Volume 15 Number 24 1987 Nucleic Acids Research

Rate of degradation of [α]- and [β]-oligodeoxynucleotides in *Xenopus* oocytes. Implications for anti-messenger strategies

Christian Cazenave, Marianne Chevrier, Nguyen T.Thuong[1] and Claude Hélène

Laboratoire de Biophysique, Muséum National d'Histoire Naturelle, INSERM U.201, CNRS UA.481, 61 rue Buffon, 75005 Paris and [1]Centre de Biophysique Moléculaire, CNRS, 45071 Orléans Cedex 02, France

Received August 21, 1987; Revised and Accepted November 23, 1987

ABSTRACT

End-labelled oligodeoxynucleotides were injected into **Xenopus laevis** oocytes and their degradation products were analysed by high-performance ion-exchange liquid chromatography after various times of incubation. The oligonucleotides were synthesised with either the natural [β] anomers or the synthetic [α] anomers of deoxynucleotide units. Oligo-[β]deoxynucleotides are short-lived inside oocytes (half-life ≈ 10 min). Covalent attachment of an intercalating agent to the 3'-phosphate and of a methylthiophosphate group at the 5'-end protects oligodeoxynucleotides against 3'- and 5'-exonucleases, respectively. The half-life of such substituted oligodeoxynucleotides is increased to 40 minutes. Oligo-[α]-deoxynucleotides are quite resistant to both endo and exonucleases inside **Xenopus** oocytes. After 8 hours only 40 % of a 16-mer oligo-[α]-deoxynucleotide were hydrolysed. The rapid degradation of oligo-[β]-deoxynucleotides suggests that efficient inhibition of translation in **Xenopus** oocytes involves an RNase H-induced hydrolysis of mRNAs hybridized to oligo-[β]-deoxynucleotides.

INTRODUCTION

The recent discovery that anti-sense RNAs are involved in various natural processes in prokaryotic cells stimulated many experiments using this alternative approach to classical genetics in order to selectively block the expression of specific genes inside both prokaryotic and eukaryotic cells (1). Among the cells used in such experiments **Xenopus** oocytes provided evidence for the occurrence in the cytoplasm of a living cell of the so-called hybrid-arrested translation previously observed **in vitro** (2). Melton reported on the inhibition of translation of rabbit globin messenger RNA with long anti-sense RNAs (3) whereas Kawasaki showed that the translation of interleukin messenger RNA could be blocked by oligodeoxynucleotides (4). We recently described the inhibition of translation of rabbit globin mRNA microinjected in **Xenopus** oocytes by oligodeoxynucleotides either unmodified (5) or modified by covalent linkage to an intercalating agent (6). In the course of this study we observed that no inhibition could be detected when the anti-messenger oligodeoxynucleotide was injected one to three hours prior to the injection of rabbit globin

mRNA whereas significant inhibition was observed when the oligonucleotide was delivered together with or after messenger RNA injection. This raised the question of the stability of anti-messenger oligodeoxynucleotides inside oocytes as one hypothesis for the observed failure to inhibit translation when they were injected prior to messenger RNA was that the major part of the oligonucleotide was degraded during the delay between the two injections. The present study was undertaken to elucidate this point.

MATERIALS AND METHODS

Oligonucleotides

An unmodified oligodeoxynucleotide $d^{5'}[TTGTGTCAAAAGCAAGT]^{3'}$ complementary to nucleotides 3-19 of β-globin mRNA was synthesised and purified as previously described (6). It was labelled either at the 5'-end with ATPγ^{32}P and T4 polynucleotide kinase or, alternatively, at the 3'-end with dideoxy α^{32}PATP, and terminal transferase. Unreacted ATP was removed by gel-filtration on a G50 Sephadex column (Pharmacia) equilibrated in TE buffer (Tris-HCl 10 mM, pH 7.6, EDTA 1 mM).

An octathymidylate was covalently linked to 2-methoxy, 6-chloro, 9-aminoacridine (Acr) as previously described (7). A pentamethylene chain (m_5) was used to link the 3'-phosphate group of the octathymidylate to the 9-amino group of the acridine derivative. This derivative will be abbreviated as **T8m5Acr.** A thiophosphate group was introduced at the 5' end by reacting the 5'-OH group with bis-cyanoethyldiisopropylaminophosphite activated by tetrazole, followed by sulphuration of the intermediate phosphite with sulphur in pyridine. After deprotection and purification the thiophosphate group (1 μmole) was reacted with ^{14}C-methyl iodide (CEA, France) (3 μmole) in DMSO/H_2O/$NaHCO_3$ 5 % (2:2:1 v/v) to form the methylthiophosphodiester. The radioactive oligonucleotide was purified by ion-exchange chromatography on polyanion HR5/5 Pharmacia columns. The specific activity of the final product was 9.8 x 10^{13} cpm.mol^{-1}. This compound will be abbreviated as **me(sp)T8m5Acr.**

The modified nonamer $d^{5'}[TAATAGCGT]^{3'}m_5$Acr was synthesised as previously described (7). The oligo-[α]-deoxynucleotide [α]-$d^{5'}[CACCAACTTCTTCCAC]^{3'}$ containing the [α] anomers of deoxynucleotides instead of the natural [β] anomers was synthesised as previously described (8). An octathymidylate lacking 5'-phosphate was purchased from PL-Biochemicals. These oligonucleotides were labelled at their 5'-end with γ^{32}P-ATP and T4 polynucleotide kinase and chromatographed on a G50 Sephadex column. As a

substantial amount of unreacted nucleotide was present in the excluded fraction containing the labelled oligo-[α]-deoxynucleotide, further purification was achieved by electrophoresis on a sequencing gel containing 20 % acrylamide. The band containing the oligonucleotide was excised, the oligonucleotide was eluted from the gel, precipitated with ethanol and recovered in sterile water.

Microinjection into **Xenopus** oocytes

80 nl of radiolabelled oligonucleotide were injected into the cytoplasm of stage VI oocytes. In some experiments oocytes were enucleated as described by Jessus **et al.** (9). Immediately after injection oocytes were rinsed with modified Barth's saline (MBS) (10 ml per 10-20 oocytes) and then incubated in 100 µl MBS in the wells of microtitration plates, at 18°C for the indicated times (10). At the end of the incubation oocytes were lysed according to Colman (11) in homogenisation buffer (0.1 M NaCl, 1 % Triton X100, 1 mM PMSF, 20 mM Tris-HCl, pH 7.6) (200 µl per 10 oocytes) and centrifuged for 5 minutes at 10,000 g to remove yolk platelets. The supernatant was collected, (taking care to avoid contamination by the lipid bilayer) and stored at - 80°C until HPLC analysis was performed.

The amount of cpm usually recovered in homogenates was greater than two-thirds of the injected radioactivity. The radioactivity associated with yolk platelets never exceeded 5 % of the total radioactivity recovered. Therefore any observed loss took place during the microinjection process. We checked that no degradation of the oligonucleotide occurred during the homogenization of the oocyte or subsequent processing. Even after two hours of incubation in the oocyte extract, either crude or clarified by centrifugation, no degradation was detected. No degradation of labelled oligonucleotides was observed when the oligonucleotides were added to the homogenisation buffer in which the oocytes were lysed.

HPLC analysis

Homogenates were loaded on a DEAE 5PW TSK column (7.5 x 75 mm) and eluted with a linear gradient of KCl (0-0.5 M) in 10 mM potassium phosphate, pH 7.0, during 40 min at a flow rate of 1 $ml.min^{-1}$. Under these conditions retention times of AMP, ApAp, ApApAp and ApApApApApAp were 11,18,22 and 26 min, respectively, whereas retention times of TMP, pTp and pTpT were 10, 16 and 14.5 min, respectively.

Two methods were used to determine the degradation profile of the oligodeoxynucleotides :

i) in the first experiments fractions of 0.5 ml were collected directly in

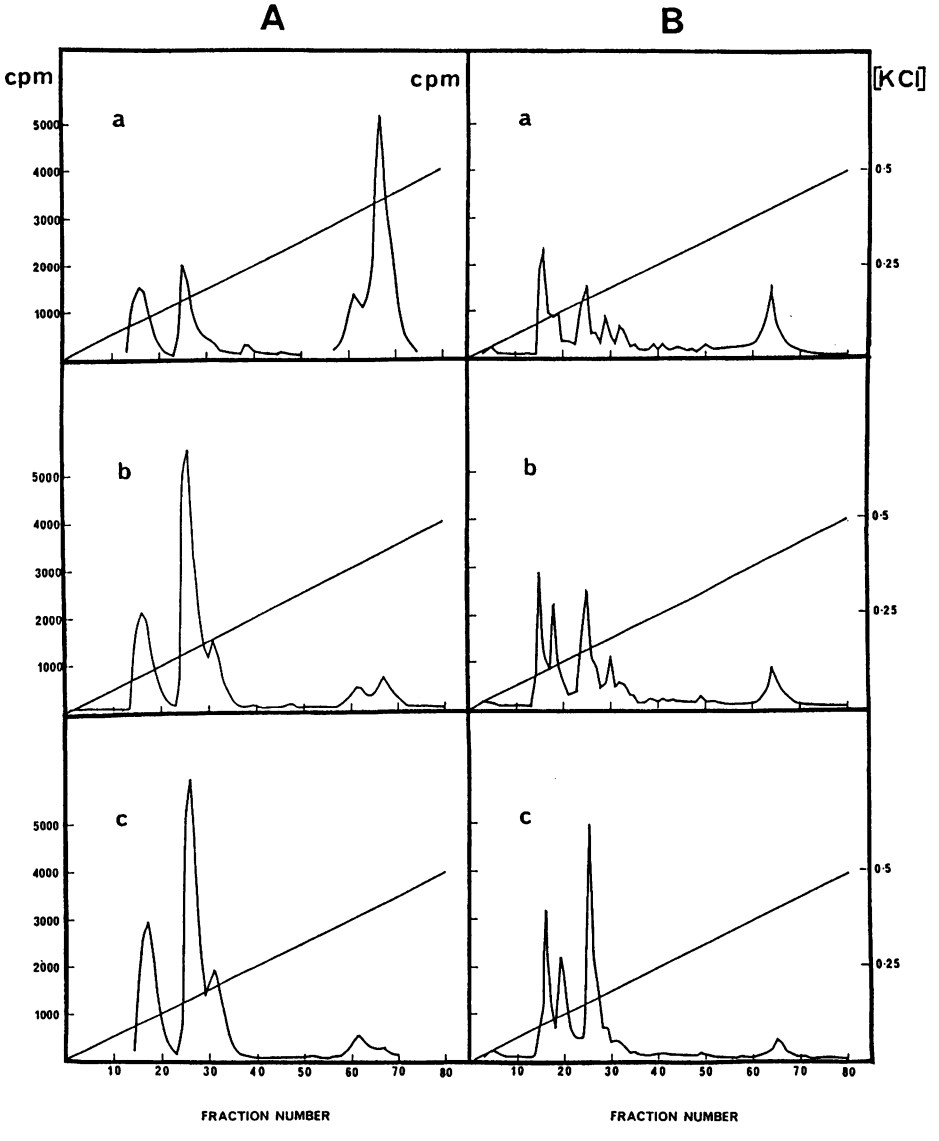

Figure 1 : Time-course of degradation of ^{32}P-labelled 17-mer injected into **Xenopus** oocytes.
A. Chromatograms from lysates of oocytes injected with ^{32}P 5'-labelled 17-mer injected into **Xenopus** oocytes and incubated for 5 min (a), 1 h (b) and 3 h (c) prior to homogenisation and HPLC analysis.
B. Chromatograms from lysates of oocytes injected with ^{32}P 3'-labelled 17-mer injected into **Xenopus** oocytes and incubated for 5 min (a), 1 h (b) and 3 h (c) prior to homogenisation.

Exp	Oligonucleotide	Activity	concentrations in the injection needle	% radioactivity in oligonucleotide peak prior to injection	% intact oligonucleotide				
					5 min	30 min	1 hour	2 hours	3 hours
1	(^{14}C)pT$_8$m$_5$Acr	70 000 cpm/µl	7.1 x 10^{-4} M	95	76.5	59	33.4	-	2.5
2	"	"	7.1 x 10^{-4} M	95	64	48	-	-	5.5
3	"	7 000 cpm/µl	7.1 x 10^{-5} M	95	79	-	-	2.6	-
4[a]	"	70 000 cpm/µl	7.1 x 10^{-4} M	95	94	-	-	-	37.4
5[a]	"	"	7.1 x 10^{-4} m	95	85	-	-	-	16.4
6	"	14 000 cpm/µl	1.4 x 10^{-4} M	95	77.2	-	-	-	8.7
7[b]	"	"	1.4 x 10^{-4} M	95	84.5	-	-	-	24
8	^{32}P-17mer	70 000 cpm/µl	≈ 3 x 10^{-8} M	78	38.1	-	7.1	-	2.6
9	"	"	≈ 3 x 10^{-8} m	78	57.1	-	7.3	-	2.4
10	"	"	≈ 3 x 10^{-8} M	78	46.6	-	8.1	-	4.3
11	17mer-^{32}pA	"	≈ 3 x 10^{-8} M	53	19.6	-	11.4	-	7.2
12	^{32}P-9mer m$_5$Acr	16 000 cpm/µl	1.5 x 10^{-7} M	96	88	9.5	3	-	0
13	"	"	"	83	64	7.5	1.2	-	0
14	(^{32}P)T$_8$m$_5$Acr	100 000 cpm/µl	≈ 10^{-6} M	85	74	22	16	-	0
15	"	"	"	85	72	15	5.5	-	0
16	(^{32}P)T$_8$	10 000 cpm/µl	1 x 10^{-7} M	87	68.5	9.3	2	-	0

Percentage of radioactivity recovered in the intact oligonucleotide peak at various times after injection into **Xenopus** oocytes. The percentage of intact oligonucleotide was determined by dividing the sum of cpm present in the peak of intact oligonucleotide by the total amount of cpm eluted during the HPLC run.
a. experiment 4 was performed with enucleated oocytes prepared according to the procedure described by C. Jessus **et al.** (9) whereas experiment 5 was performed with "mock"-enucleated oocytes (oocytes which have been submitted to the experimental conditions used for enucleation but which have not been incised for nucleus extraction.
b. the oligonucleotide was injected together with poly(rA) 1 mM.

scintillation flasks in which 5 ml of Aqualuma plus (Radiomatic) were added and then counted in a rack minibeta LKB liquid scintillation counter ;

ii) in subsequent experiments a Flo-one beta radioactive flow detector was connected to the end of the HPLC column. The volume of the flow cell was 0.5 ml and discriminators were set for Cerenkov counting.

RESULTS

Hydrolysis of unsubstituted oligo-[β]-deoxynucleotides

An unmodified 17-mer oligodeoxynucleotide d$^{5'}$[TTGTGTCAAAAGCAAGT]$^{3'}$ complementary to the 5'-end of rabbit β-globin mRNA was 5'-^{32}P-labelled and injected into oocytes. This oligonucleotide was previously shown to be efficient in inhibiting translation in the oocyte (6). Homogenates obtained after various incubation times were analysed as described in Materials and Methods. Resulting chromatograms are presented in figure 1A. The peak of intact oligonucleotide eluting in fraction 68 (retention time = 34 min) decreased with time whereas degradation products eluting at lower ionic strength increased concomitantly. The estimated half-life of the 17-mer oligonucleotide was estimated to be 10 minutes. After one hour less than 10 % remained intact (Table 1) . No degradation products were detected between fractions 35 and 59. The major degradation product eluted at fraction 27 (retention time = 13.5 min). It was already observed immediately (i.e. 5 min) after injection when the amount of intact oligonucleotide was only 38 % of the total recovered

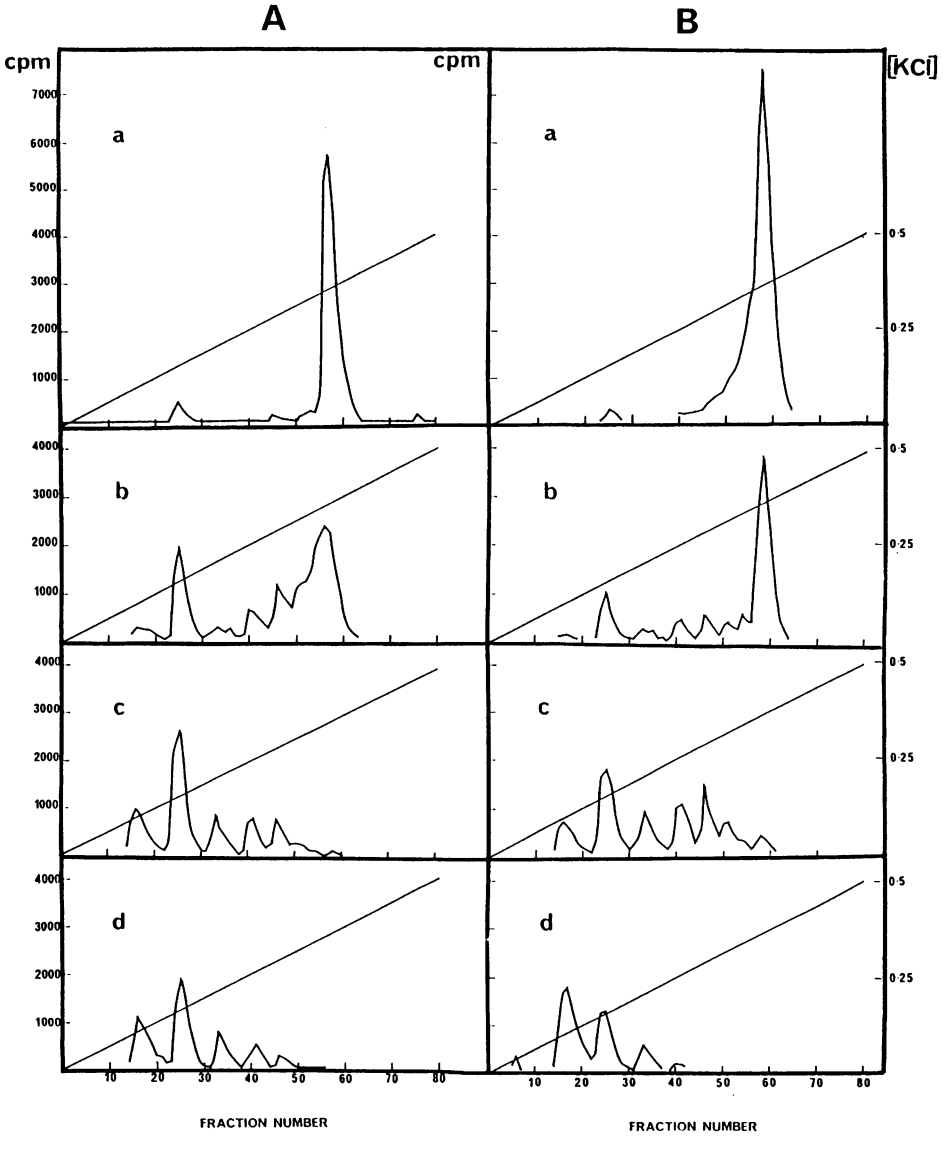

Figure 2 : Time-course of degradation of (^{14}C)me(sp)T$_8$m$_5$Acr injected into **Xenopus** oocytes. Figure 2A and 2B report two independent experiments performed with two different batches of oocytes.
A. Chromatograms from lysates of oocytes injected with (^{14}C)me(sp)T$_8$m$_5$Acr and incubated for 5 min (a), 1 h (b), 3 h (c) and 5 h (d) prior to homogenisation and HPLC analysis as described in Materials and Methods.
B. Chromatograms from lysates of oocytes injected with (^{14}C)me(sp)T$_8$m$_5$Acr and incubated for 5 min (a), 30 min (b), 3 h (c) and 12 h (d) prior to homogenisation.

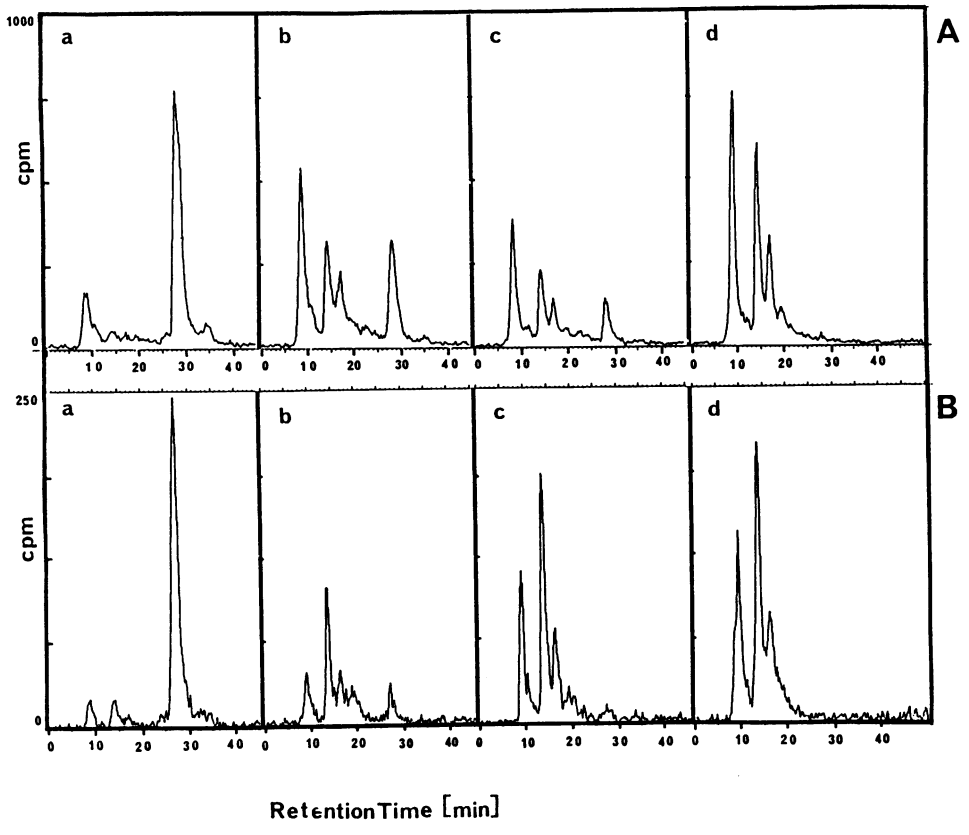

Figure 3 : Time course of degradation of ^{32}P-labelled oligonucleotides carrying an acridine derivative covalently linked to their 3' end.
A. Chromatograms from lysate of oocytes injected with ^{32}P 5' labelled octathymidylate linked to the acridine (T_8m_5Acr) and incubated for 5 min (a), 30 min (b), 1 hour (c) and 3 hours (d).
B. Chromatograms from lysates of oocytes injected with ^{32}P 5' labelled nonamer linked to the acridine ($d^{5'}[TAATACGT]^{3'}m_5Acr$) and incubated for 5 min (a), 30 min (b), 1 hour (c) and 3 hours (d).

radioactivity (see table 1). In an attempt to further identify the peaks eluting at low ionic strength, 40 oocytes were microinjected and an homogenate prepared after three hours of incubation at 18°C. Half of the homogenate was injected on the DEAE column. Fractions were collected and counted as described above to determine the degradation pattern. The second half was then injected on the DEAE column. Fractions were collected and those corresponding to the top of each peak were further injected on a reverse phase column (TSK ODS-120 T 5µm 4.6 x 250 mm) eluted with 100 mM ammonium acetate, pH 5.0, containing

10 % methanol, at a flow rate of 1 ml.min^{-1}. Fractions were collected, counted and retention times compared with those obtained for known standards (^{32}P, pT, pTp and pTpT) injected under the same conditions. We found that the major peak eluting between fractions 25 and 29 (retention time = 13.5 min) corresponded to pTp whereas the faster eluting peak was a mixture of ^{32}P (80 %) and ^{32}pT (20 %). In order to determine the effect of oligonucleotide concentration on the rate of hydrolysis the 5'-end ^{32}P-labelled 17-mer (3 x 10^{-8} M) was injected in the absence and in the presence of 10^{-4} M unlabelled 17-mer. The time course of the disappearance of intact labelled oligonucleotide was quite similar in both cases indicating that the rate of hydrolysis was directly proportional to the oligonucleotide concentration.

The inhibition of rabbit β-globin mRNA translation in microinjected **Xenopus** oocytes was achieved by oligodeoxynucleotides carrying a free 5'OH group and either a 3'OH or an acridine derivative substituted at their 3'-end (5,6). The nuclease digestion experiments reported above dealt with a 5'-^{32}P-labelled oligodeoxynucleotide. In order to assess the possible protecting effect of a 5'-phosphate, the oligonucleotide was 3'-end labelled (see Materials and Methods) and microinjected into oocytes. Chromatograms obtained at different times after injection are shown on figure 1B. Degradation was very fast during the first five minutes since more than 50 % of the oligonucleotide disappeared and generated very short fragments (figure 1B and table 1). The rate of degradation slowed down significantly at longer times (see table 1). The pattern of degradation products observed between fraction 10 and 35 was more complicated than that observed when the oligonucleotide was 5'-labelled. This was likely due to the position of the labelled phosphorus which was located in the last internucleotidic linkage so that several labelled short fragments could be generated under the action of both endo and exonucleases : pT^{32}pA, T^{32}pA, pT^{32}p and ^{32}pA.

Hydrolysis of acridine-substituted oligo-[β]-deoxynucleotides

We have previously described the synthesis of oligodeoxynucleotides covalently linked to an intercalating agent (12). These oligonucleotides bind selectively to their complementary sequence ; the intercalating agent provides an additional binding energy which stabilises the complex. Such oligonucleotide-acridine conjugates inhibit translation in microinjected oocytes (6). In order to assess the role of acridine substitution on the sensitivity of oligonucleotides to nuclease digestion, a comparison was made between an insubstituted octathymidylate T_8 and its acridine derivative T_8m_5Acr. An acridine-substituted nonanucleotide d[TAATAGCGT]m_5Acr was compared to T_8m_5Acr in order

to determine whether the base sequence had any effect on nuclease digestion. The three oligonucleotides were 5'-end labelled as described under Materials and Methods. In addition an octathymidylate modified both at the 5'- and 3'-ends was included in the comparison. This oligonucleotide, me(sp)T_8m_5Acr (see Materials and Methods), carried a 5'-end labelled ^{14}C-methyl group. 80 nl of a 7 x 10^{-4} M solution of the oligodeoxynucleotides were injected per oocyte and after various incubation times batches of 20 oocytes were lysed according to the procedure described in Materials and Methods. 200 µl of the resulting homogenates were fractionated on a DEAE anion-exchange column. Chromatograms obtained from two independent series of injections are presented in figures 2A and 2B for me(sp)T_8m_5Acr. They clearly show a gradual decrease with time of the peak corresponding to intact oligonucleotide eluting at fraction number 59 (retention time = 29.5 min) and a concomitant increase of degradation products eluting at lower ionic strength. The concentration of me(sp)T_8m_5Acr inside **Xenopus** oocytes was about 1.1 x 10^{-4} M (if one assumes a free diffusion compartment of 0.5 µl per oocyte), a concentration at which partial inhibition of protein synthesis was observed (unpublished results). Therefore an additional experiment was performed using a solution of me(sp)T_8m_5Acr diluted ten times. Similar degradation patterns were obtained (table 1, lane 3).

Chromatograms of acridine-substituted oligodeoxynucleotides are shown in figure 3. The nonanucleotide was degraded more rapidly than the octathymidylate. However different rates of dephosphorylation were observed in different experiments. The concentration of intact oligodeoxynucleotide was therefore higher than calculated from the radioactivity under the peak of the oligonucleotide. Dephosphorylation can be taken into account to calculate the concentration of intact oligonucleotide if it is assumed that the rate of hydrolysis of the oligonucleotide does not depend on whether or not its 5'-end is phosphorylated. These corrected values are reported in figure 4 where a comparison is made between different oligonucleotides including unsubstituted T_8. A semi-logarithmic plot of intact oligonucleotide concentration **versus** time was linear (figure 4, inset) in agreement with a first-order kinetic process. Several conclusions can be drawn : i) acridine substitution at the 3'-end protects the octathymidylate (most probably against 3'-exonucleases) ; ii) substitution of the 5'-end by a stable methylthiophosphate group further protects against 5'-exonuclease attack ; iii) the base sequence of the oligonucleotide plays an important role (compare nona and octanucleotides).

In order to determine the role of the different cellular compartments in oligonucleotide degradation the oocytes were enucleated as described

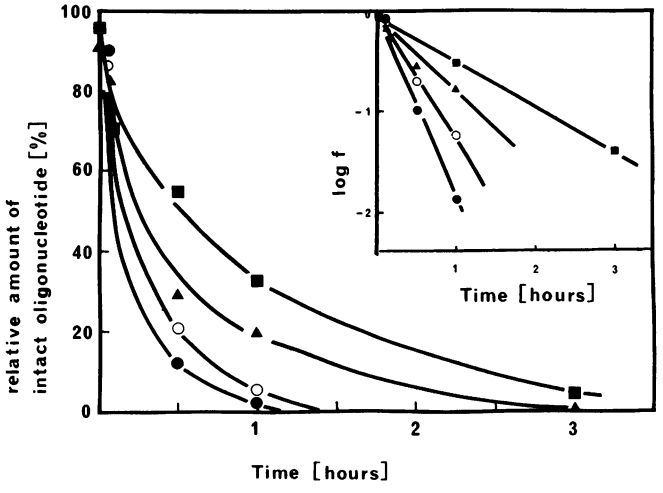

Figure 4 : Percentage of remaining intact oligonucleotide at various times (average of two experiments) for ^{14}C-me(sp)T8m5Acr (■) and 5'end labelled T8m5Acr (▲) (average of two experiments) T8 (O) and d[TAATAGCGT]m5Acr (●). Values reported for the 5'end labelled oligonucleotides were corrected to take in account the level of dephosphorylation.
Inset : plot of logarithm of fraction f of intact oligonucleotide versus time.

by Jessus **et al.** (9). The rate of degradation of me(sp)T8m5Acr was markedly reduced as shown in table 1 (compare lanes 1,2 and 4). However it should be noted that oocytes which had been submitted to the treatment preceeding the enucleation step exhibited a lower rate of degradation even though the nucleus was not removed (table 1, lane 5).

Nuclease resistance of oligo-[α]-deoxynucleotides

Our laboratory is currently investigating the properties of oligo-[α]-deoxynucleotides which have been shown **in vitro** to be much more resistant to nucleases than natural oligo-[β]-deoxynucleotides (14,15). The 5'-end-labelled 16mer oligo-[α]-deoxynucleotide d[CACCAACTTCTTCCAC] displayed strong resistance to nuclease attack after injection into the oocyte (figure 5). Eight hours after injection, 30 % of the radioactivity remained at the position of the original oligonucleotide. In contrast to what was observed with the oligo-[β]-deoxynucleotides, only one main peak was found with a retention time of 10 min and trace amounts of intermediate products eluting between 12 and 25 minutes. Identification of the main peak was performed using reverse phase chromatography. All the radioactivity could be attributed to

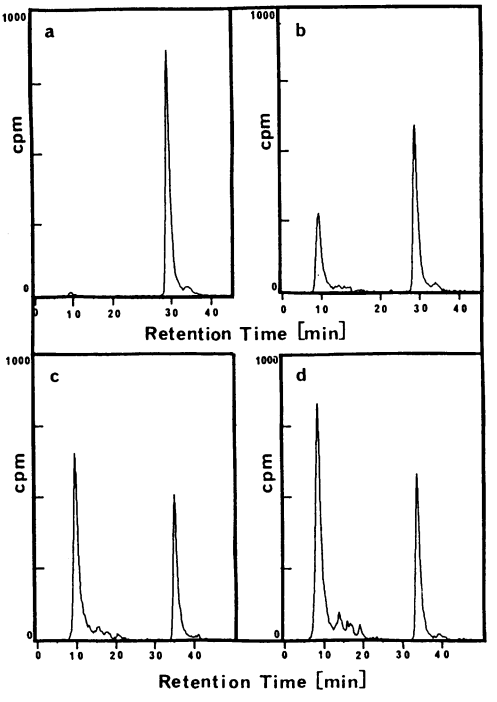

Figure 5 : Time-course of degradation of ^{32}P-labelled 16mer [α] oligodeoxynucleotide. Chromatogram of the oligonucleotide prior to injection in the oocyte (a) and 5 min (b), 1 hour (c) and 4 hours (d) after the injection.

labelled phosphate ^{32}P. The apparent decrease of the amount of intact oligodeoxynucleotide observed on the chromatograms (figure 5) was mainly due to a dephosphorylation rather than a degradation by nucleases. The major part of the oligo-[α]-deoxynucleotide remained intact in the oocyte long after injection. Integration of the radioactivity counts eluting between the peaks of ^{32}P and intact labelled oligo-[α]-deoxynucleotide allowed us to estimate the extent of degradation taking dephosphorylation into account as described above for oligo-[β]-deoxynucleotides. In figure 6 the time-dependence of the concentration of intact 16-mer oligo-[α]-deoxynucleotide is compared to that of the 17-mer oligo-[β]-deoxynucleotide. It can be clearly seen that the oligo-[α]-deoxynucleotide is very resistant to the nucleases present in **Xenopus** oocytes as previously observed with purified enzymes **in vitro** (13,14). At least 60 % of intact oligo-[α]-nucleotide remained in the oocyte eight hours after the injection.

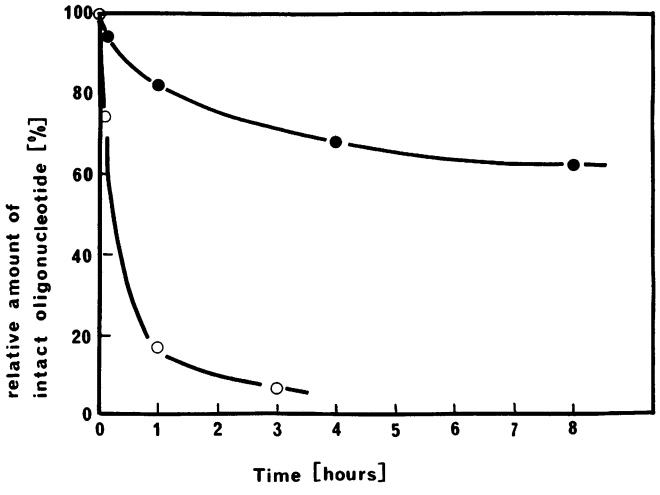

Figure 6 : Percentage of remaining intact oligonucleotide at various times for 16 mer [α]-oligodeoxynucleotide (●) and for 17 mer [β]-oligodeoxynucleotide (O) (mean of two experiments). Values reported were corrected to take dephosphorylation into account.

DISCUSSION

The above experiments clearly demonstrate that oligo-[β]-deoxynucleotides are short-lived inside **Xenopus** oocytes. The rate of degradation was faster for the 5' or 3'-labelled 17-mer than for me(sp)T8m5Acr. Acridine substitution at the 3'-end and the presence of a methylthiophosphate group at the 5'-end protected this oligonucleotide against 3' and 5'-exonucleases, respectively. The half-life of this protected oligonucleotide was about 40 minutes in the oocytes. The lifetime of the 17-mer was much less (≈ 10 minutes). After one hour only 10 % remained intact as compared to 30 % for me(sp)T8m5Acr (Table 1). No degradation products of intermediate length were detected in the case of the labelled 17-mer whereas such products existed in the case of me(sp)T8m5Acr and T8m5Acr. This behaviour reflects a base sequence dependence of nuclease hydrolysis as also demonstrated by the different rates of degradation of the acridine-substituted octa and nonanucleotides (figure 4). Enucleated oocytes were much less active in hydrolysing the oligonucleotides : 35 % of me(sp)T8m5Acr remained intact after 3 hours (Table 1). This could indicate that part of the degradation took place in the nucleus.

A burst of radioactive phosphate was observed immediatly after injection of both oligo-[β]-deoxynucleotides and oligo-[α]-deoxynucleotides. The rate of dephosphorylation was strongly decreased within a few minutes as

shown in the experiments using the nuclease-resistant oligo-[α]-deoxynucleotide. A similar behaviour was observed when the 17-mer was 3'-end labelled indicating that the initial fast hydrolysis is not restricted to dephosphorylation. These observations might reflect the redistribution of the oligonucleotides within cellular compartments which occurs upon dilution of the small volume of injected solution. This observation is in agreement with the hypothesis that the rate of oligonucleotide hydrolysis might be limited by the rate at which oligonucleotides diffuse into different cellular compartments. Fluorescence microscopy of thin fixed sections of oocytes revealed that part of the oligonucleotide linked to the acridine dye (responsible for the fluorescence) was trapped in yolk platelets (C. Cazenave, unpublished results).

Oligo-[β]-deoxynucleotides have been shown to be efficient inhibitors of mRNA translation in **Xenopus** oocytes. Inhibition of rabbit β-globin synthesis was observed 5 hours after co-injection of globin mRNA and 5 to 10 μM of an unmodified 17-mer or an acridine-substituted 11-mer (5,6). However, at that time, most of the oligonucleotide was degraded so that no inhibition of globin synthesis should have occurred. Two explanations for this paradoxical situation are plausible. First, small amounts of intact oligonucleotide were sufficient to exert an inhibitory effect. This could explain the discrepancy between concentrations needed to inhibit β-globin synthesis in wheat germ extracts (0.2 to 0.5 μM) and in oocytes (5 to 10 μM) (6). A small fraction of the oligonucleotide could have been made resistant upon hybridisation to its target RNA. However control experiments performed with me(sp)T_8m_5Acr coinjected with poly(rA) showed that only a very limited protection was observed when the oligonucleotide was bound to a complementary sequence (table 1, lane 7). In the second hypothesis an irreversible process occurred during the five hours lag between the injection and the beginning of protein labelling with ^{35}S-methionine. Previous experiments demonstrated that a major part of the messenger RNA was degraded when co-injected with a complementary oligonucleotide. This could be the result of an RNase H activity present in the cytoplasm of the oocyte (6).

Altogether our results explain why injection of oligo-[β]-deoxynucleotides **prior** to messenger RNA failed to inhibit globin synthesis. One goal for the future of anti-messenger oligonucleotides will be to design molecules resistant to nuclease attack. As mentioned above covalent attachment of an intercalating agent to either end of an oligodeoxynucleotide protects it against exonucleases but does not prevent endonuclease action (12). Oligonucleotides containing phosphonates in the internucleotidic linkage (13) or

alpha-anomers of the nucleosides (14,15) have been previously synthesised and shown to be much more resistant to nuclease attack. The results presented above for oligo-[α]-deoxynucleotides show that the increased resistance to nucleases previously reported **in vitro** is also observed in a living cell. One might expect that lower concentrations of these modified oligonucleotides would be required to obtain complete inhibition as compared to natural unmodified oligodeoxynucleotides. However due to the chemical modification, hybrids of oligo-[α]-deoxynucleotides with mRNAs are less susceptible to RNase H attack (unpublished results). Therefore the efficiency of mRNA translation inhibition will be decreased if mRNA degradation is an important component of the inhibitory process as described above. Such a limitation can be partially overcome if the modified oligonucleotide is covalently linked to a group which can be activated either chemically or photochemically to induce irreversible reactions in the target sequence (for reviews see references 16 and 17). A subtle compromise between these different requirements has to be reached in order to design nuclease-resistant but efficient mRNA translation inhibitors.

ACKNOWLEDGMENTS.

We thank Pr. R. Ozon (Laboratoire de Physiologie de la Reproduction, Université Paris VI) and his collaborators for kindly providing **Xenopus laevis** oocytes.

REFERENCES

1. Green, P.J., Pines, O. & Inouye, M. (1986) Ann. Rev. Biochem. **55**, 569-597.
2. Stephenson, M.L. & Zamecnik, P.C. (1978) Proc. Nat. Acad. Sci. USA **75**, 285-288.
3. Melton, D.A. (1985) Proc. Nat. Acad. Sci. USA **82**, 144-148.
4. Kawasaki, E.S. (1985) Nucleic Acids Res. **13**, 4991-5004.
5. Cazenave, C., Loreau, N., Toulmé, J.J. & Hélène, C. (1986) Biochimie **68**, 1063-1069.
6. Cazenave, C., Loreau, N., Thuong, N.T., Toulmé, J.J. & Hélène, C. (1987) Nucleic Acids Res. **15**, 4717-4736.
7 Asseline, U., Thuong, N.T. & Hélène, C. (1986) Nucleosides & Nucleotides **5**, 45-63.
8. Sun, J.S., Asseline, U., Rouzaud, D., Montenay-Garestier, T., Thuong, N.T. & Hélène, C. (1987) Nucleic Acids Res. **15**, 6149-6158.
9. Jessus, C., Thibier, C. & Ozon, R. (1987) J. Cell Science, **87**, 705-712.
10. Gurdon, J.B. & Wickens, M.P. (1983) in "Methods in Enzymology" (Wu R., Grossman L. & Moldave K., eds.) **101**, 370-390 (Acad. Press, London).
11. Colman, A. (1984) in "Transcription and translation : a practical approach" (Hames B.D. & Higgins S.J., eds.) 49-70 (IRL Press, Oxford).
12. Asseline, U., Delarue, M., Lancelot, G., Toulmé, F., Thuong, N.T., Montenay-Garestier, T. & Hélène, C. (1984) Proc. Natl. Acad. Sci. USA. **81**, 3297-3301.
13. Miller, P.S., Agris, C.H., Aurelian, L., Blake, K., Murakami, A., Reddy, M.P., Spitz, S.A. & Ts'o P.O.P. (1985) Biochimie **67**, 769-776.

14. Morvan, F., Rayner, B., Imbach, J.L., Thenet, S., Bertrand, J.R., Paoletti, J., Malvy, C. & Paoletti, C. (1987) Nucleic Acids Res. 15, 3421-3437.
15. Thuong, N.T., Asseline, U., Roig, V., Takasugi, M. & Hélène, C. (1987) Proc. Nat. Acad. Sci. USA 84, 5129-5133.
16. Knorre, D.G. Vlassov, V.V. (1984) Prog. Nucl. Ac. Res. Mol. Biol. 32, 291-320.
17. Hélène, C. and Thuong, N.T. (1987) in "Nucleic Acids and Molecular Biology" vol. 2, Eckstein, F. and Lilley D.M.J., eds., Springer-Verlag, in the press.