N6-SUBSTITUTED ADENINE DERIVATIVES AND RNA PRIMITIVE CATALYSTS

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Abstract. In our search for primitive RNA catalysts, we noticed that N6-ribosyl-adenine, a compound easily synthesized under presumed prebiotic conditions, has a free imidazole group. We showed that it is, as a catalyst, a potential analogue of histidine. Furthermore, among the chemical groups involved in protein catalysis, the imidazole ring of histidine has no equivalent in the RNA world. We have synthesized aliphatic amino groups containing polymers with adenine rings linked to macromolecules by their 6-amino group. These polymers exhibit pronounced catalytic activities in the hydrolysis of p-nitrophenylacetate. We discuss here the fact that in primitive catalysis the imidazole group could have been replaced by N6-substituted adenine derivatives.

1. Introduction

One of the most important questions about the origin of life concerns the structure of primitive catalysts.

Small peptides or proteins, or minerals such as clays have been proposed as the earliest catalysts (Paecht-Horowitz and Katchalsky, 1973; Barbier and Brack, 1988). Some new hypotheses have been formulated upon the discovery of ribozymes (Cech, Zaug and Grabowsky, 1981; Guerrier-Takada *et al.*, 1983). It is possible that small nucleic acids have replicative and catalytic functions (Orgel, 1968; Joyce and Orgel, 1986). However, primitive nucleic acids probably differed from RNAs (Joyce *et al.*, 1987). Some chemical groups which are involved in classical protein catalysis can be found in natural or modified RNA (Orgel, 1986; Benner *et al.*, 1987). The nucleotidic structure of many coenzymes with basic metabolic functions is also suggestive of their possible role in prebiotic catalysis (White, 1976; Trémoliéres, 1980). Some of these structures currently involved in catalysis could have been constitutive elements of primitive nucleic acids.

2. A Modified Adenosine as Catalyst

When condensation of adenine and ribose is carried out under presumed prebiotic conditions, the ribose reacts preferentially with the 6-amino group of adenine to

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form N6-ribosyl-adenine (Fuller *et al.*, 1972). In this nucleoside, the imidazole ring of the purine could act like the proton-transfer of histidine, and we have experimentally shown N6-ribosyl-adenine to be equivalent to histidine in the model reaction of *p*-nitrophenyl acetate (PNPA) hydrolysis (Maurel and Ninio, 1987). Under the same conditions (buffered water, pH 7.7) the catalytic efficiencies (defined as the optical density increase per minute) of N6-ribosyl-adenine and histidine are 0.6 and 1 respectively.

Extensive work has been devoted to N-3 substituted purine derivatives, considered as plausible constituents of a prebiotic monomer. Hill *et al.*, (1988) have demonstrated the suitability of N3-ribosyl-adenine 5′ phosphate as a monomer for non-enzymatic poly(U)-directed oligomerization. Wächtershäuser (1988) has proposed a model of an ancestral all-purine nucleic acid containing 3-bonded purine analogs xanthine and isoguanine. These compounds which possess a proton-donating imidazole ring could have catalytic properties similar to N6-ribosyl-adenine. N6-substituted adenine derivatives could undergo self-hydrogen bonding or hydrogen with uracil rings for example. This base-pairing which could involve N3 and N9-H of adenine is necessary for non-enzymatic replication of nucleic acids incorporating such modified nucleosides. Due to their potential replicative and catalytic functions, N-3 and N-6 substituted purine derivatives could have been essential links between the protein and the nucleic acids worlds.

With this in mind, the products of the reaction between ribose and the 6-amino group of adenine have been further characterized (Maurel and Convert, 1990). Four isomers differing in the sugar (furanose or pyranose) and in the α or β configuration were observed by ^{1}H NMR spectroscopy. Same amounts of each anomer were obtained, and in buffered water, at pH 7.7 and 25 °C, the pyranose form was the predominant one.

Thus we pursued this 'N6-N3 line' of investigation in studying nucleic acid-like structures which might have had replicative and catalytic functions.

3. Catalytic Groups Containing Polymers

There have been a number of attempts to polymerize ribonucleotides from unblocked monomers, in particular on montmorillonite (Ferris and Ertem, 1992). In our laboratory, the 'pseudo-histidine' (namely the four isomers of N6-ribosyl adenine) was polymerized using tri(imidazol-1-yl) phosphine as the condensing agent. A complex mixture of products was obtained and it was not possible to purify and characterize oligomers in order to measure their catalytic activity.

Thus we looked for polymers which were more easily prepared. Adenine has been attached to a polymeric chain by reaction of 6-chloropurine 1 with poly-(allylamine) 2. The polymer obtained PALAD 3 was characterized by ^{1}H NMR and UV spectrometry. The composition of this polymer, one adenine ring for about four aliphatic primary amino groups, was determined by UV measurements from the molar extinction coefficient of 6-methylaminopurine (Maurel and Décout, 1992).

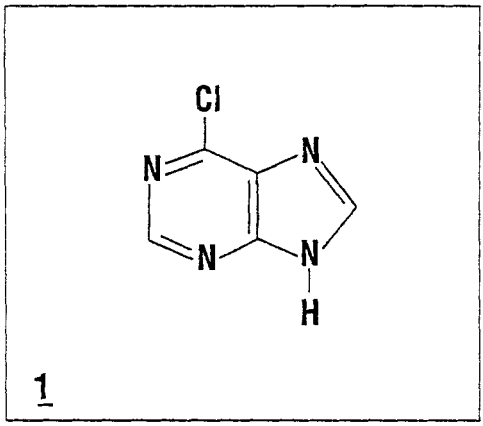


Fig. 1a: Structure of 6-chloropurine.

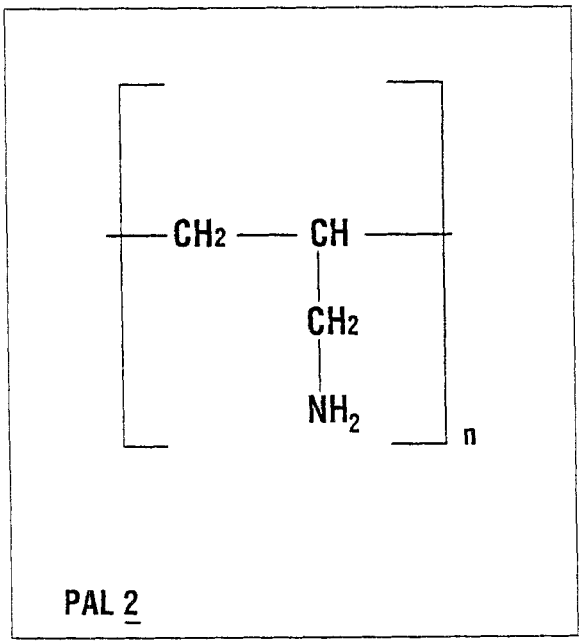
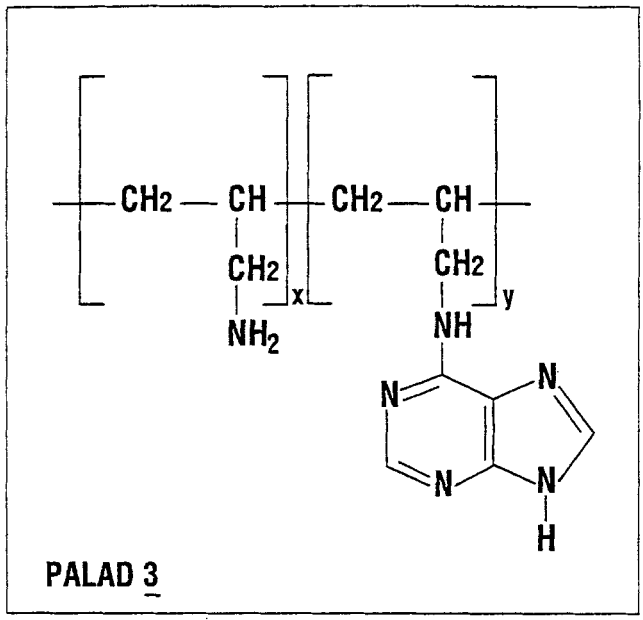
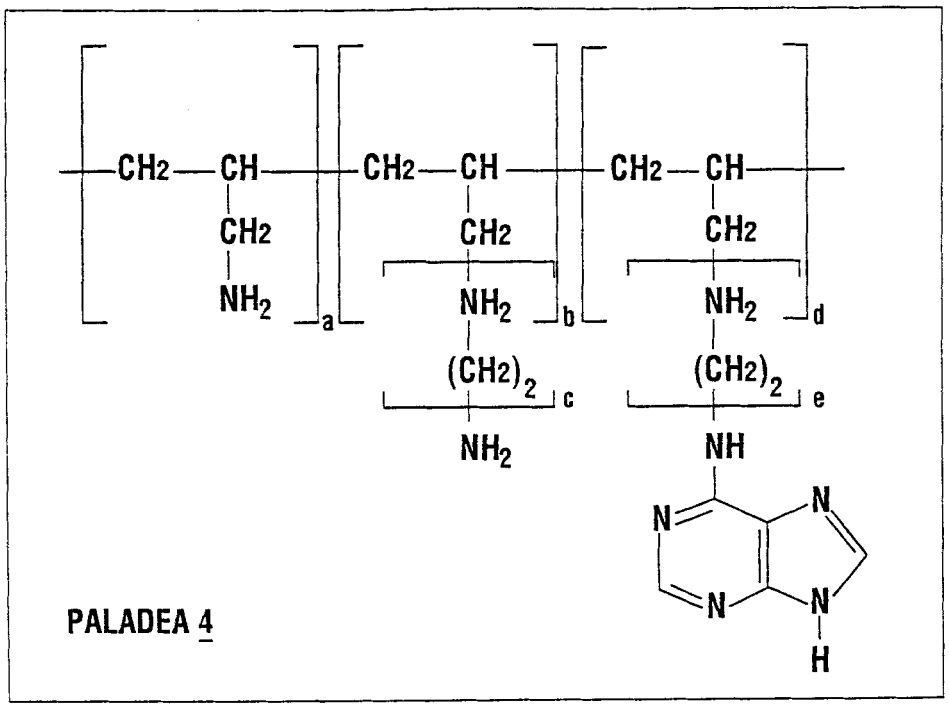


Fig. 1b: Structure of poly(allylamine).



PALAD 3

(c)



PALADEA 4

(d)

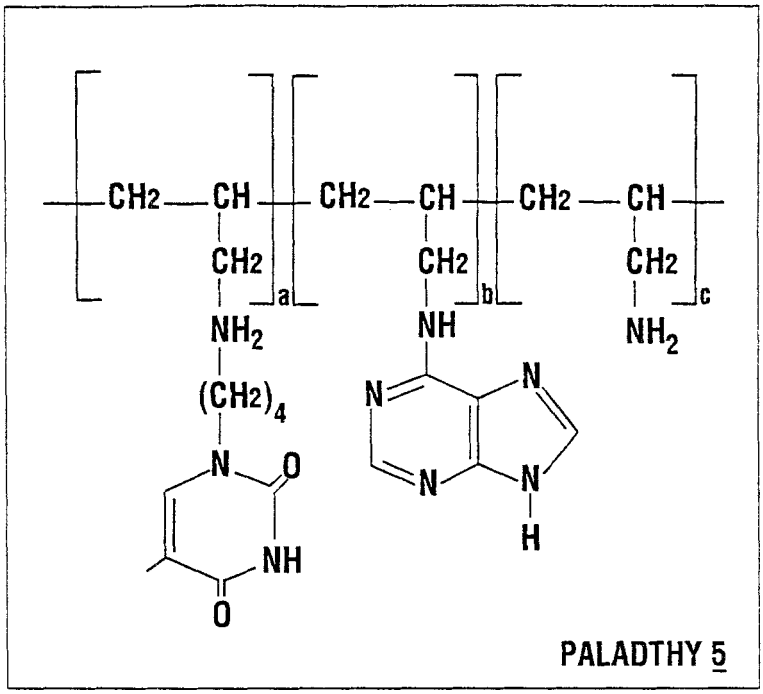


Fig. 1c, d, e. Structures of polymers studied.

Two other polymers were prepared, PALADEA 4 with side chains containing primary amino groups and PALADTHY 5 containing adenine thymine residues (Figure 1a–e).

We have studied the hydrolysis of p-nitrophenylacetate (PNPA) in the presence of catalytic amounts of these polymers at different pH. Catalytic activities were observed at pH higher than 6.5. These activities significantly increased when pH increased. For all pH values higher than 7.5, the catalytic efficiencies were higher than those of poly(allylamine) 2 alone, adenine alone or their mixture (Figure 2).

In conclusion, the synthesized polymers exhibit a catalytic activity remarkably increased in mild basic conditions: at pH 8.5 about 100 times the activity of the free adenine. This behaviour could be explained by a cooperative effect between the unprotonated primary amino groups and the adenine rings in the polymer (Figure 3). As a matter of fact, a primary amino group could deprotonate the adenine ring (adenine: pKa N_9 = 9.8) and could induce a proton transfer from a water molecule which leads to the hydrolysis. The formed adenylate ion could also react directly with PNPA.

4. Discussion

N6-ribosyl-adenine which is formed under presumably prebiotic conditions, exhibits

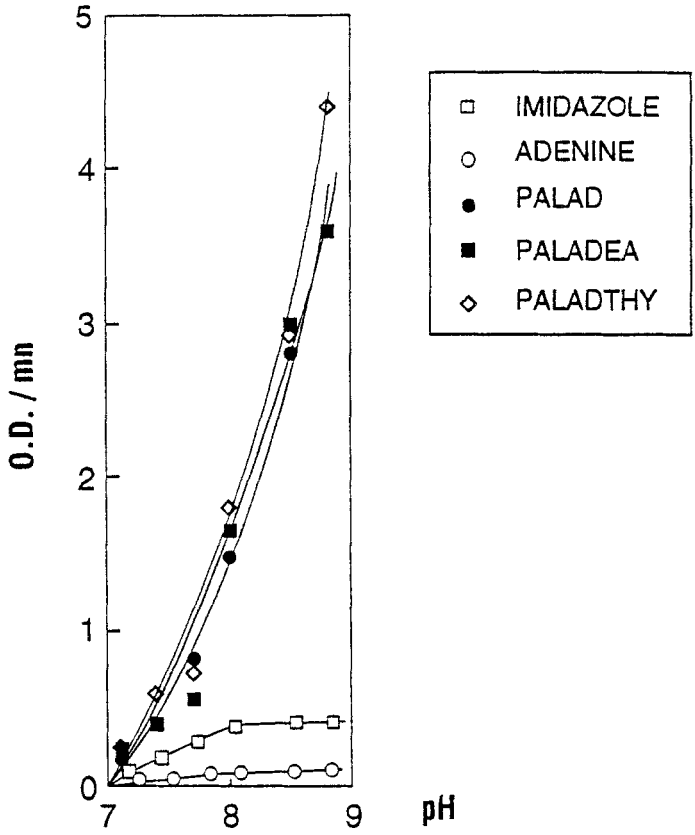


Fig. 2. Activity as a function of pH.
Tris buffer 0.02 M, (S) = 10^{-4}M, (Catalyst) = 5.10^{-5} M, 25 °C. Activity is defined as the optical density increase per unit of time, normalized to a molar concentration of substrate and catalyst.

a catalytic activity in the PNPA hydrolysis with the same efficiency as histidine. Moreover, the catalytic activity of the so-called 'pseudohistidine' puts us on the trail of the origin of the contemporary aminoacids. Histidine is unique among modern aminoacids because its biosynthesis is initiated by a purine base. It is also one of the few amino acids which cannot be easily produced under prebiotic conditions (but it is possible to form imidazole derivatives for example in the prebiotic chemistry of hydrogen cyanide). This suggests a primeval metabolic connection between histidine and purine. Catalytic groups, in nucleic acid components, could have been incorporated in the course of evolution into aminoacids and became specialized for a type of catalysis. This hypothesis infers that structural analogs of coenzymes containing adenine (namely, coenzyme B12, flavin adenine dinucleotide, conenzyme A, dihydronicotinamide-adenine dinucleotide etc...) could be functional precursors, a kind of fossil of nucleic enzymes (Eakin, 1963; White, 1976). Among the chemical groups involved in protein catalysis, the imidazole ring of histidine has no equivalent in the RNA world.

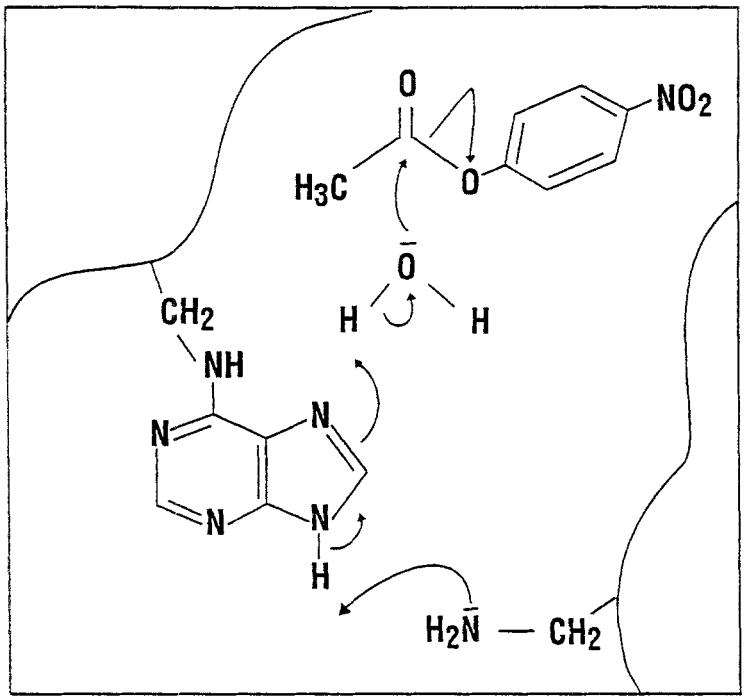


Fig. 3. Model for the mechanism of hydrolysis of PNPA by the polymer PALAD 3.

We suggest here that this group could have been replaced by N6-substituted adenine derivatives in primitive catalysis.

Aliphatic amino groups containing polymers and adenine rings linked by their 6-amino group are also active as catalysts in PNPA hydrolysis, particularly under mild basic conditions.

The search for new substrates sensitive to this type of catalysis is under investigation. Preliminary results have revealed a good catalytic activity for polymers in the hydrolysis of a substrate analog of phosphodiesterase. On the other hand, the polymers PALAD and PALADEA accelerate the hydrolysis of oligoribonucleotides (Barbier and Brack, unpublished results). N6-adenine derivatives clearly have catalytic properties.

Such an adenine ring could have been incorporated into the primitive nucleic acids from N6-ribosyl-adenine and/or into peptides or proteins from N6-amino-acid containing adenine. Accordingly, the prepared polymers, in which the nucleobases are linked to an aliphatic amino group backbone can be related at once to the 'protein world' and/or to the 'nucleic acid world'.

It has been shown that such compound indeed exist in many cells. One can note that N6-amino-acid derivatives of adenine have been isolated from plant transfer RNA (Dyson *et al.*, 1970). Oligonucleotide analogs with a peptide-like backbone and the natural base exhibit very stable complementary structures (Egholm *et al.*, 1992). Recently two new compounds have been identified in unfractioned tRNAs

from two thermophilic bacteria and from six hyperthermophilic archaea. Both compounds contain the amino acid 3-hydroxynorvaline covalently bound to the 6-amino group of adenosine by a urea bridge (Reddy *et al.*, 1992). This discovery may be related to the recent proposal that early life developed in a thermophilic environment (Achenbach-Richter *et al.*, 1987).

The next line of investigation, now under way, is to study the catalytic activity of polymers with a phosphodiester backbone containing adenine rings.

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