Polyelectrolyte Surfactant Complexes as Improved Models for Esterolytic Enzymes

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Abstract. Cationic surfactants enhance the catalytic activity of imidazole containing polymers of isocyanides in the hydrolysis of activated esters.

Imidazole containing macromolecules have extensively been studied as models of esterolytic enzymes. Micelle forming surfactants have also been investigated for this purpose. We are not aware of studies in which macromolecules and surfactants have been combined to mimic enzymatic catalysis. We now wish to report such combinations which show appreciably increased esterolytic activity.

Imidazole containing isocyanides, R-N=C, were prepared starting from imidazole containing dipeptides and tripeptides by converting the free α-amino function of the latter molecules into an isocyano function. These isocyanides were polymerized and copolymerized in the presence of nickel(II) chloride to give, after removal of protective groups, imidazole, carboxylic acid and hydroxymethyl containing polymers and copolymers, (R-N=C<) , 1 (Table). These compounds are soluble in water. The molecular weights are approximately $M_n = 20,000$ for the polymers having tripeptide side chains and $M_v = 200,000$ for the remaining polymers. The configuration of the polymers is that of a rigid helix with 4 repeating units R-N=C per helical turn (Figure 1a).

Addition of the cationic surfactants 1-cetylpyridinium chloride, CePyCl, or cetylundecyldimethylammonium bromide (CeUnDAB) in low concentrations to solutions of polymers 1 in 30% aqueous ethanol led to the formation of precipitates. Higher concentrations of these surfactants (ratio of surfactant concentration to concentration of polymer repeating unit > 3) gave stable solutions of polymer-surfactant complexes. The latter complexes were compared with polymers 1 as catalysts in the hydrolysis of 2,4-dinitrophenyl acetate (DNPA) in 30% v/v ethanol-water at 25°C. The reactions were followed by measuring the release of the 2,4-dinitrophenolate ion spectrophotometrically. The second order catalytic rate constants $k_{\text{cat}}$ were calculated from experiments with catalyst ($k_{\text{obsd}}$) and without catalyst ($k_{\text{blank}}$) according to the equation $k_{\text{cat}} = (k_{\text{obsd}} - k_{\text{blank}})/[\text{Im}]$ in which [Im] is the concentration of imidazolyl functions. Hydrolysis of DNPA by polymers 1 in the presence of various concentrations of cationic surfactant gave similar rate profiles. The second order rate constant $k_{\text{cat}}$ increases with increasing concentration of surfactant up to a plateau value. This plateau value is reached at a surfactant to polymer repeating unit ratio of approximately 6. At this point the surfactant concentration is far above its CMC value. The $k_{\text{cat}}$-plateau values of the various polymers at pH 8.93...
Table Catalytic rate constants, $k_{\text{cat}}$ (dm$^3$·mol$^{-1}$·s$^{-1}$), of poly(iminomethylene) and poly(iminomethylene)-1-cetylpyridinium complexes in the hydrolysis of DNPA.

<table>
<thead>
<tr>
<th>R in (R-N=C&lt;) (l)b</th>
<th>$k_{\text{cat}}$</th>
<th>$pK_{\text{ImH}}$</th>
<th>$pK_{\text{ImH}}^+$</th>
<th>$IP^c$</th>
<th>$IP^c$</th>
<th>$k_{\text{cat}}$</th>
</tr>
</thead>
</table>
| hp (A$_L^L$
| 9.1 5.4 0.40 | 7.4 5.4 10.8 |
| hp (A$_D^H$
| 8.1 5.5 3.80 | 7.8 4.5 8.8 |
| cp (A$_1^S$
| 9.7 3.8 0.85 | 7.7 5.1 21.6 |
| cp (A$_2^S$
| 9.6 5.2 1.60 | 7.7 5.3 9.3 |
| cp (A$_2^S$
| 8.4 5.6 0.54 | 7.3 5.6 7.7 |
| hp (A$_L^S$
| 7.1 5.2 1.1 | 6.8 5.2 3.3 |
| hp (A$_D^S$
| 8.7 7.4 2.3 | 7.4 6.0 6.4 |

a In 30% ethanol-water at 25.00°C; 0.2 mol/dm$^3$ Tris buffer; [CePy$^{+}$Cl] $1.58 \times 10^{-4}$ mol/dm$^3$; initial [DNPA] $6.7 \times 10^{-5}$ mol/dm$^3$; pH 8.93.

b hp = Homopolymer; cp = copolymer; A, H and S stand for alanyl, histidinyl and serinyl residues, respectively; subscripts indicate the ratio of residues in the copolymer; Dod = 2-dodecyl isocyanide; thus hp(A$_L^L$N=CC)n is [(L,L)-ImCH-N(COOH)NHC(O)CH(CH$_3$)N=C<]$_n$.

c $IP$ = isoelectric point.

with CePy$^{+}$Cl$^-$ surfactant are listed in the Table, together with the data for the reaction without added surfactant. As can be seen from the Table, appreciable rate enhancement is observed for all polymer-CePy$^{+}$Cl$^-$ complexes.

The anionic surfactant sodium dodecyl sulfate (SDS) showed no accelerating effect in the hydrolysis of DNPA by polymers. Negative results were also obtained with L-histidine as low molecular weight catalyst: addition of CePy$^{+}$Cl$^-$, CeUnDAB or SDS gave no or only a small increase of its second order rate constant.

Under our conditions the polymers are above their isoelectric points (see Table) and, therefore, have a net negative charge. Cationic surfactant molecules will direct themselves towards the polymer chains and initially form a hydrophobic mantle. This causes precipitation of the polymer surfactant complex. At higher concentration of surfactant, structures can be formed (Figure 1b) which make the complex soluble. The substrate molecules will prefer the new polymer-surfactant pseudophase. Thus, the substrate concentration in the neighbourhood of the imidazole functions increases, which in part explains the observed rate accelerating effect. The pseudophase also affects the degree of protonation of the imidazole nuclei as can be concluded from $pK$ measurements. The apparent $pK_{\text{ImH}}$ values of the polymers are lowered by 0.3-1.9 units in the presence of CePy$^{+}$Cl$^-$ surfactant (see Table). Thus, at a certain pH value the number of unprotonated, catalytically active imidazole groups are increased and so the rate of hydrolysis.

References

4. In the absence of polymer addition of surfactant did not appreciably affect the rate.

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