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Phosphoroselenoate oligodeoxynucleotides: synthesis, physico-chemical characterization, anti-sense inhibitory properties and anti-HIV activity

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ABSTRACT

Oligodeoxynucleotides with a phosphorus atom in which one of the non-bridging oxygen atoms is substituted by selenium were prepared and investigated with respect to their antisense properties. A general synthesis of phosphoroselenoate analogs of oligonucleotides is described using potassium selenocyanate as the selenium donor. The compounds, characterized by ^{31}P NMR, were shown to decompose to phosphate with a half-life of ca. 30 days. Melting temperatures of duplexes between poly(rA) or poly(rI) with oligo(dT) and oligo(dC), respectively, indicate diminished hybridization capability of phosphoroselenoate oligomers relative to both the unmodified phosphodiester oligomers and the phosphorothioate congeners. A phosphoroselenoate 17-mer is a sequence specific inhibitor of rabbit β-globin synthesis in wheat germ extract and in injected Xenopus oocytes. In contrast phosphoroselenoate analogs are potent non-sequence specific inhibitors in rabbit reticulocyte lysate. In vitro HIV assays were carried out on a phosphoroselenoate sequence and compared with a phosphorothioate analogue that has previously been shown to exhibit anti-HIV activity (Matsukura et al., Proc. Natl. Acad. Sci. (1987) 84, 7706−7710). The phosphoroselenoate was somewhat less active, and was much more toxic to the cells.

INTRODUCTION

Several classes of phosphate-modified oligodeoxynucleotides have been shown to exert control over gene expression through an antisense mechanism (1−3). Phosphorothioate analogues of oligonucleotides, in which one non-bridging oxygen of the phosphate is replaced by sulfur, have recently been shown to selectively inhibit translation of rabbit β-globin mRNA in wheat germ extract and in injected Xenopus oocytes (4) and to block CAT activity in CV1 transfected cells (5). These compounds display an improved resistance to DNases as compared to unmodified phosphodiester oligomers, and they also elicit RNaseH activity, an enzyme which plays a key role in the antisense effect by cleaving the mRNA strand bound to the oligodeoxynucleotide. Moreover phosphorothioate analogs were demonstrated to possess significant in vitro anti-HIV activity partly due to a non-sequence specific effect (6−11). Unfortunately these compounds are potent non-specific inhibitors of translation in rabbit reticulocyte lysate at any concentration and in wheat germ extract at high concentration. They are also toxic to Xenopus oocytes (4) and to cultured cells (6). Because of these findings we were interested in testing other phosphorochalcogenoate derivatives, namely phosphoroselenoates, in which a selenium atom (selenium lies one row below sulfur in the periodic table) replaces one of the phosphate oxygens (Figure 1). In order to do this it was necessary to develop a convenient synthetic method. Previously only short phosphoroselenoate analogs of oligonucleotides (dimers, trimers) have been synthesized (12,13). In our experiments, we were able to employ the
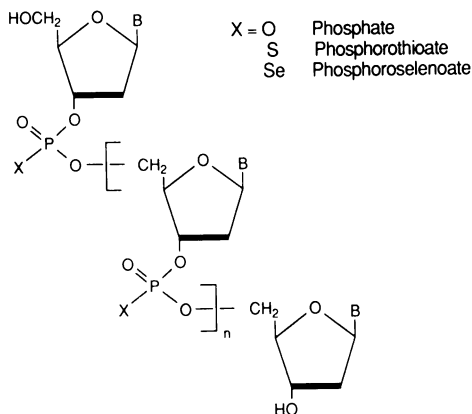
Figure 1: The structure of phosphorochalcogenate oligodeoxynucleotides. B=nucleotide base (A, C, G, or T). Phosphoroselenoate oligodeoxynucleotides are formed when X=Se. The oxygen, sulfur and selenium atoms each bear a negative charge which may be partially delocalized.

Applied Biosystems (ABI, Foster City, CA) hydrogen phosphonate method with standard automatic synthesizer technology to construct the molecular backbone. This approach leaves all the phosphorus atoms in the synthetic oligomer in the P(III) oxidation state. Substitution of a selenizing reagent in the oxidation step provides a potential approach to these compounds. Here we describe the synthesis, some of the physico-chemical properties, observations in translation systems and the anti-HIV assay results for phosphoroselenoate oligomers.

MATERIALS AND METHODS

Synthesis of phosphoroselenoate oligodeoxynucleotides: Potassium selenocyanate was used as the selenizing reagent: A KSeCN-saturated solution of 95% pyridine/5% triethylamine was made by heating the mixture with excess KSeCN at 60°C and allowing it to slowly cool to room temperature (14). After the conventional hydrogen phosphonate synthesis of the oligomer was completed, the 1 μM column was washed several times with acetonitrile and then removed from the ABI synthesizer. About 1 ml of the selenizing reagent was passed through the column and then discarded. The column was then refilled with the selenizing reagent, making sure that air was excluded, and kept at room temperature in the dark for 3−4 days. Every 3−4 hours during the daytime, fresher reagent was introduced into the column from the reservoirs held in the syringes attached to the column openings. Reverse phase HPLC on a PRP-1 column was accomplished in 0.1M triethylammonium acetate buffer (pH 7)-acetonitrile (20%, increase to 40% at 20 min) (15). Then, a stream of nitrogen was passed over the sample to evaporate the acetonitrile. Before drying in vacuo, 20 μl of triethylamine were added. (Failure to do this led to red streaking of the collected solids, presumably due to extruded elemental selenium).

Detritylation of phosphoroselenoate homo-oligomers of thymidine was accomplished by standard procedures. Detritylation of the homo-oligomers of cytidine (phosphorothioate or selenoate), however, is complicated by their insolubility in 3% acetic acid. This may lead to incomplete detritylation. Instead, detritylation was accomplished by layering ca.

150 μl of a 3% solution of dichloroacetic acid/dichloromethane (supplied by ABI) over the collected solid. The mixture immediately turned orange-red, and was vortexed gently for 45 seconds. The solvent was evaporated in a stream of dry nitrogen, and extracted with 150 μl of ethyl acetate. The residual ethyl acetate was evaporated in a stream of dry nitrogen, and the solid dissolved in 1M NaCl. In order to solubilize the oligomer, ammonia vapor (**not** aqueous ammonia) that had been drawn up into a Pasteur pipette was introduced by compressing the pipette bulb 3−4 times. This solubilized the oligomer, and the product was precipitated by the addition of 3.5 volumes of ethanol. Detritylation was 100% complete, as shown by HPLC. However, the yield of 28-mer, after ethanol/1 M NaCl precipitation was only about 5−10%. The yield of 14-mers was about 40%. Samples were stored either as solids or frozen in aqueous solution at −20° C in the absence of light.

Phosphoroselenoate derivatives of oligo(dT), oligo(dC) and of oligonucleotides complementary to either the rabbit β-globin mRNA or part of the HIV genome were prepared using this procedure.

Melting temperatures: Poly(rA) and poly(rI) were obtained from Pharmacia. All optical measurements were made on a Shimadzu UV-160 recording spectrophotometer coupled to a CPS Controller thermostat. Values of absorbance were recorded at 260 nm in 10 mM sodium cacodylate/140 mM NaCl buffer (pH 7.0). Duplexes could be formed in 1:1 mixtures of phosphoroselenoate with its complement. After heating at 75°C for about 10 minutes, samples were allowed to reanneal.

Data analysis: Data obtained from melting curves were analyzed using the MLAB program on the DEC PDP10 computer of the NIH Computer Center. A sigmoid curve of the form

$$OD(T) = \epsilon_A/(1+k) + \epsilon_B/(1-k) \qquad \text{(Equation 1)}$$

where $k = \exp[\Delta H(T-Tm)/(RTm^2)]$, and ΔH is the van't Hoff enthalpy, was fitted to the melting curves (normalized absorbtion vs. temperature; details are given in reference 16).

Electrophoretic analysis of oligonucleotides: 20 pmoles of O-d, S-d and Se-dC$_{28}$ were 3' end-labeled with terminal deoxynucleotidyl transferase by Lofstrand Laboratories. About 10^5 cpm were loaded onto a 15% polyacrylamide/6M urea gel and run at 25 mA for about 3−4 hours. Autoradiography was accomplished overnight at −80°C.

Cell-free translation of rabbit β-globin mRNA: Rabbit reticulocyte lysate and wheat germ extract were purchased from Genofit (Geneva). Oligomer was added at the desired concentration to the translation mixture containing amino acids (including 15μCi of [^{35}S]-methionine, Amersham) and 50μg of rabbit globin mRNA. The rabbit β-globin mRNA concentration was 3.9 and 4.7 nM in the wheat germ and in the reticulocyte lysate, respectively. Translation was allowed to proceed either for 1h at 25°C (wheat germ) or for 45 min. at 30°C (reticulocyte lysate). Labelled proteins were then analysed on 15% polyacrylamide gel containing 0.15% Bis acrylamide, 8 mM Triton X100, 6M urea and 5% acetic acid (17).

Translation in Xenopus oocytes: Full grown stage 6 oocytes were selected after defolliculization of the whole ovary by collagenase treatment routinely performed in the laboratory of Dr. Ozon (Université Paris VI). Oocytes were maintained in modified Barth's saline at 18°C (18) until microinjection with 80 nl of a 1/1 (v/v) mixture of globin mRNA

Table 1. Retention Times (min) of $X\text{-}dT_n$ on PRP1 Reversed Phase HPLC

Oligo	X = O	X = S	X = Se
dT_{14}	17.6	21.0	22.6
		22.4	23.7
dT_{28}	16.1	19.9	21.3
	16.9	21.3	22.3
dC_{14}	14.6	18.0	18.7
dC_{28}	13.2	16.3	17.3

(50 μg/ml) and oligomer dissolved in sterile water. Assuming a free diffusion compartment of 0.5 μl in the oocyte, the final intracellular concentration of β-globin mRNA is about 16 nM. Ten hours after injection, oocytes were incubated in the presence of $50-100\mu$Ci [^{35}S]-methionine for 6 hours, homogeneized in 20μl (per oocyte) of 20mM Tris, HCl buffer pH 7.6 containing 0.1 M NaCl, 1% Triton X100 and 1mM PMSF (19). This homogenate was analysed by electrophoresis on a 13.5% polyacrylamide/SDS gel.

Translation in intact rabbit reticulocytes: Reticulocytes were obtained on day 7 from an adult New Zealand white rabbit which received daily (from day 1 to 5) subcutaneous injection (0.5 ml/kg) of 1% phenylhydrazine in neutralized aqueous solution. The cells were collected by low speed centrifugation (2000 rpm for 5 min.), washed 3 times with 5 volumes of phosphate buffered saline (PBS) buffer and resuspended at 6×10^9 cells/ml in Dulbecco's growth medium, containing 10% fetal calf serum. Reticulocytes were incubated during 1 hour at 37°C in the presence of the desired oligonucleotide, rinsed by 10 volumes of PBS and resuspended in an equal volume of fresh Eagle medium lacking methionine, in the absence of oligonucleotide. Cells were incubated for 1 more hour at 37°C in the presence of 10 μCi of [^{35}S]-methionine. During the incubation the reticulocytes were kept in suspension by continuous rotation. After lysis by addition of
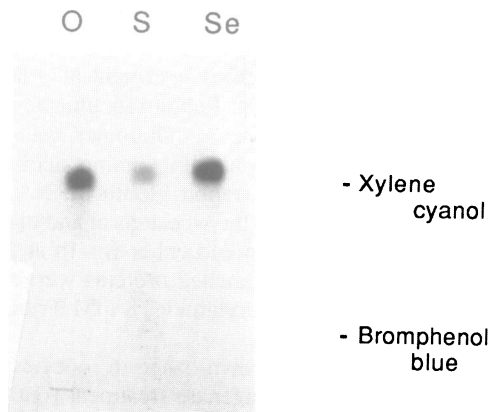

Figure 2: Autoradiogram of a 6M urea/15% polyacrylamide gel containing about 10^5 cpm of ^{32}P 3' end-labeled O-, S-, and Se-dC$_{28}$. The location of xylene cyanol, which migrates at the same rate as a 28-mer, and of bromophenol blue (8-mer) are indicated.

1 volume of 0.2% SDS, proteins were analyzed by gel electrophoresis as described for experiments in the reticulocyte lysate (see above).

Inhibition of the HIV cytopathic effect: For the assessment of anti-HIV activity, the cytopathic effect inhibition assay reported previously was employed (6). Briefly, the immortalized T-cell line, ATH8, were exposed to 500 HTLV-III$_B$ particles for one hour and then incubated in the presence of the phosphoroselenoate oligonucleotides. On day 7 after HIV exposure, viable cells were counted by the dye exclusion method.

RESULTS:

Analysis and physicochemical properties of phosphoroselenoate oligomers: The phosphoroselenoate derivatives were analysed by HPLC and ³¹P NMR spectroscopy. The
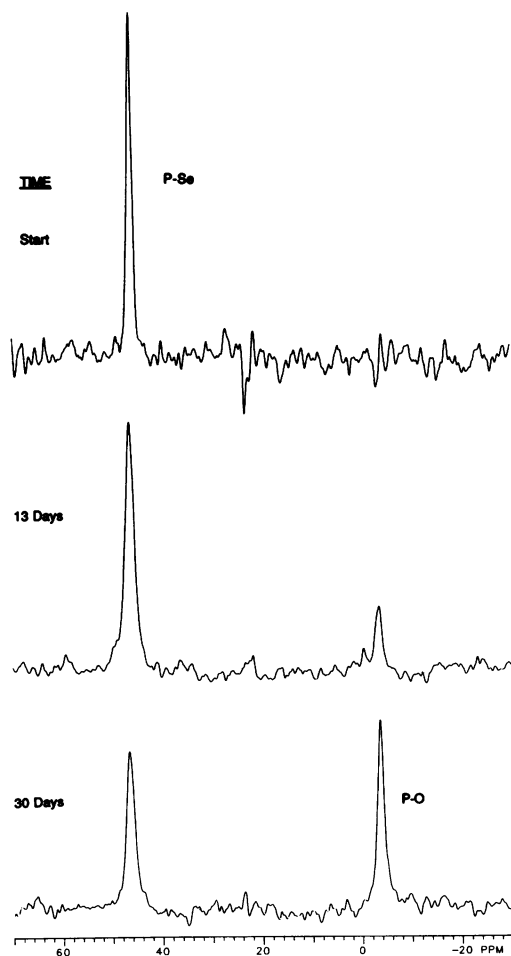

Figure 3: ³¹P NMR spectrum of Se-dC$_{28}$, 0.1 mM in D$_2$O. The downfield peak at 46.69 ppm corresponds to the PSe resonance. The increasing PO resonance with time is indicative of 'washout' of selenium from the molecule.

HPLC elution characteristics of phosphorochalcogenoate oligonucleotides are presented in Table 1. Phosphoroselenoate derivatives elute at slightly longer retention times than do phosphorothioate analogues, which, in turn, elute at still longer times than normal oligomers (20). However, this difference in polarity is not reflected in the autoradiogram derived from a polyacrylamide/urea gel (Figure 2), where it can be seen that the oxygen, sulfur and selenium analogues of dC_{28} all have the same migration pattern and appear essentially as single bands. The presence of two slow-eluting HPLC peaks containing full-length homopolymer thymidine phosphorothioate oligomer has been ascribed (20) to slight retention differences between the 5' terminal Rp and Sp forms. This pattern is also seen in the HPLC traces of the oligothymidine phosphoroselenoates (Table 1), and for both classes of phosphorus-substituted oligomer, the peaks are of equal area. However, the finding that the achiral $O\text{-}dT_{28}$ also appears as two poorly defined peaks under these elution conditions (PRP-1, reversed phase) may indicate that factors other than just the interaction between the column and the 5' phosphate group contribute to the elution characteristics. ^{31}P NMR spectra at 162 MHz of a 28-mer phosphoroselenoate ($Se\text{-}dC_{28}$, 0.1 mM in D_2O) showed a peak at 46.69 ppm downfield from 1% trimethyl phosphate (Figure 3). A small peak at ca. 4 ppm corresponded to less than 1% phosphate. By day 8, this peak had risen to about 10% of the starting material, and by 30 days, the two peaks were roughly equivalent (PSe, 44%; PO, 56%; Figure 3).

Melting curves of duplexes formed by poly(rA) and either unmodified dT_{28} or phosphorochalcogenoate analogs are shown in Figure 4. The presence of a two-state
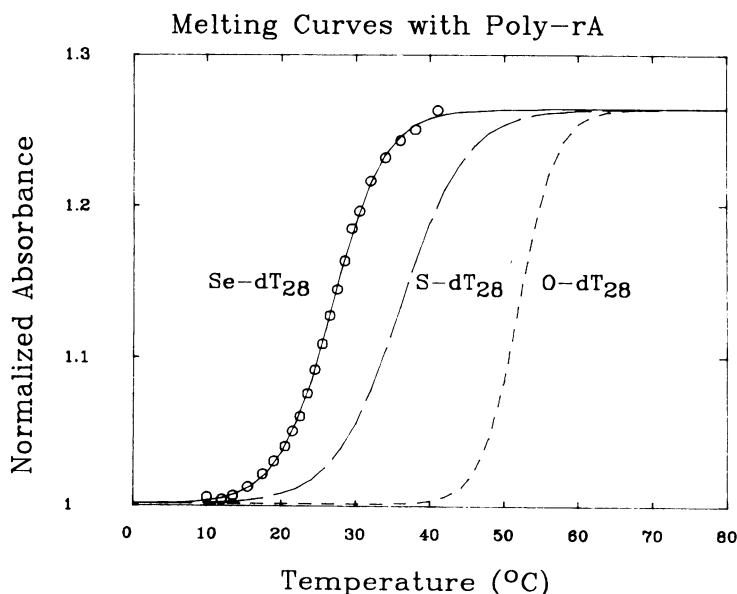

Figure 4: Melting curves of $(dT)_n$ with poly(rA). Absorbance at 260 nm was normalized and fitted using the MLAB program to a signoidal curve shown in equation 1. Measurements were performed in 10 mM Na cacodylate/140 mM NaCl (pH 7.0). Open symbols are phosphoroselenoate; the curve to the right is phosphorothioate, and the remaining curve is normal DNA. The data for the PS and PO oligomers are taken from ref. 15. See Table 2 legend for oligomer concentrations.

transition may be reliably inferred from the ability of our data to fit equation 1. Furthermore, no biphasic melting curves were seen under the concentration conditions used thus making the possibility of triple helix formation unlikely (21). The stability of the complexes increased in the order PSe < PS < PO. This holds true for shorter oligonucleotide sequences (Table 2) : under our conditions (150 mM Na$^+$), the ΔTm between melting temperatures obtained for unmodified oligomers and phosphoroselenoate analogues were 25 and 30°C for dT$_{28}$ and dT$_{21}$, respectively. The melting curves were broader and flatter for phosphoroselenoate oligomers implying increased local melting at any given temperature and diminished cooperativity : all phosphoroselenoate oligomers tested led to markedly decreased values of ΔH as compared to homologous unmodified oligomers (Table 2).

We have also compared the 'melting' of single-stranded phosphorochalcogenoate homopolymers of cytidine at neutral pH. Solutions of these materials showed increasing UV absorption as temperature increased, and this is ascribable to unstacking of the cytidine bases and the formation of a random coil (22). The increasing substituent size and decreasing electronegativity as the chalcogen row is descended seems to have little effect on this transition (Table 2). However, because the Tm of Se-dC$_{28}$/poly(rA) duplexes occurs in the same temperature range as the transition between stacked and unstacked forms of single-stranded Se-dC$_{28}$, the value of the former as given in Table 2 must remain in some doubt. A similar problem exists for Se-dC$_{14}$. It should be noted that single-stranded Se-dT$_{28}$,

Table 2. Melting temperatures Tm (°C) and enthalpies ΔH (kcal/mole) of substituted and unsubstituted oligonucleotides with polyribonucleotides in 10 mM Na cacodylate/140 mM NaCl (pH 7)§

Oligo	Substituent	Polymer	Tm	ΔH
dT$_{14}$	O	poly-rA	39	57
	S	poly-rA	20	22
	Se	poly-rA	7	
dT$_{21}$	O	poly-rA	48	75
	Se	poly-rA	18	56
dT$_{28}$	O	poly-rA	52	84
	S	poly-rA	36	43
	Se	poly-rA	27	50
dC$_{14}$	Se	none	8	26
		poly-rI	11*	44
dC$_{15}$	O	poly-rI	29	83
	S	poly-rI	20	85
dC$_{28}$	O	none	24.2	114
		poly-rI	41	
	S	none	24.5	90
		poly-rI	31	
	Se	none	23	50
		poly-rI	23*	47

* Uncorrected for the hyperchromicity of the single strand
§ The approximate concentrations of the oligodeoxynucleotides were: 28-mers; 5.0 μM: 21-mers; 3.75 μM: 14- and 15-mers; 2.5 μM.

however, does not base-stack in a similar manner to the cytidine homopolymers; thus Tm for the duplexes are more accurately determined.

Translation in cell-free systems: Translation of rabbit globin mRNA in cell-free extracts (wheat germ extract or rabbit reticulocyte lysate) gives rise to two bands corresponding to the α- and β-chains. We performed two sets of experiments using either a non-complementary phosphoroselenoate oligomer, Se-dC_{28}, or a 17-mer (17-PSe-as) whose sequence ($^5{}'CACCAACTTCTTCCACA^3{}'$) is complementary of nucleotides 113 to 129 of the b-globin mRNA. This region, located in the coding sequence, was previously targeted with other oligonucleotide analogs (4, 23). We also synthesized the sense oligomer (17-PSe-s; $^5{}'TGTGGAAGAAGTTGGTG$ $^3{}'$).

Both the sense (17-PSe-s) and antisense (17-PSe-as) compounds were assayed in wheat germ extract (Figure 5). Translation of β-globin mRNA was blocked by the antisense oligomer at concentrations below $1\mu M$; at these concentrations 17-PSe-as did not affect the translation of the α-globin mRNA. The sense analogue, which was complementary to none of the rabbit globin mRNAs did not induce such a decrease of protein synthesis under these conditions. These results indicate that the observed inhibition of β-globin synthesis in the presence of 17-PSe-as depended on the formation of a mRNA/oligonucleotide complex. However at higher concentrations of 17-PSe-as ($>2\mu M$)
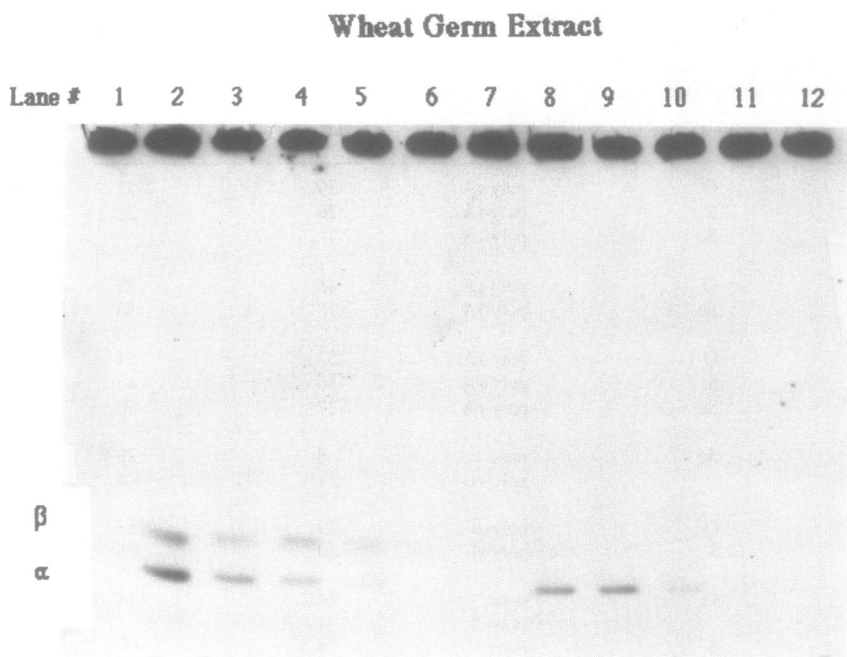

Figure 5: The effect of sense (17-PSe-s) and antisense (17-PSe-as) phosphoroselenoate oligonucleotides on the translation of rabbit globin mRNA in wheat germ extract. The sequence of the oligomers is as defined in the text. Autoradiograph of ^{35}S-labelled proteins analyzed on a 12.5% polyacrylamide/acetic acid gel (see Materials and Methods). Lanes 2—7 correspond to proteins synthesized in the presence of 17-PSe-s at a concentration of 0, 1, 2, 5, 10 and 15 mM, respectively. Lanes 8—12 correspond to samples with 17-PSe-as at a concentration of 1, 2, 5, 10 and 15 μM, respectively. Lane 1 contains no added globin mRNA.

both α- and β-globin synthesis were affected. As a computer search did not reveal the presence of a complementary sequence with a match above 70% on the α-message this should be ascribed to a non-specific effect on translation. Moreover both the sense and the antisense derivatives were inhibitory by about 80% at 5μM (Figure 5). Also relevant is the fact that Se-dC$_{28}$, the phosphoroselenoate analogue of dC$_{28}$, prevented rabbit globin mRNA translation : control levels of protein synthesis are not achieved at concentrations above 125 nM (data not shown).

In contrast to the situation in wheat germ, we were not able to detect a concentration window in which translation of only the β-globin mRNA was blocked by 17-PSe-as in the rabbit reticulocyte lysate system. Above a concentration of about 2 μM α- and β-globin synthesis were decreased (data not shown). Moreover a dose dependent inhibition was induced by both phosphoroselenoate 17-mers. Therefore oligophosphoroselenoate are non-sequence specific inhibitors of mRNA translation in the reticulocyte lysate.

Translation in Xenopus oocytes: β-globin is efficiently synthesized in *Xenopus* oocytes following micro-injection of mRNA (Figure 6; lanes 1 & 2). Upon co-injection of 17-PSe-as the synthesis of rabbit β-globin was reduced; the translation of the target mRNA was almost abolished at an intra-oocyte concentration of 8 μM whereas it was unaffected by 17-PSe-s (Figure 6; lanes 3 & 4). At this concentration, the synthesis of endogenous *Xenopus* proteins was not diminished. This sequence-specific inhibition was dose dependent, 50% inhibition being achieved at an internal concentration of about 200 nM. Moreover the inhibition was still observed when the injection of 17-PSe-as was delayed 8 hours following the injection
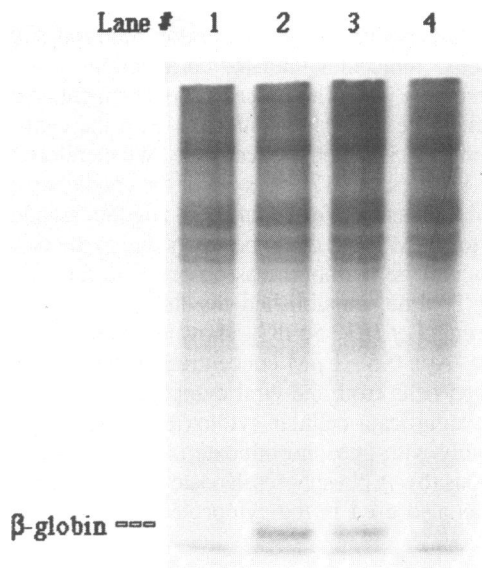

Figure 6: The effects of 17-PSe-s (sense) and 17-PSe-as (antisense) on the synthesis of rabbit β-globin in micro-injected *Xenopus* oocytes. The position of β-globin is indicated by the three bars. ^{35}S-methionine labeling was accomplished from 10 to 16 hours after injection. Lane 1 corresponds to non-injected ocytes. In lane 2 no oligomer was injected. Lanes 3 and 4 contain the sense and antisense compounds, respectively at a concentration of 8 μM. (See Materials and Methods section and ref. 6 for experimental conditions).
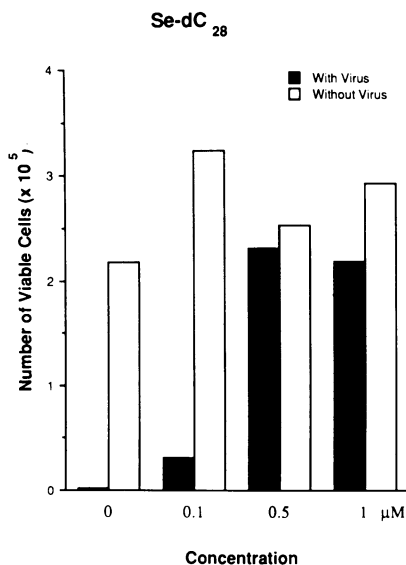
Se-dC $_{28}$

Figure 7: The anti-HIV activity of Se-dC$_{28}$. Filled columns show cellular viability using cells exposed to virus, and open columns show viability in the absence of virus.

of the message; under these conditions 50% inhibition required 800 nM oligonucleotide, i.e. 4 times more antisense molecules than for co-injection.

Effect on intact rabbit reticulocytes: The successful experiments performed with injected cells suggested that it might be possible to interfere with the expression of the β-globin gene in its natural recipient cell, i.e. rabbit reticulocyte. We therefore incubated reticulocytes in the presence of either 17-PSe-as or 17-PSe-s under the conditions described in 'Materials and Methods'. We failed to detect any effect of these oligonucleotides on protein synthesis at any concentration up to 30μM. This could be partly due to the fact that these derivatives are trapped into the reticulocyte membrane as observed in the case of phosphorothioate analogues (Boiziau and Toulmé, unpublished results).

Effect on the cytopathic effect of HIV: Se-dC$_{28}$ showed potent antiviral activity against the cytopathic effect of HIV. At a 0.5−1 μM concentration, the oligomer almost completely protected the ATH8 target cells from the viral cytopathic effect (Figure 7). However, at a 10 μM concentration, significant cellular cytotoxicity was observed (data not shown). This compares unfavorably with phosphorothioate oligomers, which are essentially non-toxic at these concentrations (6). A phosphoroselenoate 28-mer complementary to a sequence of the HIV *rev* gene was also used in the cytoprotection assay and gave similar results (not shown).

DISCUSSION

We have synthesized phosphoroselenoate analogs of oligodeoxynucleotides, anticipating antisense compounds of higher efficiency and lower toxicity than the chemically modified oligonucleotides presently available (1−3). We took advantage of what was known about phosphorothioate oligomers. Some of the interesting differences between phosphate and

thiophosphate groups has been pointed out by Saenger (24). These include a longer PS than PO bond (1.85 vs 1.61 Å in phosphorus oxysulfide (25), and a larger Van der Waals radius for sulfur (1.85 vs 1.40 Å). These trends are more pronounced for selenium, and lead to an incrementally increased Van der Waals radius (2.00 Å), and longer PSe bond. This contributes to decreasing hydrophilicity in the order O > S > Se > > Te. Due to the presence of a bulkier substituent the S-P-O bond angle is greater than the O-P-O bond angle (26), a trend that would be exacerbated with a Se-P-O linkage. This in turn would cause distortions in the spatial relationships of the sugars and their attached bases. However this apparently does not affect intramolecular stacking as indicated by the thermal transitions for the single-stranded homopolymers of cytidine which are almost independent of the substituent on phosphorus.

On the basis of these facts, the observed decrease in hydrophilicity and diminution of Tm relative to phosphorothioate and normal oligonucleotides is predictable. Phosphoroselenoates, as well as phosphorothioates are chiral at phosphorus. Since the automated synthesis is not stereospecific, our products are complex mixtures of enantiomers. Eckstein has presented evidence that if enantiomerically pure phosphorothioate oligomers are used, the value of Tm may approach that of normal oligodeoxynucleotides (27, 28). Similarly, it may be speculated that if optically pure phosphoroselenoate oligos were to be synthesized, their behavior might more closely approximate that of phosphate oligonucleotides.

Our proof of the synthesis of phosphoroselenoate oligodeoxynucleotides is based on two pieces of data. First, the ^{31}P-NMR spectrum showed only one peak, and this peak corresponded to a PSe resonance (Figure 3). Second, the polyacrylamide-urea sizing gel of the ^{32}P-cytidine end-labeled product revealed a single band consistent in length with a 28-mer. If the selenization reaction was not complete at all phosphorus atoms, the reaction conditions would have caused chain cleavage and the formation of a ladder on the gel. In our hands, as determined by NMR spectroscopy, the 'wash out' of selenium from these compounds in aqueous solution proceeded with a half-life of about one month, although after one week, about 10% of the selenium had been lost. Problems with selenium 'wash out' noted by other investigators may have been due to sample contamination by trace metals or by photochemical oxidation (13).

A 17-mer phosphoroselenoate analogue specifically inhibited translation of the target mRNA in wheat germ extract and in injected *Xenopus* oocytes. It was previously reported that these two expression systems contain an RNase-H activity (23). These enzymes which cleave the RNA part of a DNA/RNA hybrid were shown to play a key role in the inhibition of polypeptide elongation by antisense oligodeoxynucleotides (23, 29−31). Conversely, α-oligonucleotides and oligomethylphosphonates that do not elicit RNase-H do not prevent translation (4). Our results suggest that the oligonucleotide 17-PSe-as, which is complementary to the coding region of the β-globin mRNA, form an hybrid which is degraded by RNase-H. This hypothesis about the mechanism by which the antisense phosphoroselenoate oligomer works is further supported by the fact that this oligonucleotide had no selective inhibitory effect in rabbit reticulocyte lysate; in fact this cell-free extract has a poor RNase-H content (29).

The results that we obtained with these derivatives should be discussed with respect to the behaviour of the phosphorothioate (PS) analogues. In a previous study it was demonstrated that 17-PS, the homologous phosphorothioate 17-mer targeted to the region 113−129, was a very efficient translation inhibitor of β-globin synthesis in wheat germ

extract and in oocytes (4). It was also shown that this derivative elicited RNase-H activity. However, 17-PS was by far the better inhibitor of protein synthesis than 17-PSe: β-globin synthesis in injected oocytes was reduced by 50% in the presence of 200 nM and 3 nM antisense 17-PSe and 17-PS, respectively.

Phosphorothioate and phosphoroselenoate oligomers also share the ability to act as non-sequence specific inhibitors of protein translation in cell free systems (4). Phosphorothioate oligonucleotides are capable of binding irreversibly to ribosomes (Stein and Neckers, unpublished results). Presumably, this is a property shared by phosphoroselenoates, and the cellular cytoxicity seen in the HIV experiments may be reflective of this.

At concentrations in the low micromolar range, Se-dC$_{28}$ is cytoprotective to de novo infected ATH8 cells, as is its phosphorothioate congener. Majumdar, et al. have previously shown that phosphorothioate oligos inhibit reverse transcriptase III by competitively inhibiting template primer binding (32). It is possible that this is the origin of the cytoprotective effect. Se-dC$_{28}$ may well be acting by a similar mechanism.

Finally, in order to fully characterize phosphoroselenoate oligomers, experiments designed to evaluate DNAse resistance, DNA/RNA complex stability, and the ability to elicit RNAse H activity would be of interest. Completion of such work awaits the solution of technical production problems. While the organic chemistry of selenium exhibits a unique richness and diversity (33), phosphoroselenoate oligonucleotides do not appear, at this point, to be as advantageous either as potential anti-HIV materials, or as sequence specific inhibitors of gene expression, as the far more readily available phosphorothioates.

Abbreviations PSe— phosphoroselenoate; PS— phosphorothioate; PO— phosphate analogues of oligodeoxynucleotides. O-, S- and Se-dC$_{28}$: phosphate, phosphorothioate and phosphoroselenoate analogs of dC$_{28}$.

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