SEARCH FOR CATALYTIC PROPERTIES OF SIMPLE POLYPEPTIDES

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Abstract. Simple polypeptides were used as possible supports for nucleotide polymerization, in the absence of any preformed polynucleotide template. Sequential copolymers of alanine and glycine, water soluble polypeptides based on arginine and poly(Glu-SerGlu) have been tested. No catalytic effect has been found although poly(Glu-Ser-Glu) favors the 2′–5′ internucleotide linkage. More interestingly, polypeptides containing arginine residues strongly accelerate the hydrolysis of oligoadenylic acids. The influence of pH, temperature, nature of the buffer and polypeptide sequence was investigated.

1. Introduction

It has been previously shown that homochiral peptides can emerge in water from the products of an organic chemistry working at random (Brack, 1987). On the other hand, experiments by Orgel and his associates on template-directed polymerization of activated nucleotides (Bridson *et al.*, 1981, Inoue and Orgel, 1983 and Joyce and Orgel, 1986) have demonstrated the feasibility of a non-enzymatic polynucleotide replication. Moreover, the Qβ replicase experiments of Eigen *et al.* (1981) showed that enzymes are able to polymerize nucleotides without any preformed matrix. However, selective prebiotic synthesis of oligonucleotides without preformed templates and enzymes is still an unsolved challenge. It is therefore tempting to examine if simple polypeptides can act as helpers for nucleotide condensation. Two types of catalytic activity were investigated i.e. a direct effect on adenosine 5′-phospho-2-methylimidazole (2-MeImpA) polymerization and an effect on oligonucleotide hydrolysis.

Three polypeptide families were used to enhance nucleotide polymerization or oligonucleotide degradation.

The first family of polypeptides consisted of polymers made of glycine and alanine which are the most abundant natural amino acids formed in Miller's experiments (Miller and Urey, 1953) and found in meteorites (Cronin and Pizzarello, 1983). Insoluble sequential copolymers of alanine and glycine (Brack and Spach, 1972) with the general formula poly(Ala_x-Gly_y) were used with $x = 0$, 1 or 2; $y = 0$, 1, 2 or 3.

The second family included water soluble polycationic copolypeptides able to adopt different conformations. Strict alternation of hydrophilic and hydrophobic

* *Abbreviations*: All amino acids listed are of the L-configuration; poly(A), polyadenylic acid; 1–25 oligo(A)s, oligoadenylic acids up to the 25-mer; 2-MeImpA, adenosine 5′-phospho-2-methylimidazole.

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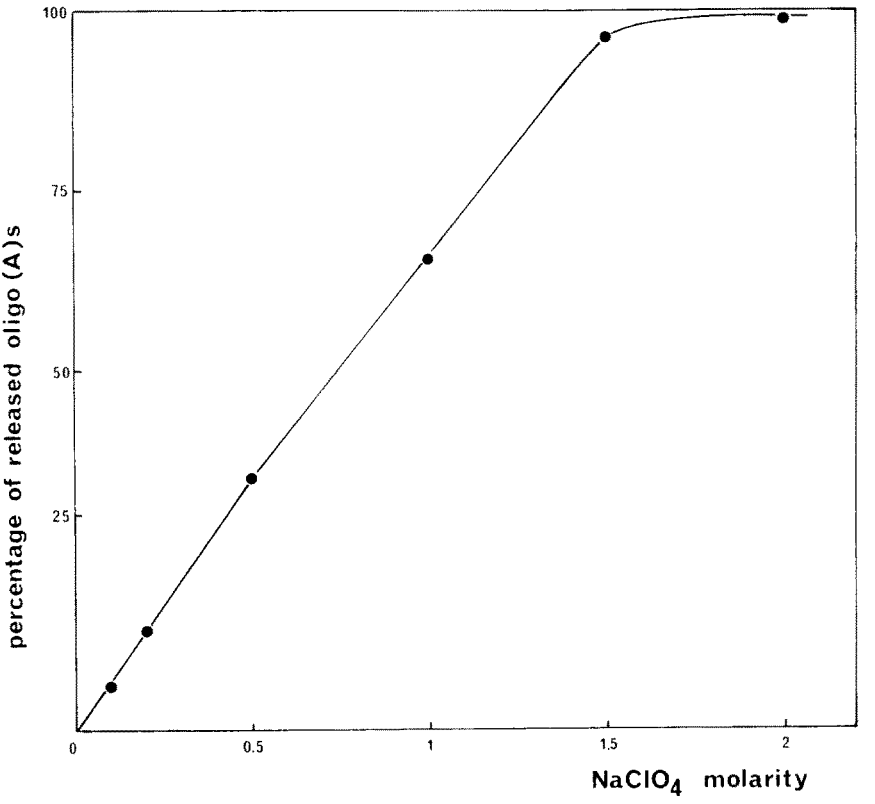

Fig. 1. Percentage of oligo(A)s released from poly(Leu-Arg-Arg-Leu)/1–25 oligo(A)s complexes (Arg/PO_4^{2-} of 2.5) as a function of $NaClO_4$ molarity. Oligo(A)s concentration was determined by UV recording of the supernatent after centrifugation.

residues induces a β-structure formation in water in the presence of salt (Brack and Orgel, 1975; Brack and Caille, 1978; Barbier *et al.*, 1984). With poly(Val-Lys), a bilayer formation with a hydrophobic interior and a positively charged hydrophilic exterior has been described. Such positively charged surfaces may direct the condensation of nucleotide due to phosphate-amino acid side-chain interactions (Brack and Orgel, 1975). For this study, arginine was prefered to lysine in order to avoid any covalent bonding of the nucleotides onto the lysine side-chain as phosphoramidates.

Periodic poly(Leu-Arg-Arg-Leu) was also synthesized. Due to the lack of alternation, the copolypeptide adopts an α-helix conformation (Barbier and Brack, 1986).

The last polypeptide family included polypeptides which may form ternary complexes with oligonucleotides and divalent metal ions (Hélène and Maurizot, 1981). To test this hypothesis, the following polymers have been used: poly Glu, poly(Glu-Leu), poly(Arg-Glu-Glu) and poly(Glu-Ser-Glu) (Chaves and Trudelle, 1975).

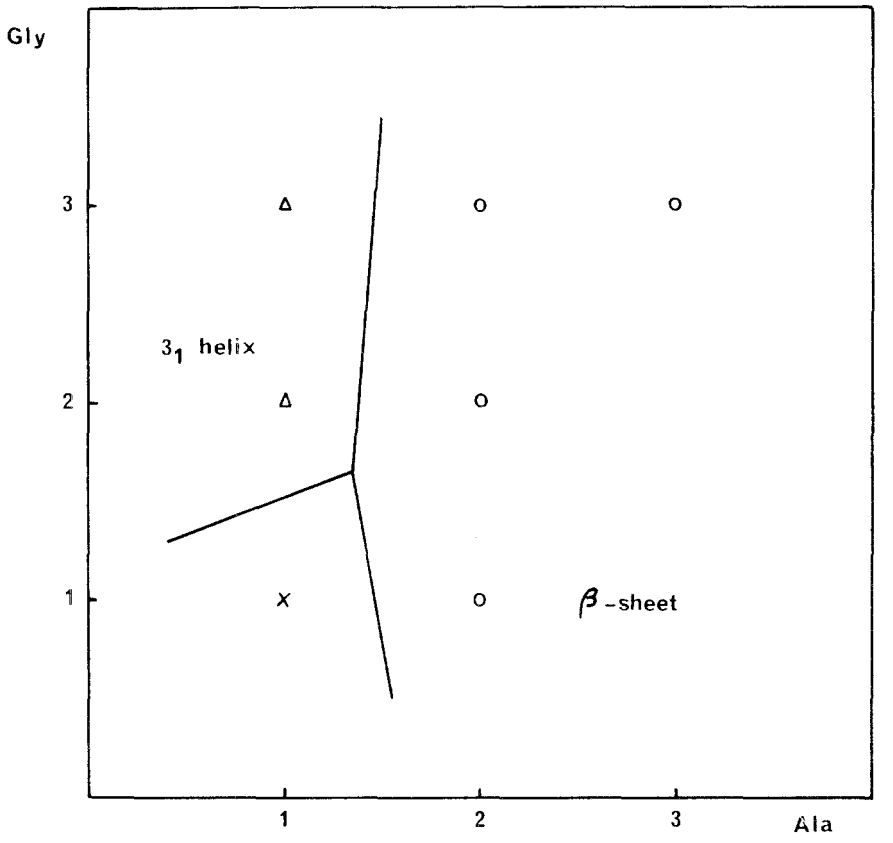

Fig. 2. Conformations obtained when periodic poly(Ala_x-Gly_y) copolypeptides are precipated with water. Poly(Ala-Gly) adopts a silk I conformation (Lotz and Keith, 1971).

2. Experimental

2.1. Materials

Poly(A) was obtained from Miles and glycyl-glycine from Ega Chemie. Nucleosides, nucleotides and polyarginine were purchased from Sigma. 2-MeImpA was prepared as described by Joyce *et al.* (1984). Poly(Arg-Leu) with a number average molecular weight of 4200 and poly(Leu-Arg-Arg-Leu) with a number average molecular weight of 6300 were obtained by condensation of the p-nitrophenyl esters of the corresponding peptides as already described by Barbier *et al.* (1984) and Barbier and Brack (1986). Poly(Glu-Ser-Glu) (Chaves and Trudelle, 1975) was a gift from Dr Y. Trudelle. Copolymers of glycine and alanine were prepared as described by Brack and Spach (1972). Oligo(A)s were analysed by high performance liquid chromatography using a Hitachi 655 A HPLC system and RPC5 column packing (Pearson *et al.*, 1971; Joyce *et al.*, 1984). The 2′–5′ linked isomers were identified by coinjection with commercial $(pA)_3^{2'-5'}$ (PL Biochemicals inc.) and by comparison with previously published HPLC elution profiles (Lohrmann *et al.*, 1980).

2.2. METHOD

Polymerizations of 2-MeImpA were run at 4 °C for 2 weeks according to a general procedure described by Fakhrai *et al.* (1981). 2-MeImpA (0.05 M), $Mg(NO_3)_2$ (0.25 M) and $Pb(NO_3)_2$ (0.01 M) were dissolved in a low ionic strength buffer (0.1 M 2-MeImidazole/HNO_3, pH 8.0) in stoppered silionized Eppendorf tubes. Amino acid residues and nucleotide phosphates were used in a 1:1 ratio and control reactions were carried out without peptides. Reactions were stopped by addition of EDTA and $NaClO_4$.

For the oligonucleotide degradation experiments, oligo(A)s up to the 25-mer, obtained by partial basic hydrolysis of commercial poly(A), were used as substrate (Lohrmann and Orgel, 1980). 1-25 oligo(A)s were treated at 22, 35 or 50 °C at various pHs between 7.5 and 12, using different buffers at low ionic strength, in the presence and the absence of peptides. At the end of the reaction, the mixtures were frozen at − 80 °C. Just before HPLC analysis, the samples were thawed and $NaClO_4$ was added to dissociate the oligonucleotide-oligopeptide complexes. From a preliminary study (Figure 1), we found that a 2M $NaClO_4$ concentration was necessary to obtain a complete dissociation.

The concentration of each oligomer was calculated from the area of the corresponding peak on the HPLC chromatogram with a correction for hyperchromicity (Michelson, 1963). In the hydrolysis experiments, the extent of hydrolysis was obtained by counting the overall phosphodiester bonds of the oligomers (1 bond for the dimer plus 2 bonds for the trimer, etc...).

3. Results and Discussion

3.1. NUCLEOTIDE POLYMERIZATION

Polypeptides based on glycine and alanine can adopt different conformations when precipitated with water (Figure 2) according to their composition and sequence. Addition of these polypeptides to the reaction mixture containing 2-MeImpA, $Mg(NO_3)_2$ and $Pb(NO_3)_2$ did not lead to any marked modification of the nucleotide polymerization. Similar studies with polyalanine and polyglycine (β-form and polyglycine II modification) showed no activity.

When polypeptides with arginine and leucine residues were added to a solution of 2-MeImpA in the buffer, a nucleotide-polypeptide complex precipitated. In these experiments, magnesium and lead were added after the formation of the complex. Although, there is a strong interaction between the polypeptides and the nucleotides (Figure 1), no significant effect on 2-MeImpA polymerization could be detected.

Polymers with Glu-X-Glu sequences were also tested. They may form ternary complexes with oligonucleotides and divalent metal ions. No catalytic effect has been detected, but they favor the 2′−5′ internucleotide linkage (Figure 3). This is particularly true for poly (Glu-Ser-Glu) under the random coil conformation. Although there is no real catalytic effect, it is interesting to underline that even simple

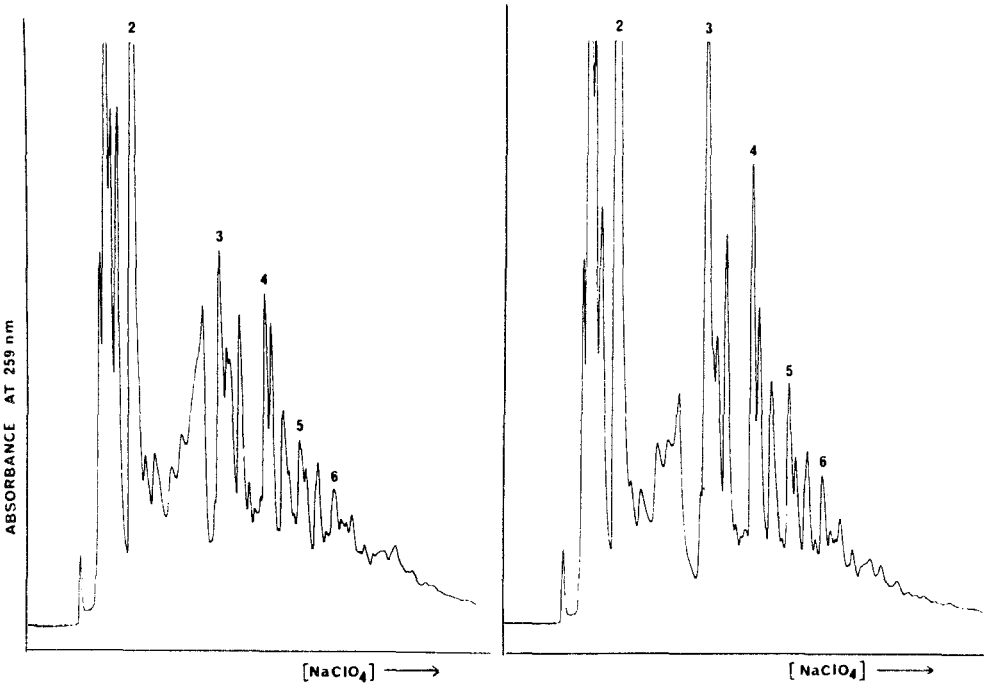

Fig. 3. HPLC elution profiles of the condensation products obtained when 2-MeImpA is polymerized in the absence (left) and in the presence (right) of poly(Glu-Ser-Glu). The number above selected peaks are the degree of polymerizations of the corresponding [2′–5′] isomers. Reaction conditions: 0 °C; 2 weeks; 0.1 M 2Me-Imidazole/HNO_3 buffer (pH 8.0), 0.25 M $Mg(NO_3)_2$, 0.01 M $Pb(NO_3)_2$, with 2-MeImpA: 0.05 M and poly(Glu-Ser-Glu): 0.05 M in amino acid. HPLC conditions: ion exchange chromatography with linear $NaClO_4$ gradient at pH 12 (0 to 0.02 M $NaClO_4$, 30′).

polypeptides with only one or two different amino acids can modify the course of nucleotide oligomerizations.

3.2. Oligonucleotide degradation

Charged water soluble polypeptides i.e. polyanions based on glutamic acid and polycations based on arginine were used to enhance oligonucleotide hydrolysis.

In a first set of experiments, the hydrolysis were run at pH 12 and room temperature using a 5:1 amino acid residue to phosphate ratio. Arginine containing polymers with a net positive charge significantly enhance oligo(A)s hydrolysis (Table I). It is interesting to note that no hydrolysis acceleration occurred with poly (Arg-Glu-Glu) despite the presence of arginyl residues. It is likely that negative charges of glutamic acid weaken the complex formation. Free arginine alone did not modify the reaction. To be active, arginine must be incorporated into a polypeptide chain.

Standard conditions were then established for arginine containing polypeptides with respect more precisely to the arginyl/phosphate ratio. Thus, 1-25 oligo(A)s were

TABLE I

Percentage of hydrolyzed phosphodiester bonds of 1–25 oligo(A)s at pH 12, 22 °C after 2 h in the precence of polypeptides

None	poly (Glu-Leu)	Poly (Glu-Ser-Glu)	Poly (Arg-Glu-Glu)	Free arginine	Poly (Arg-Leu[3], Leu-Leu[1])[a]	Poly (Leu-Arg-Arg-Leu)	Poly (Arg-Leu)
2.5	4.3	4.1	4.6	5.0	59.97	100	100

[a] Obtained by cocondensing p-nitrophenyl dipeptide esters Arg-Leu-ONp and Leu-Leu-ONp in the ratio 3:1. *Reaction conditions*: Oligo(A)s: 1.5×10^{-2} M; peptides: 7.5×10^{-2} M in amino acid residues.

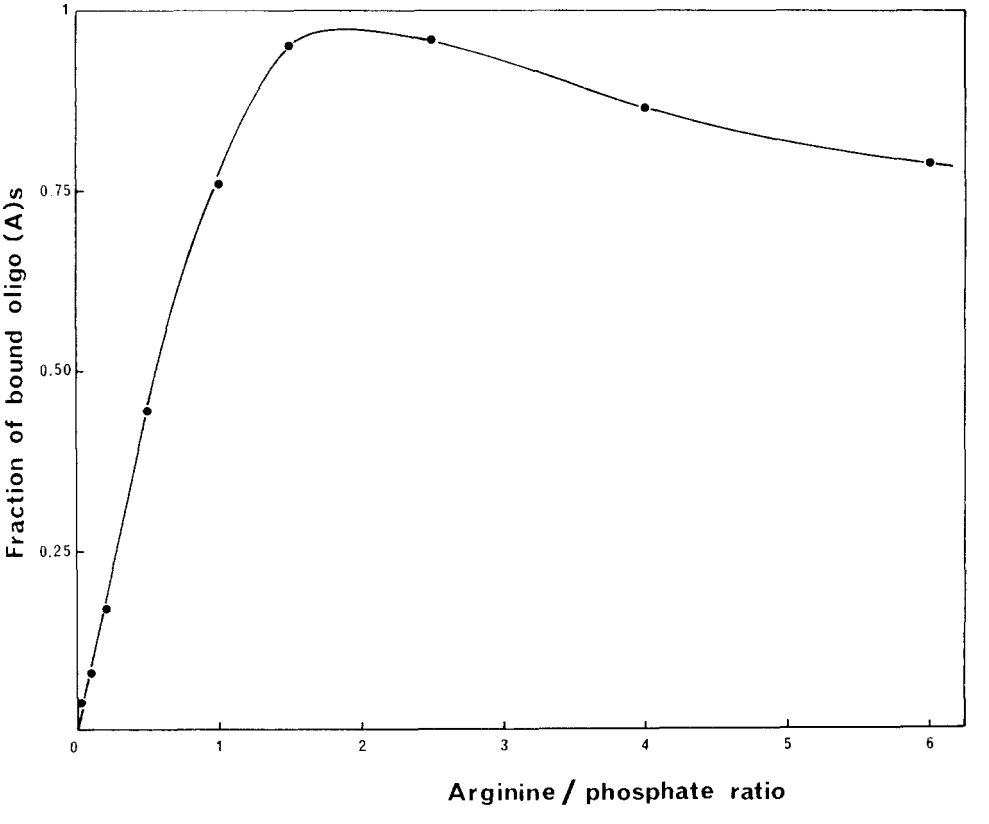

Fig. 4. Fraction of oligo(A)s bound to poly(Leu-Arg-Arg-Leu) as a function of arginine to phosphate ratio.

TABLE II

Percentages of hydrolyzed phosphodiester bonds of 1–25 oligo(A)s at pH 8.5, 35 ° C after 8 days in the presence of polypeptides

Buffers	None	Poly(Arg)	Poly (Leu-Arg-Arg-Leu)	Poly (Arg-Leu)
Tris/HCl 0.1 M	0.19	25.5	43.8	41.3
Glycine/NaOH 0.1 M	0.65	19.8	35.7	37.5

Reaction conditions: Oligo(A)s: 1.85×10^{-2} M; polypeptides: 4.625×10^{-2} M in arginine residues.

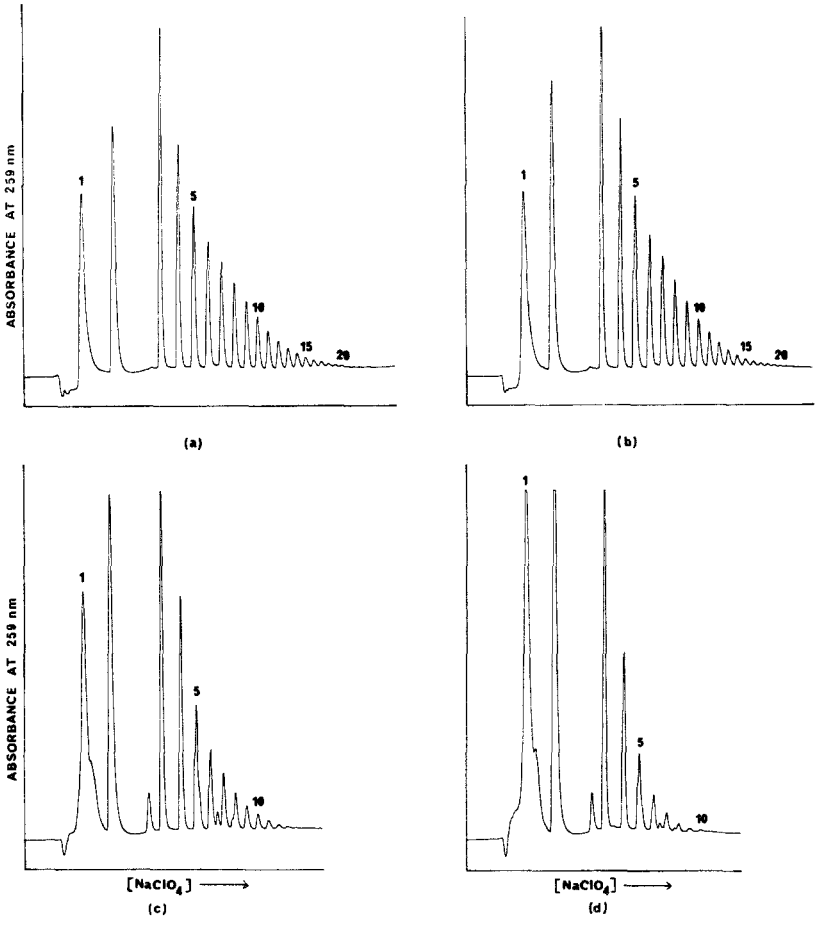

Fig. 5. HPLC elution profiles of selected 1–25 oligo(A)s used as substrates in the hydrolysis reaction (a). Hydrolysis products obtained in the absence of added polypeptides (b), in the presence of poly(Arg) (c), and in the presence of poly(Arg-Leu) (d). Reaction conditions: 35 °; 8 days; 0.1 M Tris-HCl/buffer pH 8.5; 1.85×10^{-2} M 1–25 oligo(A)s, peptide concentration calculated for a 4.625×10^{-2} M arginine concentration. The numbers above selected peaks are the length of corresponding oligo(A)s. HPLC conditions: ion exchange chromatography with linear gradient at pH 12 (0 to 0.04 M $NaClO_4$, 45′).

TABLE III

Percentages of hydrolyzed phosphodiester bonds of 1–25 oligo(A)s at pH 7.5 and 8.0, 50 °C, after 14 days in the presence of poly(Leu-Arg-Arg-Leu)

Buffer	pH 7.5		pH 8.0	
	None	Poly(Leu-Arg-Arg-Leu)	None	Poly(Leu-Arg-Arg-Leu)
Phosphate 0.05 M	4.7	21.2	5.65	35.9
Glycylglycine/NaOH 0.05 M	0.95	36.5	4.2	61.2
Tris/HCl 0.05 M	4.5	28.9	10.5	53.4

Same reaction conditions as in Table II.

complexed to different polymers such as poly (Arg-Leu), poly (Leu-Arg-Arg-Leu) or poly (Arg-Leu[3], Leu-Leu[1]) using different arginine/nucleotide ratios. Aqueous solutions of oligo(A)s and of polypeptides were mixed, stirred vigorously and left for 30 min. After centrifugation, the supernatant was collected and mixed with an equal volume of 4 M $NaClO_4$ to precipitate some uncomplexes short polypeptide chains. After a second centrifugation, the supernatant was read at 259 nm.

The results shown on Figure 4 indicate that a 2.5 Arg/PO_4^{2-} ratio is adequate. For higher peptide concentrations, the fraction of oligo(A)s complexes to poly (Leu-Arg-Arg-Leu) decreases. This may be due to a competitive polypeptide-polypeptide aggregation. To evaluate the influence of the pH, experiments were run at pH 8.5, 8.0 and 7.5 in appropriate buffers. We selected NaH_2PO_4/Na_2HPO_4, glycine/NaOH and glycylglycine/NaOH buffers for their prebiotic plausibility. For comparison, we have also taken Tris/HCl buffer which is usally used in enzymatic reactions. Two temperatures (35 °C and 50 °C) were chosen for the hydrolysis.

The results are summarized in Tables II and III. Hydrolysis in Gly-Gly buffer are shown on Figure 5. The results of Table II and III confirm that arginine containing polymers catalyze oligo(A)s hydrolysis. Moreover, the catalytic effect is not only due to arginine residues incorporated into a polypeptide chain since polyarginine and poly(Arg-Leu) behave differently under the same conditions. This is to be compared to the inefficiency of poly(Arg-Glu-Glu) (Table I). These results underline the importance of arginine residues associated to hydrophobic amino acids in a polypeptide chain.

As far as the buffer is concerned, the largest difference between the reaction run in the presence of peptides and the control was obtained with the Gly-Gly buffer. Phosphate buffer is less efficient, most probably due to a competitive effect of buffer phosphates (0.05 M) with oligo(A)s phosphates (0.018 M) in polypeptide complexation.

The conformational tendency of the polypeptides containing arginine and leucine does not play a crucial role in the hydrolytic activity since β-sheet forming poly(Arg-Leu) and α-helix forming poly(Leu-Arg-Arg-Leu) behave similarly. It must however be noted that no conformational study of the polypeptide engaged in the complexes has been undertaken, so far.

4. Conclusion

Although the tested peptides have no real catalytic effect on 2-MeImpA polymerization, it can however be noted that simple polypeptides such as $(Glu\text{-}X\text{-}Glu)_n$ modify the course of nucleotide polymerization. More interestingly, copolypeptides containing arginine markedly enhance the hydrolysis of oligoadenylates at pHs between 7.5 and 12 and at temperature below 40 °C. At 35 °C the hydrolysis is about 40 times faster in the presence of polypeptides. From a prebiotic point of view, it is important to note that the tested polymers are the first simple polypeptides exhibiting a marked hydrolytic activity on oligonucleotides *via* the formation of an electrostatic complex. In such complexes, it will be helpful to look for a possible stereochemical fit between guanido groups and phosphate residues. Prior to the complex formation, the polypeptides are in the random coil conformation and oligo(A)s occur as single stranded helical structures (Szenger *et al.*, 1975). During the complex formation, it will be interesting to follow the conformation of the polypeptide in order to see if a transition into an ordered conformation (β-sheet or α-helix) occurs.

Finally, looking at reverse activity of enzymes such as ribonucleases (Barker *et al.*, 1957; Bernfield, 1965; Bernfield, 1966) we will focus our efforts on a possible reversal of the hydrolytic activity to end up with a polymerase activity.

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