# N-Benzylated Poly(vinylamine): Synthesis, Characterization, and Catalytic Activity in Ester Cleavage

**Bernard Martel,† Alain Pollet,‡ and Michel Morcellet*,†**

*Laboratoire de Chimie Macromoléculaire and Laboratoire de Chimie Organique Physique, Université des Sciences et Techniques de Lille, URA 351 du CNRS, 59655 Villeneuve d'Ascq Cedex, France*

*Received February 8, 1994; Revised Manuscript Received June 6, 1994®*

ABSTRACT: Poly(vinylamine) (**2**) and copolymers (**3**) resulting from various degrees of substitution (DS) with benzyl groups were synthesized. The viscosity of the polymers measured in Tris buffer ($\mu$ = 0.05 M) at different pH values decreases with the increase of the hydrophobic character, and **3** takes a very tight conformation for DS > 50%. A fluorescence study was carried out using potassium 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) as the hydrophobic probe. We showed the existence of an apolar microdomain which is at the origin of the solubilizing power of **3**. The hydrolysis of *p*-nitrophenyl acetate (PNPA) carried out at 25 ± 0.1 °C in Tris buffer solution (pH = 8.74; $\mu$ = 0.05 M) was accelerated up to 20-fold compared to the **2** catalyzed reaction. Fluorometry, in agreement with kinetic measurements, showed that the solubilizing power and catalytic properties increase with DS. From these remarks, it was concluded that the substrate is entrapped in the power microdomain and undergoes the nucleophilic attack of the vicinal $NH_2$ groups. A kinetic study in the presence of cyclodextrin (CD) was carried out and resulted in an inhibition effect.

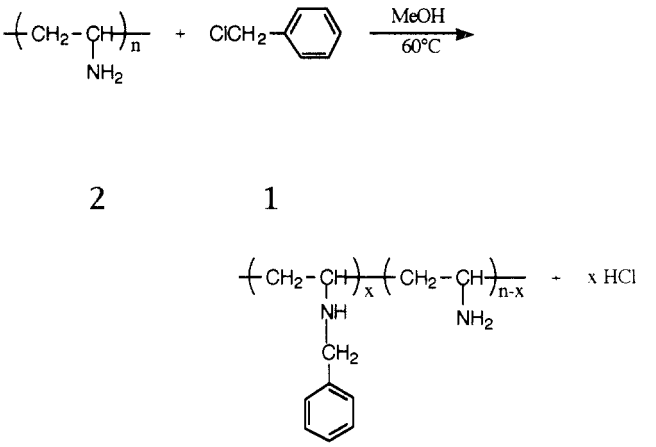
## Introduction

It is well-known that the unequaled performances of natural enzymes are in relation to their specific and complementary properties. Their efficiency results from the cooperative activity of the binding and the catalytic sites. The binding properties are usually of electrostatic or hydrophobic origin and the catalytic site often consists of a nucleophilic group. Various synthetic polymers bearing both characteristics have been tailored to mimic enzymatic reactions.[1-12] Many investigators used polyamines because of their nucleophilicity and their convenience for chemical modification, particularly the grafting of alkyl or benzyl groups. Most of the reports show that the catalytic properties of the polymers are improved with the growth of their hydrophobicity. Such a functionalization leads to a contraction of the macromolecules, enhancing their sorption ability, the consequence being that the substrate is concentrated in the vicinity of the nucleophilic groups. Considering these facts, we carried out the benzylation of poly(vinylamine) and studied its catalytic power in the esterolysis of *p*-nitrophenyl acetate, taking into account the influence of the chemical modification and its consequences on the conformation of the polymer.

## Experimental Section

**Reagents.** $\beta$-Cyclodextrin, TNS, and benzyl chloride were commercial products. Compound **2** was synthesized from polymerization and acid hydrolysis of *N*-vinyl-*tert*-butylcarbamate (NVTBC) obtained by the method of Hart,[13] adapted by Hugues and Saint Pierre.[14] The molecular weight of **2** ($M_n$ = 36 600) was obtained from viscosimetric measurements in 0.1 M NaCl/0.1 M NaOH using the relation $[\eta] = 6.2 \times 10^{-3} M_n^{0.88}$.[15]

**Synthesis of N-Benzylated Poly(vinylamine) (3).** The reaction is described in Figure 1. This reaction was used by Saegusa et al.[16] and Pshezhetskii et al.[17] for the benzylation of poly(ethyleneimine) (PEI) and by Seo et al.[18] for poly(allylamine) (PAA). Smets et al.[19] used a similar reaction for introducing dodecyl chains onto poly(vinylamine) (PVAm, **2**). We used the method reported in ref 18: **2** (0.5 g) was dissolved in 25 mL of methanol. Various quantities of **1** were added, and the mixture




| name | DS (%) |
|---|---|
| 3a | 22 |
| 3b | 34 |
| 3c | 46 |
| 3d | 52 |

**Figure 1.** Reaction scheme for the synthesis of **3** from **2**.

was heated to reflux during various reaction times in order to obtain different degrees of substitution (DS) of the amino groups by **1**. Polymer **3** was precipitated in ethyl ether, washed with a mixture of ether and hexane, redissolved in water, and freeze dried. DS was determined from the ratio C/N obtained from elemental analysis.

**Viscosimetric measurements** were performed in Tris buffer ($\mu$ = 0.05 M) for pH values below 9 and borax buffer for values above 9, with a capillary automatic viscosimeter (Schott Geräte AVS 400) at $T$ = 25 °C. The concentration of the polymer was $10^{-2}$ M in repeat units. In the following, the polymer concentration will be given on this basis.

**Fluorescence measurements** were performed with a Perkin-Elmer LS 50 spectrophotometer. Excitation of the TNS fluorescence was done at 315 nm, and the integration of the emission was made between 340 and 640 nm. The scan rate was 400 nm/min, and the band width was 5 nm in excitation and 2.5 nm in emission.

**Determination of the Dissociation Constants.**[20] The dissociation constant ($K_d$, M) was obtained by changing the

† Laboratoire de Chimie Macromoléculaire.
‡ Laboratoire de Chimie Organique Physique.
® Abstract published in *Advance ACS Abstracts*, July 15, 1994.

0024-9297/94/2227-5258$04.50/0 © 1994 American Chemical Society

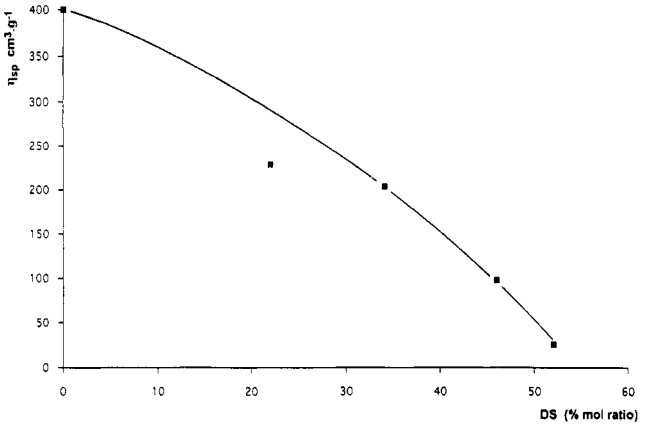



**Figure 2.** Variation of reduced viscosity versus DS. Conditions: pH = 8.05, Tris buffer 0.05 M.

polymer concentration and keeping the fluorescent probe concentration constant ($5 \times 10^{-6}$ M). The system consists of a single equilibrium expressed by

$$P + F \rightleftarrows P{\cdot}F$$

where P, F, and P·F represent the polymer, the probe, and the polymer–probe complex, respectively.

If the concentration of the probe is $C_f$ and its concentration entrapped in the polymer domain is $C_b$, eq 1 is obtained when $C_p \gg C_o$ and $C_o = C_f + C_b$:

$$K_d = \frac{[P][F]}{[P{\cdot}F]} = (C_f(C_p - C_b))/C_b \cong (C_pC_f)/C_b \qquad (1)$$

Bearing in mind that the mole fraction of the probe entrapped in the polymer domain is $X_b = (I - I_o)/(I_{max} - I_o)$ and $C_b/C_f = (X_b)(1 - X_b)$, eq 1 gives

$$I = K_d(I_o - I)/C_p + I_{max}$$

where $I$ is the observed fluorescence intensity at a given $C_p$ and $I_o$ and $I_{max}$ are respectively the intensities at $C_p = 0$ and at the complete complexation of TNS. $K_d$ was obtained from the slope of the straight line of the plot of $I$ versus $(I - I_o)/C_p$, and the intercept on the $I$ axis gave $I_{max}$. Since the fluorescence intensity $I_o$ in pure aqueous solution is much lower than that in the presence of **3** ($^*I \gg I_o$), I was plotted against $I/C_p$ instead of $(I_o - I)/C_p$.

**Kinetic Measurements.** The reaction was carried out at 25 ± 0.1 °C in Tris buffer solution (pH = 8.74; $\mu$ = 0.05 M). A 15-$\mu$L aliquot of a $2 \times 10^{-2}$ M solution of PNPA in acetonitrile was added to 3 mL of the buffer solution so that the initial concentration of the substrate was $1 \times 10^{-4}$ M. The reaction was monitored at 400 nm for the nitrophenolate ion and the absorbance was recorded with a Uvikon 930 spectrophotometer. In all cases pseudo-first-order kinetics were observed. The pseudo-first-order constant $k_{obs}$ was obtained from the slope of the linear plot of $\ln(A_\omega - A)$ where $A$ and $A_\omega$ are the absorbance for the reaction at a time $t$ and for the complete reaction, respectively. Deviations from first order occurred only at high percent conversion due mainly to uncertainties on the $(A_\omega - A)$ term. $k_{obs}$ values were obtained from data corresponding to 0–30% conversion. When $k_{obs}$ increases linearly with the concentration in catalyst, the second-order constant can be obtained according to the equation $k_{obs} = k(\text{cat}) + k_{un}$ where $k_{un}$ is $k_{obs}$ for $k(\text{cat}) = 0$. The concentration range for the polymer amine catalyst was $(0–7) \times 10^{-3}$ M. In these conditions the potential concentration of acetate ion generated from the ester is much lower than the concentration of amino groups and a possible N-acylation of the polymer is negligible. The elemental analysis of the polyamine after reaction was not distinctly different from that of the unreacted sample.

## Results and Discussion

**Characterization of the Polymers.** Figure 2 shows that the reduced viscosity of **3** decreases when the DS increases; this is the consequence of intrachain hydrophobic interactions relative to the formation of a microdomain enriched in aromatic groups. The intensity of these interactions annihilates the stretched conformation due to the electrostatic repulsions of the protonated primary amino groups. A very tight conformation is observed for the **3d** sample. The same behavior was reported for poly(allylamine) (PAA)[20] for a DS in the same range, while a similar conformation is obtained at a DS of only 10% for benzylated PEI.[17] As for **2** and in the same range of pH, the viscosity of **3** decreases when the pH increases (Figure 3 for sample **3d**); a compact conformation is observed at pH 9, and the polymer precipitates around pH = 9.5. This reveals the vanishing of the electrostatic repulsions accompanying the deprotonation of the amino groups and the increasing influence of hydrophobic forces.

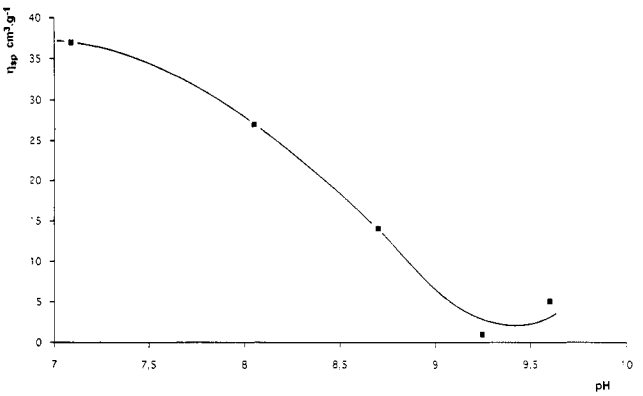



**Figure 3.** Variation of the reduced viscosity of sample **3d** versus pH. Conditions: Tris buffer for pH < 9 and borax buffer for pH > 9.

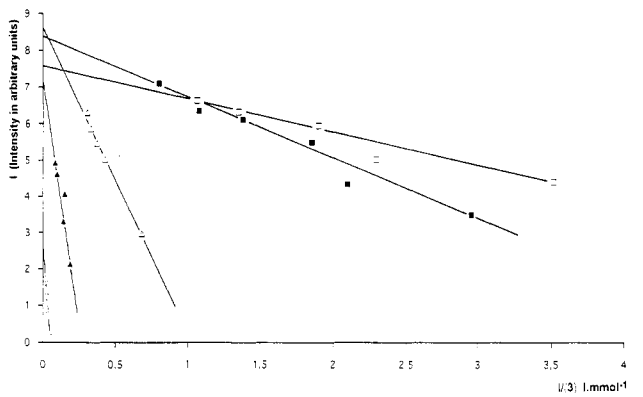



**Figure 4.** Fluorescence intensity $I$ versus $I/[3]$ for the determination of $K_d$ with TNS ($5 \times 10^{-6}$ M): (○) **3a**; (△) **3b**; (□) **3c**; (■) **3d**; (▲) **3d** + CD. Conditions: Tris buffer pH = 8.74.

The hydrophobic domain of **3** was also characterized by fluorescence measurements using TNS as a probe. The dissociation constants of the TNS/**3** complexes were determined from the method explained in the Experimental Part using the data in Figure 4. Values are summarized in Table 1. The stability of the inclusion of the probe in the apolar domain of **3** increases with DS and seems to reach a limit, within experimental error, when DS > 40%. The apparent stabilization of $K_d$ for a high DS in Figure 5 can be attributed to a very compact structure which prevents the substrate from being further included in the hydrophobic microdomain. Furthermore, we can assume that the solubilizing power of **3** depends on its benzylation degree but that it is balanced by steric strains in the interior of the hydrophobic domain that hinder the incorporation of the probe when DS > 50%. The values for $\lambda^E_{max}$ in Table 1 indicate that the benzyl units of **3** create hydrophobic regions with a more apolar

**Table 1. Fluorescence Constants for TNS in the Presence of 3, 3d + CD, and Benzylated Poly(allylamine) ([TNS] = 5 × $10^{-6}$ M, Tris buffer pH = 8.74; in Parentheses, the Uncertainty on the Measurements)**

| polymer | $10^4K_d$ (M) | $\lambda E_{max}$ (nm) | $I_{max}$ |
|---|---|---|---|
| **3a** | 45.1(4.5) | 437 | 2.5 |
| **3b** | 8.37(0.53) | 442–436 | 8.58 |
| **3c** | 0.92(0.41) | 434–437 | 7.56 |
| **3d** | 1.68(0.38) | 432–436 | 8.44 |
| **3d** + CD | 28.5(1.5) | 427–432 | 7.34 |
| PAABz (DS = 0.75)[a] | 0.31 | 432 | |

[a] Reference 20.

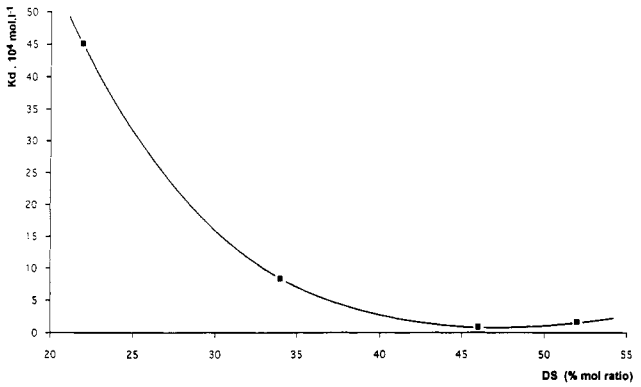



**Figure 5.** Variation of $K_d$ versus the DS of 3. Conditions: [TNS] = 5 × $10^{-6}$ M; Tris buffer pH = 8.74.

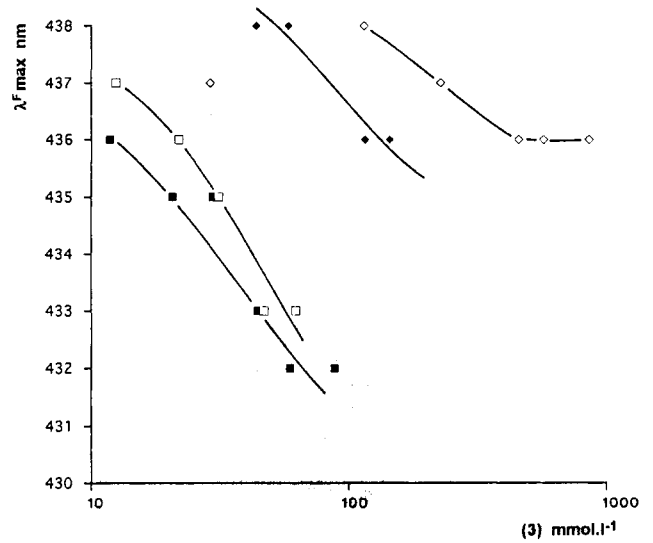



**Figure 6.** Variation of the maximum wavelength of emission of TNS against the concentration of 3: (◇) **3a**; (◆) **3b**; (□) **3c**; (■) **3d**. Conditions: Tris buffer pH = 8.74; [TNS] = 5 × $10^{-6}$ M.

character than that observed in surfactant micellar systems such as dodecyltrimethylammonium bromide and acyltrimethylammonium or dodecylamine hydrochloride.[20] The decrease of $\lambda E_{max}$ on increasing the polymer concentration (Figure 6) is related to the conformational behavior of polyelectrolytes: the repulsive intramolecular electrostatic forces decrease when the bulk concentration increases (shielding effect), and then hydrophobic interactions are responsible for the folding of the chain. In other words, the folding of the chains induces a microenvironment more and more isolated from the solvent in a reduced polarity. This effect is more pronounced for the more benzylated samples. The relative positions of the four plots in Figure 6 show that the main interactions between the polymer and the probe are of a hydrophobic nature though electrostatic interactions probably also occur

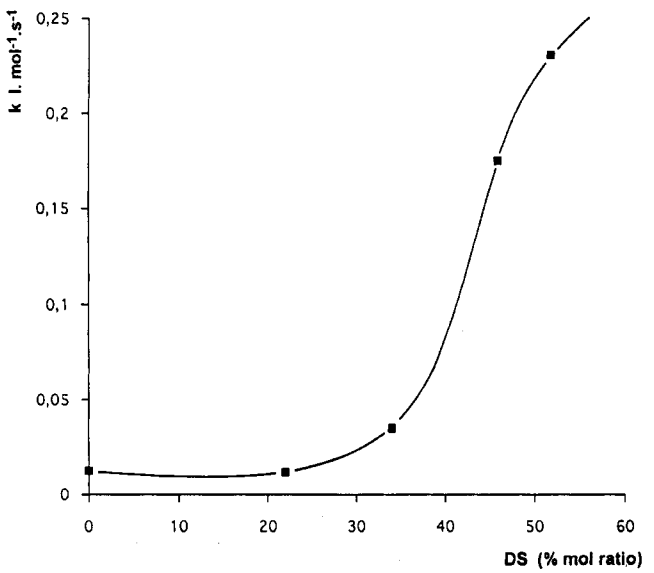



**Figure 7.** Variation of the second-order constant for the hydrolysis of PNPA versus DS of 3. Conditions: [PNPA] = $10^{-4}$ M, $T$ = 25 °C, Tris buffer ($\mu$ = 0.05 M) pH = 8.74.

between the sulfonate function of TNS and the partially protonated polymer. Data in Figure 6 are in agreement with the viscosity measurements that reported a nonmicellar conformation for the weakly substituted polymers that remain in a relatively extended conformation due to insufficient hydrophobic interactions. On the contrary, highly substituted polymers show a pronounced variation of $\lambda E_{max}$ which is related to the transition of a stretched to a folded chain. The respective evolutions of $\lambda E_{max}$ for the different 3 samples in Figure 6 are in agreement with the results obtained by Seo et al.[20] with benzylated polyallylamines: the polarity and the intensity of the hydrophobic forces in the apolar region of the polymers have an effect on their inclusion power; in other words, $K_d$ and $\lambda E_{max}$ are dependent on each other.

The values measured for the maximum of intensity emission ($I_{max}$) are low for **3a** and stabilize around 8 for a higher DS (Table 1). It is known that $I_{max}$ depends on the rigidity of the environment, as the consequence of the hindrance of intramolecular rotation of the TNS molecule around its amino group, as shown by studies of the cyclodextrin/TNS inclusion complexes[21] for example. The weakness of the apolar interactions in **3a**, which has a less rigid and compact microdomain, would allow TNS to have an important rotational mobility compared to samples of DS > 34%.

**Catalytic Experiments.** The second-order kinetic constants versus the DS of 3 for the hydrolysis of PNPA are reported in Figure 7; it shows that the catalytic activity of 3 is obvious when DS is over 30%. $k$ for the reaction catalyzed with **3d** is 20-fold that measured for the **2** catalyzed reaction. These results are in agreement with the above fluorescence measurement results: the catalytic activity is dependent on the interactions between the hydrophobic substrate and the polymer microdomain. The reaction consists of a binding step (hydrophobic interactions) and a catalytic step in which the unshared electron pair of the amino group is involved in the cleavage of the ester function, as reported previously with linear PEI carrying alkyl groups of variable lengths.[22] The catalytic power of **3a** and **3b** being close to that of **2** is attributed to the decrease in free amino groups that induces a decrease in active sites combined with the low inclusion behavior of the polymer due to its nonmicellar structure. As reported by Pshezhetskii et al.,[22] the number of catalytic

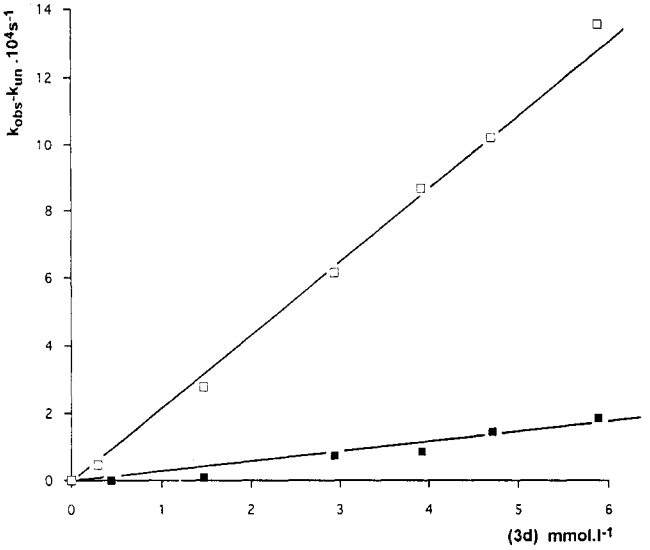



**Figure 8.** Inhibition effect of cyclodextrin on the hydrolysis of PNPA catalyzed by **3d**: (□) **3d**; (■) **3d** + CD. Conditions: [CD] = $10^{-2}$ M; $T$ = 25 °C; Tris buffer pH = 8.74 ($\mu$ = 0.05 M); [PNPA] = $10^{-4}$ M.

centers depends on the degree of deprotonation of the amino groups; alkylated PEI are reported to have lower p$K_a$'s than their low molecular weight analogues and show a difference in activity of 4–7 orders of magnitude. Futhermore, PEI substituted with alkyl groups of different lengths shows a decrease in p$K_a$ with the growth of the alkyl chain also accompanied by a variation of their activity. We compared th p$K_a$ of **3** samples with that of **2**, but no variation was observed. Viscosimetric and kinetic measurements suggest that the benzylated residues are concentrated in a hydrophobic microdomain whereas the amino groups are in contact with water. In this situation, each non-benzylated amino group would keep two ionizable groups as its neighbors with no change in the electrostatic interactions. Thus the grafting step does not affect the degree of protonation of the polyamine at a given pH. The catalytic activity of **3** depends mainly on the DS and on the polarity of the domain.

**Hydrolysis in 3-Cyclodextrin Systems.** We investigated the catalytic activity of **2** in the presence of CD ($10^{-2}$ M) and detected only a slight increase of the second-order rate constant (0.012 to 0.017 $M^{-1}$). As we reported in another paper,[23] **2** concentrates CD in its proximity and the included substrate is accumulated near the nucleophilic sites of the polymer, including the slight improvement of the hydrolysis rate. On the contrary, an important decrease of the catalytic activity of **3d** was observed when mixed with $\beta$CD, as shown in Figure 8. Such an effect has already been observed by Seo et al.[24] for the hydrolysis of PNPA catalyzed by benzylated PAA in the presence of $\beta$CD. They assumed that, on one hand, the substrate included in the CD cavity cannot react with the amino groups of the polymer. On the other hand, the hydrophobicity of the polymer is reduced because of interactions between benzyl groups and the CD cavity. The study of the conformation of **3** in the presence of CD was carried out: surprisingly, the viscosity at pH 8 decreased from 26 to 6 $cm^3/g$ when CD was added to the **3d** polymer solution. Two assumptions can be put forward: (1) CD can induce a shielding effect and annihilate the electrostatic repulsions inside the chain favorable to its folding by hydrophobic interactions. Viscosity measurements carried out on a CD/**2** mixture showed an opposite effect. (2) The encapsulation of the benzyl groups of the polymer by CD is accompanied by the establishment of a dense hydrogen bond network between the CD units. This results in a very compact structure preventing the substrate from getting into the surroundings of the amino groups. This phenomenon is under study in our laboratory, but some results obtained with polymers based on phenylalanine confirm this assumption; the addition of urea in the polymer/CD solutions destroys the compact conformation. Fluorometric data relative to the system **3d**/CD/TNS (Table 1) bring additive information: $K_d$ increases 30-fold compared to that for the system **3d**/TNS; this confirms that the substrate is hardly solubilized into the polymer microenvironment in the presence of CD. The values for $\lambda^E_{max}$ and $I_{max}$ are characteristic of the hydrophobic and rigid environment of the probe and do not correspond to the features of CD/TNS inclusion complexes.[24,25] This indicates that **3d**–CD interactions are stronger than substrate–CD interactions and confirms that CD inhibits the reaction through a change of the properties of the polymer, that is its solubilizing power.

## Conclusion

In this paper we describe the synthesis of benzylated poly(vinylamine) with various degrees of substitution. The peculiarity of these polymers is their amphiphilic properties: they contain hydrophilic groups which provide them with catalytic properties and hydrophobic groups which enable them to solubilize some hydrophobic substrates. We observed that the structure of the polymer depends (1) on the pH of the solution which changes the electrostatic intramolecular repulsions and (2) on the degree of substitution that induces by hydrophobic interactions a more or less apolar domain. These features lead to structure properties identical to those of natural enzymes: a binding site and a catalytic site. Nevertheless, the synthetic systems never reach the performances of natural enzymes. At last, we observed that CD induces the inhibition of the catalytic properties of our benzylated polymer by the suppression of its solubilizing power.

## References and Notes

(1) Overberger, C. G.; Salamone, J. C.; Yaroslavsky, S. *J. Am. Chem. Soc.* **1967**, *89*, 6231.
(2) Overberger, C. G.; St. Pierre, T.; Vorcheimer, N.; Lee, J.; Yaroslavsky, S. *J. Am. Chem. Soc.* **1965**, *87*, 296.
(3) Overberger, C. G.; St. Pierre, T.; Yaroslavsky, S. *J. Am. Chem. Soc.* **1965**, *87*, 4310.
(4) Overberger, C. G.; St. Pierre, T.; Yarovslavsky, C.; Yaroslavsky, S. *J. Am. Chem. Soc.* **1966**, *88*, 1184.
(5) Overberger, C. G.; Corett, R.; Salamone, J. C.; Yaroslavsky, S. *Macromolecules* **1968**, *1*, 331.
(6) Overberger, G. C.; Morimoto, M. *J. Am. Chem. Soc.* **1971**, *93*, 3222.
(7) Overberger, G. C.; Morimoto, M.; Cho, I.; Salamone, J. C. *J. Am. Chem. Soc.* **1971**, *93*, 3228.
(8) Pavlisko, J. A.; Overberger, G. C. *J. Polym. Sci., Polym. Chem. Ed.* **1981**, *19*, 1621.
(9) Tomko, R.; Overberger, G. C. *J. Polym. Sci., Polym. Chem. Ed.* **1985**, *23*, 265.
(10) Tomko, R.; Overberger, G. C. *J. Polym. Sci., Polym. Chem. Ed.* **1985**, *23*, 279.
(11) Nango, M.; Klotz, I. M. *J. Polym. Sci., Polym. Chem. Ed.* **1978**, *16*, 1265.
(12) Klotz, I. M.; Stryker, V. H. *J. Am. Chem. Soc.* **1968**, *90*, 2717.
(13) Hart, R. (a) *Bull. Soc. Chim. Belg.* **1957**, *66*, 229; (b) *J. Polym. Sci.* **1958**, *29*, 629; (c) *Makromol. Chem.* **1959**, *32*, 51.
(14) Hugues, A. R.; St. Pierre, T. *Macromol. Synth.* **1977**, *6*, 31.
(15) Chang, C.; Di Muccio, D.; St. Pierre, T. *J. Polym. Sci. Polym. Symp.* **1986**, *74*, 17.
(16) Saegusa, T.; Ikeda, H.; Fugii, H. *Polym. J.* **1972**, *3*, 35.
(17) Pshezhetskii, V. S.; Murtazaeva, G. A.; Kabanov, V. A. *Eur. Polym. J.* **1974**, *10*, 581.

(18) Seo, T.; Kajihara, T.; Iijima, T. *Makromol. Chem.* **1990**, *191*, 1665.
(19) Everaerts, A.; Samyn, C.; Smets, G. *Makromol. Chem.* **1984**, *185*, 1881.
(20) Seo, T.; Take, S.; Miwa, K.; Hamada, K.; Iijima, T. *Macromolecules* **1991**, *24*, 4255.
(21) Harada, A.; Furue, M.; Nozakura, S. *Macromolecules* **1977**, *10*, 676.
(22) Pshezhetskii, V. S.; Lukyanova, A. P.; Kabanov, V. A. *J. Mol. Catal.* **1977**, *2*, 49.
(23) Martel, B.; et al. Submitted for publication to *Makromol. Chem.*
(24) Seo, T.; Kajihara, T.; Miwa, K.; Iijima, T. *Makromol. Chem.* **1991**, *192*, 2357.
(25) Jobe, D. J.; Verral, R. E.; Palepu, R.; Reinsborough, V. C. *J. Phys. Chem.* **1988**, *92*, 3582.