Micelle-like Globular Polybases of the Partially Quaternized Poly[thio-1-[(*N*-R_1-*N*-R_2-amino)methyl]ethylene] Type as Catalysts for Chemical Modification of Hydrophobic Substrates in Water

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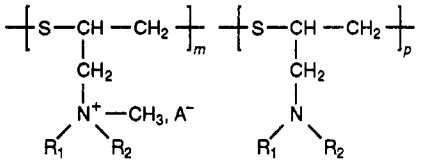
ABSTRACT: In aqueous media, bifunctional polybases of the partially quaternized poly[thio-1-[(*N*-R_1-*N*-R_2-amino)methyl]ethylene] type (Q-P(T,*N*-R_1-*N*-R_2,AE)*X*, with *X* = percentage of N-quaternization) take on a globular conformation, an extended-coil conformation, or both at equilibrium, depending on the ionization state of tertiary amine residues and on a globule-to-coil cooperative transition of the all-or-none type. It has been previously shown that micelle-like globules are able to catalyze the hydrolysis of *N*-CBZ-L-leucine *p*-nitrophenyl (PNP) ester in water at neutral pH with rate increases as high as several thousand times with respect to polymer-free solutions buffered at the same pH. To show the versatility of this catalytic activity, several compounds, namely, PNP esters of various aliphatic carboxylic acids with increasing chain length, (3-methyl-2-cyclohexenylidene)laurylamine (3-MCHLA), 2,4-dinitrochlorobenzene (DNC), and *N*-CBZ-L-leucine (CBZLeu), were allowed to react in water in the absence and in the presence of Q-P(T,*N*-R_1-*N*-R_2,-AE)15 macromolecules, with $R_1 = R_2$ = ethyl or R_1 = methyl and R_2 = *sec*-butyl, in the globular or extended-coil forms. In all cases, globules showed outstanding catalytic activities which were tentatively assigned to the distinct medium formed in water by the core of the globules. It was further shown that ionization of dyes dissolved within the core is higher than in polymer-free water at the same pH. Therefore, it was concluded that the core of the globules acts as a superbasic medium with respect to water.

Introduction

A decade ago, it was shown for the first time that partially quaternized tertiary amine polymers of the poly[thio-1-[(*N*-R_1-*N*-R_2-amino)methyl]ethylene] type (Q-P(T,*N*-R_1-*N*-R_2,AE)*X*), where *X* = the percentage of N-quaternization) exhibit an outstanding conformational behavior characterized by cooperative globule-to-coil transitions of the all-or-none type occurring in a very narrow range of pH values close to neutral, provided *X* is smaller than 20–30%.[1]



Q–P(T, *N*–R_1–*N*–R_2, AE)*X*

In aqueous media, these dibasic polyelectrolytes are characterized by a variable hydrophile/hydrophobe balance depending on the ionization state of the residual tertiary amine groups.[2] When deprotonated, each Q-P(T,-*N*-R_1-*N*-R_2,AE)*X* macromolecule is globular and composed of a hydrophobic core surrounded by an electrically charged corona which precludes aggregation and macroscopic precipitation. Micelle-like globules with molecular weights ranging between 10×10^4 and 12×10^4 had a diameter smaller than 80 Å as deduced by SAXS.[3] Since then, a theoretical approach to the ionization and conformational behaviors of weakly charged hydrophobic polyelectrolytes has been made.[4] The corresponding theory is consistent with the globular conformation of deprotonated Q-P(T,*N*-R_1-*N*-R_2,AE)*X* macromolecules and the unusual pH-dependent globule-to-coil cooperative transition which were first identified experimentally.[1]

Table I. Hydrolysis Rate Constants of Carboxylic Fatty Acid PNP Esters ($C_E = 1 \times 10^{-5}$ M) in Buffered Water (pH = 7.04) and in the Presence of Q-P(T,Me-*s*Bu,AE)15 ($C_p = 3.2 \times 10^{-2}$ M) in the Globular (pH = 7.08) and Open-Coil (pH = 6.02) States[a]

PNP ester	*n*	k_{ob}(G), 10^{-5} s^{-1}	k_{ob}(C), 10^{-5} s^{-1}	k_0, 10^{-5} s^{-1}	k_{ob}(G)/k_{ob}(C)
acetate	2	94 ± 4	7.6 ± 0.4	1.1	12.4
butyrate	4	105 ± 5	8.4 ± 0.5	0.4	12.5
caproate	8	130 ± 10	6.0 ± 0.2	<0.1	22
laurate	12	105 ± 5	3.4 ± 0.5		30
stearate	18	94 ± 4	1.0 ± 0.2		94

[a] For comparison, $k_{ob} = 3.0 \times 10^{-2}$ for CBZLeuPNP and globular Q-P(T,Me-*s*Bu,AE)15 ($C = 2.33 \times 10^{-2}$ M; pH = 6.6).[6]

In a second stage, it was shown that the core of Q-P(T,*N*-R_1-*N*-R_2,AE)*X* globules can accommodate lipophilic molecules and thus can solubilize in water compounds which have a very low aqueous solubility, e.g., progesterone or estradiol.[5] In a recent paper,[6] we reported that globular (Q-P(T,*N*-R_1-*N*-R_2,AE)15 macromolecules with $R_1 = R_2$ = ethyl (Q-(PTDAE)15) or with R_1 = methyl and R_2 = *sec*-butyl (Q-P(T,Me-*s*Bu,AE)15) can accommodate *N*-(benzyloxycarbonyl)-L-leucine *p*-nitrophenyl ester (CBZLeuPNP) and can hydrolyze it very rapidly as compared with the rates observed in the absence of polymer or when the globular state is destabilized because of protonation. Rate increases as large as 3000 times were observed, depending on the experimental conditions. An attempt was made to correlate the catalytic activity to one of the functional groups present in the Q-P(T,*N*-R_1-*N*-R_2,AE)*X* copolymer, namely, thioether, tertiary amino, and quaternary ammonium, by allowing CBZLeuPNP to react in the presence of various model compounds, namely, diethyl sulfide, triethylamine, mixtures of diethyl sulfide and triethylamine, and tetramethylammonium chloride.[6] There

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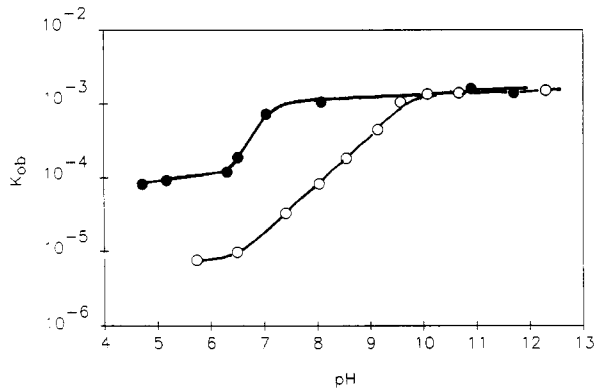


Figure 1. Variation of k_{ob} with pH in the presence of Q-P(T,-Me-*s*Bu,AE)15 (C_p = 3.2 × 10^{-2} M) (●) and in the absence of polymer (○) (ref 7) in the case of 3-MCHLA (C_i = 2 × 10^{-5} M) hydrolysis in aqueous medium at 25 °C.

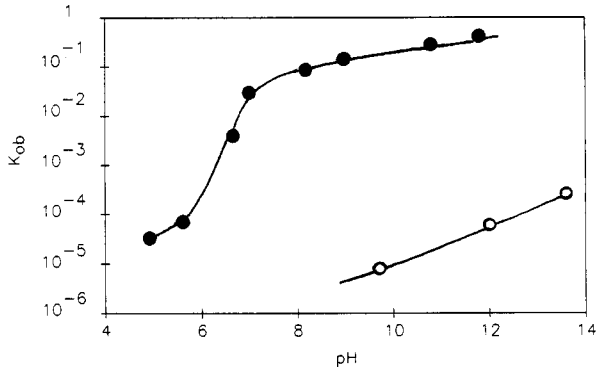
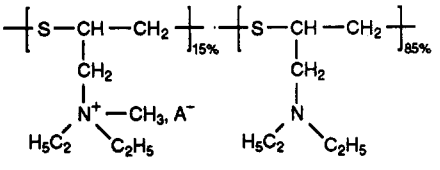
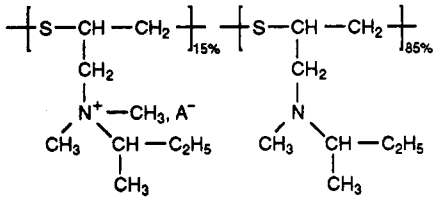


Figure 2. Variation of k_{ob} with pH in the presence of Q-P(T,-Me-*s*Bu,AE)15 (C_p = 2.1 × 10^{-2} M) (●) and in three buffer solutions respectively at pH = 9.7, 12, and 13.7 (○) in the case of DNC hydrolysis (C_s = 9.85 × 10^{-6} M) in aqueous medium at 25 °C.

were no cases of catalysis comparable to that of the globules observed. Therefore, we were forced to conclude that the rather high catalytic activity of Q-(PTDAE)15 globules was directly related to the globular conformation which acted as a macromolecular microreactor distinct from the host aqueous medium and which could thus be regarded as an enzyme-like system.[6]



Q–P(TDAE) 15



Q–P(T, Me–sBu, AE) 15

In this paper, we wish to report the results of further investigations aimed at improving the understanding of the phenomena which occur within Q-P(T,*N*-R_1-*N*-R_2,-AE)*X* globules when a hydrophobic reagent or substrate penetrates into the core and is chemically modified to form a hydrophilic compound which is finally released from the core because of partitioning with the aqueous medium. For this, a series of *p*-nitrophenyl esters of

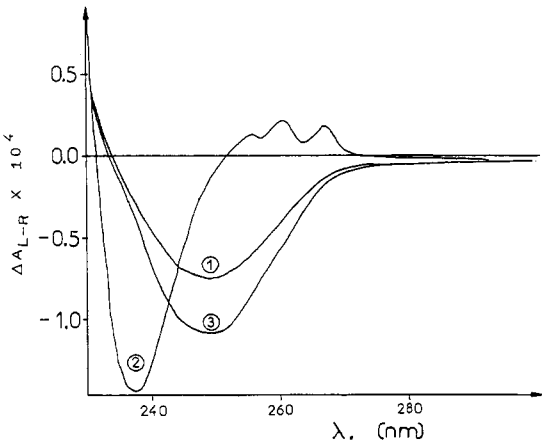


Figure 3. Circular dichroism spectra of *N*-CBZ-L-leucine and L-leucine in 75/25 v/v H_2O/MeOH under various conditions: (1) *N*-CBZ--leucine (C_s = 1.89 × 10^{-2} M) treated with Q-P(T,Me-*s*Bu,AE)15 (C_p = 1.43 × 10^{-2} M) at pH = 7.0 and 25 °C; (2) *N*-CBZ-L-leucine (C_s = 1.85 × 10^{-3} M) in polymer-free 75/25 v/v H_2O/MeOH at pH = 7.0 (phosphate buffer C_b = 10^{-2} M); (3) L-leucine (C = 2.6 × 10^{-3} M).

Table II. Calculated Equivalent pH Values within the Core of Q-P(TDAE)15 Globular Molecules (C_p = 1.93 × 10^{-2} M) As Determined Spectrophotometrically from the Behavior of Various Dyes (C_D = 1 × 10^{-6} M) in Aqueous Solutions at pH 7.02

	% ionized dye molecules			
compound	in water	in polymer	pK_a	equiv pH (calcd)
p-nitrophenol	41	96	7.4	8.4 ± 0.1
phenol red	10	69	8.0	8.2 ± 0.2
cresol red	7	41	8.2	8.0 ± 0.1
thymol blue	1	5	8.9	7.5 ± 0.2

carboxylic acids with increasing alkyl chain lengths was first considered. These compounds were allowed to react in polymer-free buffered media and in the presence of Q-P(T,Me-*s*Bu,AE)15 or Q-P(TDAE)15 in the globular or extended-coil forms. To show that the catalytic activity can be generalized to reactions other than PNP ester hydrolysis, substrates with different chemical structures, namely, (3-methyl-2-cyclohexenylidene)laurylamine (3-MCHLA), 2,4-dinitrochlorobenzene (DNC), and *N*-CBZ-L-leucine (CBZLeu) as well as ionizable dyes were also allowed to react under similar conditions. Last, but not least, the catalytic activity of the globular partially quaternized tertiary polyamines was compared with activities reported in the literature for other polymeric or micellar systems.

Experimental Section

Chemicals. Polymers. Q-P(T,Me-*s*Bu,AE)15 (M_w = 92 000 as determined by laser light scattering using a Chromatix KMX-6 SALLS apparatus) and Q-P(TDAE)15 copolymers (M_w = 92 000) were synthesized from the corresponding poly[thio-1-[(*N*-R_1-*N*-R_2-amino)methyl]ethylenes] according to the procedure described in ref 1. Since CO_2 is a major inhibitor of the catalytic activity, CO_2-free solutions of globular macromolecules were prepared as described in ref 6.

Substrates. CBZ-L-Leucine and *p*-nitrophenyl esters of alkanoic acids A_n were purchased from Sigma Chemical Co. and used without further purification. (3-Methyl-2-cyclohexenylidene)laurylamine (3-MCHLA) was prepared as described in ref 7. 2,4-Dinitrochlorobenzene (DNC) was supplied by Janssen and used without further purification. Phenol red and cresol red (purchased from Prolabo, France), thymol blue (from Janssen), and *p*-nitrophenol (from Aldrich) were used without further purification. Stock solutions of dyes were prepared in methanol.

Methods. Kinetic Measurements. Typically, 3 mL of an aqueous solution of copolymer (concentration C_p being expressed

Table III. Variations of k_{ob} and k_{cat} Rate Constants for Various *p*-Nitrophenyl Esters in the Presence of Micellar and Polymeric Catalytic Systems according to Literature data and from This Work[a]

no.	catalytic system	pH	k_{ob}, s^{-1}	k_{cat}, $L \cdot mol^{-1} \cdot s^{-1}$	ref
	p-Nitrophenyl Acetate				
1	Q-(T,Me-*s*Bu,AE)15	7.4	1.2×10^{-3}		this work
2	L-histidine N^{α}-myristate-modified CTABr micelles	7.2	4.8×10^{-3}		17
3	PEI + 10% lauryl	9	2.5×10^{-3}		18
4	PEI_{600} + 10% lauryl + 15% imidazole	7		45	18
5	poly(vinylimidazole)	7	6.7×10^{-6}	0.015	19
6	poly(iminomethylene)	7.6	6.8×10^{-2}		20
7	poly(vinylbenzimidazole)	7.6	1×10^{-4}	1.2	21
8	*N*-decylhistidine-modified DMEBr micelles	7.3		6	22
	N-CBZ-Leu				
9	Q-(T,Me-*s*Bu,AE)15	7.4	5.2×10^{-2}		this work
10	*N*-lauryl-L-histidine-modified CTABr micelles	7.4	9.2×10^{-3}	92	23
	N-t-BOC-L-Phe				
11	Q-(T,Me-*s*Bu,AE)15	7.4	4.4×10^{-2}		this work
12	*N*-lauryl-L-histidine-modified CTABr micelles	7.4	6.6×10^{-3}	66	23
	N-CBZ-L-Phe				
13	Q-(T,Me-*s*Bu,AE)15	7.4	5.8×10^{-2}		this work
14	*N*-lauryl-L-histidine-modified CTABr micelles	7.4	1.2×10^{-2}	150	23
15	CTABr micelles	7.3	3×10^{-3}		24
16	*N*-acylhistidine-modified CTABr micelles	7.3	6.8×10^{-3}		22
17	*N*-acylhistidine-modified CTABr micelles	7.3		2.75	25
18	*N*-stearylhistidine-modified CTABr micelles	7.3		2.740	25
19	DMEBr micelles	7.3	4.5×10^{-3}		22
20	*N*-decylhistidine-modified DMEBr micelles	7.3	1×10^{-1}	520	22
21	PEI + 10% lauryl + 20% imidazole	7.3	1.2×10^{-2}		24
22	modified PEI + CTABr	7.3	1.8×10^{-2}		24
23	poly(*N*-methacryloylhistidine-*co*-dodecyl methacrylate)	7.02		0.29	26
24	PEI + 10% lauryl + 13% histidine	7.3	7.6×10^{-2}		27

[a] CTABr = hexadecyltrimethylammonium bromide, DMEBr = *N*-dodecyl-*N*-methyl-L-ephedrine bromide, PEI = poly(ethylenimine). Further experimental conditions: 1,9,11, $C_p = 3.53 \times 10^{-2}$ M, 25 °C, [ester] = 1×10^{-5} M; 13, $C_p = 3.53 \times 10^{-2}$ M, 25 °C, [ester] = 7.6×10^{-6} M; 2, [CTABr] = 3.2×10^{-3} M, [L-His] = 8×10^{-4} M; 3,4, [ester] = 6.7×10^{-5} M; 5, 26% water–methanol, 26 °C, [PVIm] = 5×10^{-4} M; 6, [Im] = 3×10^{-4} M, [ester] = 6.7×10^{-5} M; 7, water/5% DMF/24% ethanol, 30 °C; 8, [DecHis] = $(0.4–5) \times 10^{-4}$ M, [ester] = 1×10^{-4} M, [DMEBr] = 5×10^{-3} M; 10,12,14, water/10% methanol/6.6% acetonitrile, [CTABr] = 4×10^{-3} M, [L-His] = 1×10^{-4} M, [ester] = 4×10^{-5} M; 15, [CTABr] = 8×10^{-4} M, [ester] = 2×10^{-5} M; 16, [CTABr] = 2×10^{-3} M, [ester] = 1×10^{-5} M; 17,18, [CTABr] = 2×10^{-3} M, [ester] = 1×10^{-5} M; 19,20, [DMEBr] = 6×10^{-3} M, [L-His] = 2×10^{-4} M, [ester] = 1×10^{-5} M; 21,22, [PEI] = 2×10^{-4} M, [ester] = 2×10^{-5} M, [CTABr] = 8×10^{-4} M^{-1}; 23, water/30% methanol, [ester] = 5×10^{-5} M, [cat.] = 5×10^{-4} M; 24, [ester] = 1.7×10^{-5} m.

in moles of polymer repeat units and not with respect to quaternary ammonium groups) and 0.025 mL of an acetonitrile stock solution of the substrate to be reacted were mixed under nitrogen in a spectrophotometric Hellma QS quartz cell with a 1-cm path length. The cell was closed with a Teflon cup and placed in the measurement beam of a Perkin-Elmer Lambda 15 spectrophotometer flushed with nitrogen, the cell holder being thermostated at 25.0 ± 0.1 °C. Values of absorbance at wavelengths typical of suitable absorbing species were deduced from UV spectra.

Results and Discussion

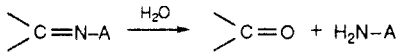
All the selected substrates, except the dyes, were hydrophobic compounds with very low solubility in water and in salt media. The increase of solubility resulting from the presence of Q-P(T,Me-*s*Bu,AE)15 globules could not be measured because of the rather rapid start of chemical reactions. These chemical modifications were performed in the presence of a large excess of polymer, as is usually reported in the literature. In the present case, addition of a buffer was not necessary because the cooperative protonation–deprotonation reaction of Q-P(T,-*N*-R_1-*N*-R_2,AE)15 tertiary amine sites occurred in a very narrow pH range and thus the polymer in excess acted as a buffer itself.[1] Under these conditions, it has been previously shown that globule-catalyzed hydrolysis of CBZ-L-leucine *p*-nitrophenyl ester obeyed a pseudo-first-order kinetics, the rate constant k_{ob} being deduced from the slope of $\ln [A_\infty/(A_\infty - A_t)]$ *vs* time plots, where A_t is the absorbance of *p*-nitrophenate ion at time *t* and A_∞ is the absorbance at infinite time, i.e., when reaction is complete.[6] Blanks were run in the absence of polymer using the same amounts of PNP esters. Corresponding rate constants were defined as k_0.

Hydrolysis of Carboxylic Acid PNP Esters. The hydrolysis of various carboxylic acid PNP esters was carried out comparatively in the presence of Q-P(T,Me-*s*Bu,AE)15 ($C_p = 3.2 \times 10^{-2}$ M) in the globular (G) (pH = 7.08) and open-coil (C) (pH = 6.02) states and in the absence of any polymer. The selected PNP esters derived from alkanoic fatty acids had 2, 4, 8, 12, and 18 carbon atoms in the aliphatic chain. Ester hydrolysis was monitored by measuring absorbance at 400 nm (λ_{max} of PNP^- ion) and at 318 nm (λ_{max} of nonionized PNP). The initial ester concentration was fixed at $C_E = 10^{-5}$ M. The hydrolysis of A_n PNP esters with $n = 8$, 12, and 18 appeared very slow, and k_0 could not be determined in polymer-free water. Consistent k_0 values were obtained for A_2 and A_4 derivatives only. Results are presented in Table I.

As compared with blank experiments, k_{ob}(G) and k_{ob}(C) values showed that both globular and open-coil macromolecules catalyzed A_n PNP ester hydrolysis. However, the catalytic effect was always larger in the presence of globular macromolecules than when the same molecules were in the open-coil state. Similar features were observed in the case of CBZLeuPNP (recalled in Table I).[6] The longer the alkyl chain, the smaller are k_{ob}(C) and k_0. Rate increases related to alkyl chain length of PNP esters mentioned in the literature were generally assigned to apolar interactions between hydrophobic substrates and catalyzing macromolecules.[8–10] Surprisingly, increasing the alkyl chain length had almost no effect on k_{ob}(G), which remained in the range of 10^{-3} s^{-1} regardless of the PNP ester. This finding suggests that apolar interactions with the core of the globules played a minor role in the case of globular (Q-P(T,*N*-R_1-*N*-R_2,AE)15. To account for the hydrolysis of *p*-nitrophenyl octanoate

(NPO) in the presence of polyionenes with long, hydrophobic alternating chain segments, Quina et al.[11-13] pointed out similarities between their polysoap-like systems, working at 9.5 < pH < 9.8, and hexadecyltrimethylammonium (CTAB) micelles for which local concentrations of OH^- ions bound to the micellar phase in buffered solutions could be very high and depended on the detergent concentration. This kind of phenomenon can hardly explain the catalytic effect of globular Q-P(T,Me-*s*Bu,-AE)15 macromolecules as this system works in buffer-free medium and close to neutral pH. Therefore, the constancy of the rate constant $k_{ob}(G)$ for PNP esters of alkanoic acids with increasing chain length was tentatively regarded as resulting from the particular distinct medium which is formed by the core of the globules. This correlation agrees well with the conclusion previously drawn from the failure in searching for a particular catalytic site to account for the rate increase in the case of the hydrolysis of CBZLeuPNP.[6] It is noteworthy that, under the conditions selected, the increase of $k_{ob}(G)/k_{ob}(C)$ with alkyl chain length of PNP esters was due to the decrease of $k_{ob}(C)$ and not to an increase of $k_{ob}(G)$. In this particular case, chain length-dependent hydrophobic interactions of substrates with the polymer backbone of the highly charged polymeric chains might account for the variations of $k_{ob}(C)$ from one ester to the other. Anyhow, $k_{ob}(C)$ was much smaller than $k_{ob}(G)$ for all the esters considered (Table I).

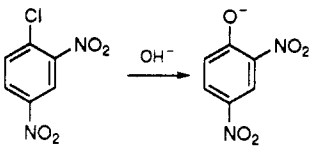
Hydrolysis of (3-methyl-2-cyclohexenylidene)laurylamine (3-MCHLA). It is well known that Schiff bases containing carbon–nitrogen double bonds can be hydrolyzed to yield corresponding aldehydes or ketones according to



For imines with A = H or small alkyl chains, the hydrolysis is easy and can be carried out in water. When A is a long aliphatic chain or an aromatic residue, the hydrolysis is more difficult and requires acid or base catalysis.[14]

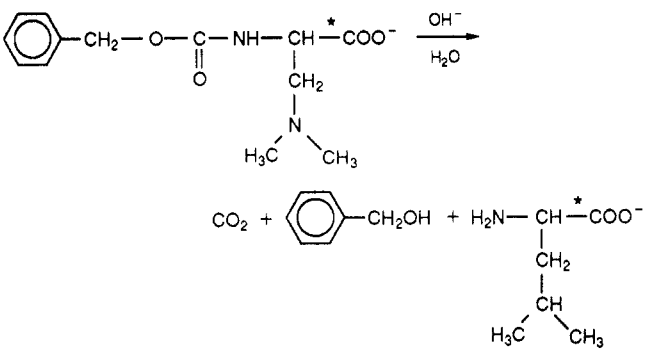
The hydrolysis of 3-MCHLA ($C = 2 \times 10^{-5}$ M) was carried out in the presence of Q-P(T,Me-*s*Bu,AE)15 in aqueous solution ($C_p = 3.2 \times 10^{-2}$ M) at various pH values and compared with polymer-free buffered solutions. The rates of hydrolysis were measured spectrophotometrically by monitoring the disappearance of the imine UV band located at 320 nm. As shown in Figure 1, in the absence of polymer, k_0 increased smoothly as pH increased, in agreement with data in ref 7. This increase was assigned to the catalytic effect of OH^- ions whose concentration increased with pH. In contrast, k_{ob} (actually $k_{ob}(C)$) was 1 order of magnitude higher than k_0 and remained almost constant below the pH of the globule-to-coil transition, i.e., when macromolecules were in the open-coil state. In the 6.6 < pH < 7.2 range where globules are known to be formed cooperatively, k_{ob} increased sharply. Above pH = 7.2, i.e., when all the macromolecules were in the globular state, k_{ob} (actually $k_{ob}(G)$) did not increase further as pH increased, thus showing that the substrate was no longer available to aqueous OH^- catalysis. In this range of high pH values, $k_{ob}(G)$ stayed at a value which corresponded to that observed at pH > 10 in polymer-free water. Similar features were previously observed for CBZLeuPNP.[6] However, for this compound,[5] the $k_{ob}(G)/k_{ob}(C)$ ratio was much larger (10^2) than for 3-MCLHA (10^1).

Hydrolysis of 2,4-Dinitrochlorobenzene (DNC). DNC is known to hydrolyze in alkaline medium at pH values higher than 9, where it yields the ionized form of 2,4-dinitrophenol according to the following equation:[15]



The hydrolysis of DNC ($C_s = 9.85 \times 10^{-6}$ M) was conducted comparatively without polymer in three buffered aqueous solutions (pH = 9.7, 12, and 13.7) and in the presence of Q-P(T,Me-*s*Bu,AE)15 ($C_p = 2.1 \times 10^{-2}$ M) at various pH values. The reaction was monitored spectrophotometrically by measuring the absorbance of the 2,4-dinitrophenate ion at 355 nm. In the absence of polymer, the rate of reaction appeared very small, with k_{ob} in the 10^{-5} range. In the presence of Q-P(T,Me-*s*Bu,AE)15 coils at pH values lower than 6.5, the formation of dinitrophenate ions was detected but k_{ob} was very small, the reaction rate being comparable to those observed at pH = 10–12 in buffered water. In contrast, k_{ob} increased rapidly by 4 orders of magnitude above pH = 6.6, i.e., in the pH range corresponding to the collapse of Q-P(T,-Me-*s*Bu,AE)15 macromolecules to the globular form (Figure 2).

Hydrolysis of *N*-CBZ-L-Leucine (CBZLeu). The hydrolysis of N-substituted aryl carbamates (such as CBZLeu) is known to occur in alkaline media and to form the parent α-amino acids, CO_2, and benzyl alcohol according to the following equation:[16]



The hydrolysis of CBZLeu ($C_E = 1.89 \times 10^{-3}$ M) was carried out at pH = 7 in the presence of Q-P(T,Me-*s*Bu,-AE)15 ($C_p = 1.43 \times 10^{-2}$ M) in a 75/25 v/v water/methanol mixture. This solvent mixture is known to maintain the globular conformation normally taken by Q-P(T,Me-*s*Bu,-AE)15 in pure water.[1] Circular dichroism was used to monitor the cleavage of the aromatic moieties through their UV electronic transitions which are optically active only when these moieties are covalently bound to chiral amino acid molecules. Figure 3 shows the spectrum of initial CBZLeu, which exhibits typical aromatic CD bands in the 250–270-nm range. In contrast, the product which was recovered after hydrolysis in the presence of Q-P(T,-Me-*s*Bu,AE)15 did not feature any aromatic bands, thus showing that cleavage of the CBZ protecting group was feasible at neutral pH. The resulting spectrum was comparable to that of L-leucine under similar conditions, the difference of magnitude being due to the difference of concentrations. Under the selected conditions, the amount of recovered compound was too small to be quantitatively analyzed.

At this stage, one could conclude that Q-P(T,Me-*s*Bu,-AE)15 in the globular form appears to be a powerful catalyst for hydrolyzing compounds in neutral water which normally are hydrolyzed at much higher pH values. These findings agree with the assumption that the core of globular macromolecules constitutes an organic microphase distinct from the surrounding aqueous medium and behaves like

an aqueous medium with a pH value higher than that measured potentiometrically.

Accordingly, attempts were made to determine the ionizing capacity or the equivalent basicity of the core of the globules by using pH-sensitive acid dyes (HD). For this experiment, Q-P(TDAE)15 was used instead of Q-P(T,Me-*s*Bu,AE)15 because of a shortage of the latter. This change did not affect the significance of the data because we have already shown that both polymers, which differ at the level of the side chain alkyl substituents only, behave similarly insofar as catalysis is concerned.[6] The pH of a solution of a UV-absorbing weak acid in water is governed by the HD $\rightleftarrows$ D^- + H^+ ionization equilibrium and can be calculated by the well-known equation pH = pK_a + log ([HD]/[D^-]) provided [HD] and [D^-] can be determined, as was the case since Beer's law was obeyed in the considered range of polymer concentrations C_p. Concentrations of ionized species D^- in contact with globular Q-P(TDAE)15 were determined spectrophotometrically by measuring the absorbance of ionized phenol red (λ_{max} = 557 nm, ϵ = 56 800), cresol red (λ_{max} = 593 nm, ϵ = 58 200), and thymol blue (λ_{max} = 593 nm, ϵ = 20 500). Nonionized species HD were determined at 430 nm for phenol red (ϵ = 21 200), at 432 nm for cresol red (ϵ = 21 200), and at 428 nm for thymol blue (ϵ = 10 300). The selected concentration of these sulfophthalein dyes was $C_D = 1 \times 10^{-5}$ M in order to use conditions close to those used for the chemical reactions described above and because at this concentration, partitioning of D^- was totally localized in the globules. Data are presented in Table II and compared with data for *p*-nitrophenol. In all cases, ionization was larger in the presence of the globular polymer than in water, and the calculated pH corresponding to an equivalent polymer-free aqueous solution was always significantly higher than the bulk pH measured for the polymer-containing solution.

Although far from being conclusive, these findings suggest that the catalytic activity of globular Q-P(*N*-R_1-*N*-R_2,AE)*X* polymers might be related to the accommodation of hydrophobic molecules within the core of the globules. Electrostatic effects due to contributions of the electrically charge corona can hardly be taken into account because most of the substrates allowed to react in this investigation were initially neutral. Therefore, it seems that entrapment within the core and polarization or spatial effects are two of the phenomena which generate the catalytic activity of our partially quaternized tertiary amine polybases, the globular microphase behaving as a superbase medium with respect to water.

For the sake of comparing the catalytic activities of globular Q-P(*N*-R_1-*N*-R_2,AE)*X* polymers to those of other polymeric or micellar systems known to catalyze the hydrolysis of amino acid *p*-nitrophenyl esters, literature data were analyzed. The comparison was difficult because the experimental conditions reported were rarely similar. Furthermore, authors discussed k_{ob} values or the so-called k_{cat} constant defined as the ratio k_{ob}/C_{sc}, where C_{sc} is the molar concentration in catalytic sites. k_{cat} could not be defined in the case of Q-P(T,Me-*s*Bu,AE)15 because of the absence of identified catalytic sites. Nevertheless, the globular Q-P(T,Me-*s*Bu,AE)15 polymer working at pH = 7.4 was tentatively compared with various systems, namely, surfactant micelles and micelles modified by addition of molecules derived from histidine, poly(ethylenimine) polymeric compounds and hydrophobized derivatives, and poly(*N*-vinylimidazoles). Data are presented in Table III.

For *p*-nitrophenol acetate (PNPA), which is the most water-soluble compound of the series, the hydrolysis rate constants were rather small in the case of simple micellar or polymeric systems ($k_{ob} \simeq 10^{-3}$ s^{-1}) whereas modified micellar systems and hydrophobized polymeric systems containing imidazole groups appeared more active ($k_{ob} \simeq 10^{-2}$ s^{-1}). Considering these k_{ob} values, it appeared that the globular Q-P(T,Me-*s*Bu,AE)15 polymer at pH = 7.4 was among the most active catalytic systems ($k_{ob} \simeq 10^{-2}$ s^{-1}), the trend being the same for the other compounds, namely, *N*-CBZ-Leu, *N*-*t*-BOC-L-Phe, and *N*-CBZ-L-Phe.

Because of their exceptional conformational and physicochemical behaviors in aqueous media, Q-P(*N*-R_1-*N*-R_2,AE)*X* polymers, and especially Q-P(T,Me-*s*Bu,AE)15, appear to be remarkable catalysts for performing various chemical reactions in water. In a following paper, globular Q-P(T,Me-*s*Bu,AE)15 macromolecules will be used as the active catalytic part of a chemical reactor able to transform substrates continuously and beyond stoichiometry as referred to C_p polymer concentration.

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