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Catalytic Activity of Microemulsion-like Globular Polybases: The Case of *N*-(Benzyloxycarbonyl)-L-leucine *p*-Nitrophenyl Ester Hydrolysis

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ABSTRACT: Hydrolysis of *N*-CBZ-L-leucine *p*-nitrophenyl ester (CBZ = benzyloxycarbonyl) was carried out in the presence of two partially quaternized poly(tertiary amines) of the poly[thio-1-[(*N*-R_1-*N*-R_2-amino)-methyl]ethylene] type, namely, Q-P(T,*N*,*N*-Et_2,AE)15 and Q-P(T,*N*-Me-*N*-Bu,AE)15, both with 15% N-methylated quaternary ammonium residues. In aqueous media, these bifunctional polybases exist either in a globular conformation or in an extended-coil form or both at equilibrium, depending on the ionization state of tertiary amine residues and on a globule-to-coil cooperative transition of the all-or-none type. In the globular conformation, Q-P(T,*N*-R_1-*N*-R_2,AE)15 macromolecules form a microemulsion-like hydrophobic microphase because globules are composed of a hydrophobic core and an electrically charged hydrophilic surface which prevents aggregation. It is shown that water-insoluble CBZ-L-leucine *p*-nitrophenyl ester is hydrolyzed much faster in the presence of globules than in the presence of the same macromolecules in the coil form or in a polymer-free solution buffered at similar pH values. The effects of pH, of substrate and polymer concentrations, of R_1 and R_2 substituents, and of relative amounts of globular and extended-coil forms are discussed. The origin of the large catalytic activity of the globular form is investigated by comparison with the activity of low molecular weight compounds modeling the contribution of the various functional groups present in the macromolecules. As none of the model compounds increases significantly the rate of hydrolysis of *N*-CBZ-L-leucine *p*-nitrophenyl ester hydrolysis, it is concluded that the high catalytic activity of Q-P(T,*N*-R_1-*N*-R_2,AE)15 polymers in the globular state is a macromolecular phenomenon directly related to the globular conformation.

Introduction

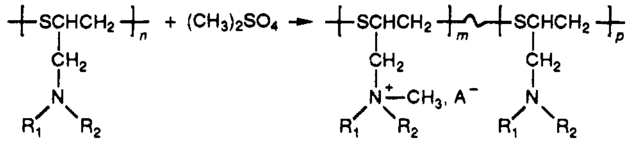
For the last 2 decades, attention has been paid to catalysis and inhibition effects on chemical reactions which can take place in submicroscopic entities or supramolecular assemblies such as macrocycles, micelles, surfactant vesicles, and synthetic macromolecular systems.[1-6]

In the case of macromolecule-based systems, most of the studies were primarily directed toward the synthesis and the catalytic activity of polymers with the aim of showing enzymelike behaviors.[7] A first class of compounds includes water-soluble polyamines which are derivatives of poly(ethylenimine),[8-15] poly(vinylimidazole),[16-20] poly(iminomethylene)[21-23] poly(allylamine),[24-26] and vinyl polymers bearing catalytic groups.[27-31] These polymeric amines were generally used to catalyze the alkaline hydrolysis of simple activated alkyl or aryl esters. It has been shown that many of them increase the hydrolysis rates and can exhibit some of the characteristics of enzymes, in particular, high reactivity, specificity of substrate, bifunctional catalysis, competitive inhibition, and saturation kinetics. The catalytic activity is sometimes regarded as due to nonspecific hydrophobic and electrostatic interactions with the substrate which give rise to an abnormally high accumulation of ester molecules around catalytic sites in appropriated ionic forms and orientations. On the other hand, cooperative effects involving interactions between neighboring catalytic functional groups covalently bound to polymers have also been regarded as responsible for the catalytic activity. A second class is formed by modified poly(4-vinylpyridines)[32,33] and hydrophobic linear alkyl ionenes[34] taken as models for micellar catalysis. In both cases, the hydrolysis of activated esters was found faster in the presence of the polymers. This catalytic activity was attributed to the solubilization of ester molecules in hydrophobic microdomains of the macromolecules and to the presence of catalytic OH^- counterions condensed in the Stern layer.

As far as the effect of polyamine macromolecule conformation is concerned, only limited information is available in the literature. The catalytic activity of benzyl-containing poly(ethylenimines) has been shown to increase in a narrow range of the degree of N-benzylation, i.e., when macromolecular coils undergo conformational changes and decrease in size which is indicated by the decrease in intrinsic viscosity only.[9] The authors assumed that the substrate is bound rather "efficiently" in the polymer globules which contain secondary and/or tertiary amine groups but no quaternary ammonium ones. It has also been mentioned that highly branched lauryl- and imidazole-containing poly(ethylenimines) assume compact, globular conformations and lead to better catalytic activity than nonhydrophobized ones.[8,35] Here again the primary structures were rather complex.

A few years ago, we reported the synthesis of a new type of bifunctional basic polyelectrolytes which exhibit unusual conformational characteristics in aqueous media due to their ability to take on a pH-dependent globular conformation and to undergo a globule-to-coil cooperative transition in a narrow range of pH values.[36]

These copolymers, (Q-P(T,*N*-R_1-*N*-R_2,AE)*X*), were obtained by partial N-methylation of some of the tertiary amine side groups present in poly[thio-1-[*N*-R_1-*N*-R_2-amino)methyl]ethylene] molecules according to the chemical reaction with *X* = percentage of quaternary ammonium



P(T,*N*-R_1-*N*-R_2,AE) Q-P(T,*N*-R_1-*N*-R_2,AE)*X*

groups ($X = 100m/(m + p)$) and A^- a counterion.

The originality of these polyelectrolytes is related to the presence of strong (quaternary ammonium hydroxide

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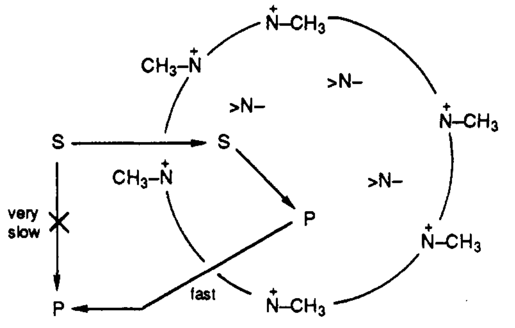
form) and weak (tertiary amine) basic groups which are more or less randomly distributed along the polymer backbone. The former are always ionized regardless of the counterion, whereas the latter can be neutral (deprotonated) or charged (protonated). When $X < 25$, macromolecules behave unusually. They can exist in two well-defined conformational states, namely, a globular conformation or an extended-coil one, depending on acid–base reaction on tertiary amine residues. In the globular state, the core of each globule forms a hydrophobic microdomain which is stabilized by the permanent quaternary ammonium charges grouped at the surface.

It has been shown that solutions of globular Q-P(T,*N*-R$_1$-*N*-R$_2$,AE)*X* macromolecules behave like microemulsions and are able to dissolve and entrap hydrophobic compounds such as steroids which are reputed water-insoluble.[37-40] This entrapment results in an apparent increase of solubility in water. Because of the globule-to-coil cooperative transition, the release of the entrapped compound can be achieved instantaneously by acid-induced destabilization of the globules. Indeed, the addition of small amounts of acids causes protonation of tertiary amine residues and triggers the all-or-none cooperative conformational transition from the globular structure to the open-coil one. The transition is reversible and occurs over a narrow range of pH values.[38]

More recently, we decided to examine the potential of the globules to act as macromolecular microreactors. The idea was to dissolve a water-insoluble organic reagent S in the hydrophobic core of the globular macromolecules and see whether the entrapped reagent can undergo a chemical modification to yield a product P as already observed in microemulsion and microemulsion-like polymeric systems.[1] If P is water-soluble, partition with water can force P to diffuse from the globule to the aqueous external continuum and thus turn on an enzymelike catalytic process. The resulting turnover has been demonstrated recently[41] and will be published separately.



S, substrate; P, product

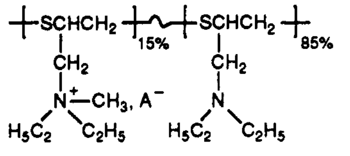
As the globular and the extended forms of the Q-P(T,*N*-R$_1$-*N*-R$_2$,AE)*X* macromolecules can exist at similar pH values, the effect of the particular globular conformation was thought to be easy to show by comparison of both states with respect to the same reaction, namely, the hydrolysis of protected amino acid *p*-nitrophenyl esters.

In this paper, we report the very first data on the catalytic behavior of two copolymers of the Q-P(T,*N*-R$_1$-*N*-R$_2$,AE)-*X* series using the hydrolysis reaction of *N*-CBZ-L-leucine *p*-nitrophenyl ester (CBZLeuPNP) as a reference for the sake of comparison with similar data present in the literature.

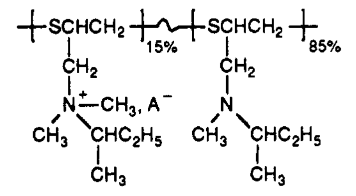
The effects of pH, degree of protonation of residual tertiary amine groups, polymer concentrations, and nature of the N-substituents on the rate of hydrolysis of CBZLeuPNP are also examined.

Experimental Part

Chemicals. Copolymers Q-P(TDAE)15 ($\bar{M}_w$ = 91 000 as determined by using a Chromatix KMX-6 SALLS apparatus) and Q-P(T,*N*-Me-*N*-sBu,AE)15 ($\bar{M}_w$ = 92 000) were prepared from the corresponding poly[thio-1-[(*N*-R$_1$-*N*-R$_2$-amino)methyl]ethylenes] according to a procedure reported in detail in ref 36.



Q-P(TDAE)15



Q-P(T,*N*-Me-*N*-sBu,AE)15

N-CBZ-L-leucine *p*-nitrophenyl ester (CBZLeuPNP) from Sigma Chemical Co. was dissolved in acetonitrile ($c = 1.2 \times 10^{-3}$ M) to form a stock solution.

Diethyl sulfide, triethylamine (99% purity), and tetraethylammonium chloride were commercial products from Janssen Chemical Co.

[(*N*-Methyl-*N*-*sec*-butylamino)methyl]thiirane was prepared according to ref 42.

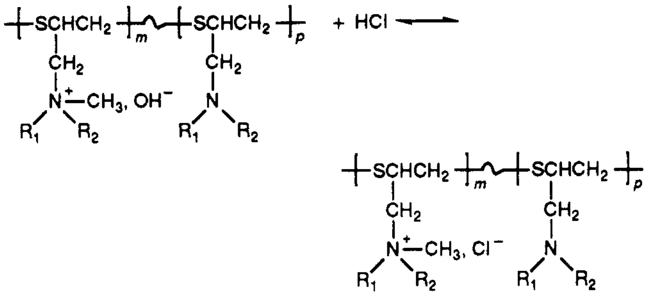
Preparation of Polymer Solutions. Typically, about 120 mg of Q-P(T,*N*-R$_1$-*N*-R$_2$,AE)15 (hydrochloride form) was dissolved in water (1–2 cm^3) and injected at the top of an ion-exchange column (L = 70 cm, ϕ = 1.2 cm) loaded with 63 cm^3 of cationic resin (Cl$^-$ form, type DOWEX-AG 1X4, 20–50 mesh).[2] The resin was conditioned to its OH$^-$ form by percolating 2000 cm^3 of 2 N NaOH[1] at a 150 cm^3·h^{-1} flow rate given by a peristaltic pump[3] (Gilson Minipulse 2) followed by CO$_2$-free distilled water up to obtaining neutral effluents. The copolymer load was eluted through the resin for 120–150 min at a 15 cm^3·h^{-1} flow rate, the collected solutions being filtered on a 0.22-μm Millipore filter[4] before being introduced in a thermostated reactor.[5] The whole setup (Figure 1) was kept flushed with nitrogen.[6] The column was then removed and replaced by a microsyringe.[7] The monomolar concentration Cp of the eluted polymer solutions was determined by acidimetric titration. Desired polymer concentrations and pH values were adjusted by dilution and by addition of 1 N HCl (Gilmont microsyringe[7]), respectively. pH values were measured by a Ag/AgCl Ingold electrode[8] connected to a Radiometer PHM 64 pH-meter. Later on, all the solutions were transferred under N$_2$ atmosphere, including for the loading of the quartz cell[9] used for UV measurements.

Kinetic Experiments. Typically, 3 cm^3 of the aqueous copolymer solution and 25 μL of the CBZLeuPNP stock elution in acetonitrile were introduced under nitrogen in a 1 cm path length quartz cell (Hellma QS), and then the cell was closed by a seal cup. The experiments were carried out at 25.0 ± 0.1 °C under N$_2$ atmosphere. Absorbances were measured by using a spectrophotometer (Perkin-Elmer Lambda 15) either at λ = 400 nm (λ_{max} of *p*-nitrophenate ion PNP$^-$) or at λ = 318 nm (λ_{max} of *p*-nitrophenol) depending on the pH of the buffered solutions.

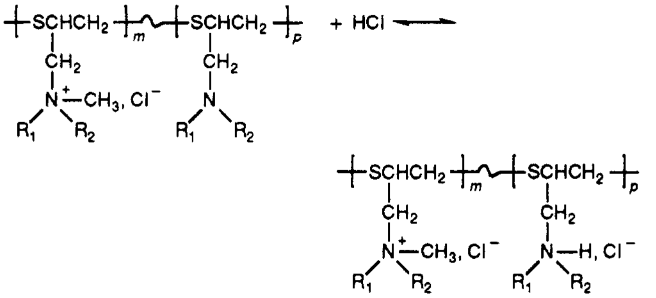
Results and Discussion

Physicochemical Behavior of Q-P(TDAE)15 and Q-P(T,*N*-Me-*N*-sBu,AE)15. In order to avoid chain degradation and amine oxidation, Q-P(T,*N*-R$_1$-*N*-R$_2$,AE)-15 samples were stored in their hydrochloride form.[42] Before use, protonated chains have to be percolated through an anion-exchange column (OH$^-$ form) in order to be converted to their dibasic form. Because prospective experiments showed that CO$_2$ decreased very much the catalytic activity of copolymers, all the experiments, including the conditioning of the ion-exchange column, had to be performed under nitrogen as described in the Experimental Part. After percolation, strongly basic quaternary ammonium hydroxide groups are ionized regardless of the pH and contribute permanently to the charge density of the macromolecule. In contrast, weakly basic tertiary amine residues are protonated more or less depending on the pH. Accordingly, protonation–deprotonation reactions can cause dramatic changes to the charge density and to the hydrophilic–hydrophobic balance of the whole macromolecule. Indeed, deprotonated tertiary amine groups are neutral and hydrophobic whereas protonated ones are charged and hydrophilic.

Because of the difference of basicity between strong base quaternary ammonium groups and weak base tertiary amine ones, the addition of an acid to a solution causes first ion exchange at quaternary ammonium sites according to



and then protonated at tertiary amine sites according to



The definition of these various states requires, at least, two parameters, namely, $\epsilon = [\text{-NR}^+\text{Cl}^-]/[\text{-NR}^+\text{Cl}^-] + [\text{-NR}^+\text{OH}^-]$, the degree of neutralization of basic quaternary ammonium groups, and $\beta = [\text{-NH}^+]/[\text{-N}] + [\text{-NH}^+])$, the degree of protonation of tertiary amine sites. As tertiary amines are weak bases, β can be considered as equivalent to the degree of neutralization $\bar{\beta}$, in the range $0.05 < \bar{\beta} < 0.95$ ($\bar{\beta} = [\text{HCl}]_{\text{added}}/[\text{-N}] + [\text{-NH}^+]$).

Figure 2 shows the potentiometric titration curves in salt-free water of dibasic Q-P(TDAE)15 and Q-P(T,*N*-Me-*N*-sBu,AE)15 copolymers. Both titration curves exhibit one of the most unusual characteristics which are typical of Q-P(T,*N*-R_1-*N*-R_2,AE)*X* copolymers with $X < 25$, namely, a high buffering effect in the β_1–β_2 region.

As previously discussed, for $\beta > \beta_2$, all the macromolecules are in the highly protonated open-coil form. For $0 < \epsilon < 1$ and $0 < \beta < \beta_1$, all the macromolecules are in the globular form.[36] In the range $\beta_1 < \beta < \beta_2$, the polymer solution behaves as a two-phase system. Protonation–deprotonation proceeds cooperatively and causes a cooperative globule-to-coil transition. Along the plateau, the copolymer solution is actually composed of two populations of macromolecules at equilibrium as shown by SAXS.[43] The proportion of these two populations can be estimated by using eqs 1 and 2

$$X_G = \beta_2 - \beta/\beta_2 \qquad (1)$$

$$X_C = \beta/\beta_2 \qquad (2)$$

where X_G and X_C are the molar fractions of the repeating units involved in globular and coiled macromolecules, respectively.

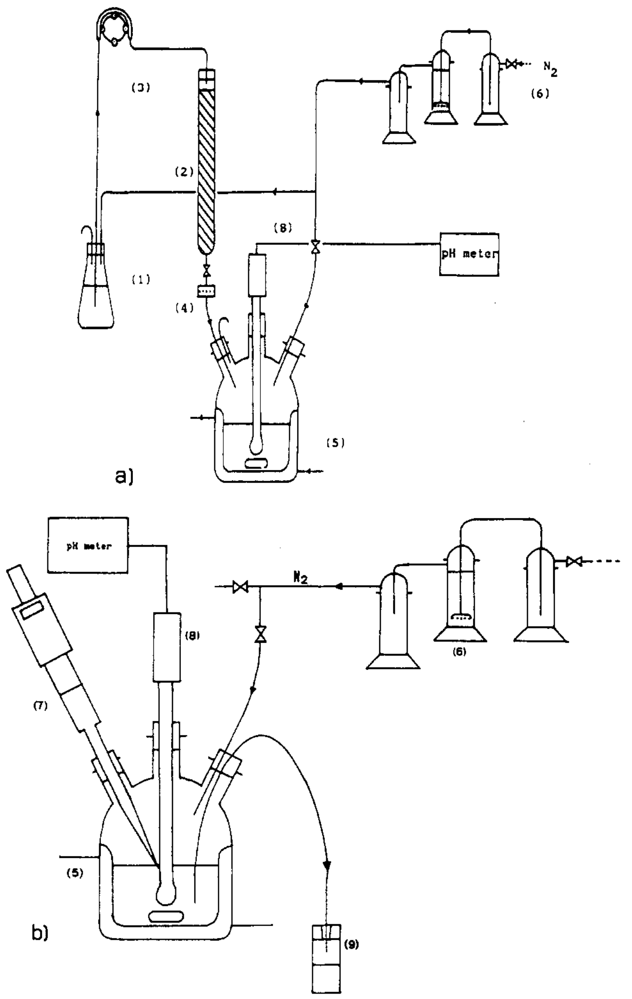


Figure 1. (a) Experimental setup for preparation of stock Q-P(T,*N*-R_1-*N*-R_2,AE)15 solutions in water: (1) 2 N NaOH, (2) anion-exchange resin, (3) peristaltic pump, (4) millipore filter, (5) thermostated reactor, (6) nitrogen flush. (b) Experimental setup for preparation of polymer solutions at desired pH and degree of protonation β for kinetic measurements: (7) 1 N HCl in microsyringe, (8) Ag/AgCl electrode, (9) quartz cell.

As is shown in Figure 2, Q-P(T,*N*-Me-*N*-sBu,AE)15 gave rise to a longer pH plateau than Q-P(TDAE)15 in agreement with the presence of more carbon atoms in side-chain tertiary amine N-substituents. Furthermore, the plateau is located at a lower pH value (6.2 vs 6.8).

Catalytic Activity of Q-P(TDAE)15 and Q-P(T,*N*-Me-*N*-sBu,AE)15. Hydrolysis of CBZLeuPNP was carried out under various conditions of copolymer concentrations and of protonation states. Copolymer monomolar concentrations Cp ranged from 2×10^{-3} to 5×10^{-2} M, whereas the concentration of the substrate CBZLeuPNP was fixed at 1×10^{-5} M, this compound being insoluble in aqueous media at higher concentrations. Because of the large excess of polymer as compared to the substrate, there was no need for using a normal buffer, the polymer itself acting as a buffering agent. For the sake of comparison, hydrolyses were also conducted in polymer-

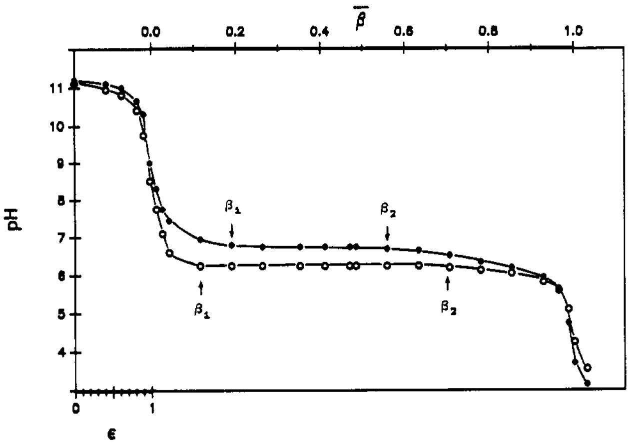


Figure 2. Potentiometric titration curves of (●-●) Q-P-(TDAE)15 and (○-○) Q-P(T,*N*-Me-*N*-sBu,AE)15 (Cp = 2.33 × 10^{-2} M) in salt-free water.

free solutions buffered at pH values suitable for achieving solutions of globular and coiled copolymer molecules. The hydrolytic reaction was monitored spectrophotometrically by measuring absorbance *A* of the predominent PNP species, namely, the *p*-nitrophenate ion PNP^- at λ_{max} = 400 nm (ϵ_{400}^{25} = 18 300) or the un-ionized *p*-nitrophenol PNP at λ_{max} = 318 nm (ϵ_{318}^{25} = 10 100), the PNP/PNP^- ratio being constant at buffered pH values as is the case in the presence of a large excess of Q-P(T,*N*-R_1-*N*-R_2,AE)15. Accordingly, the degree of hydrolysis at time *t* was given by the ratio $(A_\infty - A_t)/A_\infty$ where A_∞ is the absorbance at infinite time, i.e., when all the ester molecules are hydrolyzed.

Figure 3 shows examples of the kinetics curves obtained in the presence and in the absence of Q-P(TDAE)15. At pH = 5.9, hydrolysis appeared slightly faster in the presence of the copolymer. In contrast, at pH = 7.4, hydrolysis turned out to be more substantial in the medium containing the copolymer in the globular state than in any other media.

For the sake of comparison with other macromolecular catalytic systems described in the literature, hydrolysis kinetics curves were fitted to the general kinetic equation (3)

$$[\text{ester}]_t = [\text{ester}]_{t_0} e^{-k_{ob} t} \qquad (3)$$

where $[\text{ester}]_{t_0}$ is the initial CBZLeuPNP concentration and k_{ob} the apparent rate constant expressed in s^{-1}.

Since $[\text{ester}]_t/[\text{ester}]_{t_0} = (A_\infty - A_t)/A_\infty$, eq 3 becomes eq 4.

$$\text{Ln}\,(A_\infty/(A_\infty - A_t)) = k_{ob} t \qquad (4)$$

As reactions were carried out up to almost 100% conversion, all the kinetics curves could be fitted with eq 4 and pseudo-first-order rate contants k_{ob} were calculated from the slope of the linear Ln $(A_\infty/(A_\infty - A_t))$ vs time curves using the least-squares method with correlation coefficients better than 0.998. Blank experiments performed in the absence of polymers led to k_0 which were taken as specific constants of the pH values of the buffer solutions.

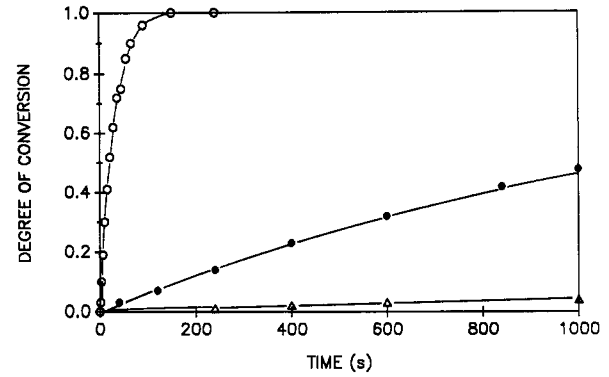


Figure 3. Degree of conversion of CBZLeuPNP in Q-P(TDAE)15 (Cp = 2.33 × 10^{-2} M) at pH = 7.4 (○-○) and at pH = 5.9 (●-●) and in phosphate buffer (C_B = 0.1 M) at pH = 7.4 (△-△) vs time *t*.

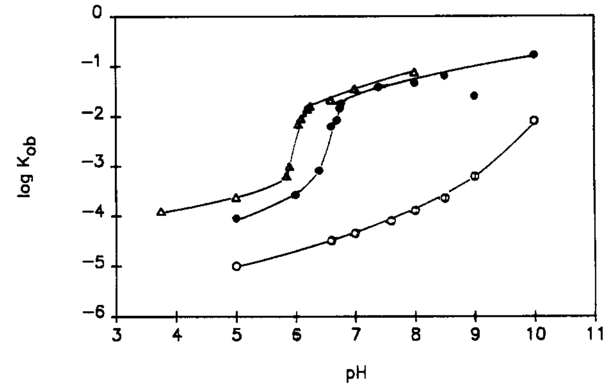


Figure 4. Variation of k_{ob} vs pH in the presence of (Cp = 2.66 × 10^{-2} M) Q-P(TDAE)15 (●-●) and Q-P(T,*N*-Me-*N*-sBu,AE)15 (△-△) and in the absence of polymer (○-○) in the case of CBZLeuPNP (C_E = 1 × 10^{-5} M) hydrolysis in aqueous medium at 25 °C.

Table I
Effect of Conformational Structures of Q-P(TDAE)15 and Q-P(T,*N*-Me-*N*-sBu,AE)15 on the Hydrolysis Rates of CBZLeuPNP in Aqueous Solutions

polymer	structure	pH at 25 °C	k_{ob}, s^{-1}	k_0,[a] s^{-1}	k_{ob}/k_0
Q-P(TDAE)15	globular	7.4	3.8×10^{-2}	5.5×10^{-5}	690
Cp = 2.33 × 10^{-2} M	open coil	5.92	2.7×10^{-4}	1.9×10^{-5}	12
Q-P(T,*N*-Me-*N*-sBu,AE)15	globular	6.6	3.0×10^{-2}	3.1×10^{-5}	970
Cp = 2.33 × 10^{-2} M	open coil	5.92	6.0×10^{-4}	1.9×10^{-5}	35

[a] k_0 = rate constant in buffered aqueous solutions (C_B = 0.1 M).

In these experiments, CO_2 turned out to be a critical factor, as very small amounts of CO_2 were able to cause dramatic variations of k_{ob}. For example, k_{ob} = 8.5 × 10^{-4} s^{-1} was found for globular Q-P(TDAE)15 at Cp = 3.6 × 10^{-2} M and pH = 7.4 when the solution was saturated with CO_2 whereas k_{ob} was equal to 2.6 × 10^{-2} s^{-1} for the same pH and the same polymer solution prepared under N_2 atmosphere according to the protocol described in the Experimental Part which prevented pollution by CO_2, CO_3^{2-}, and HCO_3^- ions (Figure 1). Therefore, it was concluded that globular polymers catalyze CBZLeuPNP hydrolysis whereas CO_2 is an efficient inhibitor.

Under these conditions, both polymer solutions exhibited catalytic activity as compared with polymer-free buffered solutions (Table I). Furthermore, globular Q-P(T,*N*-Me-*N*-sBu,AE)15 appeared to be a better catalyst than Q-P(TDAE)15 at a given pH. Hydrolysis of CBZLeuPNP was strongly catalyzed in the globular state. In contrast, open coils showed only little catalytic activity with k_{ob} 2 orders of magnitude smaller than that for globules.

Effect of pH. Figure 4 shows the effect of pH on the rate of hydrolysis for CBZLeuPNP at a fixed concentration in the presence of Q-P(TDAE)15 and Q-P(T,*N*-Me-*N*-sBu,AE)15 copolymers and in blank buffered solutions. In the case of buffer solutions, log k_0 increased progressively

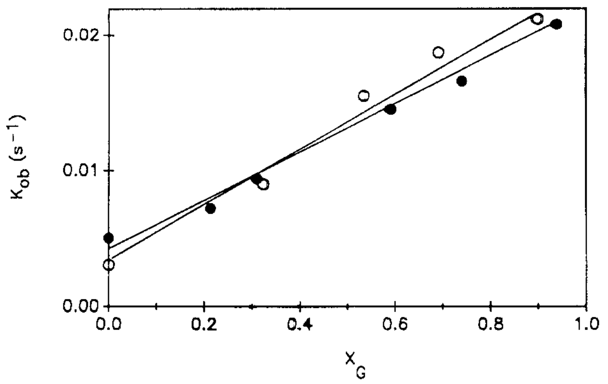


Figure 5. Effect of the molar fraction X_G of (●-●) Q-P-(TDAE)15 and (○-○) Q-P(T,N-Me-N-sBu,AE)15 (Cp = 2.66 × 10^{-2} M) globular macromolecules on the hydrolysis of CBZLeuPNP (C_E = 1 × 10^{-5} M) in aqueous solutions at 25 °C.

with pH according to the known catalytic effect of OH^-, which becomes important above pH 7. In contrast, log k_{ob} increased sharply in the narrow pH ranges corresponding to the globule-to-open coil conformational transition when polymers were present. The correlation between rate increases and globule-to-coil transition is supported by the fact that similar profiles were observed for both copolymers, with a shift on the pH scale comparable to the differences observed between corresponding pH plateaux (Figure 2).

Before the coil-to-globule transition, when copolymer macromolecules are highly protonated ($\beta > \beta_2$) and in the open-coil form, log k_{ob} values are about 1 order of magnitude greater than corresponding blank values. Similar small catalytic effects have already been observed in the case of many polyelectrolytes, and this is generally considered to be a typical polyelectrolyte effect.[44] In contrast, right after the coil-to-globule transition, log k_{ob} values at pH = 7 (globular form) are several orders of magnitude higher than log k_{ob} values at pH = 6.6 (open-coil form) and than log k_0 ones (blank). Accordingly, one can conclude that OH^- ions were not the catalytic species and that catalysis is directly related to the globular conformation.

Effect of the Molar Fraction X_G of Globules. The variation of the catalytic activity of copolymers with X_G, the molar fraction of globular macromolecules, is plotted in Figure 5. It is noteworthy that k_{ob} increased linearly as X_G increased in the very narrow pH range (6.7–6.8 or 6.2–6.3) corresponding to the globule-to-coil cooperative transition, i.e., for $0 < \beta < 0.58$ or 0.71 for Q-P(TDAE)15 and Q-P(T,N-Me-N-sBu,AE)15, respectively. These findings are in good agreement with the occurrence of the reaction in the microemulsion-like system since the average volume of globule cores varies like X_G.

On the other hand, it is of interest to note that the catalytic activity is almost independent of the N-substituents when plotted vs X_G.

Effects of Polymer Concentrations. Figure 6 shows the variation of the apparent hydrolysis rate constant k_{ob} as a function of copolymer concentration at pH = 7.4 for globular Q-P(TDAE)15 and at pH = 6.55 for globular Q-P(T,N-Me-N-sBu,AE)15, the concentrations of CBZLeuPNP being the same (C_E = 1 × 10^{-5} M). For both copolymers, k_{ob} increased linearly with the polymer concentration in the range 2 × 10^{-3} M < Cp < 3.5 × 10^{-2} M. Once again, this finding agrees well with a catalytic effect linearly dependent on the amount of microphase available. We expected k_{ob} to reach a limiting value at high polymer concentrations as in the cases of many other polymeric enzyme catalysts,[10,20,33] but our experimental conditions necessarily performed in the absence of CO_2 did not allow the detection of such a limiting value, so far.

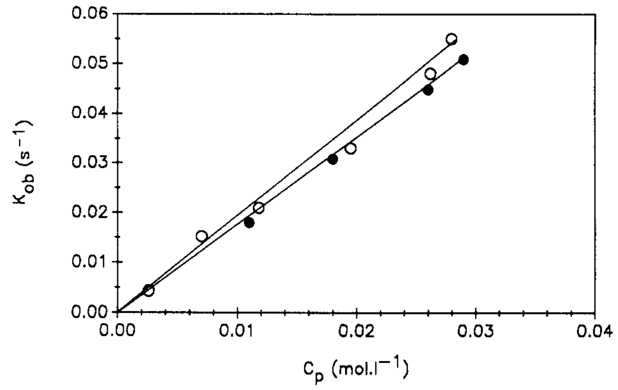


Figure 6. Effect of the globular polymer concentration on hydrolysis rates of CBZLeuPNP (C_E = 1 × 10^{-5} M) in aqueous solutions at 25 °C: (●-●) Q-P(TDAE)15, pH = 7.4; (○-○) Q-P(T,N-Me-N-sBu,AE)15, pH = 6.55.

Table II
Variations of k_{ob} in Different Model Media

model medium	k_{ob},[a] s^{-1}
diethyl sulfide	2.0 × 10^{-5}
triethylamine	2.6 × 10^{-4}
triethylamine + 1% water	2.7 × 10^{-4}
triethylamine + 2% water	2.7 × 10^{-4}
triethylamine + 3% water	8.5 × 10^{-4}
$S(C_2H_5)_2 + N(C_2H_5)_3$	1.3 × 10^{-4}
N-Me-N-sBu, AE	8.0 × 10^{-5}
tetraethylammonium chloride $[N^+]$ = 5 × 10^{-3} M, pH = 7.4	5.4 × 10^{-5}
phosphate buffer, pH = 7.4 (C_B = 0.1 M)	5.5 × 10^{-5}

[a] Remind globular Q-P(TDAE)15 (Cp = 3 × 10^{-2} M), pH = 7.4; k_{ob} = 5.2 × 10^{-2}.

In the case of an aqueous solution of globular Q-P(T,N-Me-N-sBu,AE)15 at a monomolar concentration Cp = 5 × 10^{-2} M and pH = 6.6, the hydrolysis of CBZLeuPNP was 3000 times faster than in the presence of blank aqueous solution buffered at the same pH.

Nature of Catalytic Sites. Starting from the point that different types of functional groups are present in the globular macromolecules which form the microemulsion-like catalytic system, we tried to identify whether the catalytic activity is due to one of these sites or to the globular structure itself as data seem to show. For this, hydrolysis of CBZLeuPNP was carried out in the presence of various small molecules taken as model compounds of different functional parts of the copolymer macromolecules. The thioether groups were simulated by diethyl sulfide whereas triethylamine was used to represent pendent tertiary amine ones. The effect of small amounts of water was also considered. The contribution of tertiary amine residues present in polymer chain was modeled by the [(N-methyl-N-*sec*-butylamino)methyl]thiirane monomer and by a mixture of diethyl sulfide and triethylamine. Finally, quaternary ammonium salt groups were simulated by tetraethylammonium chloride.

As shown in Table II, none of these model molecules caused any significant increase of the rate constant as k_{ob} values remained in the 10^{-4}–10^{-5} range i.e., far from the 10^{-2} value found in the presence of globular macromolecules.

Therefore, the high catalytic activity observed in the case of globular macromolecules was assigned to a pure macromolecular effect, the reaction occurring in the core of the globules and not in water. By no means was it possible to measure the solubility of CBZLeuPNP in globule-containing water because the hydrolytic reaction was always to fast. Therefore, we still do not know whether the increase of reaction rate is due solely to solubilization effects or to another phenomenon more or less related to local concentration effects or local milieu characteristics such as unusual polarization or supernucleophilicity.

In conclusion, the present work shows that Q-P(T,*N*-R_1-*N*-R_2,AE)15 bifunctional polybases act as macromolecular microreactors in neutral aqueous media when they are in the globular conformation. They can increase the rates of chemical reactions as much as several thousand times depending on the amount of globular macromolecules which are in the solution. The catalytic effect is assignable to the globular conformation and not to any of the chemical group present in the polymer chains such as tertiary amine or thioether or quaternary ammonium ones. So far, the increase of hydrolytic rate has been obtained in the presence of a large excess of globular macromolecules. The next step will be to show whether one can have the catalytic system working as a true enzymelike system, i.e., with conversion yields higher than polymer repeat unit stoichiometry, and for a significant period of time.

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References and Notes

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Registry No. Q–P(TDAE)15 (homopolymer)·Me_2SO_4, 136538-79-9; Q–P(T,*N*-Me-*N*-sBu,AE)15 (homopolymer)·Me_2SO_4, 136538-82-4; *N*-Me-*N*-sBuAE, 136538-76-6; CBZLeuPNP, 1738-87-0; Et_2S, 352-93-2; Et_3N, 121-44-8; $Et_4N^+\cdot Cl^-$, 56-34-8.