Nasseri and Germain have come to the same conclusion.[28] The mechanism by which a bound peptide exerts control over the conformational state of a class II molecule is unknown. There are several instances of ligand binding either altering or stabilizing the three-dimensional structure of a protein.[29,30]

Acknowledgment. This work was supported by a Damon Runyon-Walter Winchell Cancer Fund Fellowship, DRG-968 (S. N.W.), and by a National Institutes of Health Grant 5R01 AI 13587-13.

Registry No. Cytochrome *c*, 9007-43-6.

(28) Sadegh-Nasseri, S.; Germain, R. N. *Nature* **1991**, *353*, 167-170.
(29) Duportail, G.; Lefevre, J.-F.; Lestienne, P.; Dimicoli, J.-L.; Bieth, J. G. *Biochemistry* **1980**, *19*, 1377-1382.
(30) Khananshvili, D.; Gromet-Elhanan, Z. *Biochemistry* **1986**, *25*, 6139-6144.

Conformation-Controlled Hydrolysis of Polyribonucleotides by Sequential Basic Polypeptides

Bernard Barbier and André Brack*

Contribution from the Centre de Biophysique Moléculaire—CNRS, 1A Avenue de la Recherche Scientifique, 45071 Orléans Cedex 2, France. *Received March 5, 1991*

Abstract: Polycationic polypeptides containing basic and hydrophobic amino acids strongly accelerate the hydrolysis of oligoribonucleotides. We have examined aspects of the oligonucleotide–polypeptide interaction, as well as the relationship among amino acid composition, polypeptide conformation, and the hydrolytic effect. We conclude that, to be active, the polypeptides must present a regular distribution in space of basic groups (β-sheet or α-helix). A tentative model is given involving an alignment of the polynucleotidic chain between two parallel rows of positive charges. The experimental data for the base-induced hydrolysis are consistent with a mechanism involving two basic amino acid side chains.

Introduction

A number of studies have shown that synthetic peptides or polypeptides can exhibit enzyme-like activity. Peptides and copolypeptides with a weak esterasic activity[1-4] and glycosidic activity[5] have been reported. A strong ribonuclease activity was found for a 70-residue synthetic polypeptide analogue of ribonuclease S-protein[6] and with a 34-residue polypeptide and its dimer.[7] More recently,[8] a noticeable activity has been found with a 99-residue polypeptide of an aspartic protease.

Even very simple peptides can show such activities. For example, Lys-Trp-Lys is able to recognize and to cleave DNA strands at apuric or apurinic sites.[9-11] Other simple copolypeptides are known to exhibit weak esterasic activities[12,13] or glycosidic activity.[14]

In two previous communications[15,16] we have briefly reported that basic polypeptides interact with polyribonucleotides and accelerate their hydrolysis. In these first studies, the hydrolyses were run with mixtures of oligoribonucleotides which did not allow an accurate determination of either the rate or the stoichiometry of the reaction. Therefore, the experiments were repeated with well-defined oligoribonucleotides as substrates. In the present paper we describe a number of factors involved in the reaction and propose a model for the mechanism of hydrolysis.

Materials and Methods

$(Ap)_9A$ and poly(A) were purchased from Pharmacia. Oligo(A)s up to the 25-mer were obtained by alkaline hydrolysis of poly(A) in KOH (0.2 M) at room temperature for 10 h. The ring-opening of 2′-3′ cyclic phosphate end groups was performed in aqueous $HClO_4$ at pH 2 for 2 h. Several nucleotides and oligonucleotides were used to calibrate the HPLC chromatograms: A, A2′p, A3′p, and A > p (Sigma), ApA and $(Ap)_2$ (Waldhof), and $(Ap)_2A$, $(Ap)_3A$, and $(Ap)_5A$ (Pharmacia). $(Ap)_3$ was isolated from a mixture of oligo(A)s obtained by alkaline hydrolysis of poly(A) in KOH (0.2 M) at room temperature for 10 h. The $(Ap)_3$ fraction, isolated by ion exchange chromatography on a "strong retention" preparative PVDI 33 column (Société Française Chromato Colonne) was desalted on a G10 Sephadex column (Pharmacia) and finally lyophilized. Poly(L-lysine) and poly(L-histidine) were obtained from Bachem, and poly(L-arginine) was obtained from Sigma. The sequential polypeptides were synthesized in our laboratory: poly(Leu-Lys) and poly(Glu-Leu),[17] poly(Glu-Ser-Glu),[18] poly(Arg-Leu),[19] poly-(D,L-Leu-D,L-Lys),[20] and poly(Leu-Lys-Lys-Leu).[21] The dipeptide CH_3CO-Leu-Lys-NHC_2H_5 (Ac-Leu-Lys-NHEt) was prepared by liq-

(1) Sheehan, J. C.; Benett, G. B.; Schneider, J. A. *J. Am. Chem. Soc.* **1966**, *88*, 3455.
(2) Petz, D.; Schneider, F. *Z. Naturforsch.* **1976**, *31c*, 675.
(3) Trudelle, Y. *Int. J. Pept. Protein Res.* **1982**, *19*, 528.
(4) Nishi, N.; Morishige, M.; Tsutsumi, A.; Nakajima, B. *Int. J. Biol. Macromol.* **1983**, *5*, 42.
(5) Chakravarty, P. K.; Mathur, K. B.; Dhar, M. M. *Experimentia* **1973**, *29*, 786.
(6) Gutte, B. *J. Biol. Chem.* **1975**, *250*, 889.
(7) Gutte, B.; Daumigen, M.; Wittschieber, E. *Nature* **1979**, *281*, 650.
(8) Veber, D. F.; Nutt, R. F.; Brady, S. F.; Nutt, E. M.; Ciccarone, T. M.; Garsky, V. M.; Waxman, L.; Benett, C. D.; Rodkey, J. A.; Sigal, I.; Darke, P. In *Peptide 1988*; Jung, G., Bayer, E., Eds.; Walter de Gruyter: Berlin–New York, 1989; p 190.
(9) Behmoaras, T.; Toulme, J. J.; Helene, C. *Nature* **1981**, *292*, 858.
(10) Pierre, J.; Laval, J. *J. Biol. Chem.* **1981**, *256*, 10217.
(11) Ducker, N. J.; Hart, D. M. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 1433.
(12) Hatano, M.; Nozawa, T. *Prog. Polym. Sci., Jpn.* **1972**, *4*, 223.
(13) Noguchi, J.; Nishi, N.; Tokura, S.; Murakami, U. *J. Biochem. (Tokyo)* **1977**, *81*, 47.
(14) Mathur, K. B.; Chakravarty, P. K.; Srivastava, S.; Dhar, M. M. *Indian J. Biochem. Biophys.* **1971**, *8*, 90-93.
(15) Barbier, B.; Brack, A. *J. Am. Chem. Soc.* **1988**, *110*, 6880.
(16) Brack, A.; Barbier, B. *Origins Life* **1990**, *20*, 139.
(17) Brack, A.; Caille, A. *Int. J. Pept. Protein Res.* **1978**, *11*, 128.
(18) Chaves, J. G.; Trudelle, Y. *J. Polym. Sci.* **1975**, *52*, 125-135.
(19) Barbier, B.; Caille, A.; Brack, A. *Biopolymers* **1984**, 2299-2310.
(20) Brack, A.; Spach, G. *J. Mol. Evol.* **1979**, *13*, 35.
(21) Barbier, B.; Perello, M.; Brack, A. *Collect. Czech. Chem. Commun.* **1988**, *53*, 2825.

0002-7863/92/1514-3511$03.00/0

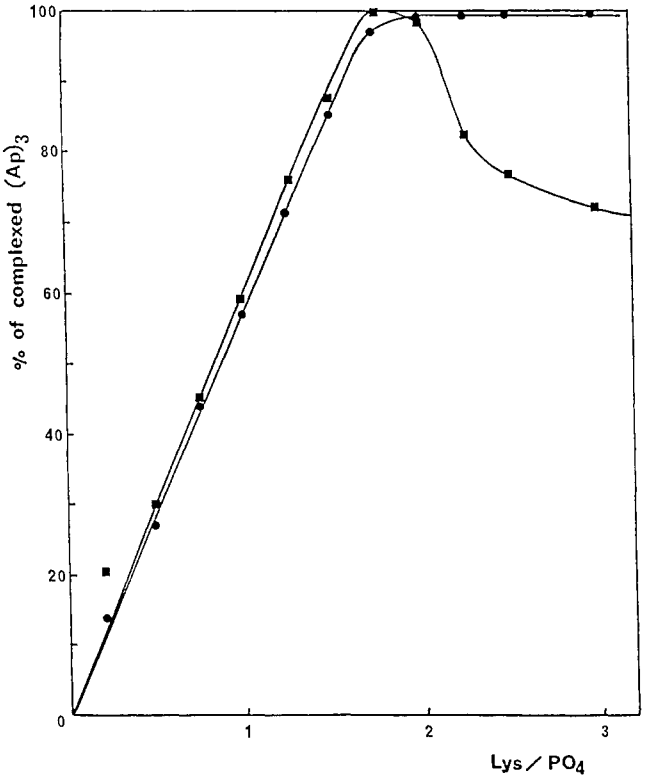

Figure 1. Percentage of $(Ap)_3$ bound to poly(Leu-Lys) (●) and to poly(Leu-Lys-Lys-Leu) (■) as a function of the lysine to phosphate ratio.

uid-phase synthesis.[22] The synthesis of poly(Ala-Lys), poly(Arg-Glu), poly(Pro-Leu-Lys-Leu-Lys), and poly(Pro-Lys-Lys-Leu) will be published elsewhere. Poly(Leu^{50}, Lys^{50})[23] has a random distribution of residues along the chain. Poly(Arg-Leu^{75}, Arg-Leu-Glu-Leu^{25}) (unpublished) was obtained by polycondensation of activated Arg-Leu and Arg-Leu-Glu-Leu in a 3:1 ratio.

Oligonucleotide–Polypeptide Interaction. For each polypeptide studied, $(Ap)_3$ was used to determine the number of basic amino acids required for the complexation of each phosphate group. Since $(Ap)_3$ has a lower affinity for polypeptides as compared to larger oligonucleotides, it will afford a lower limit for the oligoribonucleotide propensity to complex basic polypeptides. The formation of the complex was studied in 0.1 M glycylglycine/NaOH buffer at pH 7.5 and 37 °C, conditions used for the hydrolysis. Varying amounts of polypeptides were added to a 5×10^{-3} M solution of $(Ap)_3$, corresponding to Lys^+/PO_4^- ratios ranging from 0 to 5. Aqueous solutions of $(Ap)_3$ and of polypeptide were mixed and stirred vigorously. After 30 min, the complex which formed as a gel was discarded by centrifugation. $NaClO_4$ was added to the supernatant to a final concentration of 2 M to precipitate the remaining polypeptides, which were eliminated by a second centrifugation. Finally, the supernatant was diluted and the remaining amounts of uncomplexed $(Ap)_3$ remaining in solution were determined by UV absorbance measurement at 259 nm (Figure 1). For each UV measurement, the presence of a trough at 230 nm indicated that no soluble polypeptide-containing complexes remained in the supernatant.

The conformation of polypeptides complexed to oligonucleotides was studied in aqueous solution at 22–24 °C by circular dichroism (Jobin-Yvon Mark IV autodichrograph). The studies of the polypeptide conformation in the complex consume large amounts of oligoribonucleotides. Thus, mixtures of oligo(A)s cheaply obtained on a large scale from poly(A) have been used. An Arg^+/PO_4^- ratio of 10 has been taken in order to get a transparent solution.

Hydrolysis Experiments. Hydrolyses of $(Ap)_9A$ (4×10^{-4} M in phosphate) were performed in 0.1 M buffer at pH 7.5 and 37 °C, and were run in stoppered siliconized Eppendorf tubes. A freshly prepared solution of oligonucleotides in pure water, quantified by UV, was added to a solution of the polypeptide in pure water which had been stored overnight at 4 °C. Basic amino acids were in excess in comparison with phosphate groups (ratio of 2.5) to ensure a complete oligoribonucleotide complexation with basic polypeptides (Figure 1). A gel was formed upon mixing the two solutions. The gel was left for 30 min before addition of glycylglycine/NaOH buffer (0.1 M, pH 7.5). At the end of the reaction, the complexes were dissociated by addition of $NaClO_4$ to a 2 M final concentration. Under these conditions 98% of the polynucleotide component of the complex dissolves as shown by independent UV studies. The polypeptide component precipitates, and was discarded by centrifugation.

The products of the reaction were analyzed by high-performance liquid chromatography using a Hitachi 655 A HPLC system. The separation was achieved by ion exchange chromatography on a prepacked 4- × 100-mm "strong retention" PVDI column (Société Française Chromato Colonne) eluted for 10 min with a 10 mM Tris/$HClO_4$ buffer at pH 7.0 followed by a linear gradient of salt (from 0% to 80% 0.3 M $NaClO_4$ in 50 min). HPLC elution profiles were quantified with a D2000 Merck integrator. The concentration of each oligomer was calculated from the HPLC peak area with a correction for hypochromicity as a function of the pH.[24-26] Hydrolyses of $(Ap)_9A$ were followed by the disappearance of the starting oligoribonucleotides as a function of time.

Results

The hydrolytic activities of arginine- or lysine-containing copolypeptides toward oligonucleotides were compared to that of glutamic acid containing copolypeptides and to that of polyhistidine. Poly(Glu-Leu), poly(Glu-Ser-Glu), poly(Arg-Glu-Glu), and poly(L-histidine) were inactive. Poly(L-lysine), poly(L-arginine), poly(Leu-Lys), poly(Arg-Leu), copoly(Arg-Leu^{75}, Arg-Leu-Glu-Leu^{25}), and poly(Leu-Arg-Arg-Leu) displayed a significant activity. The lack of activity observed for poly(Arg-Glu-Glu) is probably due to the neutralization of the arginyl positive charges by glutamyl side chains. The formation of ionic complexes between arginine and glutamic acid has been demonstrated on several peptides.[27,28]

Poly(Leu-Lys) can be taken as an example of an active polypeptide. It cleaves $(Ap)_9A$ very efficiently at pH 7.5 and 37 °C, affording all the intermediary oligoribonucleotides (Figure 2). The polypeptide behaves as an endonuclease since no accumulation of mononucleotide is observed. The dipeptide Ac-Leu-Lys-NHEt has no activity, indicating that some repeat of the basic amino acids along the chain is required.

When the aqueous solution of poly(Leu-Lys) is mixed with the aqueous solution of oligonucleotides, a gel appears, suggesting the formation of a complex. The gel contains polypeptide chains as shown by IR measurements run on the gel recovered by centrifugation.[15] When the suspension of the isolated gel is brought to 2 M $NaClO_4$, 98% of the starting oligoribonucleotides is released in the solution. Thus, the gel can be considered as a complex between oligoribonucleotide and polypeptide chains. As described previously,[15] the hydrolytic activity is directly related to the complex formation between the basic polypeptides and the oligoribonucleotides. For example, a 2 M $NaClO_4$ concentration totally impedes the hydrolysis of the oligoribonucleotides by the polypeptides. On the other hand, the hydrolytic activity is not systematically associated with the formation of a gel. For instance, poly(Leu-Lys-Lys-Leu) has a full activity at a Lys^+/PO_4^- ratio of 10 without formation of a gel, whereas poly(Leu^{50}, Lys^{50}) provokes a dense gel formation but has a rather low activity (Figure 5). As hereafter shown, hydrolysis occurs whether or not a gel is formed if the distribution of basic groups is correct.

The study of the complexation of $(Ap)_3$ to poly(Leu-Lys) or to poly(Leu-Lys-Lys-Leu) at different Lys^+/PO_4^- ratios shows that two lysyl residues are required to complex each phosphate group (Figure 1), thus corroborating previous measurements of the complexation of oligo(A)s up to the 25-mer by poly(Arg-Leu) and poly(Leu-Arg-Arg-Leu).[29] Beyond a ratio of 2, poly(Leu-

(22) Pelerin, J. P.; Barbier, B.; Brack, A. In *Peptide 1988*; Jung, G., Bayer, E., Eds.; Walter de Gruyter: Berlin–New York, 1989; p 423.
(23) Brack, A.; Spach, G. *J. Am. Chem. Soc.* **1981**, *103*, 6319.
(24) Michelson, A. M. *The chemistry of nucleosides and nucleotides*; Academic Press: London and New York, 1963.
(25) Ayad, S. R.; Blamire, J. *Techniques of nucleic acid fractionation*; J. Wiley and Sons Ltd.: New York, 1972.
(26) Blake, R. D.; Fresco, J. R. *Biopolymers* **1973**, *12*, 775–789.
(27) Mayer, R.; Lancelot, G. *J. Am. Chem. Soc.* **1981**, *103*, 4738.
(28) Loukas, S.; Varoucha, D.; Zioudrou, C.; Streaty, R. A.; Klee, W. A. *Biochemistry* **1983**, *22*, 4567.

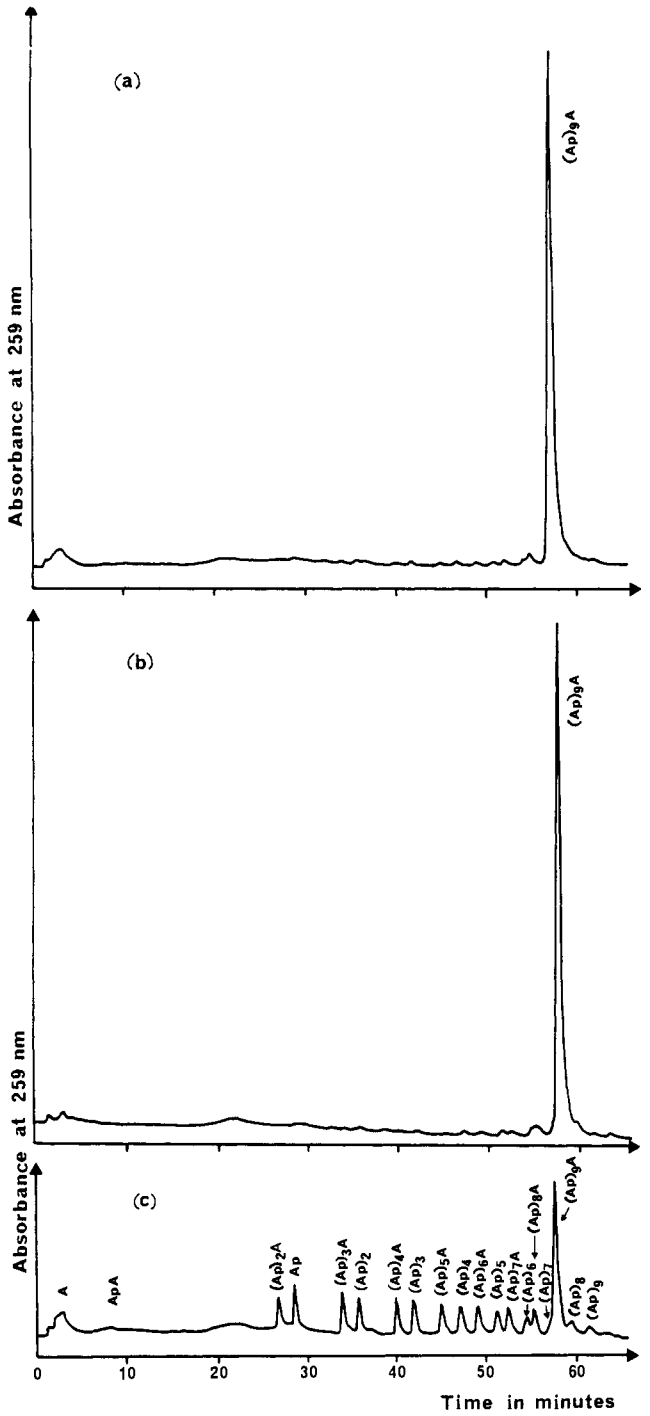

Figure 2. HPLC elution profiles on a PVDI column of the reaction products of the hydrolysis of $(Ap)_9A$: (a) control, (b) in the presence of Ac-Leu-Lys-NHEt, and (c) in the presence of poly(Leu-Lys). Reaction conditions: 37 °C, 7 days, 0.1 M Gly-Gly buffer (pH 7.5), $(Ap)_9A$ 4×10^{-4} M in phosphate, polypeptide and peptide 10^{-3} M in lysine.

Lys-Lys-Leu) exhibits a very unusual binding curve. The polypeptide seems to be unable to precipitate the totality of the trinucleotides probably because of the presence of uncomplexed lysine side chains which tend to solubilize the complex in water.

Influence of the Nature of the Amino Acids. Poly(Leu-Lys) is more active than poly(Lys) (Table I), and the same observation can be made when poly(Arg-Leu) is compared to poly(Arg). The regular insertion of leucyl residues along the chain plays an important role in the hydrolytic potentiality of the peptide. Alanyl residues have been tested in association with lysine (Table II).

(29) Barbier, B.; Brack, A. *Origins Life* **1987**, *17*, 381.

Table I. Rate Constant of the Hydrolysis of $(Ap)_9A$ by Different Basic Polypeptides[a]

polypeptide	K (min^{-1})	rate enhancement
poly(Leu-Lys)	1.7×10^{-4}	185
poly(Arg-Leu)	6.8×10^{-5}	74
poly(Lys)	3.1×10^{-5}	34
poly(His)	1.3×10^{-6}	1.4
control	0.92×10^{-7}	

[a] Experimental conditions: 37 °C, 7 days in 0.1 M Gly-Gly buffer (pH 7.5), $(Ap)_9A$ 4×10^{-4} M in phosphate, peptide 10^{-3} M in basic amino acid.

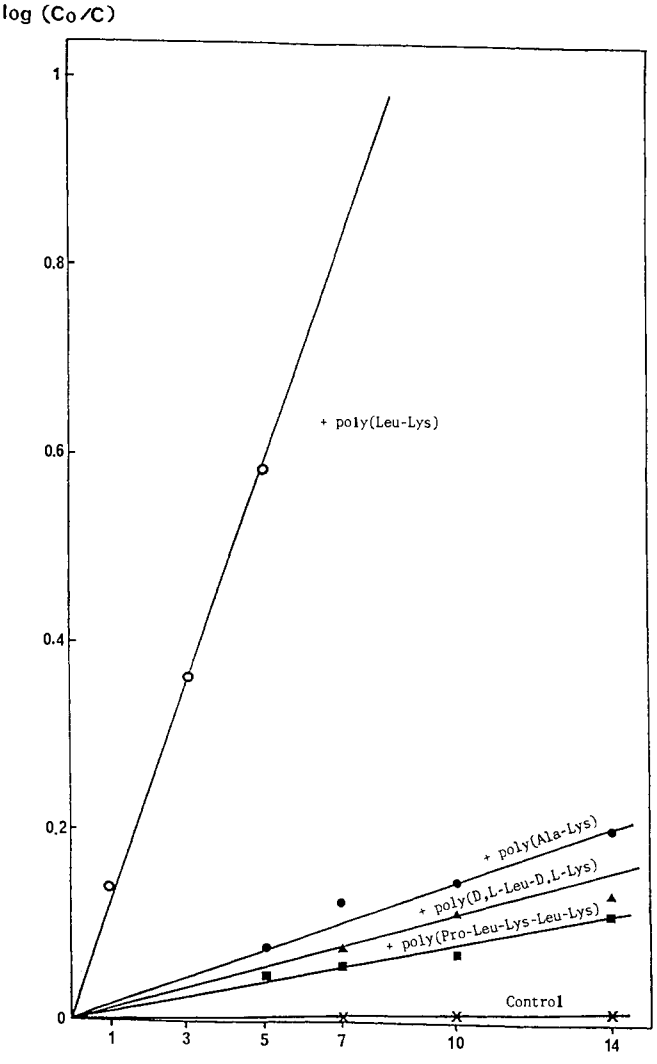

Figure 3. Kinetics of $(Ap)_9A$ hydrolysis by different polypeptides demonstrating the influence of the β-sheet conformation (C_0 = initial concentration of $(Ap)_9A$, C = concentration of the unhydrolyzed decanucleotide at a given time): without polypeptide (×) and in the presence of poly(Pro-Leu-Lys-Leu-Lys) (■), poly(D,L-Leu-D,L-Lys) (▲), poly(Ala-Lys) (●), and poly(Leu-Lys) (○). Experimental conditions: 37 °C, Gly-Gly/NaOH buffer (0.1 M, pH 7.5), $(Ap)_9A$ 4×10^{-4} M in phosphate, polypeptides 10^{-3} M in lysine.

The highest hydrolytic activity is displayed by the more hydrophobic aliphatic side chain.

Three protonatable amino acids have been compared with regard to their hydrolytic efficiencies: Arg (pK = 12.48), Lys (pK = 10.79), and His (pK = 6). Because of its insolubility in water,[19] poly(His-Leu) could not be compared to poly(Leu-Lys) and to poly(Arg-Leu). Nevertheless, we compared poly(L-histidine) to poly(L-lysine) (Table I). Poly(L-histidine) was found to be inactive at 37 °C and pH 7.5 over 7 days. L-lysyl residues are more efficient than L-arginyl residues.

Table II. Rate Constants of the Hydrolysis of $(Ap)_9A$ by Different Basic Polypeptides Exemplifying the Role of the β-Sheet Conformation[a]

polypeptide	K (min^{-1})	rate enhancement
control	1.1×10^{-6}	
poly(Pro-Leu-Lys-Leu-Lys)	1.2×10^{-5}	11
poly(D,L-Leu-D,L-Lys)	1.85×10^{-5}	17
poly(Ala-Lys)	2.2×10^{-5}	20
poly(Leu-Lys)	1.7×10^{-4}	155

[a] Experimental conditions: cf. Table I.

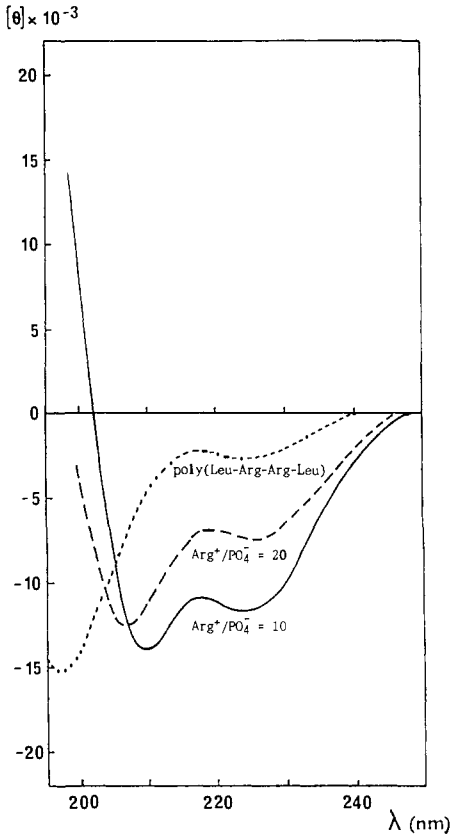

Figure 4. Circular dichroism spectra of poly(Leu-Arg-Arg-Leu) (2 mg/mL, cell path 0.1 mm), in pure water (---) and in the presence of 1-25 oligo(A)s with an Arg^+/PO_4^- ratio of 20 (– – –) and 10 (—). The oligoribonucleotide contribution has been substracted from the spectra.

Role of the Polypeptide Conformation. Polypeptides with an alternating sequence of basic and hydrophobic residues adopt a β-sheet conformation in salt-containing aqueous solution[17,30] or when complexed to oligoribonucleotides.[15] Different polymers have been used to evaluate the importance of the β-structure in the hydrolytic activity (Figure 3 and Table II). Poly(Leu-Lys) presents the highest activity. For poly(Ala-Lys), the hydrophobicity is less marked and its propensity to form a β-conformation is low. Its activity is decreased by a factor of 8 as compared to that of poly(Leu-Lys).

The simultaneous presence of both enantiomers prevents the polypeptide from adopting a β-sheet structure.[20] The racemic polymer has little activity. Finally, poly(Pro-Leu-Lys-Leu-Lys) in which prolyl residues act as β-sheet breakers is 14 times less active than poly(Leu-Lys).

In pure water poly(Leu-Lys-Lys-Leu)[21] or poly(Leu-Arg-Arg-Leu) adopts a random coil conformation due to charge repulsion. Addition of salt to the aqueous solution of the polypeptides induces a coil to α-helix transition. The conformation of poly(Leu-Arg-Arg-Leu) complexed to oligoribonucleotides has been studied by circular dichroism (CD) (Figure 4). An Arg^+/PO_4^- ratio of 2.5 does not allow the CD measurements because of gel formation. However, for a ratio of 10, the solution is transparent enough to run the CD spectrum. At such a ratio, the hydrolytic activity is retained. From the CD spectrum, it can be estimated that about 33% of the polypeptide chains undergo a coil to α-helix transition[31] when interacting with the oligonucleotides. The same behavior has been found for poly(Leu-Lys-Lys-Leu).[32] In poly(Pro-Lys-Lys-Leu), L-prolyl residues known as α-helix breakers have been introduced. The sequential polypeptide stays

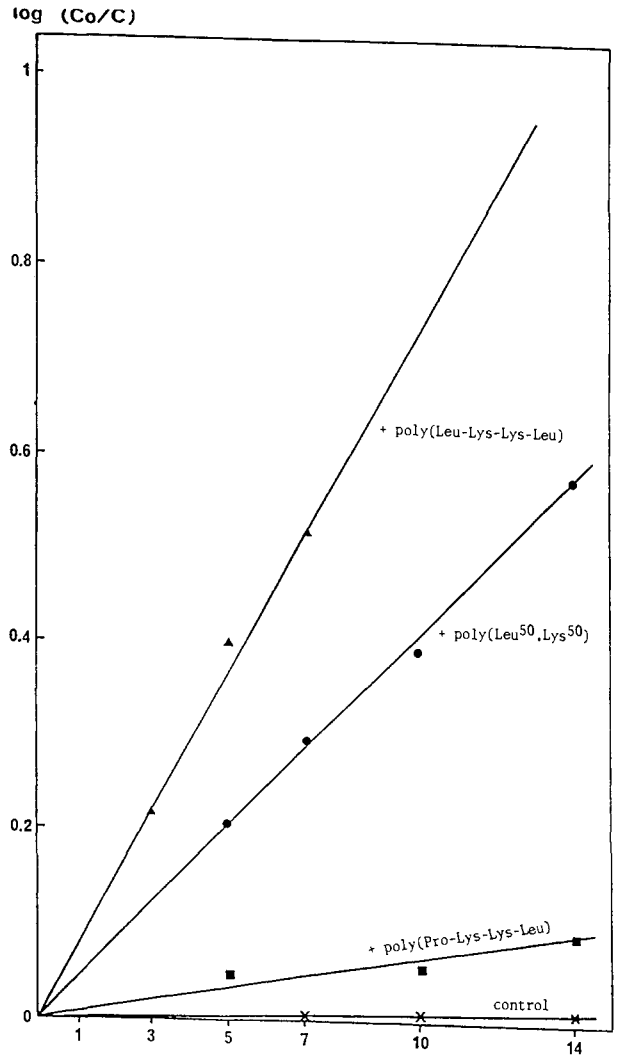

Figure 5. Kinetics of $(Ap)_9A$ hydrolysis by different polypeptides demonstrating the influence of the α-helix conformation (C_0 = initial concentration of $(Ap)_9A$, C = concentration of the unhydrolyzed decanucleotide at the time indicated): without polypeptide (×) and in the presence of poly(Pro-Lys-Lys-Leu) (■), poly(Leu^{50}, Lys^{50}) (●), and poly(Leu-Lys-Lys-Leu) (▲). Experimental conditions: 37 °C, Gly-Gly/NaOH buffer (0.1 M, pH 7.5), $(Ap)_9A$ 4×10^{-4} M in phosphate, polypeptides 10^{-3} M in lysine.

Table III. Rate Constants of the Hydrolysis of $(Ap)_9A$ by Different Basic Polypeptides Exemplifying the Role of the α-Helix Conformation[a]

polypeptide	K (min^{-1})	rate enhancement
control	1.1×10^{-6}	
poly(Pro-Lys-Lys-Leu)	4.5×10^{-6}	4
poly(Leu^{50},Lys^{50})	6.2×10^{-5}	56
poly(Leu-Lys-Lys-Leu)	1.1×10^{-4}	100

[a] Experimental conditions: cf. Table I.

(30) Brack, A.; Orgel, L. E. *Nature* **1975**, *256*, 383.

(31) Greenfield, N.; Fasman, G. D. *Biochemistry* **1969**, *8*, 4108.

(32) Perello, M.; Barbier, B.; Brack, A. *Int. J. Pept. Protein Res.* **1991**, *38*, 154-160.

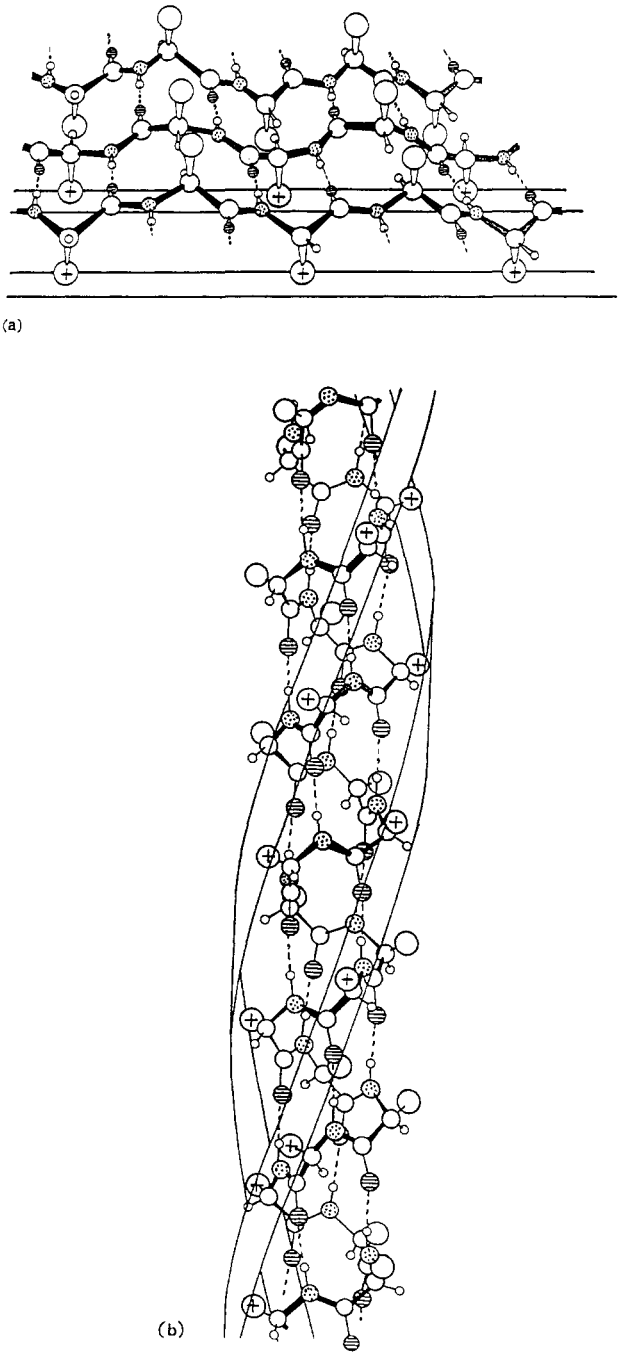

Figure 6. Illustration of the parallel rows of positive charges on the β-sheet (a) and α-helix (b) obtained with poly(Leu-Lys) and poly(Leu-Lys-Lys-Leu), respectively.

as a random coil in salt-containing aqueous solution and has very little hydrolytic activity. Poly(Leu^{50}, Lys^{50}) has a random distribution of charges and is nevertheless able to adopt an α-helix conformation. The kinetics of hydrolysis shown in Figure 5 and Table III indicate that the highest activity is displayed by a polymer which possesses both an α-helical conformation and a regular distribution of the lysyl residues.

Discussion

The hydrolytic activity of different basic polypeptides toward ribonucleic acids has been demonstrated. We were able to localize the chemical activity on the side chain function of the proton-accepting amino acids. The absence of activity of poly(L-histidine) (Table I) is noteworthy. The histidyl side function can be considered as a pseudobase since it is both a donor and acceptor of protons; histidine is present in many active sites of enzymes and particularly in ribonucleases. Many factors may be responsible for the inefficiency of poly(L-histidine), such as the conformation and the absence of strong basic groups.

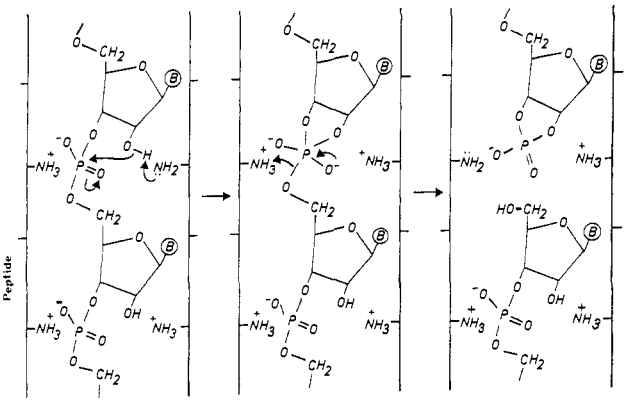

Figure 7. Tentative model for the mechanism of hydrolysis of a polyribonucleotide by poly(Leu-Lys).

The activity of basic polypeptides is not only due to the presence of lysine or arginine side chains but also to the spatial geometry of the backbone. For example, the dipeptide Ac-Leu-Lys-NHEt is inactive (Figure 2). For polypeptides with alternating hydrophobic and basic hydrophilic residues (Table I), the activity increases with the hydrophobicity, a factor known to favor the β-sheet formation of these polypeptides.[33] It is illustrated by the fact that poly(Ala-Lys) is less efficient than poly(Leu-Lys) (Figure 3 and Table II). The importance of the β-sheet geometry is confirmed by the weak activity of poly(Pro-Leu-Lys-Leu-Lys) and poly(D,L-Leu-D,L-Lys) which cannot adopt the β-sheet conformation. In the β-sheet structure, two factors may contribute favorably to the chemical activity, the rigidity of the conformation and the geometric distribution of the charges in a regular network. The distance between two charged residues, which is about 6.9 Å[34] fits with the spacing between two consecutive phosphate groups (6.2 Å) for oligoribonucleotides (from ref 35). In a tentative model it is assumed that the polypeptide is able to align the nucleotide strand along the parallel rows of positively charged side chains (Figure 6). Rows of positive charges can also be obtained with an α-helix when a strict alternation of hydrophobic (Leu-Leu) and hydrophilic (Lys-Lys) doublets is fulfilled. For such a polypeptide, the basic amino acids are arranged in two rows twisted around the helix (Figure 6). The spacing between two positive charges on one of the rows is about 6 Å (from ref 34), a distance compatible with the distance separating two phosphate groups. Here again, a stable geometry is required for hydrolytic activity. The basic polypeptides develop their nuclease-like activity only when the chemical functions are engaged in a given spatial geometry. As previously demonstrated,[15] basic peptides accelerate the classical base-induced hydrolysis of ribonucleic acids. Once the complex is formed through ionic interactions between phosphate groups and protonated basic side chains, the cleavage of the phosphodiester bond occurs via a nucleophilic attack of the free base of the side chain, affording a cyclic phosphate (Figure 7). In a second step, the 2′–3′ cyclic phosphate is hydrolyzed to give both 2′- and 3′-phosphates. Such a mechanism requires two parallel rows of basic amino acids (Figure 6) present in both the β-sheet and the α-helix which have been found to be active. Confirmation of this model will be undertaken by NMR and molecular modeling.

Acknowledgment. We thank Prof. A. W. Schwartz for careful revision of the paper.

(33) Brack, A. *Origins Life* **1987**, *17*, 367–379.
(34) Lotz, B.; Brack, A. In *Applied Fiber Science*; Happey, F., Ed.; Academic Press: London–New York, 1979; Vol. 3, Chapter 9, p 371.
(35) Saenger, W.; Riecke, J.; Suck, D. *J. Mol. Biol.* **1975**, *93*, 529.