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Studies of Nucleic Acid-like Polymers as Catalysts

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Summary. An important issue in the problem of the origins of life is whether or not nucleic acids may exert catalytic activities. In order to study the possible role of the adenine ring in catalysis, we have synthesized polymers containing aliphatic amino groups and the nucleic base adenine linked to macromolecules by its 6-amino group. These polymers exhibit pronounced catalytic activities in the hydrolysis of *p*-nitrophenylacetate. In mild basic conditions, the strong increase in the activities observed can be related to a cooperative effect between the amino groups and the adenine rings of the polymers. These properties and our previous results on the catalytic activity of N6-ribosyl-adenine are consistent with a possible role for the adenine ring in prebiotic catalysis.

Key words: Origin of life — Adenine — Catalysts — Nucleic acids — Polymers — Poly(allylamine) — *p*-Nitrophenylacetate

Introduction

What were the main organic catalysts that contributed to the emergence of the earliest biochemical pathways? RNA precursors may be included in the list, as in contemporary cells, some catalytic functions are carried out by specific RNA molecules (e.g., Cech 1987). To some extent, the activities of these natural RNA catalysts may be mimicked in vitro by smaller artificial RNAs (Uhlenbeck 1987; Koizumi et al. 1988). In a prebiotic perspective, one may also wish to investigate the properties of plausible ancestral nucleic acids that may differ substantially in their chemical structure from modern RNAs. For instance, prebiotic replication may have involved nucleic acids with a non-sugar–phosphate backbone (Spach 1984; Joyce et al. 1987; Orgel 1987) or nonstandard nucleotides (Wächtershäuser 1988). Among these, we are particularly interested in N6-ribosyl-adenine. This compound is the main product of the nonenzymatic condensation of adenine and ribose (Fuller et al. 1972). Because the sugar is linked to the N6 rather than to the N9 position of adenine, the imidazole ring of adenine is available for catalysis. Indeed, N6-ribosyl-adenine was shown to be about as efficient a catalyst as histidine in the model reaction of *p*-nitrophenylacetate (PNPA) hydrolysis (Maurel and Ninio 1987). Synthetic, N6-ribosyl-adenine is in fact a mixture of four isomers differing in the configuration (furanose or pyranose ring) and the site of substitution of adenine in the C1′ of the sugar moiety (α or β) (Maurel and Convert 1990). We have shown that the sugar moiety is mostly in the pyranose form (and not the furanose form found in biological RNAs), that it is linked to N6 of adenine, and that the α and β isomers are found in comparable amounts, roughly a 2:1 α/β ratio.

In order to study the catalytic activity of nucleic acids, or analogs, containing prebiotic elements, we have synthesized polymers already containing catalytic groups. We therefore synthesized polymers containing adenine rings through the reaction of 6-chloropurine with a polyamine.

We report here the study of the catalysis that is achieved by such products in order to better appreciate the possible role of nucleic acids catalysis in the origin of life.

Materials and Methods

Synthesis of Polymers Containing the Catalytic Groups: Materials. Poly(allylamine) hydrochloride *1* [PAL, HCl; molecular weight (Mw) = 8500–11,000], 6-chloropurine *2*, and 2-bromo-

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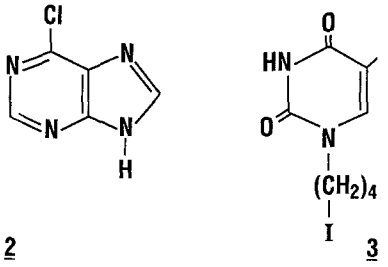
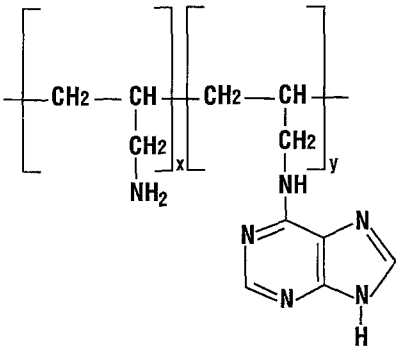


2 3

Fig. 1. 6-Chloropurine *2* and 1-iodo-4-(thym-1-yl)butane *3*.



PALAD 4

Fig. 2. PALAD *4*.

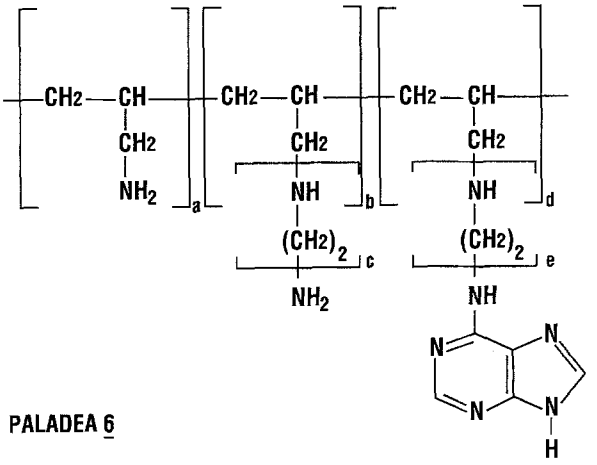
ethylamine hydrobromide were supplied by Aldrich. 1-Iodo-4-(thym-1-yl)butane *3* was prepared from thymine and 1,4-diiodobutane according to the method described previously (Décout et al. 1988).

Polymer Containing Adenine Residues (PALAD 4). The pH of a solution of *1* (0.50 g) in water (50 cm^3) was adjusted to pH 8.5–9 with aqueous sodium hydroxide (5 N). The solution was heated in an oil bath at 80°C and then 6-chloropurine *2* (0.25 g, 1.62 mmol) was added with stirring. After 1 h and 3 h of reaction at 80°C, the pH was readjusted to 8.5–9, and a second fraction of 6-chloropurine *2* (0.25 g, 1.62 mmol) was added. After another 3 h, the resulting solution was cooled, and the pH was adjusted to 6 with aqueous HCl solution. The polymer solution was purified by three dialyses against water and then evaporated to dryness under reduced pressure. The residue was dissolved in a minimal amount of water and precipitated in dry acetone.

The polymer was filtered, washed with anhydrous diethyl ether, and dried in vacuo.

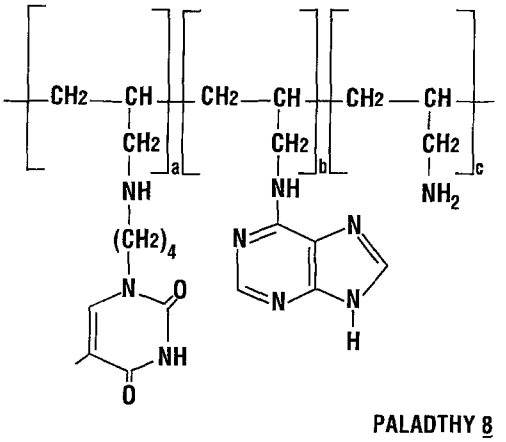
Polymers with Long Side Chains Containing Amino Groups (PALEA 5). The pH of a solution of *1* (0.50 g) and 2-bromoethylamine hydrobromide (0.70 g, 3.4 mmol) in water (50 cm^3) was adjusted to 9 with aqueous NaOH (5 N). The solution was heated in an oil bath at 80°C with stirring. After 1 h and 3 h of reaction, the pH was readjusted to 9, and then a second fraction of bromide (1.0 g, 4.9 mmol) was added. After 6 h of reaction and adjustment of the pH to 9, a third fraction of bromide (1.0 g, 4.9 mmol) was added. The reaction was continued to 80°C for 3 h. The pH of the cooled solution was adjusted to 3 with aqueous HCl. The polymer solution was purified by three dialyses against water and then evaporated to dryness under reduced pressure. The residue was dissolved in a minimal amount of water and precipitated in dry acetone. The polymer was filtered, washed with anhydrous diethyl ether, and dried in vacuo (0.7 g).

PALADEA 6. This polymer was prepared from PALEA *5* (0.5 g) and 6-chloropurine using the same procedure as for PALAD *4*.



PALADEA 6

Fig. 3. PALADEA *6*.



PALADTHY 8

Fig. 4. PALADTHY *8*.

Polymers Containing Thymine Residues (PALTHY 7). The pH of a solution of *1* (1.0 g) in water (100 cm^3) was adjusted to 8.5 with aqueous sodium hydroxide (5 N). The solution was heated in an oil bath at 80°C and 1-iodo-4-(thym-1-yl)butane *3* (0.25 g, 0.81 mmol) was added with stirring. After 3 h, the pH was readjusted to 8.5 and a second fraction of *3* (0.25 g, 0.81 mmol) was added. The mixture was heated overnight to 80°C with stirring, and then the pH of the cooled solution was adjusted to 4 with an aqueous HCl solution. The polymer solution was purified by three dialyses against water and then evaporated to dryness under reduced pressure. The residue was dissolved in a minimal amount of water and precipitated in dry acetone. The polymer was filtered, washed with anhydrous diethyl ether, and dried in vacuo (1.1 g).

PALADTHY 8. This polymer was prepared from PALTHY *7* (0.8 g) and 6-chloropurine using the same procedure as for PALAD *4*.

PNPA Hydrolysis Assays. Dry PNPA, purchased from Sigma was dissolved, just before use, in pure methanol (30 mg/50 ml) and kept on ice.

The assays were carried out in a volume of 0.8 ml containing, unless otherwise specified, 10^{-4} M PNPA, 10^{-4} M of catalyst, and 0.02 M of Tris buffer (2-amino-2-hydroxymethyl-1,3 propanediol) at pH 7.7 or 8.5 at 25°C. The reaction was initiated by the addition of PNPA, and the appearance of *p*-nitrophenol was followed in a Perkin-Elmer spectrophotometer at a wavelength

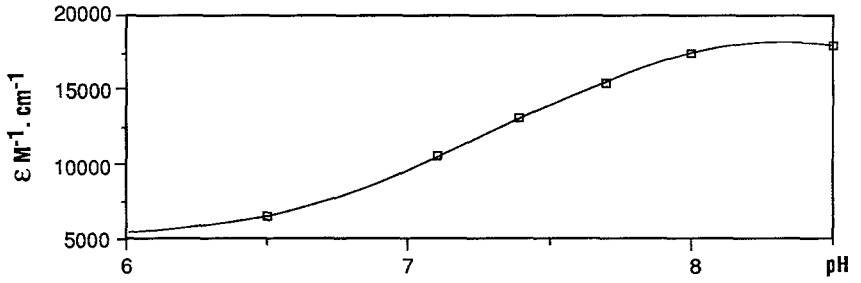


Fig. 5. Variation of molar extinction of PNP at 400 nm as a function of pH. Tris buffer 0.02 M, 25°C.

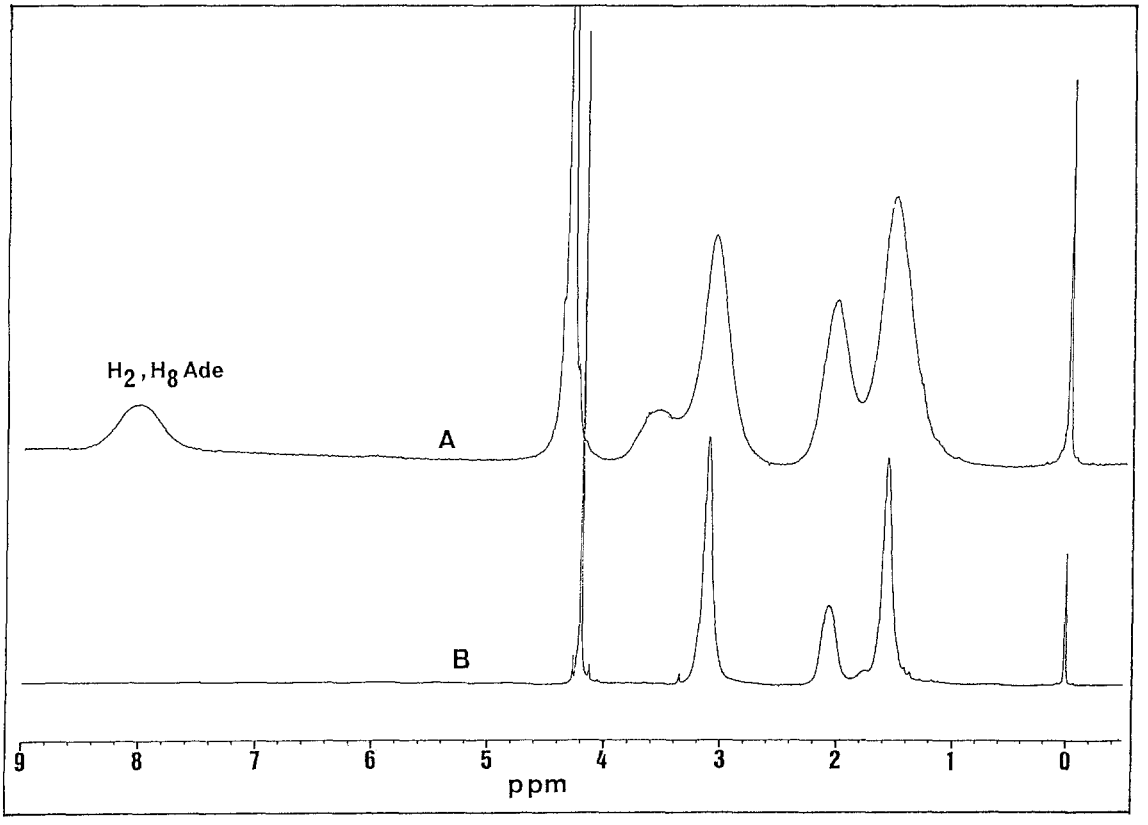


Fig. 6. ^{1}H NMR spectra of poly(allylamine) (**A**) and its derivative PALAD *4* containing adenine residues (**B**) (D_2O, 25°C, 300 MHz).

of 400 nm assuming a molar extinction coefficient of 1.8×10^4 M^{-1} cm^{-1} for the *p*-nitrophenate anion at pH 8.5 (Bender et al. 1967). The variations of this coefficient with respect to the pH were determined by following the complete hydrolysis of PNPA catalyzed by chymotrypsin (Fig. 5), and the results were corrected accordingly.

The catalytic efficiency, referred to adenine, is defined here as the ratio of optical density increases per unit of time and per mole of adenine groups in the polymers.

Numerical data of the kinetics analysis were estimated by a weighted regression procedure (Wilkinson 1961).

Results and Discussion

Synthesis and Characterization of Poly(allylamine) Derivatives Containing Adenine Residues

These polymers were prepared by reaction of a polyamine with 6-chloropurine *2* (Fig. 1) in water.

Poly(allylamine) was first modified to yield the polymer PALAD *4* (Fig. 2). The presence of the adenine ring in PALAD *4* was shown by ^{1}H NMR and UV spectrometries.

The ^{1}H NMR spectrum of *4* in D_2O reveals additional signals in comparison with the spectrum of poly(allylamine) (Fig. 6): (1) the characteristic signal of the adenine ring protons C_2H and C_8H at 8.0 ppm; and (2) a signal corresponding to the methylene attached to the 6-amino group of the adenine ring at 3.6 ppm.

The UV spectrum of *4* in water at pH 7 shows the characteristic absorption band of the adenine chromophore (λ_{max} = 264 nm). This spectrum was used to determine the adenine composition in weight: at least 35% of monomer allyladenine was estimated from the maximum molar extinction coefficient of N6-methylaminoadenine found in the literature [ϵ_{max} = 16,200 M^{-1} cm^{-1} (Venkstern and Baev 1965)].

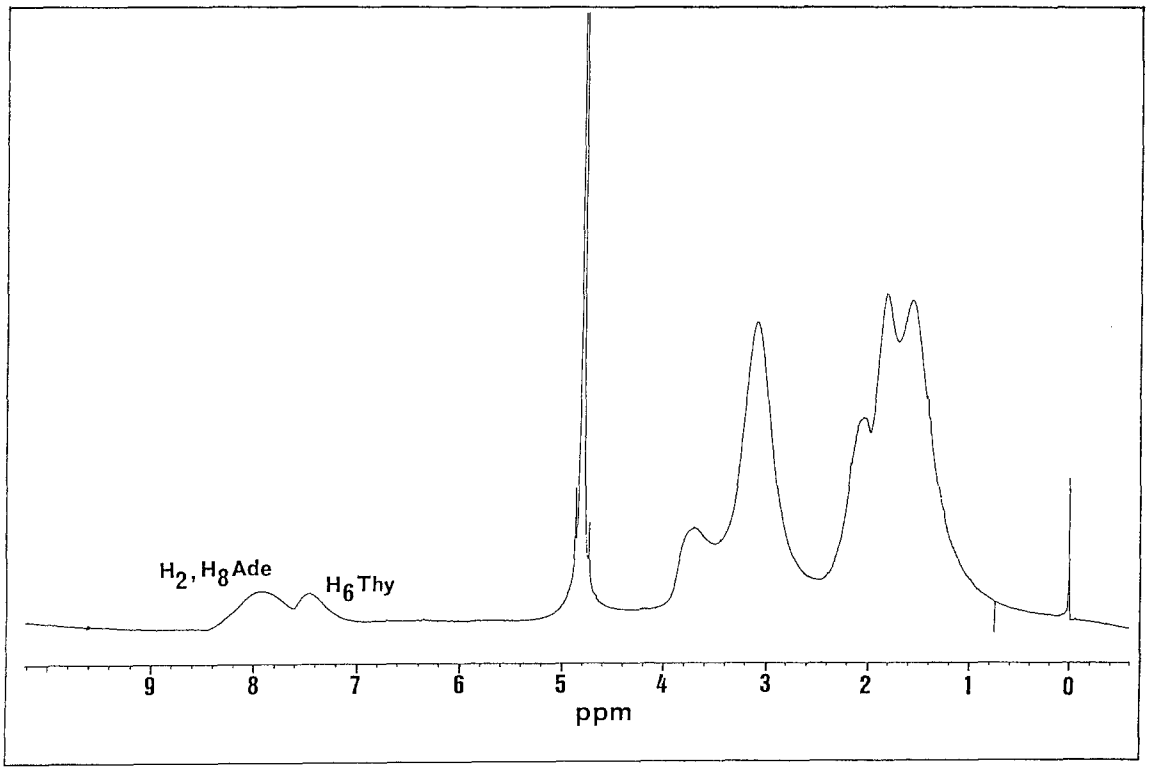


Fig. 7. ^{1}H NMR spectrum of the polymer PALADTHY *8* containing adenine and thymine residues (D_2O, 25°C, 300 MHz).

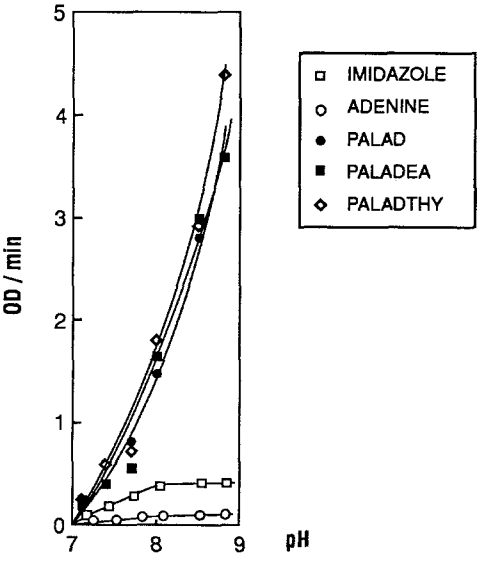


Fig. 8. Activity as a function of pH. Tris buffer 0.02 M, [S] = 10^{-4} M, [catalyst] = 5.10^{-5} M, 25°C. Activity is defined as the optical density increase per unit of time, normalized to a molar concentration of substrate and catalyst.

The percentage of protonated primary amino groups at pH 7 was not determined. On the assumption that this percentage is higher than 50%, the degree of substitution of PALAD by adenine residues can be estimated to be 20–25%.

To prepare a polymer with longer side chains containing amino groups, poly(allylamine) was modified by reaction with 2-bromoethylamine. Reaction of the polymer *5* obtained with 6-chloropurine led to the polymer PALADEA *6* (Fig. 3) containing adenine rings as observed by UV and NMR spectrometries.

Table 1. Composition in monomer allyladenine and catalytic efficiency (as defined in the Materials and Methods) of the compound studied

	Adenine (%) weight	Catalytic efficiency
Adenine	100	1
PAL	–	40
PAL + Adenine	35	40
PALAD	35	100
PALADEA	30	115
PALADTHY	20	110

[PNPA] = 10^{-3} M in methanol at 25°C, [catalyst] = 10^{-3} M, Tris buffer 0.02 M, pH 8.5

The composition of this polymer in residue allyladenine (w/w) was estimated to be at least 30% by UV spectrometry.

The polymer PALADTHY *8* (Fig. 4) with adenine and thymine rings and a hydrophobic alkyl chain was also prepared. The thymine rings were first introduced by reaction of 1-iodo-4(thym-1-yl) butane *3* (Fig. 1) with poly(allylamine). The UV spectrum of polymer *7* showed the characteristic absorption band of the thymine chromophore (λ_{max} = 264.5 nm at pH 7). The degree of substitution of this polymer was estimated to be at least 7% using

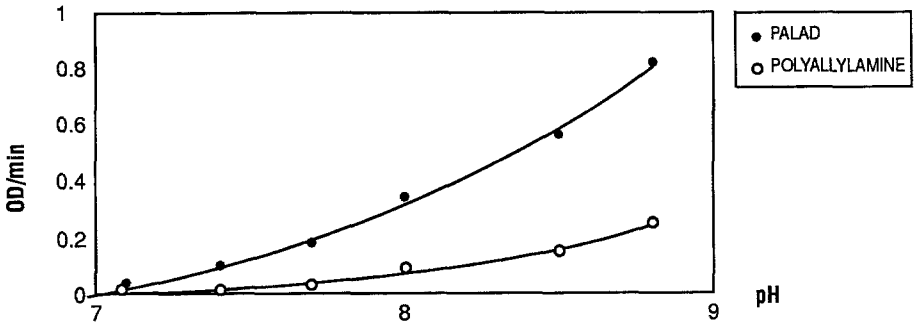


Fig. 9. Activity as a function of pH. Tris buffer 0.02 M, [S] = 10^{-4} M, [catalyst] = 1 mg/ml, 25°C. Activity is defined as the optical density increase per unit of time (for a concentration of 1 mg/ml of catalyst).

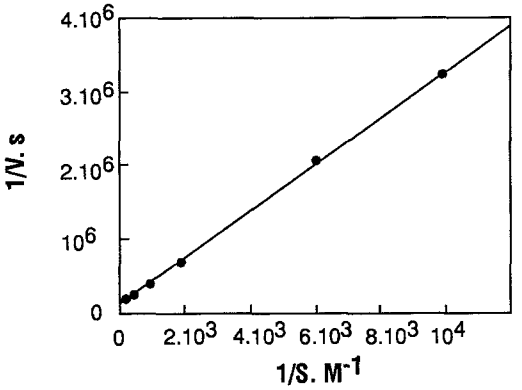


Fig. 10. Lineweaver–Burk representation of the velocity as a function of substrate concentration. Tris buffer 0.02 M, 25°C, [PALAD] = 5×10^{-5} M.

Table 2. Parameters of the hydrolysis of *p*-nitrophenylacetate

	K_{cat} (s^{-1})	K_m (M)	K_{cat}/K_m (M^{-1} s^{-1})
PALAD	2.0×10^{-2}	4.0×10^{-3}	5.0
PALADEA	2.5×10^{-2}	6.5×10^{-3}	3.7
PALADTHY	1.5×10^{-2}	2.7×10^{-3}	5.5

Substrate concentration ranging from 10^{-4} M to 6×10^{-3} M, [catalyst] = 10^{-5} M, Tris buffer 0.02 M, pH 8.5 at 25°C

the maximum molar extinction coefficient of 1-propylthymine [ϵ_{max} = 9900 M^{-1} cm^{-1} (Décout et al. 1988)].

Adenine rings were introduced in polymer *7* by reacting 6-chloropurine *3*. The ^1H NMR spectrum of polymer *8* (PALADTHY) revealed the presence of the characteristic signals of the aromatic thymine ring proton C_6H at 7.5 ppm and adenine ring protons C_2H and C_8H at 8.0 ppm (D_2O; Fig. 7). From the UV spectrum of *8* (λ_{max} = 266.5 nm, pH 7), the adenine composition was estimated to be 20% in the weight of the monomer allyladenine.

Study of the Type of Catalysis

The adenine ring incorporated in polymers *4*, *6*, and *8* presents an imidazole ring that could act as a proton transfer relay system in the catalysis of ester hydrolysis. The effects of the polymers synthesized on the hydrolysis of *p*-nitrophenylacetate were investigated. PNPA is a model substrate for proteases like papaïn (Bülow and Mosbach 1987). Simple compounds like histidine or imidazole clearly accelerate the hydrolysis of PNPA and small peptides containing one histidine were found to be 40 times more active than histidine in this reaction (Kapoor 1972).

Hydrolysis in the Absence of Catalyst

No measurable activity is observed at a pH inferior to 6.5. Above, the activity rises slowly as a function of pH, and the results were corrected accordingly.

Catalysis as a Function of pH

The effect of the pH on the rate of hydrolysis in the pH range 7–8.8 is shown in Figs. 8 and 9. A remarkable increase in the catalytic effect was observed above pH 8 for PALAD as well as for PALADEA and PALADTHY. At every pH, these compounds have a much higher effect than polyallylamine alone and adenine alone.

Catalytic Efficiencies

As shown in Table 1 and Fig. 8, at pH 8.5 and at 10^{-3} M concentration of catalyst, the polymers prepared appeared 100 times more active than free adenine. As can be seen in Fig. 9, the polymers containing adenine are more efficient than poly(allylamine) alone.

Catalysis as a Function of Substrate Concentration

The kinetic behavior of the polymers toward *p*-nitrophenylacetate exhibits a plateau at a saturation stage. At high concentrations of substrate relative to that of enzyme, the observed velocity of the reaction becomes independent of the substrate concentrations. A conventional representation of Michaelis–Menten kinetics plots $1/V$ against $1/S$ is represented in Fig. 10. This allows the evaluation of the characteristic kinetic parameters listed in Table 2. All our values are comparable to those obtained by Seo et al. (1987, 1991), with respect to the cat-

alytic effect in the hydrolysis of *p*-nitrophenyl esters by cyclodextrin covalently bound to a poly(allylamine) chain, a classical artifical enzyme used as a model compound for enzymatic action.

In summary, we have prepared derivatives of polymers containing an adenine ring that markedly enhances the rate of cleavage of a nitrophenyl ester, and which are true catalysts as one molecule of catalyst hydrolyzes one molecule of substrate in 45 s.

Clearly, the linkage of the adenine ring to a polyamine increases the catalytic efficiency (Figs. 8 and 9). This effect is notably amplified at pH values greater than 8. At pH 8.5, PALAD *4* is more efficient than adenine alone, poly(allylamine) alone, or their mixture (Table 1). These results could reveal the existence of a cooperative effect between the aliphatic primary amino group and the adenine ring. The origin of this effect could be found in the increase in the number of unprotonated primary amino groups with the pH. These groups could act as bases to deprotonate the adenine rings at the N9 position (pKa $\simeq$ 9.8 for free adenine) (Saenger 1984). The adenylate ions formed should participate in the catalysis as nucleophiles and/or in the proton transfer.

Because about half of the biological enzymes require coenzymes and most of them possess a nucleotidic part or a heterocyclic nitrogen ring derived from a nucleic base, such structures may have played a prominent role in primitive catalysis (Maurel 1992). One may envisage the primeval existence of small analogs of oligonucleotides possessing the equivalent of the catalytic site of an enzyme.

In the future, we plan to make exploratory studies with other kinds of substrates to widen the range of substrates sensitive to this type of catalyst. Another route of investigation will be to synthesize another nucleic acid-like structure closer to standard nucleic acids, but still with a simpler backbone, and possessing a binding site near the catalytic group.

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