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Oxidative damage on RNA mediated by cationic metalloporphyrin–antisense oligonucleotides conjugates †

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A manganese cationic porphyrin covalently linked to the 5′ end of an antisense oligonucleotide was shown to mediate sequence-specific oxidative lesions on a mRNA target when activated by $KHSO_5$. This manganese porphyrin cleaver did not induce direct RNA strand breaks but promoted the arrest of reverse transcription due to oxidative damage on bases of the RNA template.

Since the pioneering work of Zamecnik and Stephenson[1] on the use of antisense oligonucleotides for the regulation of gene expression, a large number of studies have been performed in this field targeting mRNA (antisense strategy) or DNA (antigene strategy).[2–8] Many different problems have limited the development of antisense therapy (oligonucleotide stability, sequence specificity, cellular uptake *etc.*),[9–11] but recent encouraging results have been obtained on animals, *e.g.* in the treatment of asthma with phosphorothioate antisense oligonucleotides.[12]

Oligodeoxynucleotides (ODNs) modified with a RNA cleaver might be useful in cellular systems deficient in RNase H[13] or when using modified oligonucleotides which are not substrates of RNase H because of structural modifications (α-ODNs or 2′-methoxy ODNs).

Our group has previously shown that the covalent linkage of a nucleic acid cleaver based on a cationic manganese porphyrin motif, manganese(III) *meso*-tris(4-*N*-methylpyridinio)porphyrin [Mn(tmpyp)], to the 5′ end of an antisense ODN induced sequence-directed DNA damage on a single- or double-stranded DNA target after activation of the metalloporphyrin core by potassium hydrogenperoxosulfate ($KHSO_5$).[14–18] Such a metal-based cleaver is also able greatly to increase the translation inhibition capacity of an antisense ODN.[19] In this latter case the metalloporphyrin–oligonucleotide conjugates were targeted against the translation initiation codon and also the elongation region of the chloramphenicol acetyltransferase (CAT) messenger RNA. The *in vitro* translation of CAT mRNA was strongly inhibited especially when the metalloporphyrin–oligonucleotide conjugate was activated by $KHSO_5$ before the translation process (for the generation of high-valent oxomanganese species in DNA cleavage, see ref. 20).

In order to elucidate the mechanism involved in this enhanced CAT mRNA translation inhibition by activated metalloporphyrin–ODN conjugates compared to the non-modified ODNs, we decided to perform experiments designed to localize and identify the damage induced by the activation of the metalloporphyrin entity of the antisense conjugates on the target mRNA. We did not observe any direct RNA breaks on the RNA target contrary to the situation observed with DNA,[14–18] but found evidence that reverse transcriptase pauses due to oxidative RNA damage.

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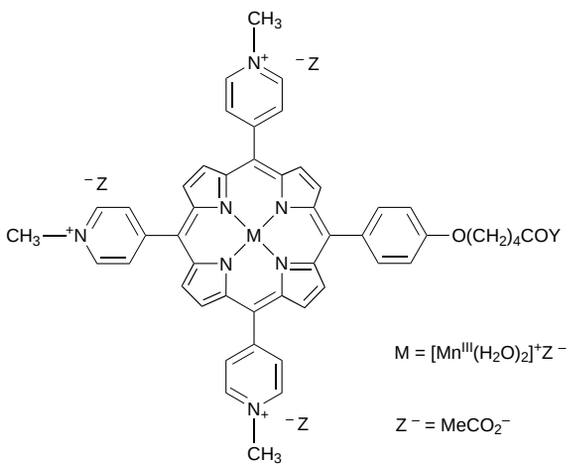

Fig. 1 Structure of the manganese(III) tris(4-*N*-methylpyridinio)-porphyrin precursor and manganese(III) tris(4-*N*-methylpyridinio)-porphyrin–ODN conjugates

Experimental

The enzymes were from Boehringer, (ribo)nucleoside triphosphates, transfer ribonucleic acid, RNasin, [α-^{32}P]UTP and [α-^{32}P]dCTP from Amersham. The compound $KHSO_5$ (Curox®) was from Interox (Belgium) and phosphoramidite precursors from PerSeptive Biosystems (France).

Antisense oligonucleotides

Antisense ODNs were synthesized by standard solid-phase β-cyanoethylphosphoramidite chemistry on a Cyclone Plus DNA Synthesizer from Milligen/Biosearch. The ODNs As-1-OH, AS-2-OH (5′-OH-26-mer) and primer ODN A (5′-OH-18-mer) were purified as described.[19]

Metalloporphyrin–oligonucleotide conjugates AS-1-Mn, S-1-Mn, AS-2-Mn and S-2-Mn (see Fig. 1 for structure) were obtained by functionalization at the 5′ end by a 1,6-diaminohexane linker of the corresponding ODNs as previously described.[19,21,22] These conjugates were equipped with an eight-base mini-loop at the 3′ position in order to inhibit their degradation by 3′-exonucleases (see Fig. 2 for the sequence of the mini-loop; the complementary sequence of these 26-mer

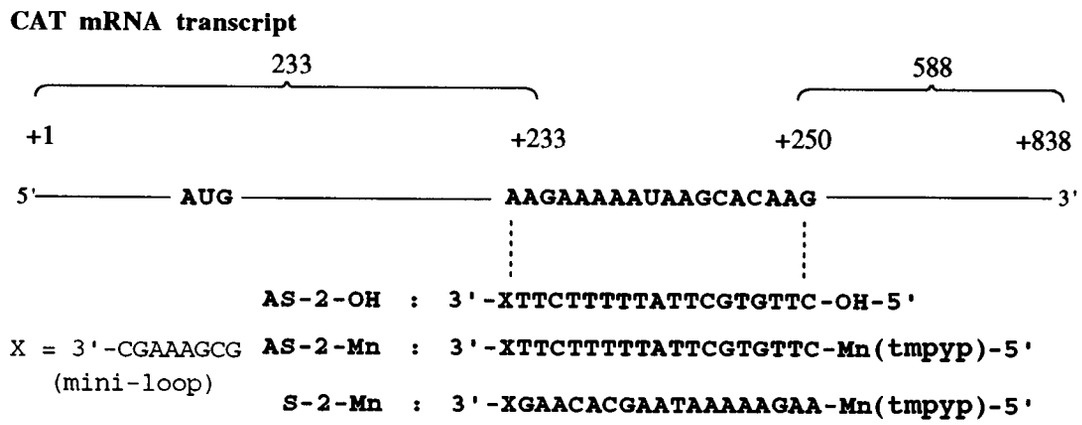

Fig. 2 Antisense oligonucleotides used for the analysis of direct cleavage of the 838-base CAT mRNA. Expected fragments of sequence-specific cleavage of RNA are about 230 and 600 bases long

conjugates corresponds to 18 bases).[17,18] These metalloporphyrin–ODN conjugates were characterized by their UV/VIS data: (i) ODNs, AS-1-OH (5′-OH or 5′-NH_2), ε_{260} = 234×10^3, AS-2-OH, 232×10^3; A, 137×10^3 M^{-1} cm^{-1}; (ii) free metalloporphyrin, $\varepsilon_{468} = 100 \times 10^3$, $\varepsilon_{260} = 30 \times 10^3$ M^{-1} cm^{-1}; (iii) conjugates, observed VIS/UV ratios A_{468}/A_{260} determined by diode-array detector spectra of the HPLC peak of the different conjugates, 0.58 for AS-1-Mn, 0.44 for S-1-Mn, 0.55 for AS-2-Mn and 0.5 for S-2-Mn. Concentrations of conjugate solutions were determined by UV absorbance at 260 nm as for single-stranded ODNs.[23] The oligonucleotide derivatives AS-1-OH, AS-2-OH, ODN A, AS-1-Mn and S-1-Mn have been characterized by electrospray ionization mass spectrometery: the recalculated masses from the spectra were 7873.2 (1.1), 7934.3 (0.5), 5428.0 (0.6), 8829.8 (0.9) and 9093.1 (0.6), respectively (standard deviations in parentheses) and corresponded to the theoretical calculated masses of 7872.2, 7933.2, 5427.6, 8828.3 and 9092.5.

In vitro transcription: synthesis of the 838-nucleotide CAT RNA substrate

The pKSCAT plasmid was kindly provided by Dr. Beatrix Bugler (INSERM U397, Hôpital Rangueil, Toulouse). It contains the chloramphenicol acetyltransferase cDNA sequence (BamH1/Hind III) under the T3 origin of transcription. A 10 μg amount was linearized by 26 units of BamH1 restriction enzyme in 50 μl of 10 mM tris(hydroxymethyl)methylamine (Tris)–HCl buffer pH 8.5, 5 mM $MgCl_2$, 100 mM NaCl and 1 mM 2-sulfanylethanol. The linearized DNA was subjected to phenol–chloroform extraction and precipitated with ethanol in the presence of sodium acetate (0.3 M final concentration). The pellet was then rinsed and dissolved in water (6 μl). The synthesis of the 838-nucleotide RNA substrate (from the +1 position of T3 origin to the BamH1 cleavage site) was then undertaken as follows. The reaction medium (total volume 50 μl) contained 10 μg of linearized plasmid pKSCAT/BamH1, 0.5 mM of the four ribonucleoside triphosphates (ATP, CTP, GTP, UTP) in 40 mM Tris–HCl pH 8, 8 mM $MgCl_2$, 2 mM spermidine trihydrochloride, 25 mM NaCl, 10 mM dithiothreitol, with 80 units of RNasin and 100 units of T3 RNA polymerase. The reaction mixture was incubated for 1.5 h at 37 °C. The linearized plasmid was then digested by RNase-free DNase I (2 μl, 20 units) for 30 min at 37 °C. The RNA was then subjected to a phenol–chloroform extraction and precipitated with ethanol in the presence of LiCl (0.5 M final concentration). The pellet was dissolved in water (100 μl) and quantified by UV spectrometry (assuming that 1 optical density unit is equivalent to 40 μg of RNA). The transcription yielded 340 μg of RNA.

For the preparation of the ^{32}P-labelled 838-nucleotide RNA substrate, the same procedure was followed but in the presence of 0.5 mM of the three ribonucleoside triphosphates (ATP, CTP, GTP), 300 μM of UTP and 10 pmol of [α-^{32}P]UTP. The yield of the labelled RNA was determined by counting, in a β-scintillation counter, 2 μl of this solution after precipitation in the presence of 0.5 mg cm^{-3} transfer ribonucleic acid from baker yeast as carrier RNA and 20% trichloroacetic acid. The transcription yielded approximately 10^{-12} mol of labelled RNA.

Degradation of RNA

For oxidative degradation of RNA, non-labelled CAT RNA (10^{-12} mol) in the presence (or in the absence) of 2×10^{-14} mol of labelled CAT RNA transcript (160 nCi; Ci = 3.7×10^{10} Bq) was incubated with the appropriate antisense ODN or antisense conjugate at various concentrations (100 nM, 500 nM, 1 μM or 5 μM) in a 20 mM phosphate buffer pH 7 (total volume 10 μl). The final concentration of RNA was 100 nM in all experiments. Annealing of complementary sequences was performed by heating samples at 70 °C for 10 min followed by slow cooling to 25 °C and further incubation at 4 °C for 1 h. In the presence of antisense conjugates, the oxidation reaction was then initiated by addition of 1 μl of freshly prepared $KHSO_5$ (100 μM final concentration) and lasted 15 min at 25 °C. The reaction was stopped by addition of 1 μl of 1 M hepes [*N*′-(2-hydroxyethyl)-piperazine-*N*-ethane-2-sulfonic acid] buffer pH 8.

For RNase H cleavage, the RNA/ODN hybridization was performed in 20 mM Tris–HCl buffer pH 7.5, 10 mM $MgCl_2$, 100 mM KCl and 0.1 mM dithiothreitol. Complementary sequences were annealed by heating samples at 70 °C for 10 min followed by slow cooling to 25 °C. One unit of RNase H from *Escherichia coli* was then added and the reaction was incubated at 37 °C for 1 h.

Damaged RNA was then subjected to phenol–chloroform extraction and RNA was precipitated with ethanol in the presence of 0.5 M LiCl. The pellet was dissolved in water (8 μl) and was either directly analysed by polyacrylamide gel electrophoresis (when RNA was labelled) or further subjected to reverse transcription (in the case of non-labelled RNA). For electrophoresis analysis of direct cleavage of labelled RNA, the samples were loaded on a 5% polyacrylamide–7 M urea gel. The gel was revealed by autoradiography with a Kodak BioMax MR film.

Primer extension of RNA by reverse transcription

Non-labelled CAT RNA transcript (10^{-12} mol) previously treated for degradation under the above conditions was allowed to incubate with primer oligonucleotide (150×10^{-12} mol, 15 μM). The RNA/primer hybridization was performed by heating the solution for 10 min at 70 °C followed by slow cooling to 25 °C in a final volume of 10 μl. The final reverse transcription reaction medium consisted of 20 μl of 250 mM Tris–HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$, 50 mM dithiothreitol, contain-

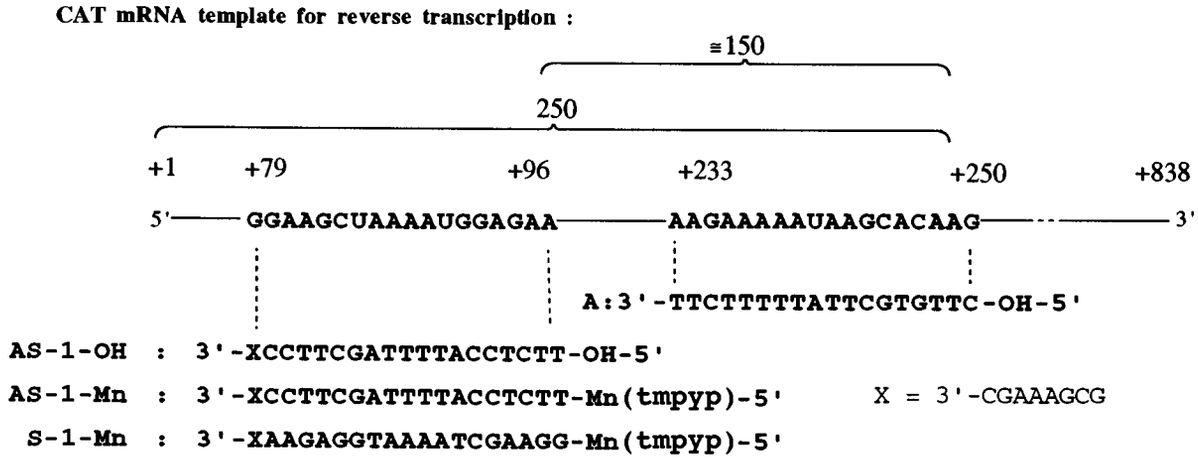

Fig. 3 Antisense oligonucleotides used for the analysis of CAT mRNA damage by reverse transcription. The maximum primer extension of ODN A was a 250-base long cDNA transcript. Antisense-induced aborted extension was expected to give a 150-base long cDNA transcript

ing 5 nmol of each of the four deoxynucleoside triphosphates, 40 units of RNasin, 100 units of murine Moloney Leucemia virus reverse transcriptase (RT) and 1.65 pmol of [α-^{32}P]dCTP. The reaction was allowed to proceed for 1 h at 37 °C and was heated at 90 °C for 5 min to inactivate the enzyme. The reverse transcripts were then subjected to a phenol–chloroform extraction before ethanol precipitation in the presence of 0.3 M sodium acetate pH 5.2. The sample pellets were then dissolved in water and loaded on a 10% polyacrylamide–7 M urea gel. The gel was revealed by autoradiography with a Kodak BioMax film.

Results and Discussion

The pKSCAT/BamH1 linearized plasmid was transcribed by T3 RNA polymerase into an RNA transcript of 838 bases that was used as substrate for the oxidative degradation mediated by antisense oligonucleotides with a metalloporphyrin complex at their 5′ end. Experiments with the corresponding non-modified antisense ODNs in the presence of RNase H were used as controls of sequence-directed cleavage of RNA target. The general structure of the various antisense ODNs used in this study is presented in Fig. 1. The sequences of the carrier ODNs and the location of their hybridization region on the 838-base RNA transcript are presented in Figs. 2 and 3.

Two types of RNA damage mediated by the metalloporphyrin entity of modified oligonucleotides were examined: first, analysis of direct cleavage products of target RNA by gel electrophoresis and secondly analysis of oxidative damage by the method of reverse transcriptase primer extension. Some lesions on the CAT mRNA (especially on the bases) may not lead to strand breaks but may induce enzyme arrests and give rise to aborted cDNA transcripts (for previous examples of detection of RNA damage by primer extensions see refs. 24 and 25).

In both types of analysis the location of either RNA strand breaks or RNA lesions observed with activated metalloporphyrin–ODN conjugates were compared to the those obtained with the corresponding non-activated antisense ODNs and non-modified ODNs but in the presence of RNase H.

Analysis of direct cleavage of the 838-nucleotide labelled RNA

Antisense ODNs used in this study were complementary to the region (+233, +250) of the CAT sequence (see Figs. 1 and 2 for structures). Modified ODNs bearing the Mn(tmpyp) motif at their 5′ end were compared to their 5′-OH analogues in their ability to mediate the degradation of the ^{32}P-labelled 838-base RNA in the presence of RNase H or $KHSO_5$. The results are shown in Fig. 4. In all these experiments, the final RNA concentration was 100 nM and the antisense ODN concentrations varied from 100 nM to 1 μM from left to right, as specified in the legend of Fig. 4. Lane 1 represents the control of the full length RNA of 838 nucleotides and lane 2 the RNA control incubated with 100 μM $KHSO_5$. Incubation of RNA with the unmodified AS-2-OH ODN (lanes 3 to 5) in the presence of RNase H led to sequence-specific cleavage of the RNA strand and generated the two expected fragments of about 600 and 250 nucleotides long (Fig. 2). The same fragments of RNA were found with AS-2-Mn conjugate (lanes 6 to 8) under the same conditions. Contrary to AS-2-OH, AS-2-Mn (lanes 6 and 7) did not elicit total RNase H cleavage at lower concentrations. This result may be due to a lower affinity of AS-2-Mn compared to AS-2-OH. The sense conjugated ODN (lanes 12 to 14) in the presence of RNase H, S-2-Mn, did not show any particular site of cleavage but induced a decrease of the intensity of the full-length RNA at the top of the gel, that might correspond to a disperse unspecific degradation due to partial random hybridization of the ODN. It is known that RNase H is able to cleave RNA on very short duplexes of four-base hybridization.[26,27]

Experiments to promote the oxidative degradation of RNA by metalloporphyrin–ODN conjugates were initiated by $KHSO_5$ at 100 μM final concentration, as described in the Experimental section. When the metalloporphyrin moiety of AS-2-Mn conjugate (lanes 9 to 11) was activated by $KHSO_5$ direct strand cleavage of RNA was not observed. In the same way, when the S-2-Mn control conjugate was annealed to the RNA (lanes 15 to 17) and activated by $KHSO_5$ the 838-base RNA band was unchanged. Moreover the free metalloporphyrin precursor (lanes 18 to 20) associated with $KHSO_5$ had no effect on RNA.

These results pointed out that metalloporphyrin–ODN conjugates activated by $KHSO_5$ were not able to mediate cleavage of RNA even though the ODN entity of these conjugates was annealed to the complementary sequence on RNA as revealed by RNase H cleavage. However in comparison with the results obtained in the oxidative degradation of DNA target by Mn(tmpyp)–ODN conjugates activated by $KHSO_5$,[17,18] we can propose an oxidative degradation of the RNA bases in the vicinity of the metalloporphyrin location without direct strand cleavage. To check this hypothesis we performed primer extension experiments with the CAT RNA by reverse transcriptase enzyme after incubation with Mn(tmpyp)–ODN conjugates in the presence of $KHSO_5$. Oxidative lesions of the bases of the RNA template should promote the arrest (or pause) of the reverse transcription in the polymerization process.

Analysis of oxidative damage mediated by metalloporphyrin–oligonucleotide conjugates on the 838-nucleotide RNA by reverse transcription

Antisense 5′-OH oligonucleotides or antisense metalloporphyrin–ODN conjugates were complementary to the region

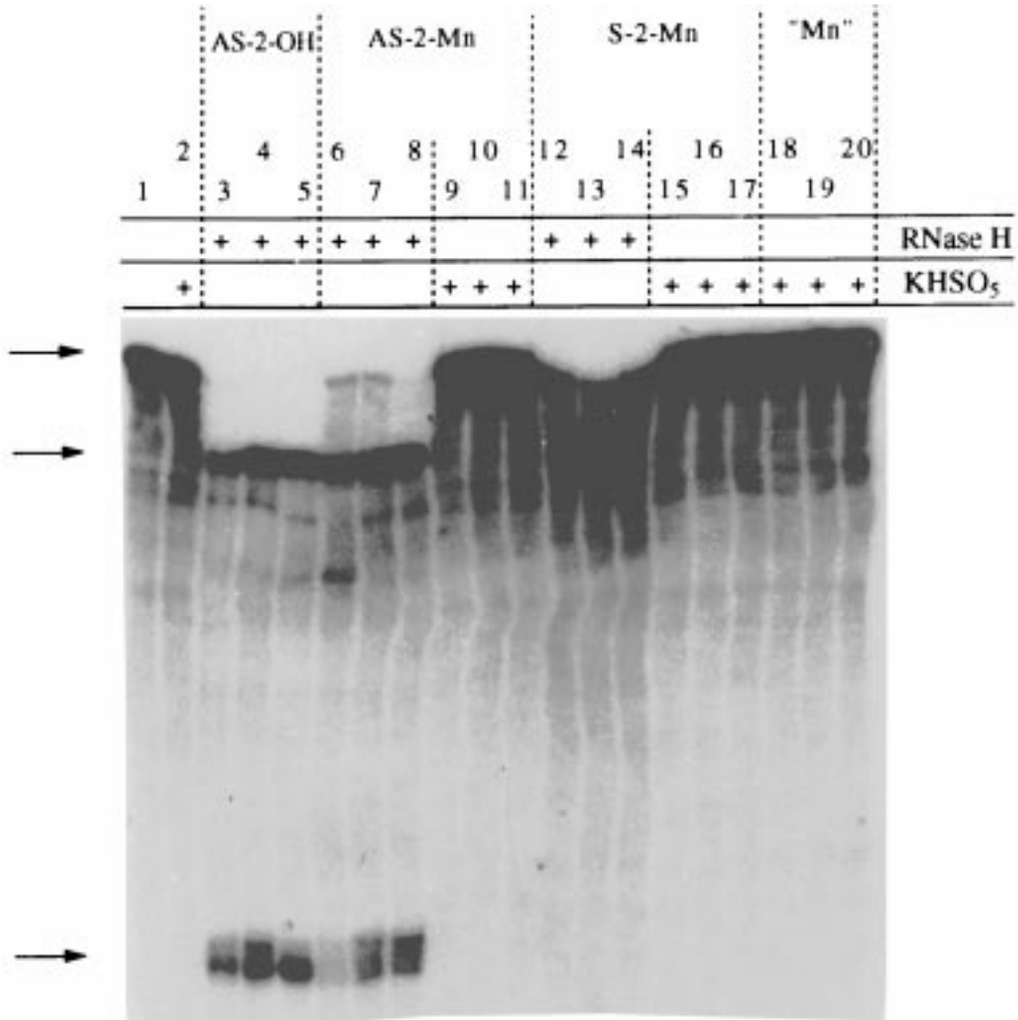

Fig. 4 Analysis of RNA cleavage by polyacrylamide gel electrophoresis. Uniformly labelled 838-base CAT mRNA (100 nM) was annealed to various antisense ODN and allowed to incubate during 15 min at 25 °C with $KHSO_5$ (100 μM) in 20 mM phosphate buffer pH 7 or with one unit of RNase H for 1 h at 37 °C in 20 mM Tris buffer pH 7.5, 10 mM $MgCl_2$, 100 mM KCl, and 0.1 mM dithiothreitol. Lanes: 1, control RNA; 2, control RNA with $KHSO_5$; 3–5, RNA incubated with 100 nM (3), 500 nM (4) or 1 μM (5) of AS-2-OH and subjected to RNase H digestion; 6–11, RNA incubated with 100 nM (6, 9), 500 nM (7, 10) or 1 μM (8, 11) of AS-2-Mn in the presence of RNase H (6–8) or $KHSO_5$ (9–11); 12–17, RNA incubated with 100 nM (12, 15), 500 nM (13, 16) or 1 μM (14, 17) of S-2-Mn in the presence of RNase H (12–14) or $KHSO_5$ (15–17); 18–20, free metalloporphyrin assayed at 100 nM (18), 500 nM (19) or 1 μM (20) in the presence of $KHSO_5$

(+79,+96) of the CAT sequence containing the translation initiation codon of the CAT protein. They were incubated with the non-labelled 838-base RNA substrate in the presence of $KHSO_5$ or RNase H and the resulting non-labelled hybrids of damaged RNA/antisense were then taken as template for the reverse transcription reaction performed with [α-^{32}P]dCTP. The primer ODN (ODN A) for the reverse transcriptase enzyme was complementary to the region (+233,+250) of the CAT sequence. So the full length of the labelled cDNA strand was expected to be 250 nucleotides (see Fig. 3). The samples where the RNA/AS-1 ODN hybrids were subjected to reverse transcription should give cDNA transcripts of about 150 nucleotides long.

The results of the reverse transcription inhibition due to the degradation of RNA in the presence of various antisense ODN are shown in Fig. 5. The RNA concentration was 100 nM and the same protocol as that described for Fig. 4 was used, except that the RNA was taken as a substrate for reverse transcription instead of being directly analysed on polyacrylamide gel. The 150 nucleotides long cDNA transcript was obtained in a control experiment by incubation of the antisense ODN AS-1-OH (5 equivalents with respect to RNA) with CAT RNA in the presence of RNase H (lanes 2 and 3) prior to reverse transcription. Full-length cDNA transcript was observed as in lane 1 in the absence or in the presence of $KHSO_5$ (100 μM final concentration).

These two RNA species are marked by an arrow on Fig. 5 (150 and 250 bases). The small cDNA band observed in lane 1 of Fig. 5 might be due to a second species of cDNA obtained by elongation of primer ODN A hybridized in the +93,+105 zone (secondary hybridization site of ODN A: 9 or 12 nucleotides). This latter cDNA should be about 110 nucleotides long. This secondary site of hybridization of ODN A overlaps with the site of specific hybridization of AS-1. Consequently this cDNA secondary species was absent in the lanes where AS-1-ODN was present, but can be detected in incubations of RNA with free metalloporphyrin (lanes 16–19, Fig. 5) or with S-1-Mn ODN (lanes 20–23). No pauses of reverse transcriptase were observed in control lanes (lanes 16–23) when the RNA was incubated with free metalloporphyrin or S-1-Mn.

Specific arrest of reverse transcriptase polymerization was observed in lanes 4 to 7 with AS-1-OH ODN (respectively 1, 5, 10 or 50 molar equivalents with respect to RNA). This effect was concentration-dependent since the 250-base full length cDNA became less intense when the concentration of antisense ODN increased. This inhibition of reverse transcription is probably due to steric hindrance during enzyme polymerization by the hybridized antisense oligonucleotide. Similar results were

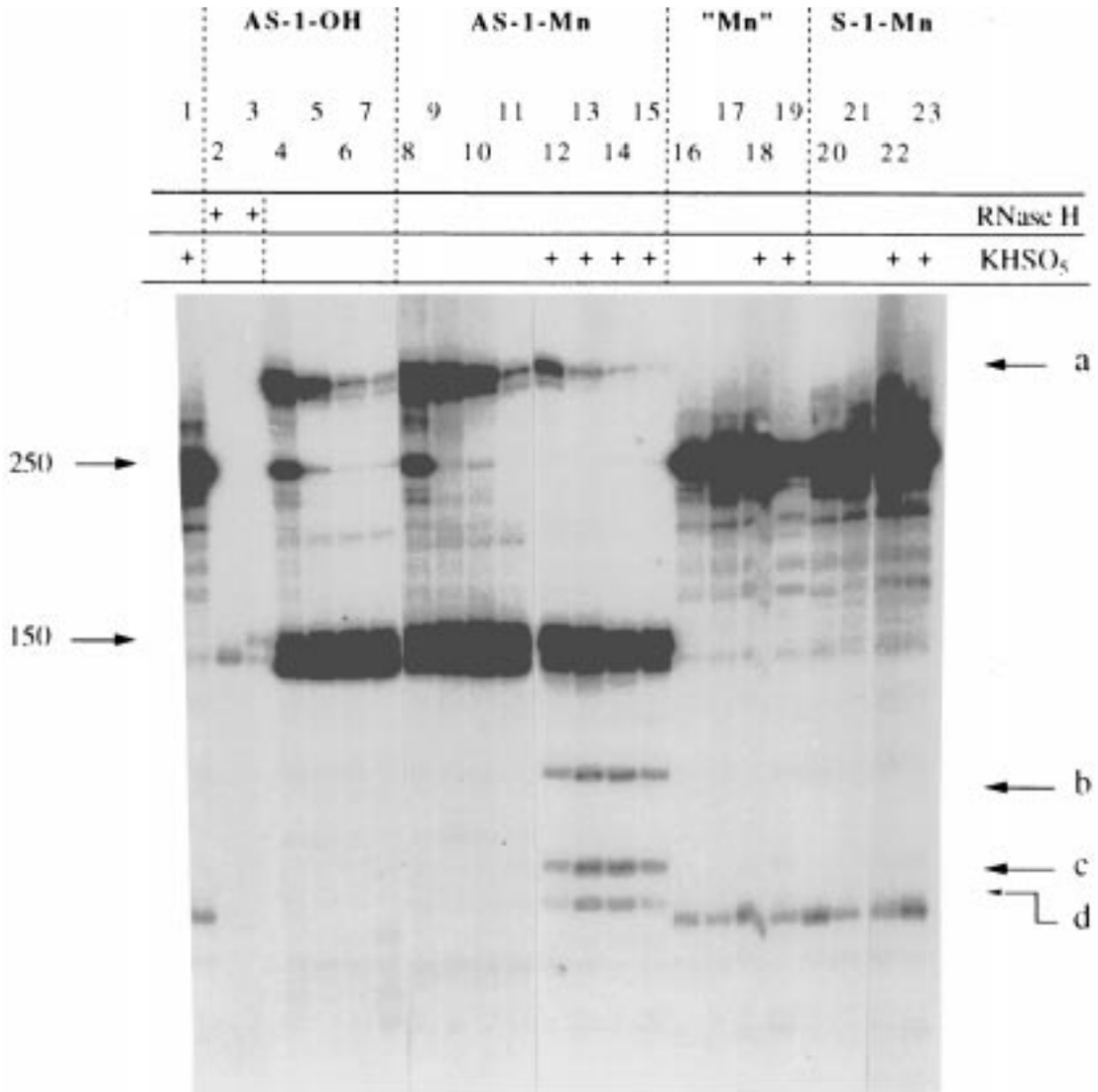

Fig. 5 Analysis of reverse transcription of the 838-base CAT mRNA by polyacrylamide gel electrophoresis. Non-labelled CAT mRNA (100 nM) was annealed to various concentrations of antisense ODNs, treated as in Fig. 4 with $KHSO_5$ or RNase H, precipitated and subjected to reverse transcription in the presence of [α-^{32}P]dCTP. Lanes: 1, cDNA obtained with CAT mRNA incubated with 100 μM $KHSO_5$ before reverse transcription; 2 and 3, incubation with 500 nM of AS-1-OH and RNase H; 4–7, incubation with AS-1-OH at 100 nM (4), 500 nM (5), 1 μM (6) and 5 μM (7); 8–15, incubation with AS-1-Mn at 100 nM (8, 12), 500 nM (9, 13), 1 μM (10, 14) and 5 μM (11, 15) without activation of metalloporphyrin (8–11) and in the presence of $KHSO_5$ (12–15); 16–18, incubation with the free metalloporphyrin at 100 nM (16, 18), 1 μM (17,19) without $KHSO_5$ (16, 17) or with $KHSO_5$ (18, 19); 20–23, incubation with S-1-Mn at 100 nM (20, 22) or 1 μM (21, 23) without $KHSO_5$ (20, 21) or with $KHSO_5$ (22, 23)

obtained with AS-1-Mn (see lanes 8 to 11). The presence of a slow migrating species (band a) in lanes 4–15, with an intensity variation following that of the full-length cDNA transcript, might reflect some particular interactions of the AS-1 sequence with the full-length cDNA in the region between +1 and +79, since this phenomenon was only observed with AS-1-OH. This cannot be a polymerization process using AS-1 as primer and starting after the ODN-A hybridization site since its intensity decreased when the AS-1-OH concentration increased. So this slow migrating species was associated with the full-length cDNA. Whereas only one arrest site of the polymerization process was visualized in lanes 4 to 11 in the presence of AS-1-OH or non-activated AS-1-Mn, the activation of the metalloporphyrin moiety of AS-1-Mn by $KHSO_5$ (lanes 12 to 15) led to three more sites of pause for reverse transcriptase primer extension. The three bands of aborted cDNA synthesis are marked as b, c and d bands on Fig. 5. These three sites of arrest of reverse transcriptase were probably due to metalloporphyrin-mediated oxidation of RNA bases on secondary sites of hybridization of the AS-1-Mn oligonucleotide. These sites of pause are not uniquely due to the steric hindrance of some secondary hybridization of AS-1-Mn ODN since they are absent in lanes 4 to 11 of Fig. 5. So the enzyme was able to displace these three non-specific hybridizations in experiments corresponding to lanes 4 to 11 even if it was not able to displace the 18 base-specific hybridization of AS-1 sequence. We found three sites of partial complementarity between the AS-1 sequence and CAT RNA. One was located at the +132,+136 site and two others in the +142,+148 and +152,+160 zone. The first should lead to a 115-base cDNA (band b) and the second to about 100 to 90 bases (bands c and d on Fig. 5). They migrate more slowly than the 110-base secondary cDNA but this latter may have altered migration properties due to a hairpin structure.

These experiments did not allow the analysis of the oxidative degradation at the site of specific hybridization of AS-1-Mn because the reverse transcriptase was stopped by the antisense oligonucleotide when it was still hybridized on its target sequence. One can just note that the total amount of full-length cDNA (250-base long band plus band a) is less important in lanes 12 to 15 compared to 4 to 11. However, the result of significant pauses of reverse transcriptase at secondary sites of hybridization of antisense ODN carrying a metalloporphyrin moiety activated by $KHSO_5$ is highly encouraging. The next

step will be to perform experiments with digestion of the antisense oligonucleotides by DNase before the RNA is subjected to primer extension in order to show that unless the metalloporphyrin of AS-1-Mn is activated by $KHSO_5$ the reverse transcriptase is able to polymerise over the site of hybridization of non-reactive AS-1-OH or AS-1-Mn ODNs and complete the reverse transcription of the template RNA.

Conclusion

These studies have further established that the oxidative chemistry of metalloporphyrin–antisense oligonucleotide conjugates can be directed onto RNA sequences targeted by the carrier oligonucleotide. The lesions mediated by the activated metalloporphyrin moiety onto the RNA target were not direct breaks but base oxidations under the experimental conditions used in the present work. Owing to the high reactivity of the cationic metalloporphyrin entity of conjugated ODNs, non-specific hybridization sites can be revealed and may induce non-specific effects of this reactive antisense ODN. This feature is reminiscent of the reactivity of RNase H on partially matched hybridization sites that was proposed to be responsible for non-specific effects of classical antisense ODNs.[14]

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References

1 P. C. Zamecnik and M. L. Stephenson, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 280.
2 *Oligodeoxynucleotides*, ed. J. S. Cohen, Macmillan, London, 1989.
3 *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*, ed. E. Wickstrom, Wiley-Liss, New York, 1991.
4 E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90**, 543.
5 P. B. Dervan, *Science*, 1986, **232**, 464.
6 N. T. Thuong and C. Hélène, *Angew. Chem.*, *Int. Ed. Engl.*, 1993, **32**, 666.
7 T. Skorski, M. Nieborowska-Skorska, P. Wlodarski, G. Zon, R. V. Iozzo and B. Calabretta, *Blood*, 1996, **88**, 1005.
8 A. J. Gutierrez, M. D. Matteuci, D. Grant, S. Matsumura, R. W. Wagner and B. C. Froehler, *Biochemistry*, 1997, **36**, 743.
9 R. A. Stull and F. C. Szoka, *Pharm. Res.*, 1995, **12**, 465.
10 O. Zelphati and F. C. Szoka, *J. Controlled Release*, 1996, **41**, 99.
11 Y. Rojanasakul, *Adv. Drug Delivery Rev.*, 1996, **18**, 115.
12 J. W. Nyce and W. J. Metzger, *Nature* (*London*), 1997, **385**, 721.
13 J. J. Toulmé, C. Boiziau, B. Larrouy, P. Frank, S. Albert and R. Ahmadi, in *DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Diseases*, ed. B. Meunier, Kluwer, Dordrecht, 1996, pp. 271–288.
14 M. Pitié, C. Casas, C. J. Lacey, G. Pratviel, J. Bernadou and B. Meunier, *Angew. Chem.*, *Int. Ed. Engl.*, 1993, **32**, 557.
15 P. Bigey, G. Pratviel and B. Meunier, *Nucleic Acids Res.*, 1995, **23**, 3894.
16 M. Pitié and B. Meunier, *J. Biol. Inorg. Chem.*, 1996, **1**, 239.
17 B. Mestre, G. Pratviel and B. Meunier, *Bioconjugate Chem.*, 1995, **6**, 466.
18 B. Mestre, A. Jakobs, G. Pratviel and B. Meunier, *Biochemistry*, 1996, **35**, 9140.
19 V. Duarte, G. Pratviel, B. Meunier, M. Berton, S. Sixou and G. Favre, *New. J. Chem.*, 1997, **21**, 55.
20 M. Pitié, J. Bernadou and B. Meunier, *J. Am. Chem. Soc.*, 1995, **117**, 2935.
21 L. Wachter, J. A. Jablonski and K. L. Ramachandran, *Nucleic Acids Res.*, 1986, **14**, 7985.
22 C. Casas, K. J. Lacey and B. Meunier, *Bioconjugate Chem.*, 1993, **4**, 366.
23 *Handbook of Biochemistry and Molecular Biology Nucleic Acids*, ed. G. Fasman, CRC Press, Boca Raton, FL, 3rd edn., 1975, p. 175.
24 T. Inoue and T. R. Cech, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 648.
25 G. J. Murakawa, C. B. Chen, M. D. Kuwabara, D. P. Nierlich and D. S. Sigman, *Nucleic Acids Res.*, 1989, **17**, 5361.
26 S. Shibahara, S. Mukai, T. Nishihara, T. Inoue, E. Ohtsuka and H. Morisawa, *Nucleic Acids Res.*, 1987, **15**, 4403.
27 B. Larrouy, C. Blonski, C. Boizian, M. Stuer, S. Moreau, D. Shire and J. J. Toulmé, *Gene*, 1992, **121**, 189.

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