first order kinetics. The difference between the first order rate constants \( k_{\text{meas.}} \) (with catalyst) and \( k_{\text{blank}} \) (without catalyst), \( k_{\text{obsd}} = k_{\text{meas.}} - k_{\text{blank}} \), is proportional to the molar concentration of imidazole groups, [Cat.].

The second order catalytic rate constants, \( k_a \) at different pH values are summarized in Tables I and II.

Table I Catalytic rate constants \( a \) of poly(carbylhistidine) (1a), l-histidine, poly(carbylhistamine) (1b) and histamine (2b) in the hydrolysis of PNPA.

| pH  | \( k_a \times 10^2 \) (1 mol \(^{-1}\) l. mol \(^{-1}\) s \(^{-1}\)) |
|-----|-----------------|---|---|---|
|     | 1a              | 2a | 1b | 2b |
| 7.6  | 6.8             | 1.5 | 5.1 | 2.5 |
| 8.2  | 10.4            | 3.5 | 7.0 | 6.2 |
| 8.6  | 13.0            | 5.1 | 9.1 | 13.3 |
| 8.8  | 14.2            | 4.9 | 9.9 | 17.8 |
| 9.3  | 24.9            | 5.1 | 16.7 | 52.0 |

\( a \) The concentrations of 1 and 2 are 3 \times 10^{-4} and 5 \times 10^{-4} mol imidazole groups/l, respectively; the initial concentration of PNPA is 6.7 \times 10^{-5} mol/l.

Table II Catalytic rate constants \( b \) of poly(carbylhistidine) (1a), l-histidine, poly(carbylhistamine) (1b) and histamine (2b) in the hydrolysis of DNPA.

| pH  | \( k_a \times 10^2 \) (1 mol \(^{-1}\) l. mol \(^{-1}\) s \(^{-1}\)) |
|-----|-----------------|---|---|---|
|     | 1a              | 2a | 1b | 2b |
| 4.5  | 7.58            | 3.70 | 11.3 | 4.72 |
| 5.5  | 15.0            | 11.5 | 20.1 | 14.5 |
| 5.9  | 27.3            | 17.0 | 32.7 | 21.6 |
| 6.0  | 39.0            | 22.2 | 47.8 | 25.7 |
| 6.3  | 45.1            | 25.1 | 45.2 | 30.2 |
| 6.6  | 56.5            | 32.3 | 59.1 | 35.9 |
| 6.7  | 68.5            | 35.3 | 92.8 | 36.8 |
| 7.1  | 99.5            | 46.5 | 193 | 52.2 |
| 7.5  | 145             | 58.1 | 243 | 71.3 |
| 7.9  | 150             | 79.8 | 103 |
| 8.3  | 177             | 108 | 252 | 148 |
| 8.9  | 230             | 174 | 247 | 280 |
| 9.3  | 259             | 185 | 264 | 292 |

\( b \) The concentrations of 1 and 2 are 3 \times 10^{-4} and 5 \times 10^{-4} mol imidazole groups/l, respectively; the initial concentration of DNPA is 6.7 \times 10^{-5} mol/l.

In a second series of experiments reactions were carried out under conditions of excess of substrate. In this series the esterolytic catalytic activity of 1a (2 \times 10^{-2} mol imidazole groups/l) towards PNPA (5-25 \times 10^{-4} mol/l) was measured at pH 8.0 by the pH-stat method. A plot of initial rate versus substrate concentration gave a straight line. From the slope of this line the second order rate constant, \( k_a = (8.5 \pm 1.0) \times 10^{-2} \) l.mol\(^{-1}\)s\(^{-1}\) was calculated. This value is in agreement with the value which is obtained by interpolating to pH 8.0 the data in Table I (\( k_a = 9.0 \times 10^{-2} \) l.mol\(^{-1}\)s\(^{-1}\)).

The catalytic activity of 1a (1.0 \times 10^{-4} mol imidazole groups/l) at pH 7.0 towards DNPA (2-25 \times 10^{-4} mol/l) showed a different behaviour. The plot of initial rates against substrate concentration leveled off at high substrate concentrations, giving rise to a Michaelis-Menten type of curve.

The rate data fitted the equation

\[ v = k[S]_0/([S]_0 + K) \]

[2] where \( k \) and \( K \) are constants and \( [S]_0 \) is the initial concentration of ester substrate. From the slope of the plot of \( v \) versus \([S]_0\), at \([S]_0 = 0\) the second order rate constant \( k_0 = (k/K) \) was calculated. This rate constant equals, within experimental error, the rate constant which can be obtained from Table II by extrapolation to pH 7.0 (\( k_0 = 0.81 \) l.mol\(^{-1}\)s\(^{-1}\)).

As appeared from our first series of experiments, the hydrolysis of DNPA catalysed by various concentrations of excess of 1a did not show saturation kinetics. Therefore, the Michaelis-Menten like behaviour at excess substrate concentrations was suspected to be the result of a change in rate-determining step, viz. from step 1 to step 2 in Scheme I.

\[
\begin{align*}
\text{(P \text{+ CH}_3\text{COOR})} & \quad \xrightarrow{k_a} \quad \text{NCOCH}_3 \\
& \quad \xrightarrow{k_d} \quad \text{(+H}_2\text{O)}
\end{align*}
\]

Scheme I

In order to confirm this idea the initial stage of the hydrolysis of DNPA (9-26 \times 10^{-4} mol/l) by 1a (2.55 \times 10^{-4} mol imidazole groups/l) was carefully followed by UV at 455 nm and an ionic strength of 0.05. Typical burst behaviour, masked in the more sluggish pH-stat technique, was now observed: an initial fast exponential liberation of 2,4-dinitrophenolate followed by a slower, steady release. The presteady state liberation can be attributed to an almost complete acylation of the catalyst and the subsequent slower release to a steady state turnover reaction.

The initial rate of the presteady state reaction appeared to be linear in substrate concentration, whereas the rate of the steady state reaction showed the substrate dependency of eqn. [2]. This result is indeed in line with a change from step 1 to step 2 being rate-determining. Scheme I predicts \( v \) to be linear in \([S]_0\), when step 1 is rate-determining and a relation between \( v \) and \([S]_0\), as in eqn. [2], when step 2 is rate-determining. In the latter case the constants of eqn.

A curve fitting procedure was used for determining the rate constants and \( k_d \). The coupled differential equations

\[
\frac{d[RO^\text{-}]}{dt} = k_a[\text{Cat.}]_0[S] + k_1[S]
\]

where \([\text{Cat.}]_0\) is the concentration of non-acylated imidazole. The term \( k_1[S] \) represents the uncatalysed reaction, \( k_d \) being obtained from a separate experiment. Applying the equalities

\[
[\text{Cat.}] = [\text{Cat.}]_0 - [\text{ImAc}]
\]

\[
[S] = [\text{RO}^\text{-}]_\text{t=0} - [\text{RO}^\text{-}],
\]

eqn. [3] can be rearranged to:

\[
\frac{d[\text{RO}^\text{-}]}{dt} = \left(k_a[\text{Cat.}]_0 + k_1 - k_d[\text{ImAc}]\right)
\]

\[
\left([\text{RO}^\text{-}]_\text{t=0} - [\text{RO}^\text{-}],\right)
\]

The time dependency of \([\text{ImAc}]\) is described by:

\[
\frac{d[\text{ImAc}]}{dt} = k_a[\text{Cat.}]_0[S] - k_d[\text{ImAc}]
\]


\[
\frac{d[\text{ImAc}]}{dt} = \left(k_a[\text{Cat.}]_0([\text{RO}^\text{-}]_\text{t=0} - [\text{RO}^\text{-}]), - [\text{ImAc}]\right)
\]

The coupled differential equations [6] and [8] were solved by numerical methods and fitted to the experimental kinetic data. A representative example is given in Fig. 1. Rate constants \( k_a \) and \( k_d \) calculated in this way from the burst experiments at several pH values and different buffer concentrations are presented in Table III. Considering the sometimes appreciable experimental error, the \( k_a \)-values of Table III agree reasonably well with the corresponding \( k_d \)-values of Table II.

![Fig. 1. Hydrolysis of DNPA by poly(carbylhistidine) at pH 9.1. The dots are experimental points; the line is computer calculated. For conditions see Table III.](image)

**Table III** Catalytic rate constants of poly(carbylhistidine) (1a) in the hydrolysis of DNPA from a computer analysis of the burst experiments.

<table>
<thead>
<tr>
<th>pH</th>
<th>( k_a \times 10^2/(1\text{mol}^{-1}\cdot\text{s}^{-1}) )</th>
<th>( k_d \times 10^4/\text{s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1</td>
<td>0.85</td>
<td>0.92</td>
</tr>
<tr>
<td>7.7</td>
<td>1.15</td>
<td>1.7</td>
</tr>
<tr>
<td>8.1</td>
<td>1.2</td>
<td>2.3</td>
</tr>
<tr>
<td>8.7</td>
<td>1.7</td>
<td>6.3</td>
</tr>
<tr>
<td>9.1</td>
<td>3.0</td>
<td>12</td>
</tr>
</tbody>
</table>

* Mean value from experiments at different buffer concentrations (0.02-0.06 mol/l); estimated error 10%.
* Extrapolated to zero buffer concentration from three experiments at buffer concentrations of 0.02, 0.04 and 0.05 mol/l, respectively; estimated error 10%.
* Phosphatc buffer.
* Tris buffer.

Hydrolysis of 4-acetoxy-3-nitrobenzoic acid anion and 3-acetoxy-N,N,N-trimethylanilinium iodide. The hydrolysis of the negatively charged substrate 4-acetoxy-3-nitrobenzoic acid anion (NABA) catalysed by excess of 1a and of 1b was measured at pH 7.5 and 8.8. Rates were determined from the release of the 4-carboxy-2-nitrophenolate ion, observed at 410 nm. The kinetic behaviour was similar to that of PNPA and DNPA under excess catalyst conditions. The rate constants are given in Table IV.

For the positively charged substrate 3-acetoxy-N,N,N-trimethylanilinium iodide (ANTI) the hydrolysis catalysed by excess of 1a and of 1b was followed at pH 8.9 using a pH-stat. The results are given in Table IV.

**Table IV** Catalytic rate constants of poly(carbylhistidine) (1a) and poly(carbylhistamine) (1b) in the hydrolysis of a positively charged ester (ANTI) and a negatively charged ester (NABA).

<table>
<thead>
<tr>
<th>Ester</th>
<th>pH</th>
<th>( k_a \times 10^2/(1\text{mol}^{-1}\cdot\text{s}^{-1}) )</th>
<th>( 1a )</th>
<th>( 1b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTIa</td>
<td>8.9</td>
<td>10</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>NABAa</td>
<td>8.8</td>
<td>10</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>NABAa</td>
<td>8.7</td>
<td>5.8</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>NABAa</td>
<td>7.9</td>
<td>5.1</td>
<td>7.9</td>
<td></td>
</tr>
</tbody>
</table>

* The concentration of both polymers is 2.3 \times 10^{-4} mol imidazole groups/l; the initial concentration of ANTI is 6.7 \times 10^{-3} mol/l; ionic strength 0.02 mol/l.
* The concentration of both polymers is 2.5 \times 10^{-4} mol imidazole groups/l; the initial concentration of NABA is 10^{-3} mol/l; ionic strength 0.02 mol/l.

**Discussion**

**Acylation.** The kinetic data suggest that the hydrolysis of activated esters catalysed by poly(iminomethylene) anchored imidazole follows a two step mechanism involving an acyl intermediate, as depicted in Scheme 1. Although evidence for this suggestion is provided only by the experiments with poly(carbylhistidine) and excess of DNPA, it presumably will be present in the other cases as well. In the present system acylation of imidazole is not preceded by a binding of substrate to the catalyst in contrast to what is observed for enzymes. This can be concluded from the linear dependency of \( k_a \) from substrate concentration under conditions of excess of catalyst as well as of excess of substrate. Catalyst-substrate binding in polymeric model systems has only been reported for long chain substrates and for catalysts modified with long chain side groups; these long chains enhance hydrophobic interactions.
Fig. 2. Catalytic rate constants of poly(carbarylhistidine), O and •, and poly(carbarylhistamine), △, in the hydrolysis of DNPA as a function of pH. O Excess of catalyst; • excess of substrate.

Fig. 3. Catalytic rate constants of L-histidine, O, and histamine, △, in the hydrolysis of DNPA as a function of pH.

In Figs. 2 and 3 the second order rate constants $k_2$ for the hydrolysis of DNPA catalysed by 1a and 1b, and by 2a and 2b, respectively, are plotted against pH. Similar plots for hydrolysis of PNPA by these catalysts do not differ significantly from the DNPA profiles but are limited to a smaller pH range due to the higher pKₐ value of 4-nitrophenol. Therefore, they will not be discussed separately.

An adequate analysis of the data of Figs. 2 and 3 requires a knowledge of the fraction of unprotonated imidazole groups ($a_{im}$). For 1a and 1b the relationships [9] and [10] between $a_{im}$ and the pH have been determined by potentiometric titrations.\(^\text{13}\)

$$\text{la PH} = 9.30 - 1.53 \log\left(\frac{1-a_{im}}{a_{im}}\right) \quad [9]$$

$$\text{lb PH} = 5.18 - 1.43 \log\left(\frac{1-a_{im}}{a_{im}}\right) \quad [10]$$

Fig. 4. Catalytic rate constants of poly(carbarylhistidine), O and •, and poly(carbarylhistamine), △, in the hydrolysis of DNPA as a function of the fraction of unprotonated imidazole groups, $a$. O Excess of catalyst; • excess of substrate.

Fig. 5. Catalytic rate constants of L-histidine, O, and histamine, △, in the hydrolysis of DNPA as a function of the fraction of unprotonated imidazole groups, $a$.

The monomers histidine (2a) and histamine (2b) both possess a pKₐ of 6.0.\(^\text{15,16}\)

In Figs. 4 and 5, $k_A$ is plotted against the $a_{im}$ value calculated for each pH. For both 2a and 2b there is a linear relationship between $k_A$ and $a_{im}$ up to $a_{im} \approx 0.8$. Both lines have slopes which are the same within experimental error (0.44 ± 0.04 l.mol⁻¹ s⁻¹). Above $a_{im} \approx 0.8$ the curves strongly deviate from linearity in upwards direction due to participation by


\(^{15}\) E. Katchalski et al., Arch. Biochem. Biophys. 88, 361 (1960).
the unprotonated amino functions of 2 (pKₐ (NH₃) = 9.1 for 2a, pKₐ (NH₂) = 9.7 for 2b)\textsuperscript{17}. Plots of these upwards deviations of kₐ against the calculated fraction of unprotonated α-amino groups appeared to be linear with a high degree of accuracy (r = 0.999); the corresponding second order rate constants are kₐ (NH₃) = 5.0 ± 0.1 l.mol⁻¹.s⁻¹ for 2a and 11.9 ± 0.3 l.mol⁻¹.s⁻¹ for 2b. The behaviour of 1a is completely different: the plot of kₐ versus aₐm curves downwards. The curve can be described by equation \[ [11] \]

\[
kₐ = kₐ,1aₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐₐ$_{\text{COOH}}$

\[
\text{pH} = 5.85 - 1.37 \log \left( \beta/(1 - \beta) \right) \quad [12]
\]

Using eqns. [9] and [12] and the rate constants kₐ in Tables II and III, the constants kₐ,1 and kₐ,2 are estimated to be (5.4 ± 0.3) l.mol⁻¹.s⁻¹ and (180 ± 20) l.mol⁻¹.s⁻¹, respectively. The solid line for 1a in Fig. 5 is the line calculated from eqn. [11]. It appears from these results that the most effective pathway for hydrolysis is the one which involves the carboxylic group. The corresponding transition state most probably is:

![Diagram of the transition state](attachment:transition_state.png)

Similar cooperative effects of imidazole and carboxyl groups are known to occur in hydrolytic enzymes\textsuperscript{2}. Unfortunately, in the present system the pKₐ values of the imidazole group and the carboxylic group differ too much for the above mentioned pathway to be predominant at higher pH's.

For polymer 1b the plot of kₐ versus aₐm curves upwards strongly (Fig. 4). A comparison of kₐ at aₐm = 1 for this compound and the corresponding rate constant of the model compound 2b, obtained by extrapolating the straight line below a = 0.8 to a = 1, shows that the anchored imidazole group is 6 times more active as a catalyst than the non-anchored imidazole group. One might wonder whether this higher activity is due to the involvement of an anionic imidazole species in catalysis by 1b. Suggestions of this kind can be found in the literature\textsuperscript{4,5} for other imidazole-containing polymeric catalysts. However, it seems unlikely that in the pH range that we have studied, even small amounts of anionic imidazole groups are present, considering the very high pKₐ,2-value of the imidazole group (estimated to be 14.5\textsuperscript{18}). Support for this noninvolvement of anionic imidazole groups is afforded by the presence of a plateau in the pH

profile of polymer 1b in Fig. 2. The observed higher activity of 1b as compared with 2b is more satisfactorily explained by a cooperative action of several neutral imidazole functions along the polymer chain:

\[ \text{Such a cooperative action might also be operative in polymer 1a. However, we could not verify this experimentally because of lack of rate data at large aₐm-values for this compound.} \]

An additional reason for the steep upward curving of the kₐ versus aₐm plot of polymer 1b might be that going from small to large aₐm-values and consequently high to low degree of protonation of imidazole functions, a reduction occurs in unfavourable electrostatic interactions in the transition state. These interactions are conceivable to appear when an imidazole group attacks the ester substrate: the positive charge developing on the attacking nucleus is destabilized by the positive charges of neighbouring protonated imidazoles. On the other hand, one might expect a stabilization of the transition state by similar interactions between these neighbouring groups and the developing negative charge on the carbonyl oxygen atom of the ester substrate. Apparently, the latter stabilization does not take place to a considerable extent. In this context it is worth noting that the rate constant kₐ,2 of polymer 1a is about 1.3–1.5 times that of polymer 1b.

### Table V Catalytic rate constants of poly(carbylhistidine) (1a), poly(carbylhistamine) (1b), poly(vinyylimidazole) (PVIm) and a copolymer of vinylimidazole and acrylic acid (PVIm–Ac) in the hydrolysis of PNPA, NABA and ANTI.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>kₐ × 10⁻³(l.mol⁻¹.s⁻¹) / pH 8.8 ±0.2</th>
<th>kₐ (1a)/kₐ (1b)</th>
<th>kₐ (PVIm–Ac)/kₐ (PVIm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>15</td>
<td>6.8</td>
<td>1.3–1.5</td>
</tr>
<tr>
<td>1b</td>
<td>10</td>
<td>5.4</td>
<td>0.15–0.25</td>
</tr>
<tr>
<td>PVIm–Ac</td>
<td>16</td>
<td>3</td>
<td>0.6–0.7</td>
</tr>
<tr>
<td>PVIm</td>
<td>70</td>
<td>20</td>
<td>0.15–0.0025</td>
</tr>
<tr>
<td>NABA</td>
<td>5.8</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>PVIm–Ac</td>
<td>9.9</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>PVIm</td>
<td>5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>ANTI</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

\* Second order rate constants for PVIm–Ac and PVIm are estimated from Figs. 3 and 5 of Ref. 23, and from Tables I and IV of Ref. 24.

\* Polymer composition 46.3% imidazole.

\textsuperscript{17} D. D. Perrin, Dissociation constants of organic bases in aqueous solution, Butterwords (London), Supplement 1972.

\textsuperscript{18} T. C. Bruce and J. L. Herz, J. Am. Chem. Soc. 86, 4109 (1964).


ten times greater than the corresponding rate constant of polymer \( \text{Ib} \), which can be calculated from the first part (up to \( a_m = 0.6 \)) of the plot in Fig. 4: \( k_{a,1} = 0.45 \text{l.mol}^{-1} \text{s}^{-1} \). This is conceivable because in polymer \( \text{Ia} \) the positively charged imidazole nuclei are partly counterbalanced, even at relatively low pH values, by the negatively charged carboxylic anions. If there is a stabilization of the transition state by positively charged neighbouring imidazole groups the \( k_{a,1} \) constant of \( \text{Ia} \) should be lower than that of \( \text{Ib} \), if other factors do not predominate.

It is of interest to make a comparison between the catalytic activities of our polymers \( \text{I} \) and those of poly(vinylimidazole) (PVIm) and a copolymer of vinylimidazole and acrylic acid (PVIm—Ac). In Table V relevant data are collected for PNPA as are data for the two charged substrates ANTI and NABA. Comparable rate constants for DNPA are not available in the literature.

The catalytic activities of \( \text{Ia}, \text{Ib} \) and PVIm—Ac towards PNPA are almost the same. PVIm is more active than the comparable species \( \text{Ib} \). For the charged substrates NABA and ANTI it is worthwhile to compare the rate ratio of \( \text{Ia} \) to \( \text{Ib} \) with the rate ratio of PVIm—Ac to PVIm. The decrease in activity towards the negatively charged NABA by the introduction of a carboxylic function is larger for the polyvinyl system than for our poly(iminomethylenes). A similar conclusion can be drawn with respect to the increase in activity towards the positively charged ANTI, although here the effect is less pronounced. This means that interactions with charged substrates are more effective for vinylic polymers than for poly(iminomethylenes). This behaviour reflects the difference in structure of both supports. The flexible polyvinyl support is more able to accommodate a positively charged substrate than is the rigid rod poly(iminomethylene) support. For the same reason the former support might be more effective in repelling a negatively charged substrate than the latter support.

**Decaylation.** The good leaving group ability of the 2,4-dinitrophenoxyide ion enables us to study the decaylation process. The first order rate constant, \( k_d \), for decaylation of acetylated 2,4-vinylimidazole is 5 times greater than that of acetyl-4(5)-vinylimidazole. Catalysis by free imidazole is not expected to be appreciable.

From the slope and intercept of Fig. 6 the contributions of the hydroxyl ion and solvent catalysed decaylation have been calculated:

\[
\begin{align*}
k_d(\text{OH}^-) & = 275 \pm 15 \text{l.mol}^{-1} \text{s}^{-1} \\
k_d(\text{H}_2\text{O})[\text{H}_2\text{O}] & = (1.2 \pm 0.3) \times 10^{-4} \text{s}^{-1}
\end{align*}
\]

The corresponding constants for hydrolysis of acetylimidazole in water at 25°C are:

\[
\begin{align*}
k_d(\text{OH}^-) & = 317 \text{l.mol}^{-1} \text{s}^{-1} \\
k_d(\text{H}_2\text{O})[\text{H}_2\text{O}] & = 0.83 \times 10^{-4} \text{s}^{-1}
\end{align*}
\]

![Fig. 6. Rate constants of decaylation of acetylated poly(carbonyllhistidine) as a function of the concentration of hydroxyl ions.](image)
The values for $k_d(OH^-)$ as well as for $k_d(H_2O)$ do not differ significantly for the two systems. The similarity of $k_d(OH^-)$ values indicates an absence of electrostatic repulsion between the hydroxyl ions and the negatively charged carboxylate groups in the polymer.

**Experimental part**

**Materials**
Poly(carbylhistidine) and poly(carbylhistamine) were prepared as described previously\(^1\). L-Histidine monohydrate and histamine dihydrochloride were obtained from Baker and Aldrich, respectively. 4-Nitrophenyl acetate (m.p. 79°C), 2,4-dinitrophenyl acetate (m.p. 70-71°C), 4-acetoxy-3-nitrobenzoic acid (m.p. 151°C) and 3-acetoxy-N,N,N-trimethylanilinium iodide (m.p. 208°C) were prepared according to literature methods\(^2\).\(^3\)\(^4\)\(^5\).

**Kinetics**

**UV-measurements.** For the experiments under conditions of excess of catalyst, $3.2 \times 10^{-4}$ and $5.0 \times 10^{-4}$ mol/l solutions of 1 and 2, respectively, were prepared in 28.5 vol. % EtOH–H$_2$O with sufficient potassium chloride to adjust the ionic strength to 0.02 mol/l. At pH 8 and higher, solutions were buffered with 0.02 mol/l Tris and hydrochloric acid; for the pH region 6 to 8, KH$_2$PO$_4$ and Na$_2$HPO$_4$ buffers were prepared in such a way that the ionic strength was 0.02 (mol/l); for the pH region below 6, solutions were buffered with 0.02 mol/l sodium acetate and hydrochloric acid. The substrates PNPA and DNPA were dissolved in 28.5% EtOH–H$_2$O to a concentration of 10$^{-4}$ mol/l. For each measurement the catalyst solution (2.8 ml) and the substrate solution (0.2 ml) were mixed in a quartz cell, which was subsequently placed in a Cary type 15 spectrophotometer, thermostated at 25.00°C. Absorbances (A$_t$) of 4-nitrophenolate at 400 nm and 2.4-dinitrophenolate at 360 nm were followed as a function of time. After at least ten half-lives the absorbance for complete reaction (A$_\infty$) was measured. Catalysis by buffer only was measured in the same fashion. For burst experiments equal volumes of a solution of substrate in 57% EtOH–H$_2$O and of catalyst in pure water (both of 25°C) were mixed (t = 0). Recording at 455 nm was started within 20 s. Experiments with the polymeric catalysts and NABA were performed in a similar way. Thus, 2.8 ml of catalyst solution and 0.2 ml of substrate solution (1.5 mol/l in acetonitrile) were mixed and the reaction was recorded at 410 nm.

**pH-Stat measurements**

The reaction solution (7 ml) contained $10^{-4}$ mol/l poly(carbylhistidine) and $(0.3-2.5) \times 10^{-3}$ mol/l DNPA in 28.5% EtOH–H$_2$O. The reaction rate at 25.0°C and pH 7.0 was obtained from the volume of titrant (0.01 mol/l NaOH in 28.5% EtOH–H$_2$O) needed to neutralize the acetic acid and 2,4-dinitrophenol formed. The titrant volume _versus_ time plot was recorded using a Radiometer Titrigraph, type SBR 2c, equipped with an automatic burette. Experiments with poly(carbylhistidine) and ANTI were performed in a similar way.

**Computer calculations**

The differential equations were solved by a numerical method devised by Bulirsch and Stoer. This method is available in the FORTRAN subroutine DREBS from IMSL\(^2\). The minimalization was worked out by the Levenberg–Marquardt method, which is the basis of the subroutine ZXSSQ, also from IMSL.

**Acknowledgement**

This research was financially supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO). We wish to thank Mr. H. A. van der Vorst of the Computer Centre of the University at Utrecht (ACCU) for his assistance in the computer calculations and Mr. G. Kers for experimental assistance.

\(^{26}\) J. J. Blanksma, Chem. Weekbl. 6, 725 (1909).